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ABSTRACTS:
IN THIS ISSUE

THE TOXIC POTENTIAL OF TRACE METALS IN FOODS. A Review. E. SOMERS. *J. Food Sci.* 39, 215–217 (1974)–Our foods contain trace amounts of a wide range of heavy metals: some of these have a biochemical function, others are contaminants. Trace metals can contaminate foods through agricultural technology, industrial pollution, geological sources and food processing. Some results from recent Canadian monitoring surveys are given with particular emphasis on data from lead and cadmium analyses. Consideration is given to the criteria that can be used to determine the priorities in the design of a monitoring program for trace metals in foods.

DENATURATION OF PLANT PROTEINS RELATED TO FUNCTIONALITY AND FOOD APPLICATIONS. A Review. Y.V. WU & G.E. INGLETT. *J. Food Sci.* 39, 218–225 (1974)–Proteins can be denatured by heat, changes in pH, organic solvents, detergents, urea, guanidine hydrochloride or other methods that modify the secondary, tertiary or quaternary structure, without breaking any covalent bonds. Physical-chemical measurements or functionality related to denaturation include solubility, viscosity, dissociation into subunits, sedimentation constant, optical rotation, association and ultraviolet spectra. The relationship between pH, temperature and rate of denaturation of wheat and soy proteins is complex. Optimum heat treatment of soy flakes, for example, inactivates nearly all biologically active components, but the protein retains most of its functionality. Knowledge about protein denaturation helps to produce food products with desirable functional properties.

SPINNING OF ZEIN. E. BALMACEDA & C-K. RHA. *J. Food Sci.* 39, 226–229 (1974)–An experimental study of the variables in the wet spinning process for zein is presented. The results indicate conditions at which spinning of zein is possible and a spinnability curve, which shows the region where spinning is possible, has been defined. The spinnability curve tends to be higher for larger extrusion velocities, lower dope concentrations, higher bath temperatures, and larger spinnerette diameters.

CONDITIONS FOR THE SEPARATION OF OIL AND PROTEIN FROM COCONUT MILK EMULSION. K.G. GUNETILEKE & S.F. LAURENTIUS. *J. Food Sci.* 39, 230–233 (1974)–Coconut milk extracted from fresh coconuts, was centrifuged to obtain cream and skim milk. The whole mass of cream was chilled to 17°C or below. It was established that 17°C was the critical temperature for subsequent phase separation. At 17°C, crystallization of the oil phase was observed under the microscope. On warming to 25°C, the emulsion broke with separation of oil and protein. This process differs from similar processes in that no enzymes are used and the temperature of the whole mass of cream has to be lowered only to 17°C. The oil obtained did not differ significantly from commercially available oil. Amino acid composition of different protein fractions was determined.

SWELLING STRESS AND HYDROSTATIC COMPRESSIBILITY OF GROUND CORN AND ITS CONSTITUENTS. M. KUMAR. *J. Food Sci.* 39, 234–238 (1974)–Swelling stresses and hydrostatic compressibilities of ground whole corn, endosperm and germ were determined by using the theory of swelling stresses in gels. The ground corn and its constituents were considered as gels. The magnitude of swelling stresses in the ground corn materials was in order of several thousand pounds per square inch at low equilibrium moisture contents, when subjected to increased vapor pressure. The hydrostatic compressibility for ground whole corn, endosperm and germ increased with an increase of moisture content.

AIR-FLUIDIZED TOASTING OF WHOLE KERNEL WHEAT–PROCESSING VARIABLES AND FUNCTIONAL PROPERTIES FOR FOOD APPLICATIONS. M.E. LAZAR, A.P. MOSSMAN & J.M. WALLACE. *J. Food Sci.* 39, 239–243 (1974)–Processing variables affecting toasted wheat properties were studied. For maximum gelatinization of starch, substantial moisture, up to 60% of the weight of the dry wheat, must be infused into the whole kernel and equilibrated prior to the heat treatment. Moistening, tempering and toasting optima were established. Cold paste viscosities can be reduced by over-toasting, as well as under-toasting. Heating rates must maximize starch gelatinization before moisture is evaporated. Enzyme inactivation, which affects storage stability as well as viscosity values, was not complete in 20 sec at 300°F (mass-avg kernel temp 275°F) but was complete in only 15 sec at 400°F (t_{MA} = 323°F). Toasting expands the kernel twofold or more and creates a porous endosperm structure which absorbs water readily. Several applications were tested, such as soup and sauce thickeners, breaders and cereals for domestic and foreign feeding programs.

ENRICHMENT OF TORTILLAS WITH SOY PROTEINS BY LIME COOKING OF WHOLE RAW CORN-SOYBEAN MIXTURES. F.R. DEL VALLE & J. PEREZ-VILLASEÑOR. *J. Food Sci.* 39, 244–247 (1974)–A simple and inexpensive method for enriching tortillas with soy proteins was studied. The method consisted of application of the traditional process for making corn tortillas to whole raw corn-soybean mixtures: lime cooking of a mixture, allowing the cooked mixture to stand overnight in the cooking liquor, washing the mixture with tap water, grinding the cooked mixture into a dough, and making tortillas from the dough. This method of enrichment was found to give tortillas with significantly higher protein content and protein quality than normal unenriched tortillas. PER and NPU values of tortillas containing 8% and 16% soya were found to be comparable to those obtained with tortillas enriched with soy flour or with amino acids lysine and tryptophane. The presence of soybeans in tortillas so enriched was found to be not significantly detectable by a trained panel, at least up to a soya level of 16%. Toxic factors present in raw soybeans were not detected in enriched tortillas by the urease method, having been apparently inactivated by the tortilla-making process. Enrichment by this method is cheaper than enrichment with soy flour or with amino acids. The method permits uniform introduction of soya proteins within the tortillas, and changes neither food recipes nor eating habits.

SLUSH EVAPORATION: A NEW METHOD FOR CONCENTRATION OF LIQUID FOODS. C.M. LOWE & C.J. KING. *J. Food Sci.* 39, 248–253 (1974)–Slush evaporation involves removal of water by combined evaporation and sublimation from the partly frozen state. It provides dehydration rates considerably greater than those attainable with freeze drying, but at the same time gives very good retention of volatile flavors and aroma. Experiments are reported which quantitatively examine the retention of natural volatile compounds during slush evaporation, as a function of temperature, drying rate, mode of drying, sample thickness, initial concentration, the fraction of water removed and the presence or absence of foam. A second set of experiments investigated the effect of these variables upon dissolved-solids loss, with losses of 0.1–0.5% being found under typical conditions for concentration.

EFFECTS OF pH ON QUALITY OF STORED TOMATO JUICE. R.H. DOUGHERTY & P.E. NELSON. *J. Food Sci.* 39, 254–256 (1974)–Tomato juice was prepared by the hot-break method and filled hot into cans containing sufficient concentrated HCl to adjust pH to 4.0, 3.5, 3.0, 2.5 and 2.0. Controls were also prepared with no HCl added. Product was stored at ambient (18–22°C) and 40°C up to 3 months. As pH decreased below 4.0, consistency also showed a significant decline through pH 2.0. At the same time there was no apparent pH effect on serum viscosity. Further studies indicated that this effect was noticeable after 4 hr, at pH 3.0 to 5.0. Addition of as much as 1% NaCl to tomato juice had no effect on consistency of tomato juice. Lightness (Hunter color L) decreased with pH after 3.5, redness (a_L) decreased after pH 4.0, and yellowness (b_L) decreased after pH 3.0. All storage effects were more profound at

the higher temperature. The storage of tomato juices at pH less than 4.0 does not appear to be advantageous to the processor.

pH AND ACIDIC STABILITY DURING STORAGE OF ACIDIFIED AND NONACIDIFIED CANNED TOMATOES. D.R. SCHOENEMANN, A. LOPEZ & F.W. COOLER. *J. Food Sci.* **39**, 257–259 (1974)–A study was conducted to evaluate pH stability of acidified tomato packs during storage for 1 yr at 24°C, to associate pH stability with tomato composition, and to determine the variability of pH within the canned product caused by tomato cell walls and other structural matter. Results show that pH of acidified tomatoes is as stable during heat processing and storage as that of nonacidified tomatoes.

A LABORATORY STUDY ON COUNTERCURRENT DESALTING OF PICKLES. J.L. BOMBEN, E.L. DURKEE, E. LOWE & G.E. SECOR. *J. Food Sci.* **39**, 260–263 (1974)–The technique of countercurrent leaching was applied to the desalting of salt stock pickles. Experiments and calculations showed that a spent brine of 13–14% salt can be produced in 4–6 stages and that pickles can be desalted in 48 hr. The volume of waste effluent from countercurrent leaching is considerably less than the amount from conventional leaching, and the higher concentration of salt in the effluent makes salt recovery much more practical from a cost standpoint.

YIELD, PROXIMATE COMPOSITION AND MINERAL ELEMENT CONTENT OF THREE CULTIVARS OF RAW AND ROASTED PEANUTS. N.L. DERISE, H.A. LAU, S.J. RITCHEY & E.W. MURPHY. *J. Food Sci.* **39**, 264–266 (1974)–Yield of kernels, proximate composition (moisture, crude fiber, fat, protein and ash), and content of nine mineral elements–Ca, Mg, P, Na, K, Fe, Cu, Zn and Mn–are reported for three cultivars of peanuts, raw and roasted. Roasting resulted in higher concentrations of all nutrients measured in the peanuts except moisture and sodium, whose contents decreased. Although statistically significant differences in nutrient content were noted among cultivars, these differences were of little practical importance. Data on retentions of proximate components and mineral elements with roasting of peanuts indicate that except for moisture and sodium (which were low) and ash (which was high), retentions were close to 100%.

INDIVIDUAL HEAT TRANSFER MODES IN BAND OVEN BISCUIT BAKING. C.N. STANDING. *J. Food Sci.* **39**, 267–271 (1974)–The individual modes of heat transfer, e.g., conduction, radiation and convection, are considered for the processing of products in conventional band ovens. A specific theoretical model is considered for the baking of biscuits in an indirect fired oven. Values of individual heat transfer constants in the theoretical model and major effects of the individual modes of heat transfer were determined using lab scale heating devices. Extrapolating these results to a band oven baking process, the model indicated a heat transfer profile of about 20% heat transferred by conduction, about 45% by radiation and about 35% by forced convection in the band oven, with about half the heat being absorbed as sensible heat, and about half as latent heat.

PREDICTING AN EQUILIBRIUM STATE VALUE FROM TRANSIENT STATE DATA. K. HAYAKAWA. *J. Food Sci.* **39**, 272–275 (1974)–Values of many biological, chemical and physical factors approach their respective equilibrium state values when constant reactive potentials are applied to these factors. These equilibrium state values are frequently required for analyzing their transient processes. Mathematical analyses of available data on transient state values of various factors revealed that most of these values could be estimated by using an exponential formula. A procedure was developed for the reliable prediction of equilibrium values through the proper application of the formula to transient state values. For this development, it was assumed that transient state values were given at equal time intervals. According to a number of experimental computations, errors of equilibrium state values predicted by the developed procedure were less than 5%. The procedure estimated equilibrium values with less than 50% the time effort required by conventional procedures and will be of great use for reliably predicting equilibrium state values, especially when a factor converges very slowly to its equilibrium condition.

EVALUATION OF THERMAL PROCESSES FOR CONDUCTION HEATING FOODS IN PEAR-SHAPED CONTAINERS. J.E. MANSON, C.R. STUMBO & J.W. ZAHRADNIK. *J. Food Sci.* **39**, 276–281 (1974)-A numerical model was developed to study transient heat conduction in a pear-shaped can. The model was extended to perform bacterial lethality and nutrient retention calculations for foods processed in pear-shaped containers. Transient temperature distributions and lethality predictions obtained from the numerical model when applied to finite cylinders, compared favorably with previously accepted solutions. Calculations of integrated F_s values of processes for pear-shaped containers indicated up to 50% overprocessing by the single point lethality concept. An "equivalent cylinder" was defined as one having a geometry index and characteristic heat transfer length corresponding to those of a pear-shaped container. Process evaluations for "equivalent cylinders" were in agreement with solutions obtained for the pear-shaped model.

CHARACTERIZATION OF MECHANICALLY DEBONED HOT AND COLD MUTTON CARCASSES. R.A. FIELD, M.L. RILEY & M.H. CORBRIDGE. *J. Food Sci.* **39**, 282–284 (1974)–Bone from one side of chilled mutton carcasses and bone from one side of hot mutton carcasses were physically separated by hand while the other sides were mechanically deboned. Yield of boneless carcass was similar for hot machine-boned and hot hand-boned carcasses. Cold machine-boned carcasses had a slightly lower yield of lean meat than cold hand-boned carcasses. Approximate analysis of meat from cold hand-boned, cold machine-boned, hot hand-boned and hot machine-boned carcasses was similar. However, calcium and hydroxyproline contents were lower for hot machine-boned than for cold machine-boned carcasses. Emulsion stability and total pigment concentration were higher for mechanically deboned meat. Organoleptic evaluations favored bologna made with hand-boned meat.

INFLUENCE OF YIELD ON CALCIUM CONTENT OF MECHANICALLY DEBONED LAMB AND MUTTON. R.A. FIELD, M.L. RILEY & M.H. CORBRIDGE. *J. Food Sci.* **39**, 285–287 (1974)–Mutton carcasses and lamb carcass cuts were mechanically deboned at different settings to give variable yields of mechanically deboned meat. Higher yields of mechanically deboned meat were related to higher calcium and fat percentages. Lamb breasts had the lowest bone percentage of any of the cuts and the mechanically deboned meat from breasts had the lowest calcium content when compared to deboned meat from other lamb cuts or mutton carcasses. Necks had the highest bone percentage and the highest calcium content in the mechanically deboned meat. Data are presented which show that mechanically deboned meat is not homogenous as it is extruded from the cylinder. Palatability of bologna made with mechanically deboned meat increased as the size of cylinder holes through which the meat was extruded decreased.

EFFECTS OF SOY CURD ON THE ACCEPTABILITY AND CHARACTERISTICS OF BEEF PATTIES. V. YEO, G.H. WELLINGTON & K.H. STEINKRAUS. *J. Food Sci.* **39**, 288–292 (1974)–Soy curd-beef patties were made containing 0%, 5%, 10%, 20%, 75% and 100% (w/w) of curds which had been pressed at 300 psi, 600 psi and 1100 psi during manufacture. Taste panel tests showed that by increasing pressure on the soy curd or by the addition of flavoring to the curd before patty formation, increases in soy concentration became less detectable and the acceptability of the patties was drastically increased. Soy curd-beef patties made with 1100 psi curd and with color and flavor added were favorably accepted even at levels as high as 75% curd (w/w). The easy detectability of flavored soy in patties did not greatly change the high acceptability of the patties. The chemical composition, functional characteristics and physical properties of the soy-beef patties were also studied.

SPECIFICITY OF LIPOLYSIS DURING DRY SAUSAGE RIPENING. D. DEMEYER, J. HOOZEE & H. MESDOM. *J. Food Sci.* **39**, 293–296 (1974)–The amounts of total and individual fatty acids present in triglycerides (TG), free fatty acids (FFA), diglycerides (DG), monoglycerides (MG) and polar lipids (PL) were determined at various stages of dry sausage ripening using a combination of thin layer and gas chromatography. Total FFA increased from 1 to 5% of total fatty acids and DG fatty acids from 0.5 to 4%, whereas TG fatty acids showed a corresponding decrease. The rate of liberation of FFA was in the order 18:2 > 18:1 > 18:0 > 16:0 while MG and DG were enriched in 16:0. These results suggest specificity of lipolysis.

ABSTRACTS:
IN THIS ISSUE

STOICHIOMETRY OF CARBOHYDRATE FERMENTATION DURING DRY SAUSAGE RIPENING. A. De KETELAERE, D. DEMEYER, P. VANDEKERCKHOVE & I. VERVAEKE. *J. Food Sci.* 39, 297–300 (1974)–During ripening of dry sausage, disappearance of carbohydrates and production of lactate, volatile fatty acids, pyruvate and carbonyls was followed. When expressed as mmoles/100g of dry matter, carbohydrate disappearance (ca. 19 mmoles/100g dry matter) could be accounted for by production of lactate (ca. 17 mmoles) and acetate (ca. 2 mmoles) in two similar experiments. No differences were observed due to the presence of a starter culture in one experiment. In a third experiment, carbohydrate disappearance (ca. 16 mmoles) could only partly be accounted for by lactate (ca. 19 mmoles) and acetate (ca. 2 mmoles) production. Oxidative dissimilation of carbohydrates by micrococci during the early stages of ripening in the third experiment is offered as possible explanation.

CHANGES IN NONPROTEIN NITROGEN COMPOUNDS DURING DRY SAUSAGE RIPENING. N. DIERICK, P. VANDEKERCKHOVE & D. DEMEYER. *J. Food Sci.* 39, 301–304 (1974)–Concentration changes for ammonia, total and individual free amino acids, total peptides, nucleotides, nucleosides and some individual amines were followed during ripening of dry sausage, with and without "starter culture." A decrease was observed for peptides, nucleotides, glutamic acid, histidine, tyrosine and ornithine, an increase for all other compounds, being most intense for total free amino acids during the first days of ripening. The rate of free amino acid production exceeded the rate of ammonia production. The presence of a starter culture intensified free amino acid production and peptide disappearance. A tenfold increase in the concentrations of histamine, tyramine and putrescine was observed in the presence of a starter culture.

THE ROLE OF LIGHT AND SURFACE BACTERIA IN THE COLOR STABILITY OF PREPACKAGED BEEF. L.D. SATTERLEE & W. HANSMEYER. *J. Food Sci.* 39, 305–308 (1974)–When intact beef was packaged in a PVC film, stored at 5°C and illuminated with 250 ft-c light, a significant alteration in color stability was observed when compared to storage in the dark. Fluorescent lights, emitting light of lower wavelengths, were most detrimental to color, in comparison to incandescent lights. Upon prolonged storage large bacterial numbers on the surface created a reducing environment and caused the conversion of surface MetMb to reduced Mb, which was responsible for the purple color that developed. Gel electrophoresis indicated that no significant alterations occurred in the structure of myoglobin or hemoglobin (color pigments) upon storage at 5°C for as long as 18 days. Color instability of meat stored under soft white fluorescent light is almost entirely due to the effect of the light, whereas color instability when stored under incandescent light is due to a combination of light energy and bacterial growth.

CONTINUOUS MICROWAVE STERILIZATION OF MEAT IN FLEXIBLE POUCHES. J.A. AYOUB, D. BERKOWITZ, E.M. KENYON & C.K. WADSWORTH. *J. Food Sci.* 39, 309–313 (1974)–Feasibility studies originated earlier in this laboratory, on microwave energy thermal processing of foods, were continued. A means of measuring temperature distribution, as well as a photometric method for determining time/temperature integration within thermally processed foods, were developed. A knowledge of the processing parameters involved in the preservation of foods by microwave energy was obtained. Feasibility of the process was demonstrated.

EFFECT OF FRYING AND OTHER COOKING CONDITIONS ON NITROSOPYRROLIDINE FORMATION IN BACON. J.W. PENSABENE, W. FIDDLER, R.A. GATES, J.C. FAGAN & A.E. WASSERMAN. *J. Food Sci.* 39, 314–316 (1974)–Nitrosopyrrolidine (NO-Pyr) formation in bacon is primarily dependent on frying temperature and not time. Cooking methods affect the amount of NO-Pyr formed: pan frying produces the highest level of NO-Pyr with variable concentrations formed on baking, broiling and cooking in a "baconer." Microwave oven treatment produced the lowest amount of NO-Pyr. A model system study of the decarboxylation of nitrosoproline shows this precursor, which may be present in bacon, is maximally converted to NO-Pyr at 185°C, near the recommended temperature for frying.

RELATIONSHIP OF AGE TO GENERAL COMPOSITION, SKIN THICKNESS, PHOSPHOLIPID CONTENT AND ENZYMATIC ACTIVITY IN TURKEYS. R.M. WANGEN & W.W. MARION. *J. Food Sci.* 39, 317–320 (1974)–Meat samples from Large White male turkeys, taken at 4-wk intervals between 12 and 24 wk, indicated that "finish," estimated by measuring skin thickness, increased significantly between 20 and 24 wk. The amounts of moisture and lipids, but not protein, changed with age of turkeys. The phospholipid portion of total lipid also changed (decreased) with increasing age. Means for lactate dehydrogenase and β-hydroxybutyrate dehydrogenase generally were smaller for Pectoralis major than for Biceps femoris or Gastrocnemius. Glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase were detected only on occasion in the muscles used. Variation in the enzymatic activity and the composition of muscles imply differences in the capability of muscles to accumulate lipid as the turkey increases in age.

EFFECT OF ESSENTIAL MINERALS ON CADMIUM TOXICITY. A Review. M.R. SPIVEY FOX, *J. Food Sci.* 39, 321–324 (1974)–Cadmium (Cd) is a toxic element that has no known beneficial effects in living organisms. At levels several-fold above the average intake of 50 μg/day for man in the U.S., Cd can interfere with the metabolism of the essential elements iron, calcium, zinc, manganese and copper. Antagonism of the first two elements has been observed in man and the last three in experimental animals only. When Cd was fed to young Japanese quail at levels ranging from 0.78 to 80 mg/kg diet, Cd was markedly concentrated in duodenal tissue at the lowest intake levels. Maximal duodenal Cd concentrations were obtained with 20 mg Cd/kg diet. Dietary Cd caused decreased concentrations of essential mineral elements in duodenal and other tissues. A major effect of high levels of dietary Cd appears to be interference with absorption of essential minerals. Excess dietary intakes of essential minerals can either decrease or eliminate some of these effects of Cd, as well as result in decreased Cd concentrations in the kidney, a target organ for Cd accumulation and functional damage. Environmental toxicants such as Cd cannot be completely avoided. It is important, therefore, to define low intake levels of essential nutrients at which toxicity of Cd is exacerbated and high intake levels of essential nutrients at which toxicity of Cd is minimized.

COLOR OF ANTHOCYANIN SOLUTIONS EXPRESSED IN LIGHTNESS AND CHROMATICITY TERMS. Effect of pH and Type of Anthocyanin. J.P. VAN BUREN, G. HRAZDINA & W.B. ROBINSON. *J. Food Sci.* 39, 325–328 (1974)–The combined effects of pH (3.0–3.8) and anthocyanin concentration on the color and spectral characteristics of aqueous solutions were studied with a number of anthocyanin glucosides. Color data, obtained with a Hunter Color Meter, were treated by plotting the "L" values against the corresponding "a," "b," hue angle and saturation chromaticity values, showing that maximum chromaticity values occurred at middle "L" values, and that these chromaticity values were increased when the pH was decreased. The predominantly red hues of anthocyanin solutions had more purple character at very low or very high "L" values, and when the pH was raised, or the anthocyanin had a greater degree of hydroxylation or methoxylation or when the anthocyanin was glycosylated in the 5 position as well as the 3 position. The range of hues, from orange to purple, obtainable with individual anthocyanins was dependent on the type of anthocyanin. At equal concentrations and pH the 3-monoglucosides gave darker solutions (lower "L" values) than 3,5-diglucosides. Wavelengths of maximum absorbance decreased as the an-

thocyanin concentration increased. The results might find application in standardizing the color of beverages containing anthocyanins.

ANTHOCYANINS IN BARBERA GRAPES. H.C. SAKELLARIADES & B.S. LUH. *J. Food Sci.* **39,** 329–333 (1974)–The anthocyanins of Barbera grapes were extracted with methanol containing 0.1% HCl and purified by adsorption on cation-exchange resin. The individual pigments were separated by paper chromatography. The anthocyanins were identified by their R_f values, sugar moiety, alkaline degradation products and spectral characteristics. The pigments were identified as delphinidin-3-monoglucoside, cyanidin-3-monoglucoside with caffeic acid, petunidin-3-monoglucoside, cyanidin-3-monoglucoside with p-coumaric acid, malvidin-3-monoglucoside, malvidin-3-monoglucoside with p-coumaric acid, peonidin-3-monoglucoside, peonidin-3-monoglucoside (acylated) and malvidin-3-monoglucoside (acylated). Knowledge of the composition and inheritance pattern of the anthocyanin pigments in grapes is extremely valuable to the plant breeders in developing new grape varieties with the desirable characteristics. Barbera grapes may be used for blending with other varieties poor in red color to make red wines of more attractive color.

COLOR STABILITY OF BETANIN. J.H. von ELBE, I-Y. MAING & C.H. AMUNDSON. *J. Food Sci.* **39,** 334–337 (1974)–Some chemical and physical factors were evaluated for their effects on stability of betanin in model systems and in beet juice. Results indicate that the stability of betanin color is pH sensitive and generally is less than that of artificial dyes. The color of betanin is most stable between pH 4.0 and 6.0. Thermostability of betanin was pH dependent and was greatest between pH 4.0 and 5.0. The half-life of betanin at pH 5.0 and 100°C was 14.5 min, and increased in an unprotected model system to 1150 min at 25°C. The rate at which betanin degraded in model systems was affected by air and/or light. The cumulative effect of these two conditions was a reduction in the half-life of betanin by 28.6%. The half-life of betanin in beet juice at pH 5.0 was greater than in model systems. Despite limited stability of betanin in model systems, there are many foods in which betanin could be used as a colorant. Foods to be colored with betanin should have a pH between 4.0 and 7.0, be exposed to low temperatures and be protected from air and/or light to achieve maximum color stability.

INACTIVATION AND REGENERATION OF IMMOBILIZED CATALASE. S.S. WANG, G.E. GALLILI, S.G. GILBERT & J.G. LEEDER. *J. Food Sci.* **39,** 338–341 (1974)–Catalase was immobilized on collagen membrane. The inactivation of immobilized catalase by 0.01M and 0.1M H_2O_2 was reported. After the initial stage of inactivation, a stable catalatic activity as measured in a continuous flow of 0.01M H_2O_2 through a modular reactor was observed for longer than 20 days (2.6 min residence time). The regeneration of catalatic activity from the 0.1M H_2O_2 inactivated catalase occurred after incubating the inactivated modular reactor with 0.01M phosphate buffer, pH 6.8. The amount of activity regenerated is directly proportional to the time of incubation.

SCANNING ELECTRON MICROSCOPE STUDIES ON DRY BEANS. Effects of Cooking on the Cellular Structure of Cotyledons in Rehydrated Large Lima Beans. L.B. ROCKLAND & F.T. JONES. *J. Food Sci.* **39,** 342–346 (1974)–The scanning electron microscope was used to make a photographic comparison between the cellular characteristics of raw, partially cooked, and completely cooked, water-soaked as well as quick-cooking beans. The cooking process involved gelatinization of starch granules contained within integral cell units and concurrent dispersion of intercellular components of the middle lamella which facilitated separation of intact cells without rupture of cell walls. Mechanical stresses due to starch gelatinization, protein denaturation, swelling and heat convection may have promoted cell separation. Except for differences in the rates at which these processes progressed there were no conspicuous differences between the structural characteristics of the water-soaked beans and the quick-cooking beans.

L-ASPARTYL-L-PHENYLALANINE METHYL ESTER (ASPARTAME) AS A SWEETENER. M.R. CLONINGER & R.E. BALDWIN. *J. Food Sci.* **39,** 347–349 (1974)–In aqueous solutions, L-aspartyl-L-phenylalanine methyl ester (aspartame) was 182 times sweeter than 2% sucrose but only 43 times sweeter than 30% sucrose according to rank analyses of scores from 20 judges. In buffer solutions (pH 3.2), pH was elevated by 0.025% and 0.12% aspartame and not by 4% or 12% sucrose, but no effect on sweetness equivalents or sourness was detected. Sweetness of 0.025% aspartame was enhanced by gelatin (1.5%) and methocel (1%). Enhancement also occurred when gelatin was combined with 0.12% aspartame. Sweetness ranks were not significantly affected by 1% carboxymethylcellulose or gum arabic. Viscosity was not a reliable indicator of differences in sensory response for thickness.

CARBONYL RETENTION IN MODEL SYSTEMS AND BERMUDA ONION JUICE DURING LYOPHILIZATION: EFFECT OF SIMPLE CARBOHYDRATES, BINARY CARBOHYDRATE MIXTURES AND SUCROSE INVERSION. R.P. OFCARCIK & E.E. BURNS. *J. Food Sci.* **39,** 350–353 (1974)–Information leading to the improvement and understanding of volatile retention was developed. This was accomplished by studying the effects of selected process variables on retention of carbonyl components in model carbohydrate-pyruvic acid systems and in Bermuda onion juice. Variables studied were carbohydrate type, concentration, binary mixtures and sucrose inversion. Increases in carbohydrate concentration increased retention in both model systems and onion juice. Increases in retention were greater at low carbohydrate levels (5–10%) than at high concentration (15–20%). Distinct differences in retention were found in model systems, but not in onion juice. A synergistic retention effect was observed in binary mixtures of lactose and glucose in model systems. Retention appeared to be a function of percent hydrolysis of sucrose in model systems. At low inversion levels, retention was high and uniform. At high hydrolysis levels, retention decreased to a low retention level which remained uniform to 100% inversion.

RELATIONSHIPS AMONG TITRATABLE ACIDITY, pH AND BUFFER COMPOSITION OF TOMATO FRUITS. K.N. PAULSON & M.A. STEVENS. *J. Food Sci.* **39,** 354–357 (1974)–Relationships among pH, titratable acidity (TA) and buffer composition of tomato fruits were studied using analytical data, from 25 divergent accessions, and from previous work. Citric and malic acids, and phosphate account for about 93% of the TA of tomatoes. An increase in phosphate concentration results in a decrease in $[H^+]$, whereas an increase in citric and malic acids increases $[H^+]$. These relationships probably exist because of differences in the origins of phosphate and the two acids. Variation in phosphate concentration can be of practical value in efforts to maintain a safe processing pH in low sugar, low acid varieties because phosphate has a relatively large effect on $[H^+]$ with a minimal effect on TA.

STARCH AND PECTIC SUBSTANCES AS AFFECTED BY A FREEZE-THAW POTATO GRANULE PROCESS. B. OORAIKUL, G.J.K. PACKER & D. HADZIYEV. *J. Food Sci.* **39,** 358–364 (1974)–Changes in total content and fractions of pectic substances and starch were studied in a freeze-thaw process for potato granule production consisting of peeling, steam cooking, hot mashing, freezing and thawing, pre-drying, granulation, drying, cooling and sifting. Pectic substances in raw potatoes (expressed as mg uronide/netted Gem 100g dry wt) were 202.4 for water soluble and only 80 for the calgon-soluble fractions. Water soluble pectic substances increased sixfold after cooking, and calgon-soluble fraction about threefold. Little change was observed in either fraction due to subsequent granule processing steps. There was no indication that pectic substances present in the extracellular matrix could have influenced the processing steps in granule production. Total starch comprised 68.4%/dry wt of raw potato, decreasing slightly after cooking, increasing up to the pre-drying step and stabilizing at a level of 83.7%. In contrast, free water-soluble starch, depended on the processing steps, being lower in cooked unmashed potatoes than in conditioned raw potatoes. Mashing slightly increased starch content while pre-cooling to 5.5°C and freezing to −20°C with subsequent thawing to room temperature substantially decreased starch content. Mechanical forces applied in subsequent steps brought about some increase while in the final product the content was related to particle size of the granules. The percentage of broken cells in cooked potatoes mashed at various temperatures appears to be dependent on the free extracellular starch present in cell binding matrix. Broken cell counts were substantially decreased when mashing was performed immediately after cooking at high temperature or when the mash was frozen and thawed. In the latter case determination of Blue Value Index suggested that a decrease in cell binding strength of the matrix occurred due to soluble starch retrogradation.

GLUTAMINE AS A PREDICTIVE MEASUREMENT IN THE QUALITY ASSESSMENT OF PROCESSED CARROT PUREE. T.C. BIBEAU, F.M. CLYDESDALE & F.M. SAWYER. *J. Food Sci.* **39,** 365–367

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(1974)—The aim of this investigation was the development of a simple quality test whereby knowing the amount of glutamine present in a fresh sample of carrots and the processing temperature, the amount of PCA and perhaps the acceptability of the final product might be determined. Two different varieties of carrots from two different geographical locations were packed in TDT tubes, flushed with nitrogen and sealed. A process of F_0 = 4.9 was given to each series of tubes at temperatures ranging from 240–300°F with 20°F increments. Following this, analyses were conducted for glutamine, pyrrolidone-carboxylic acid (PCA) and pH to determine the effect of initial concentration of glutamine and process temperature upon the formation of PCA. Taste thresholds for PCA were also determined both in model systems and in processed carrot puree. PCA formation was found to exhibit an inverse linear semilogarithmic plot with process temperature such that amounts could be predicted from initial glutamine contents for a given batch. Sensory studies indicated that the levels of PCA found in samples at the lower temperatures were near the taste thresholds established for this compound. With such results it is possible to predict the best process time and temperature for carrot puree based on the initial concentration of glutamine in order to minimize off-flavors due to PCA formation.

CERTAIN FUNCTIONAL PROPERTIES OF SUNFLOWER MEAL PRODUCTS. M.J.Y. LIN, E.S. HUMBERT & F.W. SOSULSKI. *J. Food Sci.* 39, 368–370 (1974)—Certain functional properties including water absorption, fat absorption, emulsification, whippability and foam stability were determined on the sunflower flour, protein concentrates and isolate. The results were also compared to those obtained on soy products. Data on water and fat absorption studies suggest that soy products are more hydrophilic in nature while sunflower material exhibited greater lipophilic properties than the soy products. Emulsification tests showed that sunflower flour was superior to all other soy and sunflower products. In general, whipping properties of soy and sunflower isolates were similar, while less whippability was observed for the soy flour and protein concentrates. Whipped foams produced by soy and sunflower protein isolates and sunflower flour were more stable than soy flour, soy and sunflower protein concentrates.

NUTRITIVE VALUE OF BREAKFAST CEREAL-MILK COMBINATIONS. M. WOMACK, D.A. VAUGHAN & L.R. MILLER. *J. Food Sci.* 39, 371–373 (1974)-The protein quality of 11 breakfast cereal-milk mixtures combined as normally eaten (1 oz cereal and 4 oz fluid milk) was determined by using a modified slope-ratio assay. Slope ratios (milk = 100) of three of the mixtures (oatmeal-milk, cream of rice-milk and shredded wheat-milk) were higher than that of milk alone. Using protein content and slope ratios it was calculated that 1 oz of the various cereals could increase potential protein value over that of milk alone by 26 to 117%, and that some of the mixtures could supply as much as 22% of the recommended daily allowance for an 8–10-yr-old child. It is suggested that information of this sort combining the protein quality of food mixtures as eaten with the protein content of a serving would be welcomed by consumers.

HYDROLYSIS OF LACTOSE IN ACID WHEY BY LACTASE BOUND TO POROUS GLASS PARTICLES IN TUBULAR REACTORS. L.E. WIERZBICKI, V.H. EDWARDS & F.V. KOSIKOWSKI. *J. Food Sci.* 39, 374–378 (1974)—Partially purified lactases (β-galactosidase, EC3.2.1.23) from *Aspergillus niger* were covalently bound to acetone-silanized, diazotized porous glass particles (mean pore diam, 86.5 nm; particle diam, 75–125 μm). The temperature (~55°C) and pH (3.5–4.0) optima were established in acid whey containing 5% total whey solids. Lactose hydrolyzing activity was stable during 43 days of semicontinuous operation at 55°C with reconstituted acid whey (pH = 4.5) at total solids (TS) concentrations varied between 4 and 25% and to which 5 ml/liter toluene had been added to retard microbiological contamination. Kinetic experiments with acid wheys gave results reproducible when assayed by both thin layer chromatography (TLC) and glucose oxidase (GS) procedures, although the TLC method gave systematically higher values at intermediate conversions and high TS concentrations. The kinetics of lactose hydrolysis by columns of lactase bound to porous glass (LBG) of 1.6 cm diam and lengths of 1, 5 and 10.5 cm showed some evidence for reduction of the rate of lactose hydrolysis by film diffusion resistances. Calculations using correlations for packed beds also suggest the presence of diffusional effects. Lactose was hydrolyzed slightly more rapidly in whey than in deproteinized whey. Lactose hydrolysis rates in both types of reconstituted whey increased as the TS concentrations increased from 4 to 25%. The data did not obey any of a number of integrated reaction rate equations, including a rate equation which accounted for competitive product inhibition of Michaelis enzyme kinetics. Failure of simple models is due in part to diffusional resistances and in part to the large range of concentrations studied. The LBG preparation retained appreciable activity after more than 8 months of frequent use at a wide variety of conditions.

EFFECT OF ENZYMATIC HYDROLYSIS ON SOME FUNCTIONAL PROPERTIES OF WHEY PROTEIN. C.A. KUEHLER & C.M. STINE. *J Food Sci.* 39, 379–382 (1974)—A study was undertaken to further elucidate the functional properties of whey protein with respect to foaming and emulsifying capacities and to observe the effect of enzymatic hydrolysis on these properties. Emulsion capacity decreased as proteolysis continued suggesting there is an optimum mean molecular size of the proteins involved which is lower than that of casein. Heat treatment of the reconstituted protein concentrate was necessary for foam stability; specific volume and foam stability increased directly with temperature of heating. Re effect of pH on whippability, data indicate that the greater the net charge the greater the tendency to foam. A limited amount of hydrolysis appears desirable to increase foaming but greatly decreases foam stability.

ATLANTIC QUEEN CRAB (Chionoecetes opilio), JONAH CRAB (Cancer borealis), AND RED CRAB (Geryon quinquedens). Proximate Composition of Crabmeat from Edible Tissues and Concentrations of Some Major Mineral Constituents in the Ash. B.H. LAUER, M.C. MURRAY, W.E. ANDERSON & E.B. GUPTILL. *J. Food Sci.* 39, 383–385 (1974)—The gross composition of the edible tissues of Atlantic Queen crab, Jonah crab and Red crab was determined by standard analytical methods and results compared to those obtained for analysis of edible tissues in other species of crab. In addition, the concentrations of various cationic constituents of the ash (Na^+, K^+, Ca^{++} and Mg^{++}) and phosphate were estimated in hydrogen peroxide-sulfuric acid digests of meat samples of each of the three crab species. The nutritive value of crabmeat from these species is discussed in terms of protein content, mineral composition and overall calorific value.

AMINO ACID, FATTY ACID AND PROXIMATE COMPOSITION OF SNOW CRAB (Chionoecetes bairdi). R.A. KRZECZKOWSKI & F.E. STONE. *J. Food Sci.* 39, 386–388 (1974)—The proximate analysis and fatty acid content of the lipids were determined on four separate types of cooked snow crabmeat plus composite samples of the cooked meat and a commercially canned product. The amino acid, sodium and potassium content were determined on a commercially canned product. These results show that snow crabs contain a considerable amount of meat (33.7%) which is high in protein (18.8%) and nutrition. The fatty acid content is over 50% polyunsaturated. Fatty acid 20:5 was the predominant acid (26.0–30.0%) followed by 18:1 (17.0–19.3%), 22:6 (15.0–16.6%) and 16:0 (12.5–13.5%). Amino acid content is similar to dungeness and Chesapeake Bay blue crab meat.

HEAT STABILITY OF CHICKEN ACTOMYOSIN. L.L. YOUNG. *J. Food Sci.* 39, 389–392 (1974)–The heat sensitivity of chicken actomyosin was determined under varying conditions of the pH and ionic environment and in the presence and absence of various additives. The emulsifying capacity of the protein was used as the index of heat damage. As the pH of the solvent was increased above 8.5, the stability of the protein increased. It was more stable in 0.6M KCl than in 0.2M KCl. Up to 6% sucrose significantly improved the stability. Polyphosphates were beneficial in stabilizing the actomyosin. This information should facilitate development of methods of heat pasteurizing mechanically deboned chicken meat.

RELATION BETWEEN ISOMETRIC TENSION, POSTMORTEM pH DECLINE AND TENDERNESS OF POULTRY BREAST MEAT. A.W. KHAN. *J. Food Sci.* 39, 393–395 (1974)–Postmortem changes in isometric tension, pH and shear force of poultry breast meat were studied to determine the relation between rigor contraction and tenderness. Strips of pectoralis major muscle immersed in isotonic saline solution held at 25°C developed measurable tension when their pH dropped to 6.1–6.3. The tension gradually increased until the pH dropped to a final value of 5.5–5.8, then started to decline. Muscle cooked at the start of tension development had the highest shear force. After that the shear force of the cooked meat gradually declined to a minimum value. The results indicate a high correlation (r=0.97) between ultimate shear force and degree of maximum tension developed during rigor.

EFFECTS OF PRE-RIGOR TENSION ON TENDERNESS OF INTACT BOVINE AND OVINE MUSCLE. D.R. BUEGE & J.R. STOUFFER. *J. Food Sci.* 39, 396–401 (1974)–Mechanical and weighted methods of applying pre-rigor tension to longissimus muscle of ovine and bovine carcasses conventionally hung by a hind shank were studied. The effect of tensioning of carcasses by adding 4.5 kg weights or mechanically stretching the back 5% in length was significant ($P < 0.01$) and as effective as greater forces in decreasing shear force values, decreasing fiber diameters and increasing sarcomere lengths. Chops and steaks from tensioned carcasses at 48 hr postmortem were as tender as those from nontensioned carcasses which had been aged 168–240 hr. Further aging of longissimus muscle of tensioned carcasses continued to decrease shear force values at approximately the same rate at which shear force was decreasing in nontensioned carcasses. The pre-rigor tension was also effective in reducing animal-to-animal tenderness variability.

TENDERNESS VARIATION IN OVINE LONGISSIMUS MUSCLE. D.R. BUEGE & J.R. STOUFFER. *J. Food Sci.* 39, 402–405 (1974)–31 lamb carcasses were split after slaughter and one side of each had pre-rigor tension applied to the longissimus muscle by weighted or mechanical methods. Warner-Bratzler shear values were obtained on each of three core positions within rib and within loin chop samples from each nontensioned and tensioned side. Differences in shear values between cores were significant ($P < 0.001$), and the interactions of muscle location, tension treatment and core were also significant ($P < 0.001$). The lateral position was the least tender in rib chops while the dorsal position was least tender in loin chops of the nontensioned sides. Pre-rigor tension made core positions more uniform in tenderness in the rib chops, while considerable tenderness variation persisted in tensioned loin chops.

THE AROMA OF CANNED BEEF: PROCESSING AND FORMULATION ASPECTS. T. PERSSON & E. VON SYDOW. *J. Food Sci.* 39, 406–413 (1974)–The influence of processing techniques and formulations on chemical and sensory aroma properties has been studied with the purpose of finding ways to improve the flavor of canned meat. HTST-sterilization, "aseptic" canning and sterilization in various packaging materials including flexible pouches have been investigated. It was found that "aseptic" canning and, especially, HTST-sterilization had a pronounced positive effect on aroma, in the latter case for samples packed in thin layers (flexible pouches). It was shown that addition of e.g., fumarate or maleate in small amounts (0.06–0.15%) before sterilization decreased the concentrations of hydrogen sulfide and mercaptans, and the additions of certain amino acids, e.g., arginine, decreased the concentrations of the aldehydes. Both types of ingredients resulted in an improved aroma. Storage changes have also been investigated. It was found that there was a tendency towards a more accelerated change in flexible pouches compared with rigid cans.

EFFECT OF CONCURRENT CALORIC INTAKE ON THE RESPONSE TO ORAL MONOSODIUM L-GLUTAMATE IN SUSCEPTIBLE SUBJECTS. R.A. KENNEY. *J. Food Sci.* 39, 414–415 (1974)–A study was undertaken to assess the susceptibility of a population of proven reactors to a standard 5g dose of MSG given as part of a lunch-type snack. The study was also designed to test the response to naturally occurring L-glutamic acid in amounts comparable with the test dose of MSG. Results show the latency of symptom onset to be consistently longer when MSG is administered in juice accompanied by a snack. Response rate to snacks containing 5g MSG is significantly lower for high protein, high natural L-glutamic acid and high carbohydrate snacks than for the high-fat snack or MSG administered without a snack. The high-fat snack (also low in bulk) afforded no protection.

COMPARATIVE SENSORY EVALUATIONS OF TWO CULTIVATED MUSHROOMS: *A. bisporus* AND *A. bitorquis*. J.A. ABBOTT & J.P. SAN ANTONIO. *J. Food Sci.* 39, 416–417 (1974)–Comparative sensory evaluations were made between sauteed mushrooms of commonly cultivated *Agaricus bisporus* and related *A. bitorquis*, being considered for cultivation, to determine whether nontrained persons could distinguish them, which was preferred, and how they differed. Modified triangle tests showed the species distinguishable. Half the correct responses preferred *A. bisporus*; 1/3, *A. bitorquis*. A texture profile panel found *A. bitorquis* firmer, crisper and chewier. Comments on flavor indicated that *A. bitorquis* had a stronger flavor than *A. bisporus*.

DIFFERENTIATION OF PERFECTION, ALSWEET AND ALASKA VARIETIES BY TOTAL STARCH AND AMYLOSE CONTENT. W. THERAVUTHI, C.E. JOHNSON & J.H. von ELBE. *J. Food Sci.* 39, 418–419 (1974)–The USDA grading system assigns different quality attributes according to two types of peas, wrinkled and smooth skinned. Since crosses between these types exist which exhibit characteristics of both types, a method of identification between types is needed to apply the correct grading standards. Perfection, Alsweet and Alaska varieties were evaluated for percent starch and amylose content in starch within sieve size classification or tenderometer value grouping. Perfection peas are easily differentiated from Alsweet and Alaska peas of similar sieve size or tenderometer values by their high amylose content. Alsweet has the lowest percent amylose in starch; however, there exists an overlap in content between Alaska and Alsweet varieties making differentiation uncertain. Results indicated that differentiation was possible between Alaska and Alsweet within sieve size or tenderometer values on the basis of total starch content. The Alaska variety has a higher total starch content compared to the Alsweet variety.

FLUORESCENT QUANTITATION OF BIOLOGICALLY ACTIVE AMINES IN FOODS WITH 7-CHLORO-4-NITROBENZOFURAZAN (NBD-Cl). M.N. VOIGT & R.R. EITENMILLER. *J. Food Sci.* 39, 420–421 (1974)–The usefulness of 7-chloro-4-nitrobenzofurazan (NBD-Cl) for the quantitative fluorescence detection of biologically active amines commonly found in fermented foods was studied. The reagent was found to be advantageous when compared to ortho-phthaldehyde and ethylene diamine due to the simplicity of derivative formation and ability to form fluorescent derivatives with a variety of biologically active amines. NBD-Cl was shown to form quantitative derivatives with tyramine, tryptamine and histamine. There was a limited linear response for tryptamine.

TRYPSIN INHIBITORS IN SORGHUM GRAIN. J. XAVIER FILHO. *J. Food Sci.* 39, 422–423 (1974)–The presence of trypsin inhibiting substances was shown in aqueous and acid extracts of sorghum grain powder. Gel filtration of the acid extract showed that the trypsin inhibitors have a broad distribution of molecular weight with the most significant peak of activity centered around 15,000 daltons. It was also shown that aqueous extracts of the inhibitors adjusted to pH 4.0 were stable to heat treatment of 100°C for 30 min.

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INFLUENCE OF ODOR SOURCES ON THE ODOR AND FLAVOR OF BEEF. R.J. BORTON, H.W. OCKERMAN, B.D. VAN STAVERN & J.P. HADDEN. *J. Food Sci.* 39, 424–425 (1974)–Research was conducted to determine the influence of odor sources present in the vicinity of meat during slaughter, storage, shipping and handling, on the odor and flavor of beef. In most cases, outside samples and ground composites scored higher in off-flavor intensity than did inside samples. Fat sample results were similar but exhibited greater variation. Odor scores followed a similar pattern. Ammonia was found the easiest source of odor and flavor to detect; cigarette smoke, butts and ashes, automobile exhaust, kerosene and paint were also easily detected. Spoiled meat could be detected as imparting an off-odor and off-flavor, but the source was not easily identified. Other variables (e.g., silage, soap, rancid grease) showed little evidence of causing off-flavor or off-odor to the meat. The influence of storage time and exposure pre- or post-rigor seemed negligible in most cases.

VITAMIN B_6 IN PORK MUSCLE COOKED IN MICROWAVE AND CONVENTIONAL OVENS. J.A. BOWERS, B.A. FRYER & P.P. ENGLER. *J. Food Sci.* 39, 426–427 (1974)–Loin sections of pork muscle were heated to 75 or 85°C internal temperatures in microwave or conventional electric ovens. Cooking losses and time, percentage moisture and total vitamin B_6 were determined. Cooking time was longer, total cooking loss less and moisture content greater for pork cooked in an electric oven than for that cooked in a microwave oven. Differences in vitamin B_6 due to type of oven or internal temperature were small and not significant when calculated on a cooked weight basis. When calculated on a dry weight basis samples cooked in the conventional oven contained more vitamin B_6 than did those cooked by a microwave oven.

APPLICATION OF SDS-ACRYLAMIDE GEL ELECTROPHORESIS FOR DETERMINATION OF THE MAXIMUM TEMPERATURE TO WHICH BOVINE MUSCLES HAVE BEEN COOKED. Y.B. LEE, D.A. RICKANSRUD, E.C. HAGBERG & E.J. BRISKEY. *J. Food Sci.* 39, 428–429 (1974)–The sodium dodecylsulfate-acrylamide gel electrophoresis was evaluated as a method to determine the cooking temperature to which beef has been cooked. Water soluble protein extracts from bovine muscles cooked to the final temperatures of 65, 70, 75, 80, 85 and 90°C were applied on SDS-acrylamide gels. The extracts showed characteristic electrophoretic patterns for each cooking temperature examined. The thermoprofiles were highly reproducible among a number of experimental variables. The high degree of accuracy and reproducibility of the method described herein makes it possible to determine the precise temperature to which beef has been cooked, within ± 5°C.

APPLIED SCIENCE and ENGINEERING

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THE TOXIC POTENTIAL OF TRACE METALS IN FOODS. A Review

INTRODUCTION

HEAVY METALS are widely distributed throughout the earth's crust and oceans and it is inevitable that traces of these metals can be detected in virtually all plant and animal organisms—and hence in our food. The rapid development of trace analytical methodology has exacerbated this problem so that sensitive techniques, such as neutron activation analysis, allow trace elements to be measured at concentrations that previously we never even knew existed.

Some of these metals (e.g., cobalt, copper, zinc, manganese) are essential to our health; others (such as vanadium, nickel and tin) we now learn are possibly essential. Many metals, however, have no proven function in life processes. Toxicity and the resulting threat to human health are, of course, a function of concentration: hence metals as essential as copper and cobalt can be dangerous at sufficiently high levels. A few years ago 20 people died in Quebec City from cardiomyopathy induced by the heavy consumption of beer that contained added cobaltous sulphate as a foam stabilizer (Mercier and Patry, 1967).

In Canada statutory tolerances for three metals—lead, copper and zinc—in a variety of foods are incorporated in the Food and Drug Regulations. A temporary guideline has been set for a fourth metal, mercury, at 0.5 ppm in fish.

There is no doubt that food represents our most intimate medium of chemical pollution for all three primary components of the environment—air, water and soil—are involved. Food reaches the consumer as the end-product of a long chain of preparation and processing operations during which it can be contaminated by trace metals. The metals may be present as the unavoidable result of technological operations as well as from environmental pollution. Essentially there are four sources of the contamination of foods by trace metals:

1. **Agricultural technology**: where the metals can be components of pesticides (e.g., lead arsenate, maneb) or contaminants (e.g., cadmium in phosphate fertilizers).
2. **Industrial pollution**: here the possibilities are legion in view of the myriad compounds released by modern industrial and mining technology. The hazards attending the presence of such industrial effluents as lead, cadmium and mercury have been well publicized in recent years.
3. **Geological sources**: the natural presence of zinc, mercury, chromium, nickel and so forth provides a means whereby these metals can enter the food chain. We have, in fact, no clear idea as to the contribution these geological sources make to the total metal content of foods. It could well be appreciable; with mercury, for example, it has been estimated that the discharge to water from rocks is of the same order as that from man-made sources (Anonymous, 1970).
4. **Food processing**: either as contaminants of food additives or from physical or chemical contact with equipment and vessels. Thus, acid foods will leach lead and cadmium from glazed pottery; abrasion of machinery gives metal particles in flour and chocolate.

These are some examples of the sources of trace metals in foods. I would like now to give some recent results obtained, in Canada, by the Health Protection Branch.

RESULTS & DISCUSSION

TOXICOLOGICALLY it is of paramount importance to know the daily intake of trace metals by our population. To assess this intake we have analysed by atomic absorption spectrophotometry composites of the 12 major food classes (dairy products, meats, cereals, potatoes, leafy vegetables, legumes, root vegetables, garden fruits, fruits, oils and fats, sugar products and drinks) for copper, lead, zinc, nickel, manganese, cadmium, chromium, cobalt, iron and mercury. The foods were prepared as by the housewife. That is, they were washed, trimmed and cooked, as appropriate, before being analysed. The average diet of Canadians has been estimated by Smith (1971) from food disappearance tables as being nearly 1800g/person/day. Hence the average daily intake of metals can be calculated: the average values obtained from surveys in 1970 and 1971 are shown in Table 1 (Kirkpatrick and Coffin, 1973a).

The joint FAO/WHO Expert Committees on Food Additives have established a maximum daily load for copper and provisional tolerable weekly intakes for lead, mercury, and cadmium (Lu, 1973; Joint FAO/WHO Expert Committee on Food Additives, 1972). For a 60 kg man on a daily basis these intakes are 30,000 μg for copper, 430 μg for lead, 43 μg for mercury and 57–72 μg for cadmium. With the exception of cadmium all the daily intake results from food shown in Table 1 are well below these overall limits. It is generally acknowledged that ingestion from food provides our major intake of trace metals.

Variations between total diet intake values for trace elements determined in different countries are often simply a measure of the different diet volumes used in these studies. In the U.S.A., for example, the total diets based on the eating habits of 16–19 year old males give an intake of 4 kg/day (Duggan and Weatherwax, 1967). Thus care must be exercised when making international comparisons.

However, recent monitoring surveys in the United Kingdom (Survey of Lead in Food, 1972) on a similar daily food in-

take (1500g) to that of Canada show a higher daily intake of lead, some 200 μg/person, than found in Canada (Table 1). This higher overall intake is reflected in higher U.K. values for the lead content of meat, fish and vegetables.

In our laboratories, Kirkpatrick and Coffin (1973b) have analysed some 190 samples of cured meats for cadmium, lead and mercury. The cadmium content of the meats averaged 0.02 ppm and mercury 0.006 ppm; the average concentration of lead was 0.05 ppm except in canned meats where the mean was 0.16 ppm. A similar effect has been reported by Bogen (1968) who found canned vegetables to have 3.6 times the lead concentration of fresh In limited analyses of baby foods we have found the average lead content of the food in cans to range from 0.1–0.3 ppm whilst that in the jars was from 0.04–0.08 ppm. It would seem, therefore, that there is some dissolution of the lead in the can solder although tinlead alloys exhibit a positive potential in relation to tin and iron (Joint FAO/WHO Expert Committee on Food Additives, 1972). The highest lead values from canned foods are well below our legal tolerances.

Recent surveys of some 545 food samples, by the Winnipeg Regional Laboratory of the Health Protection Branch, representing the major 15 staple foods have shown average lead and cadmium levels of 0.10 and 0.02 ppm, respectively. The mercury content of all but two samples was < 0.01 ppm, a pork at 0.01 ppm and a beef at 0.20 ppm being the exceptions. The highest level of cadmium reported was 0.36 ppm in a sample of tomatoes and the highest lead was 1.06 ppm in a cabbage sample.

These results relate to average concentrations of trace metals; however it is well to bear in mind the following:

(1) There may be appreciable variations from the total diet, as calculated by "food disappearance," with members of different age groups, ethnic populations, geographically sited populations, and so forth. In Canada the results of our recent nutritional survey—soon to be published—will provide a sounder basis in the future for determining the trace metal contribution from different types of diet.

(2) Local geographical and geological factors as well as urban and industrial pollution can produce wide variations in the trace metal content of foods. Some interesting studies have been carried out by Warren (1972) who reports concentration ranges of up to 300 with lead and molybdenum contents of vegetables from different localities. In this respect it is important to note that heavy metal contamination is not simply related to a particular industrial operation—in this case mining. Warren et al. (1971) have shown, for example, that urban contamination can lead to higher levels of metal content in local vegetables than those grown in the vicinity of mining operations.

Table 1—Average daily intake (μg/person) of trace metals from foods for 1970–1971

Cadmium	67
Chromium	144
Cobalt	50
Copper	2100
Iron	16600
Lead	138
Manganese	3300
Mercury	13
Nickel	460
Zinc	16900

The toxicological significance of these levels of metal in foods is determined by the appropriate national or international (e.g., FAO/WHO Expert Committee) authorities, and tolerances, maximum acceptable intakes, or provisional tolerable intakes have been established for many metals. Most of our present information relates to the acute or short-term effects of metals and it is only in recent years that attention has been given to the assessment of the carcinogenic, teratogenic, and mutagenic effects. The Health Protection Branch (1973) has recently published a comprehensive appraisal of the methods available for the testing of these effects. In fact much more research is required: we need screening tests that will enable priorities to be set amongst the vast number of chemical compounds that must be tested for carcinogenicity. Only the compounds that fail these screening tests will be subjected to expensive and time-consuming long-term studies. Three tests that offer promise in screening tests will be subjected to expensive and time-consuming long-term studies. Three tests that offer promise in this area are: mutagenesis, DNA repair synthesis, and in vitro cell transformation.

There is an undoubted need to coordinate programs on the monitoring of all trace contaminants, including metals, in foods—both nationally and internationally. This need was made visible at the 1972 U.N. Conference on the Human Environment in Stockholm in the form of a definite recommendation (No. 78). A specific FAO/WHO proposal has now been advanced as a means to achieve these ends and one would hope that an embryonic scheme for the monitoring of contaminants in foods may well be in operation by 1974. However, before embarking on too grandiose a scheme it is essential to define the priorities; to determine, that is, the relative ranking order of the trace metals to be analysed. There are six criteria required to establish a priority listing for the monitoring of trace metals:

1. **Biological role of the metal**, i.e., the human requirement. This can be expressed in terms of μg/kg body weight/day.
2. **Toxicity of the metal**. This comprises such components as: absorption, metabolism, accumulation, excretion, acute and chronic effects; carcinogenic, teratogenic and mutagenic effects. These can be expressed in terms of an acceptable daily intake (ADI), μg/kg body weight, or a provisional tolerable weekly intake.
3. **Actual intake of the metal**—particularly in the toxic form if known, e.g., methyl mercury—in food, air and water. This can be expressed as μg/kg body weight/day.
4. **Extent of use of the metal**, e.g., industrial, agricultural, household. This answers the question as to how much is used in a particular country or region. The annual use of a metal may be expressed as kg/year.
5. **Occurrence and availability of the metal in the environment**. This is concerned as to whether the metal is evenly dispersed or whether it is locked-up and safe. Thus, lead is released from gasoline but not from batteries. The units are again kg/year.
6. **Persistence of the toxic form of the metal** e.g., where appropriate methyl mercury rather than total mercury. This factor is best expressed in comparative terms, i.e., high, medium, low, or negligible.

Using these criteria tentative rating tables can be constructed that will illuminate research and information needs. The full use of these tables will enable us to more effectively plan our monitoring programs and prevent the gathering of irrelevant data.

The food examined in a monitoring program should be selected at random and samples to be analysed considered within three components:

1. **The mixed total diet as eaten**: this must be determined on the basis of national and regional food consumption data. Composites of the major food classes should be prepared as for consumption at the table. From knowledge of the average daily diet the average intake of a specific metal can be calculated.
2. **Staple foods**: these are foods that form the major component of the diet, e.g., cereals, milk, potatoes, rice, bread, fish, meat, and will vary with national, regional or ethnic groups. These foods must be analysed separately, for their individual contribution could escape detection in the diluted mixed total diet survey. By

comparing the value for a specific contaminant with that for a particular food, a ranking can be obtained for each contaminant-staple food combination.

3. Indicator foods: analysis of these foods can give valuable early warning of potential food contamination problems. Foods that accumulate contaminants, (e.g., mollusca), or that are high on the food chain, (e.g., fish such as pike, raptorial birds), serve as valuable indicators.

By the development of food monitoring programs on these bases–using ranking criteria for the metals and for the food groups–it will be possible to more effectively establish the toxic potential of trace metals in our foods–both in our own countries and internationally.

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DENATURATION OF PLANT PROTEINS RELATED TO FUNCTIONALITY AND FOOD APPLICATIONS. A Review

INTRODUCTION

ALTHOUGH DENATURATION of protein has been studied by a number of investigators, relatively few studies have been directed to plant proteins. Reviews on protein denaturation in general were published by Kauzmann (1959), Scheraga (1961), Joly (1965) and Tanford (1968, 1970); on wheat proteins by Bourdet (1956); and on soybean proteins by Wolf (1972).

Denaturation may be defined in general terms as any modification of the secondary, tertiary or quaternary structure of the protein molecule, that does not break covalent bonds. A change in protein structure is usually associated with changes in physical-chemical and functional properties.

METHODS OF DENATURATION

Heat

The degree of heat processing of soy flour affects the properties of the products. Although soy flours can have a water-dispersible protein as high as 90% without heat treatment, after a high heat treatment the water-dispersible protein can be below 10%.

Some correlations have been found between functional characteristics of soy flour in various applications and nitrogen solubility index (NSI is the percentage of total nitrogen in a sample that is water dispersible based on the method used) (Johnson, 1970). The results of a study to correlate NSI of a soy flour and its water-absorption characteristics are shown in Table 1 (Dippold, 1961). Apparently as NSI decreases, water absorption increases to a point and then decreases with decreasing NSI.

Soy protein concentrates produced by different manufacturers may have NSI values ranging from 5–70% (Johnson, 1970). Generally, the higher the water-dispersible protein in a soy protein concentrate the better its emulsifying action. Whipping properties of soybean products, expressed by foam expansion and foam stability, correlate with water-dispersible nitrogen, and the resultant foams were stable when the dissolved proteins were native (Yasumatsu et al., 1972).

When isolated soy proteins are prepared from a starting raw material receiving a minimum heat treatment and having water-dispersible protein in a range of 80% or more, such proteins do not have gelling properties like those of proteins heat-treated to be 50–60% water dispersible.

Heat and moisture cause progressive insolubility of the protein in soybean meal (Beckel et al., 1942), and this denaturation is nearly quantitative in 2.5 hr at 127°C and 100% relative humidity. The effect of heat and other factors on gelation of soy protein dispersions was reported by Circle et al. (1964). Heat causes thickening and then gelation of aqueous dispersions above 7% by weight. Temperature, time of heating and protein concentration determine rate of gelling and gel firmness.

Watanabe and Nakayama (1962) found that distinct changes in ultracentrifugal patterns resulted from heating a solution of water-extracted protein of soybean at pH 7.0 for 10 min at 80°C, that the main components disappeared and that new ones which were formed had higher sedimentation velocity. A series of "native" defatted soy flakes was subjected to both dry heat (130°C) and wet heat (steaming at 100°C) for different times (Pour-El and Peck, 1973). When these samples were analyzed for protein solubility by two accepted methods, wet heat had a harsher effect than dry heat. General functionality dropped with the length of treatment for either.

Table 1—Correlation between NSI and water-absorption characteristics of soy flour[a]

NSI	Water absorption (%)
85	270
70	385
55	370
15	290

[a] Dippold, 1961

The effects of heating time on soybean 11S protein at 100°C in pH 7.6 solution were determined at different ionic strengths with varying levels of mercaptoethanol by Wolf and Tamura (1969). Heating at 0.5 ionic strength rapidly converted 11S protein into a fast-sedimenting aggregate and a 4S fraction. The soluble aggregate increased in size and precipitated on continued heating. Soybean 11S protein is dissociated into subunits by exposure to temperatures above 70°C. The thermal aggregation of these subunits was investigated by turbidity measurements (Catsimpoolas et al., 1970a). The rate and extent of aggregation were enhanced by low ionic strength but depressed at extreme acidic or alkaline pH values, as well as at high ionic strength. The antigenicity of soybean 11S protein disappeared rapidly at temperatures between 70 and 90°C, and the immunological inactivation at different temperatures was a time-dependent phenomenon (Catsimpoolas et al., 1971b).

Kunitz (1948) studied the kinetics and thermodynamics of reversible denaturation of crystalline soybean trypsin inhibitor and found that the protein is stable over a wide pH range at temperatures below 30°C. The protein was gradually denatured if heated to higher temperatures. Also, denautration is accompanied by a loss in trypsin-inhibiting power and by a change in solubility. Pepsin readily digests the denatured protein in contrast to the native protein. The heat-denatured protein, when cooled, is gradually reversed to its native soluble form. Irreversible denaturation is caused by prolonged heating, brief heating of the protein solution at its isoelectric point or adding salt. Wu and Scheraga (1962) observed a rather sharp thermal transition of soybean trypsin inhibitor from ultraviolet difference spectra measurements. The transition is reversible at pH 6.6 and 9.0 at low ionic strength but irreversible at pH 1.3. The transition temperature depends on pH and ionic strength.

Heat inactivation of soybean hemagglutinin at different pH values was reported by Liener (1958). Maximum stability toward thermal inactivation was between pH 6 and 7. Mustakas et al.

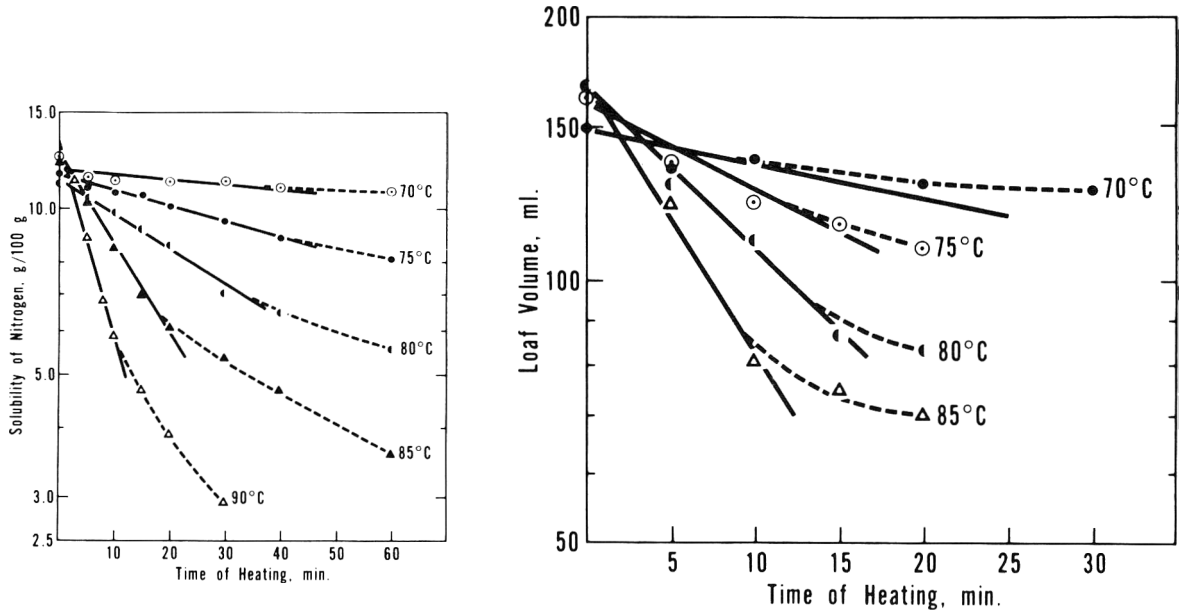

Fig. 1—Denaturation of wet, gum gluten by heat at various temperatures as measured by (A) loss of solubility in dilute acetic acid and (B) decrease in loaf volume of reconstituted doughs. Gluten from commercial flour no. 1. Straight lines calculated by method of least squares (Pence et al., 1953).

(1969) evaluated various heat treatments for effectiveness of lipoxygenase deactivation. Dry heat to 100°C, steaming, or both, deactivated lipoxygenase to give flours with good flavors after 2 years' storage.

Pence et al. (1953) studied the effects of time, temperature, moisture content, pH and salt concentration on the denaturation of wheat gluten by heat ranging from 70–90°C. Denaturation was measured by loss of solubility in dilute acetic acid as a function of time from 70–90°C (Fig. 1A). Little decrease in solubility was seen at 70°C for up to 60 min, but loss in solubility of gluten was considerable at 75°C for the same length of time. The decrease in solubility was much more rapid at higher temperatures, and at 90°C most of the gluten became insoluble in 30 min.

The denaturation of wet, gum gluten by heat from 70–85°C as measured by the decrease in loaf volume of reconstituted doughs is plotted in Figure 1B. Some loss in loaf volume was observed at 70°C for 20 min, and the loss was more pronounced at higher temperatures until less than half the original loaf volume resulted at 85°C for 20 min. The relation between temperature and rate of denaturation of wet, gum gluten by heat as measured by the baking test and by the solubility method is shown in Figure 2. Gluten denaturation was essentially a first-order reaction with an energy of activation of approximately 35,000 calories per mole by the baking-test method and 44,000 calories by the solubility method.

The effect of moisture content upon rate of denaturation of gum gluten by heat is seen in Figure 3A. The solubility method (curve 1) shows that denaturation is rapid at 90°C as long as the moisture content remains above 35%. Below this value the rates diminish rapidly until no measurable denaturation can be detected for heating periods up to 1 hr at 5% moisture. At 80°C, gluten from a second flour showed a well-defined maximum rate of denaturation around 40% moisture by both baking-test and solubility method (curves 2 and 3). Curves 2 and 3 are similar in shape, but values obtained with the baking-test method are larger than those obtained with the solubility method at the same moisture levels.

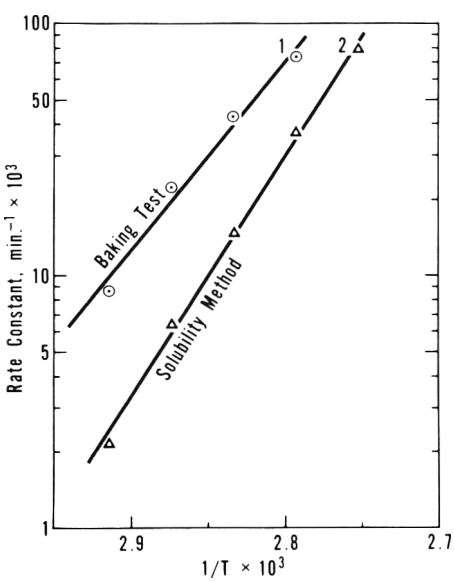

Fig. 2—Relation between temperature and rate of denaturation of wet, gum gluten by heat as measured by the baking-test method (curve 1) and by the solubility method (curve 2). The energy of activation (E = slope × R) equals 34,600 calories per mole by the baking test, and 43,800 by solubility (Pence et al., 1953).

The effect of pH on rate of denaturation of gluten by heat is plotted in Figure 3B. Denaturation proceeds slowly at pH 4 but increases rapidly as pH rises to about 5 (curve 1). The rate remains constant until about pH 6 where another sharp increase occurs, and the rate again becomes fairly constant between pH 6.5 and 7.5. At 80°C, gluten from the second commercial flour (curves 2 and 3) behaves similarly, except that more pronounced minima were seen between pH 4.5 and 6. The low rate of denaturation observed with the solubility method for gluten heated at 90°C near pH 4 (curve 1) was confirmed by the baking-test method.

Variations in salt concentration had no effect on rate of denaturation of gluten by heat but may influence the damage to baking properties caused by low pH conditions. The rates of denaturation of glutens from flours of different quality

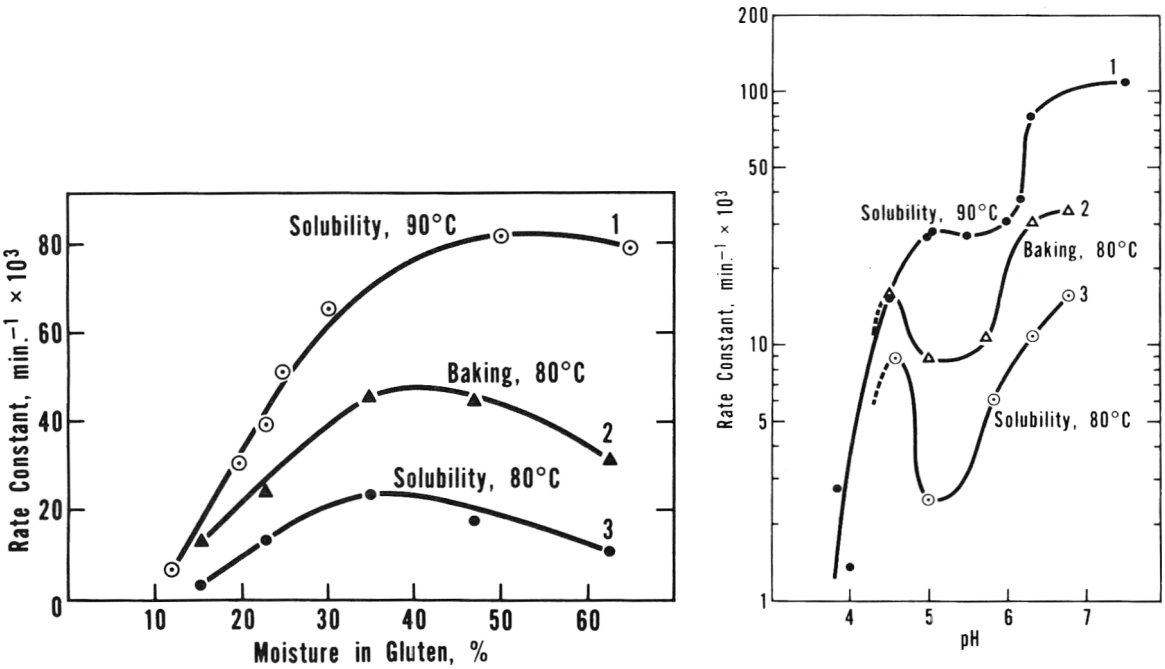

Fig. 3—The effect of (A) moisture content and (B) pH on rate of denaturation of gluten by heat. Curve 1, solubility method on gluten from commercial flour no. 1 heated at 90° C; curve 2, baking-test method on gluten from commercial flour no. 2 heated at 80° C; curve 3, solubility method on gluten from commercial flour no. 2 heated at 80° C (Pence et al., 1953).

varied significantly, but there is no correlation between rate of denaturation of gluten and baking quality.

The rate of denaturation of the gliadin fraction of wheat gluten at 90°C by the solubility method is displayed in Figure 4. An induction period of about 10 min occurs before denaturation starts. After this interval, the denaturation attains a first-order rate about one-third as large as that of gluten under similar conditions.

The excellent parallelism between baking-test and solubility results indicates that the solubility method can be used with confidence for studies on heat denaturation of gluten. The heat denaturation of gluten is a good example where denaturation correlates well with functionality.

The degree of denaturation of protein in vital gluten was measured by viscosity and optical rotation of the dispersion (Dalek et al., 1970). Studies of drying methods demonstrated that freeze drying and vacuum drying did not denature gluten and that spray drying and drum drying changed molecular structure significantly. The heat denaturation of wheat grain proteins greatly increased the capacity of the grain to bind methylene violet, malachite green and brilliant green from 0.001% water solution (Kotlyar, 1967). The safe temperature limit for drying of grain depends on moisture content; at 19% moisture grain may be dried at 60°C, whereas at 26% it may be dried only at 50° without loss of vitality.

Rohrlich (1955) studied the influence of drying wheat grain on enzyme activity of wheat and gluten protein. Catalase activity remains relatively unaffected by heat up to 65°C, but at 65°C activity begins to decrease rapidly, and at 100°C it amounts to only about 30% of its original value. Activity of protease decreases proportionately with increase in temperature, and at 100°C only about 30% of the original activity remains. The β-amylase activity decreases with rising temperature to about 40% at 100°C. Up to a temperature of about 65°C, the soluble portion of endosperm protein in normal acetic acid increases by about 5%, and then up to 100°C it decreases by about 19%.

Moran et al. (1968) found that hemagglutination and antitrypsin activities, originally high, were negligible after autoclaving wheat germ meal at 121°C (15 psi) and drying at 50°C or after toasting in a rotary drum drier at 121°C. Kasarda et al. (1968) measured optical rotatory

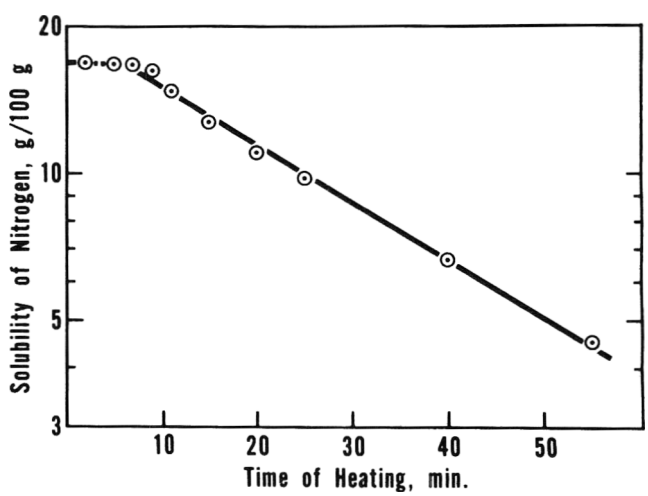

Fig. 4—The rate of denaturation of gliadin at 90° C as measured by loss of solubility in dilute acetic acid (Pence et al., 1953).

dispersion and circular dichroism of α-gliadin between 4 and 90°C. Above 30°C the helical content decreased gradually, but even at 90°C about 65% of the helical structure present at 25°C remained intact.

The effect of dry roasting at 145°C on the stability of peanut proteins was reported by Neucere et al. (1969). Solubility of the proteins in phosphate buffer was reduced to less than one-half, and both dissociation and association occurred as shown by sedimentation analysis. The major reserve protein, α-arachin, increased in electrophoretic mobility, and all other proteins showed modified physico-chemical properties. Neucere (1972) also studied the effect of wet and dry heats (110–155°C) on peanut proteins. Only two proteins out of approximately 14 antigenic constituents remained antigenic after heating peanuts with 40% moisture at 110°C. On the other hand, dry heat induced sequential antigenic destruction between 110 and 155°C. Disc electrophoresis showed greater difference in protein migration for the dry- than for the wet-heated seed. Protein solubility was inversely proportional to temperature with dry heat, but a sigmoid curve was observed after wet heat. The maximum solubility of α-arachin after wet heat occurred at 120°C.

Heat denaturation of corn proteins was examined as a function of moisture, temperature and duration of heating (Braterskii, 1969). No change occurred below 40°C; complete denaturation occurred at 58°C with 58% moisture within 30 min, with 27% moisture at 60°C within 60 min and with 14% moisture at 70°C within 60 min. The rate of denaturation is first order above 40°C, and the rate increases 1.8- to 7.4-fold for salt-soluble proteins in the endosperm and 1.4- to 6.8-fold for those in the embryo, for each 10°C increase. The intensity of hydrolytic and oxidation-reduction processes in corn during drying was studied by changes in activity of lipase and dehydrogenase and in the level of water-soluble proteins (Bukhantsov and Sirko, 1969). The level of water-soluble proteins decreased with increased heating rate and temperature. The decrease in solubility was an indirect measure of decrease of enzymatic activity.

The degree of denaturation of proteins during roasting of cottonseed pulp is influenced by moisture, temperature, time and degree of crushing (Koneva and Rzjekhin, 1967). Denaturation is linearly dependent on degree of crushing at near 80°C for 50 min. Denaturation occurred mainly during cooking and preliminary pressing of cottonseed (Markman and Karavaeva, 1969). The amounts of water- and salt-soluble proteins decreased while alkali-soluble and insoluble proteins increased. Heat treatment caused a decrease in solubility of bean protein in water and in salt solution and increased its solubility in NaOH (Lobanov and Treneva, 1966). Treating sunflower seed at 60, 80 or 100°C for 10, 20 or 30 min decreased the albumin fraction and increased the globulin and glutelin fractions (Segal, 1968).

Heating ground rye, wheat and rice products at various temperatures for long periods inactivated the enzymes lipase, acetylesterase, peroxidase and lipoxygenase (Rothe and Stoeckel, 1967). The temperature effect becomes apparent for each enzyme at a specific temperature level, and linear relations exist between inactivation temperature and moisture content of the products. The inactivation-limiting temperature at 0% water for wheat germ and rice peroxidases is 108°; rye acetylesterase, 106°; rye and rice lipase, 94°; rice acetylesterase, 92°; and wheat germ lipoxygenase, 67°C.

Two sweet-tasting proteins, thaumatin I and II, have been isolated from katemfe, a tropical African fruit, and one, monellin, from serendipity berry (van der Wel, 1973). Circular dichroism measurements show that these proteins undergo reversible conformational changes as temperature increases. At a certain temperature depending on pH, however, irreversible heat denaturation occurs. Circular dichroism, proton magnetic resonance and ultraviolet difference spectroscopy measurements reveal that tyrosine residues, and at least one disulfide, are involved in this conformational change. Since these irreversible changes coincide with a loss of sweetness, the groups underlying the conformational change also help generate the sweet taste.

Acid or alkali

Almost all proteins are affected by hydrogen or hydroxyl ions. The effect of pH and ionic strength on the behavior of soybean 11S protein in acid systems was studied by ultracentrifuge (Rackis et al., 1957). The 11S protein in acid solution is a freely reversible association-dissociation system containing three resolvable fractions having sedimentation coefficients of approximately 2, 7 and 13S. An unresolvable fraction having a sedimentation coefficient value of more than 13S is also present. The relative amounts of the fractions depend upon pH, ionic strength and type of salt. Low pH and low ionic strength favor dissociation primarily into the 2 and 7S fractions.

Ultracentrifugal studies indicate that soybean 11S globulin is capable of undergoing conformational changes probably involving dissociation into subunits which appear to be one-half and approximately one-eighth the size of the 11S molecule (Wolf and Briggs, 1958). These changes occur not only at low ionic strength at alkaline pH values, but also at moderate ionic strengths at acid pH values. The conformational changes generated at low ionic strength and alkaline pH are reversible on increasing the ionic strength, but those generated at acid pH are irreversible. Effect of pH and ionic strength on the 11S protein suggest that dissociation is due to forces of electrostatic repulsion between the subunits (Wolf et al., 1958). Changes with time in some systems were dissociation, aggregation without precipitation or aggregation with precipitation. Dissociation is accompanied by an increase in levorotation, a condition that indicates configurational changes of the subunits take place and that these changes may be responsible for irreversibility of dissociation and for aggregation under certain conditions.

The exposure of acid-precipitated soy protein to sodium hydroxide at high pH

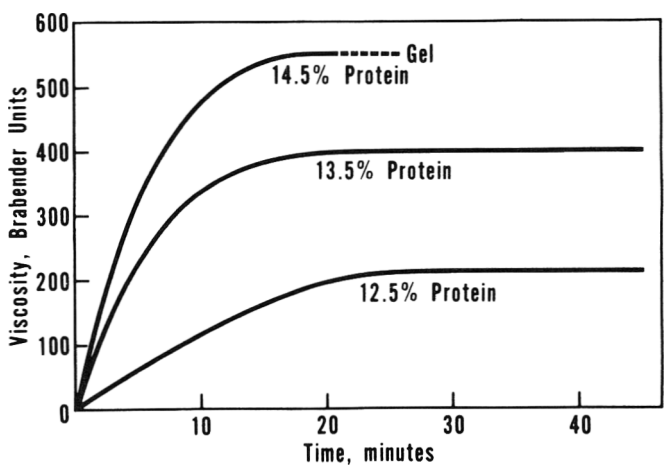

Fig. 5–Effect of time and concentration of soy protein, in 0.95% sodium hydroxide, on viscosity of dope solution (Kelley and Pressey, 1966).

markedly increases its relative viscosity and rapidly shifts the sedimentation constants of the 2, 7, 11 and 15S ultracentrifuge components to essentially 3S (Kelley and Pressey, 1966). The shape and magnitude of the viscosity curve depend on the concentration of protein and sodium hydroxide as well as time.

The effect of time and concentration of soy protein, in 0.95% sodium hydroxide, on viscosity of dope solution is plotted in Figure 5. The viscosity of the pH 12 suspension increases rather sharply during the first 15 min and then levels off. The concentration of protein determines the magnitude of the curves as well as the time at which the solutions reach maximum viscosity. Protein concentrations of 14.5% or higher form gels, which cannot be spun, whereas concentrations lower than about 12.5% yield solutions having viscosities too low for spinning.

The concentration of sodium hydroxide during dope formation sets the practical limits within which the protein content can be varied. The viscosity curves in Figure 6 display a suitable concentration (0.95% sodium hydroxide) along with higher and lower levels. At 0.81% sodium hydroxide, solutions of low viscosity that increases slowly with time are produced. However, a concentration of 1.1% induces maximum viscosity rapidly, which then slowly decreases, presumably because of protein degradation.

Conversion of soy protein into coagulated fibers, which can be formulated into textured food products, usually demands a pH in the range 9 to 13.5 but in practice at least 10.5 (Tombs, 1972). A serious disadvantage of these processes is their use of a high pH, which tends to degrade the protein and requires complex procedures to ensure uniformity in the alkaline solution subjected to spinning. A different process avoids a high pH and dissolves the plant protein at pH 3–9 by a water-soluble salt, such as 0.2M NaCl. An edible protein fiber is produced by extruding the aqueous protein into an aqueous medium above 80°C to get a heat-set product, in contrast to the conventional method of extruding a strongly alkaline protein solution into an acid-salt coagulation bath.

In solution with lower pH than the isoelectric point, soybean 7S protein dissociated into two components in low ionic strength, but above ionic strength 0.1 it gave a 7S sedimentation pattern (Koshiyama, 1968). On the other hand, in solution with higher pH than isoelectric point, this protein aggregated to a 9S isomer below ionic strength 0.1. The mixture of 7S and 9S forms existed between ionic strength of 0.1 and 0.5, and the protein kept a stable 7S form above ionic strength 0.5. These reactions were reversible, and the 9S isomer was considered to be a dimer of the 7S protein based on molecular weight studies.

Ultraviolet difference spectra indicated that both tyrosine and tryptophan groups are exposed by treating soybean 11S protein with acid at pH 2 (Catsimpoolas et al., 1969). Acid at pH 2 and alkali at pH 11 cause dissociation of the 11S protein into subunits and subunit association products according to disc electrophoretic data. Koshiyama (1972) compared the acid-induced conformational changes between 7 and 11S soybean proteins by ultraviolet difference spectra, ultracentrifugation and optical rotatory dispersion. Maximum denaturation occurred at approximately pH 2 in both proteins, and simultaneously, dissociation of the proteins into subunits and unfolding of the polypeptide chains were observed. However, both proteins showed apparent differences in their readiness to undergo acid-induced denaturation, and the differences were particularly remarkable in the presence of 0.1 ionic strength sodium chloride.

Time of acid treatment and extremes of acidity caused denaturation of water extract of soybean meal (Nash et al., 1971). Loss of solubility in a pH 7.6 buffer, as compared to a nonacidified control, served as a criterion of denaturation. Acidification of water extracts for 2 hr to pH 4.5 decreased solubility and total ultracentrifuge area of the globulin fraction by about 12%. The 11S protein showed marked sensitivity to pH values lower than 4.5. Both 7S and 11S fractions decreased in water extractability with aging of the meal.

Organic solvents

Both hot and cold methanol or ethanol extraction of soybean meal reduced the subsequent extractability with water or salt solutions of all the protein components, but the effect was most pronounced on globulin components (Mann and Briggs, 1950). Hot extractions were more effective in reducing protein extractability. Denaturation of protein in soybean meal by various concentrations of methanol, ethanol, isopropanol and acetone has been investigated in the temperature range of 30–75°C for various time intervals (Smith et al., 1951). Denaturation was measured by the change in water dispersibility of the protein before and after treatment of the meal with solvents. Water is less effective as a denaturant under certain conditions than pure organic solvents, and alcohol-water solutions, at 40–60% concentration, are the most effective. Denaturation of the proteins is nearly complete in 5 min.

Of the four components of soybean globulins resolvable in an analytical ultracentrifuge, the 7S component is rapidly denatured (rendered insoluble in phosphate buffer at pH 7.6, ionic strength 0.5) when the wet curd is brought into contact with an ethanol-water mixture of 20% or more alcohol (Roberts and Briggs, 1963). However, the rate of denaturation of 11S and 15S components is slow, and the 2S component is not denatured at all. Effects of isopropanol concentration, and time and temperature of isopropanol treatment on solubility of soybean globulins in buffer at pH 7.5, ionic strength 0.5 were studied (Wolf et al., 1964). Insolubility of the proteins in this buffer was used as a criterion of denaturation. Ultracentrifugal and chromatographic analyses of the proteins soluble in buffer showed that the 7S, 11S and 15S components were denatured by isopropanol at 25°C but that the 2S component was not. Maximum denaturation in 2 hr at 25°C occurred with 40% isopropanol, and the

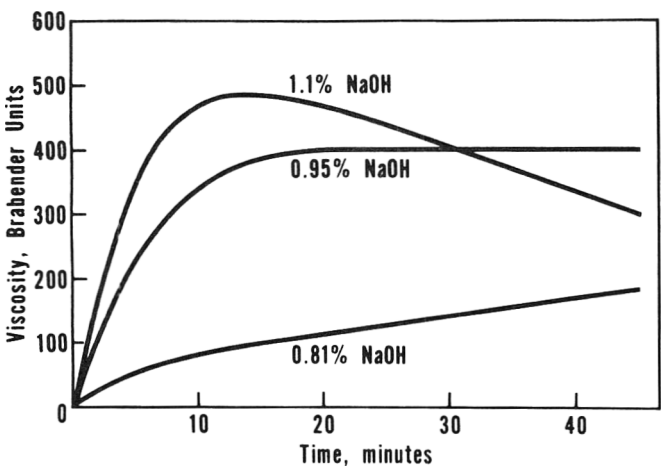

Fig. 6—Effect of time and concentration of sodium hydroxide on viscosity of dope solution. Protein concentration was 13.5% in all three samples (Kelley and Pressey, 1966).

7S component was most susceptible to denaturation. Increasing temperature of the alcohol treatment increased the rate of denaturation and also caused the 2S fraction to be denatured.

A systematic study was made of the denaturing ability of around 30 kinds of organic solvents toward soybean proteins (Fukushima, 1969). Usually the denaturing ability of organic solvents depended on their hydrophobicities and their degree of dilution by water. Highly hydrophobic solvents had little denaturing power toward proteins, even at high temperatures. The denaturing power of solvents increased with addition of water and that of water also increased with addition of solvents. Lower alcohols were much stronger denaturants than other solvents studied. The denaturing ability of alcohols at low concentration increased with the hydrophobicities of alcohols, whereas the reverse was found at high concentrations.

The denaturing effect of heptane, carbon tetrachloride and acetone on peanut proteins was studied by comparing the chromatograms of solvent-treated proteins with those from nonsolvent-treated proteins (Neucere and Ory, 1968). Some proteins become more insoluble, some appear to be partially dissociated, while others seem to form families of proteins with similar chromatographic properties. The albumin and the conarachin fractions showed the most change in solubility properties.

Detergents

Soybean 11S protein undergoes conformational changes probably involving dissociation into subunits in the presence of sodium octylbenzene sulfonate (Wolf and Briggs, 1958). The conformational changes generated by free detergent concentration of 0.42×10^{-3} M or less are reversible on removal of the detergent and an increase in the ionic strength at neutral pH. However, conformational forms of the protein generated at free detergent concentration of 7.3×10^{-3} M are irreversible. Soybean 7S protein was dissociated into subunits by treatment with 0.025 to 0.25% sodium dodecyl sulfate (Koshiyama, 1970). The subunit had a sedimentation coefficient of 2S and a molecular weight of 34,000. The detergent treatment contributed new partial formation of an α-helical conformation for the subunits. Conformational studies of soybean 7S and 11S proteins by optical rotatory dispersion and circular dichroism indicated that the formation of α-helix accompanying its dissociation into subunits and that changes of molecular ellipticity by treatment with sodium dodecyl sulfate were less in the 11S than in 7S proteins (Koshiyama and Fukushima, 1973).

The effect of sodium octyl-, decyl- and dodecyl sulfates and of sodium dihexyl- and dioctyl sulfosuccinates on soybean trypsin inhibitor was investigated (Jirgensons, 1962). The surface active anions enhanced the viscosity of this protein and caused a negative shift of b_0. The optical rotatory dispersion curve of the native protein was smooth in the far ultraviolet, and the surface active anions produced a Cotton effect at 230–240 nm. Evidently, the hydrophobic groups of the anions interacted with the hydrophobic interior of the protein, and the native nonhelical conformation of the protein changed into a different order. This change is opposite to that observed in denaturation of helical proteins. Circular dichroism of solutions of soybean trypsin inhibitor and papain was determined in the presence and absence of sodium dodecyl sulfate (Jirgensons, 1972). The circular dichroism bands in the 240–320 nm spectral zone were almost completely eliminated by the detergent, a condition indicating that tertiary folding was disturbed. The circular dichroism bands in the far ultraviolet were modified by sodium dodecyl sulfate in a manner that indicated increase of the α-helix content in these proteins. Reconstructive denaturation is suggested for this type of denaturation in which the helix content of the protein is increased by the denaturing agent.

Urea and guanidine hydrochloride

The viscosity of native soybean protein and urea-denatured protein increased with protein concentration, and the urea-denatured protein displayed great concentration dependence (Shibasaki et al., 1969). The protein increasingly dissociated into subunits with increasing urea concentration and time of exposure to urea. Sedimentation coefficient of the subunits obtained by treating soybean 7S protein with 8M urea was 1.35S and molecular weight was 22,500 (Koshiyama, 1970). Urea treatment almost completely unfolded the subunit structure on the basis of optical rotatory dispersion.

Soybean 11S protein undergoes dissociation into subunits in the presence of relatively low concentrations of urea (Wolf and Briggs, 1958). The 11S protein is irreversibly dissociated by urea, and dissociation products remain in solution and do not show significant association after removal of the urea (Vaintraub, 1967). Chromatography of dissociated 11S protein reveals four main fractions, each of which is characterized by a particular N-terminal amino acid and a particular group of electrophoretic zones. Therefore, the 11S protein contains at least four different kinds of subunits.

Ultraviolet difference spectra indicated that urea and guanidine hydrochloride at increasing concentrations caused progressive exposure of tyrosine and tryptophan residues in soybean 11S protein (Catsimpoolas et al., 1970b). Electrometric and spectrophotometric titrations of the 11S protein in 0.4M KCl in the presence of 6M guanidine hydrochloride and 6M urea showed that conformational changes occurred at alkaline and acidic pH values (Catsimpoolas et al., 1971a).

A relatively small change in viscosity and polarization fluorescence of soybean trypsin inhibitor occurs in 9M urea (Edelhoch and Steiner, 1963). At high temperatures or at alkaline pH, a transition to a largely unorganized structure occurs in the presence of 9M urea. In addition, a blue shift develops in the difference spectrum, and major changes in viscosity and polarization of fluorescence take place.

Hydrogen-ion titration curves of wheat gluten in 2M and 4M guanidine hydrochloride at 25°C indicated that the empirical electrostatic factor w at acid pH was considerably larger than it was at alkaline pH, and a larger decrease in w was observed in alkaline than in acid solutions when the solvent was changed from 2M to 4M guanidine hydrochloride (Wu and Dimler, 1964a). These changes in w suggest that the conformation of gluten in guanidine hydrochloride depends on pH and that the conformation in acid solution is more stable. Hydrogen ion equilibria of wheat gluten, glutenin and gliadin in 3M urea plus 0.15M KCl at 25°C showed that the empirical electrostatic factor w at acid pH was significantly larger than it was at alkaline pH for each protein, and this difference suggests that a conformational change has occurred (Wu and Dimler, 1963a, b).

Viscosity, sedimentation velocity, ultraviolet difference spectra and optical rotatory dispersion measurements were carried out on wheat gluten, glutenin and gliadin in 3M urea plus buffer at pH 3–10 at 25°C (Wu and Dimler, 1964b). An increase in intrinsic viscosity and a decrease in sedimentation coefficient for glutenin at pH 10, compared with that at pH 4, suggested an increase in asymmetry of the protein molecule. Optical rotatory dispersion results on glutenin also indicated a conformational change at pH 10.

Conformations of gluten, glutenin and gliadin were studied by optical rotatory dispersion in 3M urea plus buffer at several pH values, and in 0.002N HCl (Wu and Cluskey, 1965). Gliadin contained more α-helix than glutenin and all three proteins contained more α-helix in hydrochloric acid than in urea solutions. From optical rotatory dispersion data an α-helix content of 38% was calculated for gliadin in trifluoroethanol; 35% for glutenin. On the other hand, little or no α-helix was found when the proteins were dispersed in 8M urea solutions (Cluskey and Wu, 1971).

The number-average molecular weights

of wheat gluten and gliadin by osmotic pressure at 25°C between pH 4.7 and 7.5 are 67,300 and 46,600, respectively, and are constant at ionic strengths between 0.0025 and 0.15 (Wu et al., 1967). Gluten and gliadin are aggregated considerably in aluminum lactate buffer of pH 3.2 to 3.4, but in 3M urea plus aluminum lactate buffer the molecular weight of gluten is not much higher than 67,300. The molecular weights from ultracentrifuge data of gliadin, and gliadin with the high-molecular weight component removed by a crosslinked dextran column, were 49,200 and 30,300, respectively, in 3M urea plus 0.15M KCl at pH 3.1 (Sexson and Wu, 1972). Molecular weight for gliadin in 6M guanidine hydrochloride plus 0.1M acetic acid was 46,000 by sedimentation equilibrium (Beckwith et al., 1966).

Increase of intrinsic viscosity and decrease of turbidity of glutenin were observed with increasing concentrations of guanidine hydrochloride up to 6M, and a limiting state was attained above 6M (Hamauzu and Yonezawa, 1972). The molecular weight of glutenin in 3M guanidine hydrochloride was 3.8 million and decreased to a limiting value of 850,000 above 6M.

The molecular weights of wheat γ_1- and γ_3-gliadins in 3 and 8M urea, as well as in 6M guanidine hydrochloride, at several pH values were determined from sedimentation equilibrium data in an ultracentrifuge (Sexson and Wu, 1972). The respective minimum molecular weights for γ_1- and γ_3-gliadins, obtained in 3M urea plus 0.15M KCl at pH 3.1, were 30,300 and 34,700.

The molecular conformation of sorghum prolamin was studied by optical rotatory dispersion, circular dichroism and infrared spectra in several solvents (Wu et al., 1971). Optical rotatory dispersion data of the prolamins in 60% tert-butanol gave α-helix contents of 40–47% independent of hybrids or the color of the prolamin solution. The α-helix content of the prolamins in 60% tert-butanol + 1.5M guanidine hydrochloride is lowered somewhat to 34–40%, but is greatly reduced in 6M guanidine hydrochloride. The circular dichroism and far ultraviolet optical rotatory dispersion curves of decolorized prolamin gave an α-helix content in agreement with that from optical rotatory dispersion data. The optical rotatory dispersion properties of sorghum prolamins did not change during the formation of gels in 60% v/v tert-butanol-water solvent.

The weight-average molecular weight for 95% ethanol-soluble fraction of sorghum prolamin in neutral 6M guanidine hydrochloride decreased from 65,400 to 44,600 when the initial protein concentration decreased from 0.21 to 0.11 mg/ml (Beckwith and Jones, 1972). When the 6M guanidine hydrochloride solvent was made acidic with either 0.1M citric acid or 0.001N HCl, molecular weight was 85,200 and was the same for protein concentrations between 0.49 and 0.15 mg/ml. Molecular weight of a single component of sorghum prolamin in 6M guanidine hydrochloride decreased from 27,500 to 20,500 when the initial protein concentration decreased from 0.41 to 0.14 mg/ml.

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SPINNING OF ZEIN

INTRODUCTION

AS EARLY AS 1857, a British patent was granted for making filaments from proteins and in 1935 a commercial protein fiber was produced (Moncrieff, 1963). Lundgren (1949) has written a review article on protein fibers. He discussed aspects of solubilization and unfolding of protein molecules, molecular orientation and crystallization as related to fiber formation, and to the mechanical properties of the fibers formed. His article summarized the knowledge on protein fibers prior to 1949.

Trail (1945) has presented the "Ardil" process of producing textile fiber from groundnut protein. Several articles by Naismith and Thomson (1955) dealt with the formation of fibers from groundnut protein with emphasis on denaturation and on viscosity changes during the spinning process.

Moncrieff (1963) and Peters (1963) have outlined the technical processes (wet spinning) used in producing textile fibers from several natural protein sources such as casein, soybean and zein.

In recent years, interests have been directed to the production of edible fibers from proteins which could then be used in making synthetic and simulated foods. In 1954, Boyer (1954) developed the first procedure of producing synthetic meats from vegetable proteins (soybean, corn, peanuts) and from animal proteins such as casein and keratin. The process is essentially preparing a dope of protein, spinning into a coagulating bath to form filaments, stretching and further treatment of the filaments with binders to form the desired texture. Several other patents appeared with the same basic process which varied in the kind of protein used, the solvent for the dope, the composition of the coagulating bath and the after-treatments to which the filaments were subjected to (Noyes, 1969). A continuous process for making shaped protein products was developed by Westeen and Kuramoto (1964). A slightly different process was developed by Anson and Pader (1968). Their process uses protein gel precursors (instead of protein dope solutions) which are then gelled by heating after the extrusion procedure. Dudman (1957) recommended heating the edible fibers without using binders.

Several commercial processes are now producing simulated meats from proteins, especially vegetable protein. Articles describing these processes have appeared, but mostly dealing with the equipment and unit operations rather than a technical analysis of the process of spinning the proteinaceous material (Ziemba, 1969).

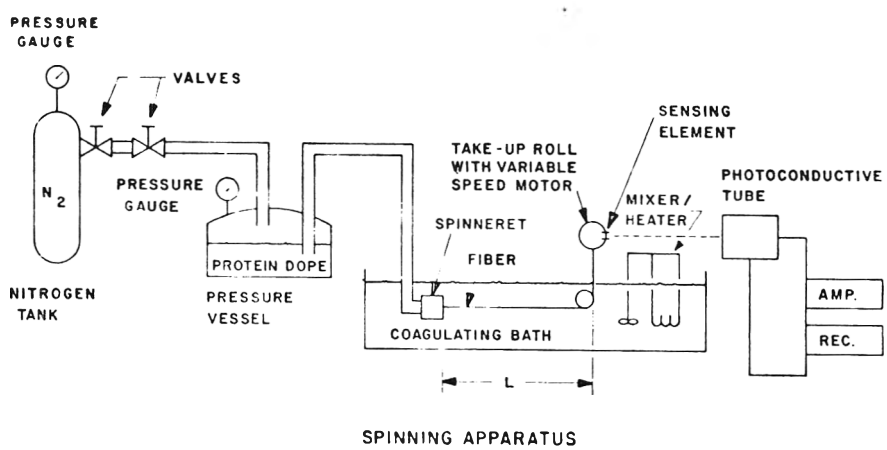
Zein stands out among the various proteins as having superior fiber-forming properties (Lundgren, 1949). It has high enough molecular weight (20,000–50,000) and an axial ratio of about 20:1 (Mertz et al., 1966). Zein which is the alcohol soluble part of corn gluten, is soluble in low alcohols. Ethyl alcohol and isopropyl alcohol seem to be the best solvents. Zein is soluble at pH 6, and is precipitated at pH 3.

Zein is deficient in lysine (Sherman, 1952) with poor nutritional value. Spinning of zein is not new, and once commercial textile fibers were produced from zein. However, up to the present, spinning dynamics of zein or any other proteins have not been studied systematically. This investigation attempts to initiate such a study to establish the relationship between some of the spinning variables which may apply to spinning of other proteins. In particular, the maximum drawing velocity is determined for increasing values of the spinning length. The effect of other variables such as dope composition, velocity through the spinnerette, bath temperature and diameter of spinnerette were also studied.

Background

All proteins are potential fiber-forming materials, because they are linear macromolecules. However, due to differences in chain length, size, shape, chemical nature and disposition of amino acid residues along the chain, differences in their fiber-forming properties exist (Lundgren, 1949).

In wet spinning the mechanism of fiber formation along the spinning line could be described as follows (Paul, 1968a; Ziabicki, 1967a, b): As the filament comes in contact with the bath, diffusion (counter-current) of the solvent out of the dope and the coagulating medium into the dope takes place. When the right concentration of the components is reached, structural rearrangement in the filament involving the solvent, the medium and the polymer occurs. A skin of precipitated polymer is then formed. The thickness of this skin grows as the filament travels along and more polymer is precipitated. At the same time, the filament is being elongated by means of tensile force applied by the drawing roller. The diameter of the filament therefore decreases along the spinning line. At a considerable distance from the spinner-



SPINNING APPARATUS

Fig. 1—Diagram showing various parts of the spinning apparatus.

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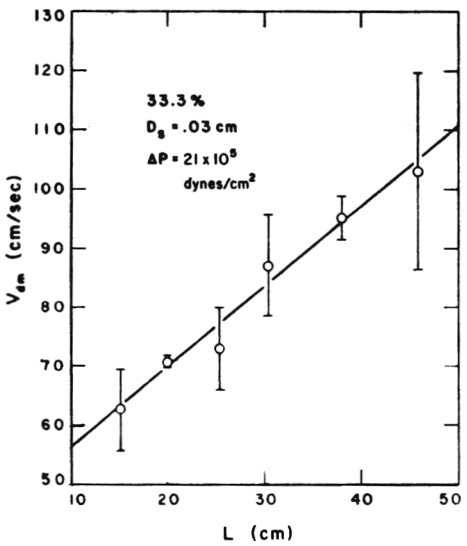

Fig. 2–Figure showing one of the spinnability curves.

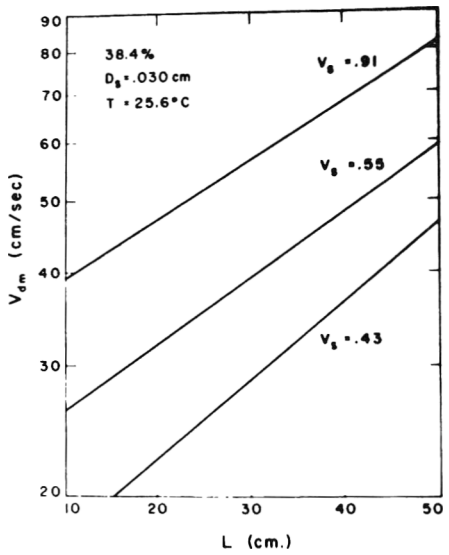

Fig. 3–Effect of extrusion velocity (V_s) on the spinnability curve.

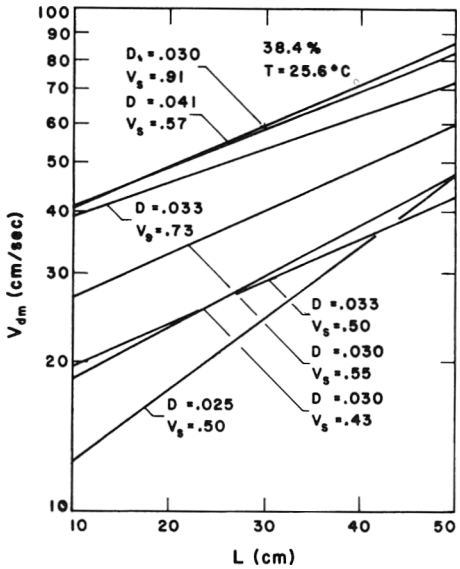

Fig. 4–Spinnability curve at different spinnerette diameters.

ette, a stable, coagulated filament is formed which could be wound up with a certain velocity of drawing.

With these considerations, the different variables and parameters that affect the spinning process are the following:

(a) Spinning variables such as spinnerette dimensions, drawing velocity (V_d), spinning length (L) which is the distance that fiber travels within the coagulation bath (see Fig. 1), and the velocity through the spinnerette;
(b) Composition and properties of the dope (uncoagulated, coagulated, and fully coagulated);
(c) Composition and temperature of the spinning bath; and
(d) Coagulation properties involving the dope and the bath.

An analysis of the interrelationships between these variables may lead to a measure of spinnability, which could be defined as the limiting condition on the spinning process.

MATERIALS & METHODS

Description of the spinning apparatus

The laboratory spinning apparatus (see Fig. 1) was designed and constructed using a Millipore dispensing vessel, connected to a nitrogen cylinder, as the dope reservoir and a Hamilton square end hypodermic syringe needle as the spinnerette. The needle is attached to the vessel by means of a luer lock connector and a connecting tube. The pressure inside the vessel is read from a pressure gage. The bath container is a rectangular plexiglass tank, fixed with a provision for adjusting the spinning length (from tip of needle to rolling drum). The rolling drum is a plexiglass cylinder which is attached to a variable speed motor. A heater and mixer assembly is used to maintain the bath temperature accurate to ± 0.1°C. The rotational velocity of the roller (and hence the linear velocity of drawing) is measured by means of a phototube recorder assembly.

Preparation of zein dope

The dope was made by dissolving a weighed amount of zein (Zein F-200, Freeman Laboratories, Tuckahoe, N.Y.) in a certain volume of 95% ethyl alcohol. The dilutions used were 50g of zein in 125, 112.5, 100, 87.5 and 75 ml of alcohol. Using the percentage purity of the zein (98% from Zein F-200 Data Sheet, Freeman Laboratories), these dilutions correspond to 33.3, 35.7, 38.4, 41.6 and 45.4% of pure protein by weight.

Procedure

For a set of spinning lengths, the maximum velocity of drawing was determined. These sets of (L vs. V_{dm}) values were determined for several values of the flow rate in the spinnerette, the spinnerette diameters, the dope concentrations and the bath temperatures.

The technique of getting the maximum velocity had to be developed. As the dope comes out of the needle, it sticks to the end of the needle such that, a glob of dope forms. The glob is then caught by a pair of tweezers and slowly pulled under the holder and attached to the roller. The motor is then turned on and the velocity is slowly increased. When the tension on the filament is great enough to overcome the interfacial tension on the needle rim with the dope sticking to it, then the dope comes out in a clean filament formation. That is, if the thread is released, the extruded dope will come out as a cylinder and not as a glob like in the beginning. Through a microscope (traversing), one could easily see the bulb stretch which is then followed by gradual reduction in diameter along the spinning length. In fact, in this experiment, this typical die swell phenomena could be observed directly even without the microscope.

Once a true filament is obtained, the velocity of drawing is then gradually increased (almost infinitesimal variation as could be done manually), until the filament breaks at the needle. This was generally done twice before a reading was taken.

The spinning length is then increased and the procedure repeated for six or seven different lengths. The replicates (5–6) were obtained by starting at the first value of (L) and then increasing L up to the highest value. Half of the replicates were also obtained by using one particular length and repeating the procedure for this length.

During the conduct of the experiment, the temperature of the bath was constantly checked for uniformity. Occasional mixing was also done to make the bath composition uniform. The bath was also changed after each set of experiments.

RESULTS & DISCUSSION

THE RELATIONSHIP between the maximum drawing velocity and the spinning length is shown in Figure 2 for 33.3% zien dope at T = 25.6 and D = (0.03 cm). The points used are the mean average of the replicates and the 95% confidence limits are also shown. A regression analysis was performed on each curve and the results showed that except for a few points, the curves approximated a straight line. An improvement was obtained by using a second degree polynomial for some of the curves, but the use of straight lines would be within statistical error and would also facilitate the analysis of the data.

The maximum drawing velocity (V_{dm}) increases linearly with the spinning length (L), except for 45.5% dope at 25.6 and 27.8°C where the velocity of drawing is

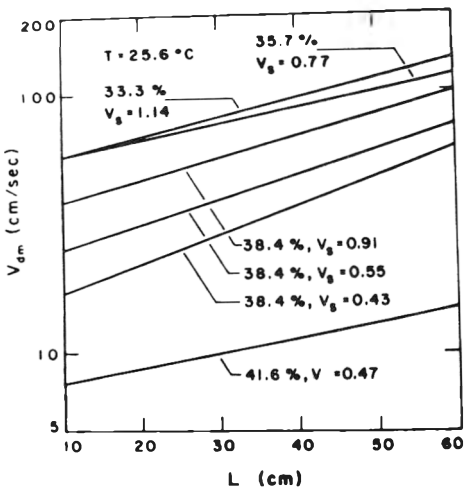

Fig. 5—Spinnability curve at different dope concentrations.

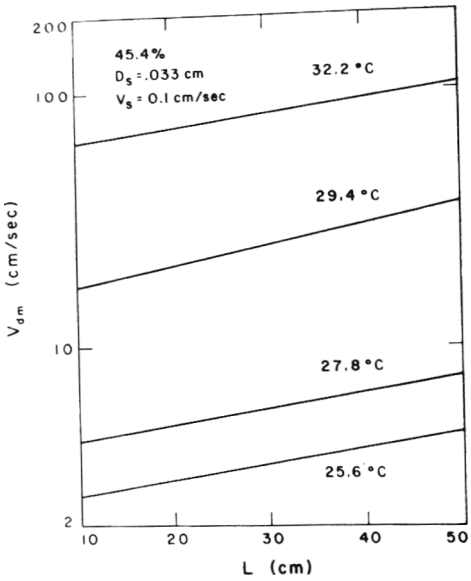

Fig. 6—Effect of temperature on the spinnability curve.

nearly constant. Considering the filament as a homogeneous fluid in elongational flow an increase in length means a decrease in the elongational strain ($=\delta V/\delta L$), hence a decrease in the tension along the filament. Thus the velocity could be increased before the tension needed to break the thread is reached. This relationship between the maximum drawing velocity and the spinning length (defined as the spinnability curve) will be examined as it is affected by the bath temperature, the composition and viscosity of the dope, the velocity of extrusion and the diameter of the spinnerette. The spinnability curve indicates the region where the spinning of zein is possible. Below the curve, one can always spin a dope under the given conditions. Above the curve, one could not obtain a continuous filament.

In Figure 3, the maximum drawing velocity (at each value of L) is found to increase with an increase in the extrusion velocity, V_s. This seems to agree with what one might actually expect. That is, if V_s increases, then for the same spinnerette diameter the volumetric flow rate (Q_s) would increase. From the continuity equation, either the velocity or the diameter at L should increase. It is more likely that the velocity would increase since the diameter could not increase indefinitely. Paul (1968b) and Han and Segal (1970) found that it is actually the free jet velocity (V_f) which determines the drawing velocity because of the Barus or die swell effect. The free jet velocity is in turn affected by the extrusion velocity, the diameter of the spinnerette or the flow rate.

The direct relationship between V_{dm} and V_s is affected by a change in the spinnerette diameter (Fig. 4). For example, using 38.4% dope at 25.6°C, for approximately the same extrusion velocities (V_s) of 0.50—0.57 cm, the highest drawing velocity (V_{dm}) was attained with the largest spinnerette with 0.041 cm diameter, lowest with the smallest diameter of 0.025 cm, and in between with 0.033 and 0.30 diameter spinnerettes. Paul (1968b) observed that V_{dm} may be affected by Q_s and D_s in an opposing manner due to momentum and elastic energy considerations and he suggested an optimum hole size where V_{dm} or the free jet velocity is maximum for a given flow rate. In Figure 4, the curves for larger diameters and higher extrusion velocities tend to be higher than those for smaller diameters and lower extrusion velocities.

The dependence of the spinning curve on the extrusion velocity is also affected by changes in dope concentration (Fig. 5). The figure shows that as the concentration increases, the drawing velocity for a particular spinning length decreases. As the concentration increases, the viscosity becomes greater while the rate of coagulation decreases. It is harder to draw a more viscous fluid than a less viscous one (Han and Segal, 1970). Paul (1968b) explained the effect of coagulation rate in terms of die swell. As the coagulation rate is increased, the skin of the filament is formed at a faster rate. This skin restrains the swelling of the filament such that the jet issues with a faster velocity. Accordingly, V_{dm} increases as the concentration is decreased. However, after 35.7%, there seems to be a leveling out. The curve for 33.3% almost coincides with that for 35.7% although the extrusion velocity is so much higher (1.14 compared to 0.77 cm/sec). This might be due to lesser elasticity for the less concentrated dope.

Figure 6 shows the effect of the bath temperature on the spinning curve. As the temperature increases, the filament could be drawn at a faster rate. At the higher temperatures, just a 3° change in temperature produces a large increase in the magnitude of V_{dm}. The effect of temperature on the mechanics of the process is twofold. It causes changes in the physical and rheological properties of the filament and it also affects the coagulation process. Higher bath temperatures give faster coagulation rates and this would increase the drawing velocity. An increase in temperature would also lower the viscosity of the filament and this again leads to greater drawing velocity. Han and Segal (1970) found that it is indeed easier to draw a viscous substance at higher temperatures than at low temperatures. This is similar to the results of Paul (1968b), both the free jet velocity and the drawing velocity increased with temperature.

CONCLUSIONS

THE LINEAR relationship between the maximum drawing velocity and the spinning length has been defined as the spinnability curve. This curve indicates a limit on the wet spinning conditions for zein.

The maximum drawing velocity is dependent on the extrusion velocity.

Higher bath temperatures give a higher spinnability curve (thus increasing spinnability region).

In general, larger diameters gave higher spinnability curves than smaller spinnerette diameters.

Dopes of lower concentrations could be drawn faster than dopes of higher concentrations.

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CONDITIONS FOR THE SEPARATION OF OIL AND PROTEIN FROM COCONUT MILK EMULSION

INTRODUCTION

COCONUTS are grown mainly in countries where there is a protein deficiency in the human diet. Oil is commercially obtained from the dried coconut kernel (copra) and the residual cake (poonac) which contains proteins, is used as animal feed. During the commercial extraction of oil, the high temperature of the expellers lowers the quality of proteins in the residual cake. The characteristics of the fresh and heat-treated proteins derived from residual cake have been examined (Samson, 1971).

Annual world production of coconuts would have a potential yield of approximately 200,000 metric tons of protein (USDA, 1971). In an attempt to obtain coconut protein for human consumption as a by-product of the oil industry, a number of wet processes for coconut oil extraction have been developed. In one process, the oil and the proteins have been separated from an aqueous extraction of kernel by heat and centrifugation (Rajasekharan and Sreenivasan, 1967). In the Robledana-Luzuriage (1956) process, the cream obtained by centrifugation is subjected to enzyme action, frozen and thawed to break the emulsion. In the Roxas (1963) process, the coconut milk obtained from the pasteurized coconut kernel is frozen to −4°C and thawed to break the emulsion. Peters (1960) reported breaking the emulsion in coconut milk by freezing and thawing. In a more recent method, the emulsion in coconut milk was broken, inversing the emulsion by shear (Hagenmaier et al., 1972; 1973).

This paper describes process conditions for breaking the emulsion in coconut milk by chilling the whole mass of cream obtained by centrifugation to 17°C or below with subsequent thawing, instead of freezing and thawing the milk or cream.

MATERIALS & METHODS

ALL CHEMICALS used were purchased from British Drug Houses Ltd, London. Fully mature coconuts harvested within 3 days were purchased from the local market in Colombo. The kernels of these coconuts had about 34% oil and about 5% protein.

Equipment

A Mince Master was used for pulping the coconut kernel. It has rotating blades which are powered by a 6.6 kw motor. There is a perforated plate below the rotating blades. The pulped material is discharged through apertures in the plate. This Mincer was purchased from Food Tech, London.

A Hander Baby Oil Expeller, a pilot-plant scale screw press, was used for expelling oil from the oil-bearing seeds. It is powered by a 0.75 kw motor. This expeller was purchased from Fukoku Kogyo Co., Japan.

A Vulcan-Laval Cream Separator was used to obtain cream from coconut milk. It has a rated capacity of 100 gal of cows milk/hr. It was purchased from Vulcan-Laval Ltd, Poona, India.

Methods

Amino acid analysis. Amino acid analysis was carried out at the Tropical Products Institute, London using an EEL 194 automatic analyzer according to the procedure described by Moore et al. (1958). Tryptophan was estimated by the Miller (1967) method after alkaline hydrolysis.

GLC of fatty acids. Methyl esters of fatty acids were analyzed in a Varian Gas Liquid Chromatograph equipped with a thermal conductivity detector. Fatty acids of oil obtained by the described process and of refined coconut oil marketed by British Commercial Co., Ceylon, were analyzed.

Oil analyses. The total oil in coconut cream or milk was estimated by the Werner-Schmid method as described by Pearson (1970). Percentage of free fatty acids, refractive index, color in Lovibond scale, saponification value, iodine value, Reichert value, Polenske value and Kirschner value of oils were determined by the standard procedures described in British Standards (1967).

RESULTS & DISCUSSION

Process conditions

Coconut kernel (undried) was disintegrated with an equal weight of water for 2 min in a Mince Master. Coconut milk was obtained by pressing the disintegrated kernel in a hand operated stainless steel press. Coconut milk was filtered through a piece of cloth and centrifuged in a "Vulcan Laval" Cream Separator.

The cream from the Separator was collected in shallow aluminium pans and chilled for 2 hr in a bottle cooler maintained at 10°C and then brought to room temperature (25°C). The emulsion broke with the formation of an oil layer on the surface of the liquid in the pans. Recentrifugation in the Cream Separator enabled the complete separation of oil (A grade) from the rest of the liquid.

The skim milk was either spray dried or the protein in the skim milk was heat coagulated. The residue after extraction of coconut milk was dried at 100°C for 2 hr and passed through an expeller to give B grade oil and meal. The isolation of skim milk proteins and the extraction of oil from the residue after the removal of coconut oil, has been described in other wet processes (Sreenivasan, 1963). The flow diagram of the wet process presented in the paper is given in Figure 1.

Two parameters of the process, namely the degree of packing of the oil globules in the centrifugation stage and the temperature to which the cream had to be lowered for the complete release of oil from the emulsion, were investigated on a laboratory scale.

Degree of packing of the oil globules

Coconut milk was centrifuged at 2000G in 50 ml tubes for varying lengths of time. After chilling them for 4 hr in a bottle cooler maintained at 10°C, the contents of the tubes were brought back to 25°C, by dipping the tubes in a water bath at 40°C. Then the tubes were centrifuged at 4000G for 5 min. The oil released was taken out with a hypodermic syringe. The amount of oil released in each case was measured and expressed as the percentage of total oil present in the emulsion. These values were plotted against time of centrifugation prior to chilling. As shown in Figure 2 (identical curves were obtained from three runs) the percentage of oil released increased with time of centrifugation prior to chilling. With about 5 min of centrifugation of the coconut milk at 2000G, prior to chilling, almost all the oil in the emulsion was released. If the centrifugal force prior to chilling was increased up to 5000G, even a few seconds of centrifugation was sufficient to release all the oil in the emulsion.

If coconut milk were chilled and

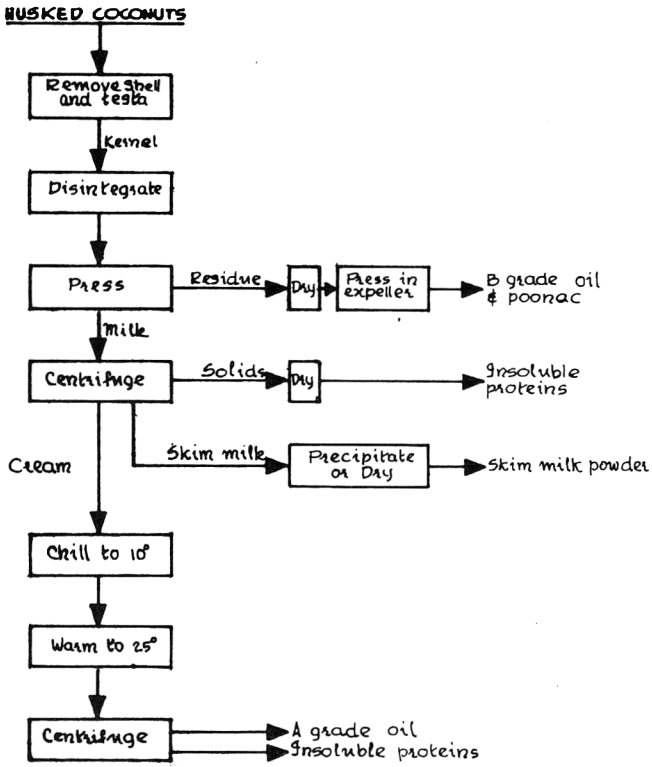

Fig. 1–Flow diagram of the new process.

thawed without prior centrifugation, there was no oil separation at all. Approximately 50% of oil in the emulsion was released if the coconut milk were centrifuged for 2 min, under the conditions described. Complete release of oil took place only after 5 min of centrifugation (2000G). These data show that centrifugation bringing about concentration of the dispersed phase in the continuous phase, is a prerequisite for breaking the emulsion under the conditions referred to.

Therefore one critical condition for the process would be adequate packing of the oil globules. On a laboratory scale, a centrifugal force of 5000G for a few seconds satisfied this requirement. Passage of coconut milk through a cream separator at the recommended flow rate (100 gal/hr) also satisfied this requirement. The oil content of the cream obtained from the cream separator varied from 60–78%. The water content of the cream varied from 20–12% (pH 6.1–6.4).

Effect of lowering the temperature of the cream

Coconut milk was centrifuged at 5000G for 5 min and kept immersed in a water bath maintained at temperatures between 5°C and 15°C for various intervals of time. The tubes were removed at definite time intervals and warmed in a water bath at 40°C until the contents reached 25°C. They were centrifuged at 5000G and the percentage of oil liberated was determined as before. Figure 3 shows the percentage of oil separated as a result of chilling at each temperature for definite intervals of time. (Identical curves were obtained from three runs.) The lower the temperature of the water bath, the greater was the release of oil. Oil was not released if the temperature of the water bath was above 17°C. These experiments show that the cream has to reach 17°C for oil separation to occur.

Even though the critical temperature for oil separation of the cream is 17°C, as shown in Figure 3, it took about 20 min of chilling at 5°C for complete oil separation. It took about 50 min of chilling at 7.5°C and about 80 min of chilling at 11°C for complete oil separation to occur. After 80 min of chilling at 15°C, only about 70% of oil was released. Oil separation will occur only in the regions where the temperature has reached 17°C or below. In a mass of cream, if only the outer layers have reached 17°C or below, there would be proportionate percentages of oil separation. At 15°C, it took 175 min of chilling to obtain complete oil separation. This requirement of longer time of chilling at higher temperatures (below 17°C, for example, at 15°C and 11°C) is caused by the low rate of heat penetration in coconut cream. When the cream in aluminium pans was chilled in a bottle cooler maintained at 10°C, it al-

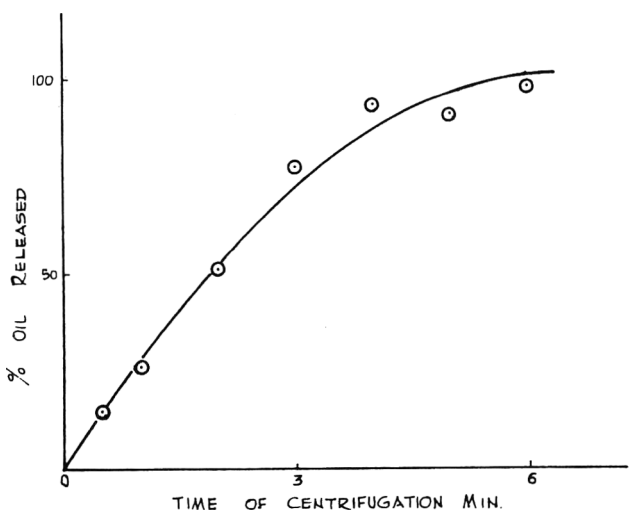

Fig. 2–Percentage of oil released after time intervals of centrifugation prior to chilling and thawing.

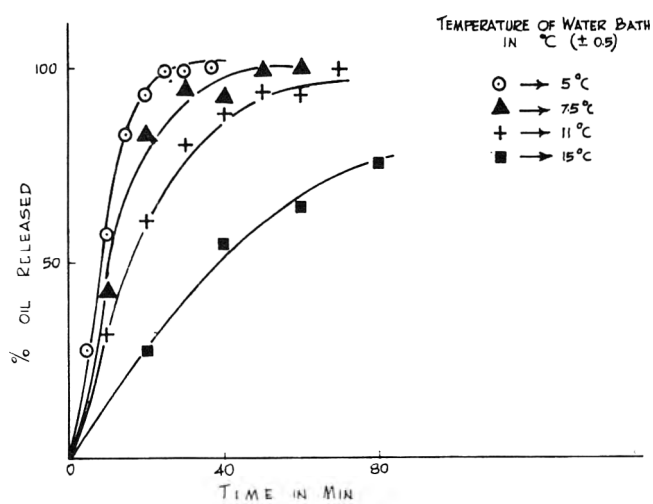

Fig. 3–Percentage of oil released after time intervals of chilling at varying temperature

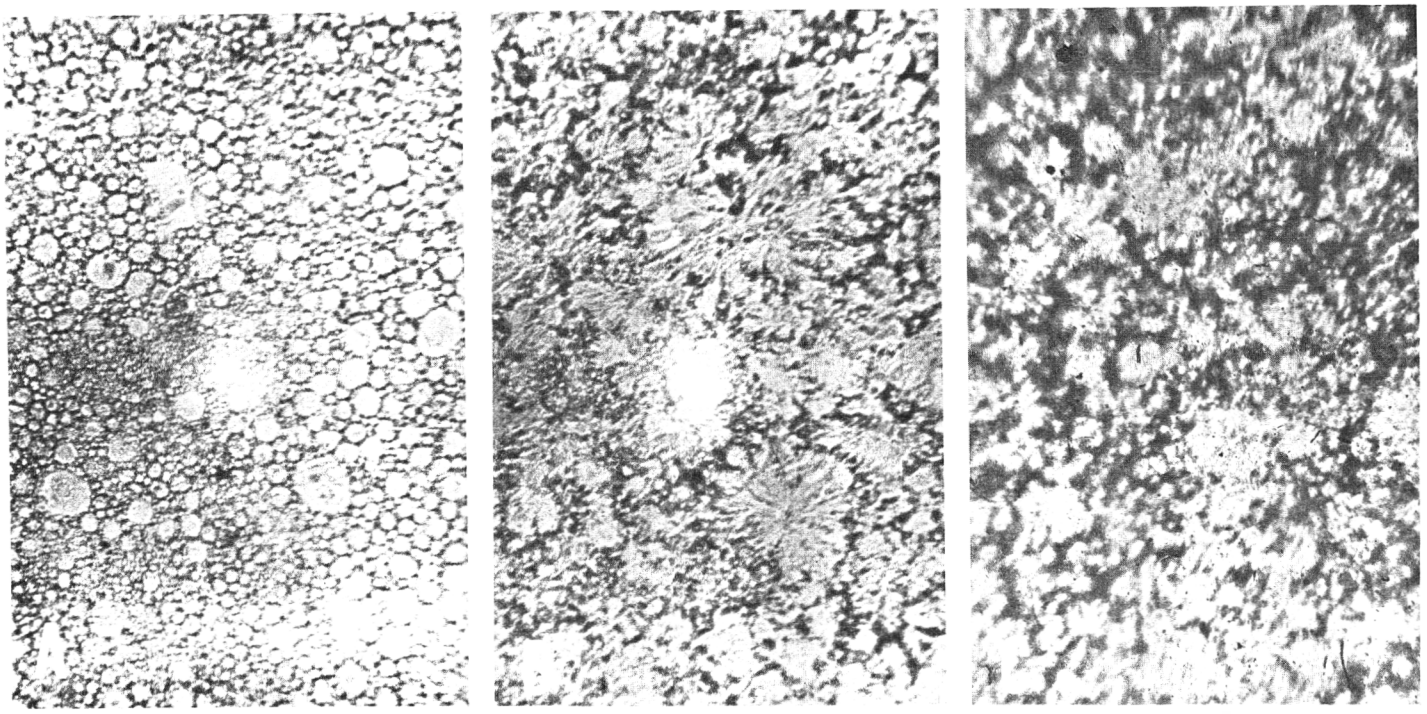

Fig. 4—Centrifuged cream (A) at 25°C; (B) at 17°C; and (C) after chilling to 17°C and warming up to 25°C. (Magnification ≈ 550X)

ways took less than 2 hr for the subsequent, complete oil separation, after thawing and centrifugation. During the 2 hr of chilling, another batch of cream was prepared and replaced the already chilled cream. Therefore, in the pilot-plant scale experiments, the cream was kept for 2 hr in the bottle cooler maintained at 10°C. In the flow diagram of the process (Figure 1) the chilling temperature is given as 10°C (2 hr), even though the critical temperature for breaking the emulsion is 17°C.

The process described differs from the other wet processes operating under similar conditions in that no enzymes are used and that it is not necessary to lower the temperature of the whole mass of cream below 17°C. Any process which requires the temperature of the whole system to be lowered to −4°C would inherently require a greater consumption of energy than a process which requires the temperature of the whole system to be lowered only to 17°C. In the former case a larger heat sink for the removal of latent heat would probably be required than the latter which only calls for the removal of sensible heat.

Microscopic view of the oil globules

The physical changes occurring at various stages of the process were examined under the microscope. As a result of centrifugation, the oil globules in the cream were tightly packed. As a result of lowering the temperature, the crystallization of oil took place. On thawing, the oil globules lost their spherical shape and coalesced to form large droplets of varying sizes.

Figure 4A shows the distribution of oil globules in centrifuged cream at 25°C. The oil globules in the centrifuged cream are densely packed and have an average diameter of 5μ at 25°C. Figure 4B shows the crystallized oil when the slide was kept for 10 min in a room maintained at 17°C. Unlike the oil globules at 25°C, the oil crystals seem to be in contact with each other. As the slide was warmed to 25°C, the oil melted and formed a more or less continuous phase as shown in the Figure 4C. Embedded in the continuous phase of oil are the proteins along with water. Figures 4A through 4C, suggest that the emulsion broke as a result of crystallization of oil in the cream.

Breaking of the emulsion as illustrated in Figures 4A to 4C, raised the question as to whether it was necessary to have the oil globules well packed during chilling for the occurrence of this phenomenon. Centrifuged cream was re-dispersed in 2, 5 and 10 volumes of water and in corresponding volumes of skim milk. The dispersions were chilled below 17°C in a water bath maintained at 10°C. On warming up to 25°C and on centrifugation, no oil separation was observed, suggesting that packing of oil globules is necessary for breaking the emulsion.

Composition of the proteins and oil

The amino acid composition of the two types of proteins available from the process was determined and compared with the amino acid composition of the coconut proteins as shown in Table 1.

Percentage of free fatty acids, refractive index, color in Lovibond scale, saponification value, iodine value, Reichert value, Polenske value, Kirschner value and the fatty acid composition of the oil obtained by the described process were determined and compared with those of the commercially available oil. There was no significant difference in the comparisons.

Generally, the oil obtained from all the wet processes, has been of superior quality (Dendy and Grimwood, 1972). The wet process described in this paper worked very well with batches of 50 kg of coconut kernel, yielding A grade oil. During disintegration and pressing of kernel, 93% of the total oil went into the emulsion and 7% remained in the the residue. Complete oil separation was obtained by breaking the emulsion which was originally formed from the 93% of the oil in the fresh kernel. Pressing of the dried dual in the conventional screw type expeller yielded B-grade oil. Pilot plant experiments would have to be conducted on a large scale to calculate the economics of the process. The oil obtained by this wet process on a laboratory scale, was stored at room temperature (25°C), in glass bottles with crown corks for 2 yr without the development of off odors or a significant amount of free fatty acids.

The secondary products of importance are the proteins from skim milk and from cream. The skim milk proteins obtained

Table 1—Amino acid composition of coconut proteins, g/16g N

Amino acid	Sample 1[a]	2[b]	3[c]	4[d]
Alanine	5.1	1.2	6.0	5.9
Arginine	16.6	1.6	14.7	15.6
Aspartic acid	9.2	1.3	12.7	10.7
Cystine	1.7	2.3	0.9	1.7
Glutamic acid	18.7	2.8	24.8	22.8
Glycine	2.2	1.7	3.5	3.1
Isoleucine	3.7	0.2	5.2	4.3
Leucine	6.9	1.2	9.7	8.9
Lysine	4.6	2.1	5.7	5.0
Methionine	1.6	0.4	1.8	1.9
Phenylalanine	4.4	0.5	8.7	6.8
Proline	3.6	1.5	4.0	4.5
Serine	5.2	1.1	5.8	5.8
Threonine	3.4	1.6	4.2	4.1
Tryptophan	0.9	1.2	1.4	1.2
Tyrosine	2.9	0.9	3.5	3.1
Valine	5.5	1.8	8.0	7.4

[a] Sample 1: Kernel after drying at 60°C for 3 hr and removing oil by solvent extraction.
[b] Sample 2: Residue after milk extraction, drying at 60°C for 3 hr and removing oil by solvent extraction.
[c] Sample 3: Skim milk proteins precipitated by heat after drying at 60°C for 3 hr.
[d] Sample 4: Cream layer proteins after the removal of oil by the described process and drying at 60°C for 3 hr.

from the wet processes have been tested in various feeding trials (Rama Rao et al., 1964). The skim milk proteins from the process which were precipitated by boiling for 10 min, and air drying at 60°C for 2 hr, contained 75.6% crude protein (N × 6.25). Air-dried proteins obtained from the cream after chilling and thawing contained 84.7% crude protein. These proteins are almost free of fiber and their use as a protein supplement is being studied in our laboratory.

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SWELLING STRESS AND HYDROSTATIC COMPRESSIBILITY OF GROUND CORN AND ITS CONSTITUENTS

INTRODUCTION

A CORN is a cellular material of complex structure and small in size. For practical purposes it is permissible to treat it as a continuous medium for calculating its elastic constants from mechanical tests. But, it is evident that constants of the solid materials of which corn is composed may be different from those of the whole kernel. Since we are interested in the elastic constants of these solid materials, the constants will be determined by the hygroscopic properties of these materials. The connection between mechanical and hygroscopic properties of corn can be established by thermodynamic methods which have the advantage of requiring no assumption about the molecular structure of the material.

Swelling and shrinkage of wood in relation to its mechanical properties was investigated by Barkas (1939, 1946). Barkas (1940) also described a method for finding the hydrostatic compressibility of wood-water aggregate. The same method was used in this investigation. White (1966) studied swelling stresses in whole corn, but, could not evaluate them because whole corn was a constrained structure and free swelling was not achieved.

The water vapor adsorption and increase in vapor pressure tends to increase the volume of the gels due to swelling. The stress exerted by gels during swelling is called swelling stress. This swelling stress could be visualized as the stress which could occur if the vapor pressure was raised to the saturation point and swelling was completely restrained.

Hydrostatic compressibility is the ability of the material to be compressed (decrease in volume) when a hydrostatic stress is applied. In this work hydrostatic stress is equal to swelling stress in magnitude but opposite in direction. Bulk modulus is the inverse of hydrostatic compressibility.

The information on swelling stress and hydrostatic compressibility is helpful in explaining the process of degerming (separation of germ and endosperm) in dry milling of corn. Furthermore, the quantitative values of swelling stresses can be utilized in determining the criterion of fracture in a corn kernel.

Theoretical consideration

The definition of a gel given by Katz (1933) and Barkas (1953) is as follows:

(1) Gels adsorb moisture from the surrounding atmosphere and hold it at an equilibrium vapor pressure lower than the saturation value at the same temperature.
(2) Gels swell on adsorption.
(3) Gels possess rigidity and can, therefore, withstand shear stresses as well as hydrostatic stresses.

Therefore, the ground corn can be considered a gel from the above definition. It is reasonable to assume that only one constituent of gel, namely water, is volatile.

In order to allow for an exact thermodynamic treatment, it is also assumed that the gel is perfectly elastic. The following derivation of hydrostatic compressibility will be based on swelling stresses in gels.

The hydrostatic compressibility is given by

$$B = -(1/\bar{V})(\partial \bar{V}/\partial p)_m \qquad [1]$$

where p is applied hydrostatic pressure, $\bar{V}$ is the specific volume of gel and m is the equilibrium moisture content, both calculated on a dry weight basis. Therefore, the hydrostatic compressibility of a gel can be calculated from the thermodynamic relationship of hydrostatic pressure, specific volume, moisture content and relative vapor pressure (p, $\bar{V}$, m, h).

Porter's equation for osmotic pressure of a solution

$$\int_{h_1}^{p_m} s_p \, dp_m - \int_{h_o}^{p_o} u \, dp_o = \int_{h_1}^{h_o} v \, dh \qquad [2]$$

gives the relationship between the hydrostatic pressures p_m and p_o to be applied to a solution of constant moisture content m and to free water, respectively, in order that their vapor pressures, initially h_1 and h_o, may be made equal (Porter, 1907). In equation [2] $s_p = (\partial \bar{V}/\partial m)_p$, specific volume increases per unit increase in moisture at constant pressure p; u = specific volume of liquid water at constant pressure p_o; v = specific volume of water vapor under pressure h, at saturation h_o.

Equation [2], being exact for any solution of which only one constituent is volatile, may be applied equally to gels fulfilling this equation. For the range of vapor pressures being used, h_1 and h_o may be considered zero in the first two integrals. For normal temperatures, v may be written as R_gT/Mh. It is assumed that compressibility is zero and $s_p = u$, then $udp_m = vdh$ or

$$p_m = -(R_gT/M\bar{u})(\ln(h_1/h_2)) \qquad [3]$$

where h_1 and h_2 are the initial and final vapor pressures, $\bar{u}$ is the mean specific volume of the water, R_g is universal gas constant, T is absolute temperature and M is molecular weight of water. At infinite dilution, the change in pressure at constant moisture content, dp_m equals the change in pressure at constant volume, $dp_{\bar{V}}$. Then, from equation [3] the pressure $p_{\bar{V}}$ required to raise a gel at constant volume $\bar{V}_o$ to any vapor pressure can be calculated so that the complete (p, h) system of curves can be constructed independently of any calculations of strains.

Also, dp_m can be obtained by equation [3] allowing for the compressibility of gel, provided the inevitable reduction in volume is counteracted by the addition of a small amount of water. This amount of water is represented by the line AB in Figure 1. It is also established that, if the counteraction of a solution is changed, while the pressure on it is continually adjusted so as to keep the vapor pressure constant, then change in volume of the solution is equal to the volume of water removed or added. This is represented by the equation

$$\bar{V}_1 - \bar{V}_2 = u(m_1 - m_2) \qquad [4]$$

This equation would be achieved in practice by slowly increasing the load on a solution in a constant vapor pressure.

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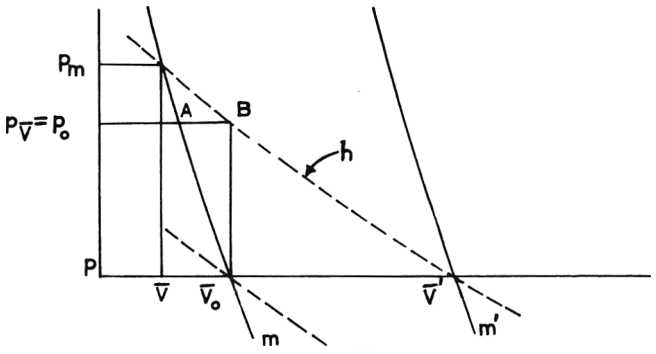

Fig. 1—Portion of (p, $\bar{V}$, m, h) diagram.

As in Figure 1, $\bar{V}_o$ is the original specific volume of a gel at constant moisture content m. This specific volume must be subjected to pressure p_m in order to raise its vapor pressure to h. Then, we have by equation [4], the change in specific volume ($\bar{V}_o - \bar{V}$), where $\bar{V}$ is given by

$$\bar{V} = \bar{V}' - u\,(m' - m) \qquad [5]$$

On the (p, h) system of curves discussed previously, the strained volumes $\bar{V}$, obtained by equation [5] are plotted on their appropriate relative vapor pressure lines. Next, the external pressure p_m is read from the graph. Finally, the hydrostatic compressibility is obtained by the slope of the resultant (p, $\bar{V}$) curves.

MATERIALS & METHODS

THE CORN used in this study was a midwest, open variety supplied by the Robinson Hybrid Corn Co., Delaware, Ohio. The pedigree of the variety was (WF9MSTXH71XO1.43RFXB37RF). The endosperm and germ were separated from whole corn by hand dissecting them after soaking overnight. The samples were ground to a size less than 60 mesh. Samples were dried in an air oven for 72 hr at 75°C.

Samples of ground whole corn, endosperm and germ were conditioned for moisture with a series of relative humidities from 11–97%. The desired relative humidities were maintained in desiccators by means of saturated salt solution methods given by Wexler and Hasegawa (1954), Carr and Harris (1949) and Wink and Sears (1950). The samples attained equilibrium moisture content in 2–3 wk, depending on the humidity shift. Mold growth was prevented in the high humidity desiccators by placing an open dish of toluene in it. The moisture content was determined by an air oven method (103°C, 72 hr), given by researchers at USDA (1959). All moisture contents in this work are presented on a dry weight basis.

Specific volume of ground corn samples was determined by the benzene displacement method (Stamm and Seborg, 1935). Benzene is used for density measurement because it will penetrate the void spaces in the cellular structure and does not add to swelling. Vacuum was applied to reduce the entrapped air. The air was removed by pulling and releasing the vacuum four to five times at 10–15 min intervals.

The specific volume of water at various relative vapor pressures, obtained from Barkas (1953), were modified for 26°C and 50°C by considering the compressibility and temperature of water. The relationship between specific volumes and relative vapor pressures was determined by the least square method (Steel and Torrie, 1960):

$$h = 1336606.40 \exp(-14.0968\,u)\ (26°C)$$
$$h = 1543257.78 \exp(-14.0968\,u)\ (50°C) \qquad [6]$$

A computer program was developed to determine the values of p, $\bar{V}$, m and h for constructing the (p, $\bar{V}$, m, h) diagram. The change in hydrostatic stress was calculated for each corresponding change of vapor pressure, using equation [3]. The interaction of the constant moisture curves and the vapor pressure curves were determined using equation [5].

The swelling stress for corn gel at constant volume was read from the intersection of the vapor pressure curves and the specific volume values at zero pressure. The hydrostatic compressibilities were determined from the slope of the constant moisture content curves at the given pressures.

RESULTS & DISCUSSIONS

The (p, $\bar{V}$ m, h) Diagram

The relationship between hydrostatic stress, specific volume, moisture content and vapor pressure for ground whole corn, endosperm and germ is illustrated in Figures 2, 3 and 4, respectively. Barkas (1940) observed similar phenomenon for

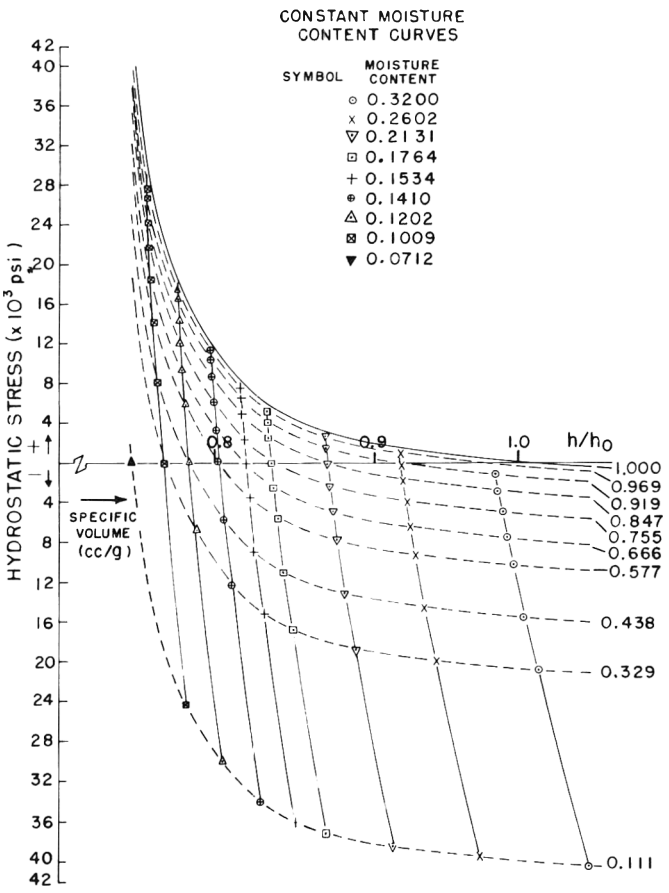

Fig. 2—Relationship between hydrostatic stress, specific volume, moisture content and relative vapor pressure for ground whole corn at 26°C.

wood gel in his experiments. The slope of (p, $\bar{V}$) curves at constant moisture contents decreased with an increase in pressure for whole corn and endosperm, while the slope for corn germ increased with an increase in pressure. The (p, $\bar{V}$, m, h) diagram indicated that ground whole corn and its constituents could be described by theoretical relationships for the swelling of gels.

It was also observed that hydrostatic compressibility (inverse slope of (p, $\bar{V}$) curves at constant moisture content in the (p, $\bar{V}$, m, h) diagram) increased with an increase in moisture content of ground whole corn, endosperm and germ. The phenomenon could be explained from molecular adsorption of water. In molecular adsorption hydroxyl anion (—OH) in the molecular structure offer active polar sites of bonding energy for the water molecules. At moisture content close to saturation, water molecules have satisfied the active polar site of hydroxyl anions. Additional moisture can be held by the formation of chains of water molecules due to dipole nature. When water chains are formed, the bonding forces between the hydroxyl anions at the end of the chains become smaller. In shear, the water molecules in the chain can jump from one anion group to another. This jump is not completely elastic because some energy is lost in the process. This slippage of anion bonds between neighboring chains was the most plausible explanation for increasing plasticity with increasing moisture content. An increase in hydrostatic compressibility would be expected with increasing plasticity. At the same time, increasing plasticity would decrease the bulk modulus of the ground whole corn, endosperm and germ.

The theoretical and experimental increase in hydrostatic compressibility for ground whole corn and endosperm at increased pressure could be explained by the investigation of Kumar (1972). The explanation is based on the fact that starch had more active polar sites than a germ material. The greater number of active polar sites would adsorb more water. Therefore, the greater number of active polar sites would increase the possibility of hydroxyl anion bond (—OH) slippage with internal shear stresses resulting from an increased external hydrostatic stress. The hydrostatic compressibility for ground germ at constant moisture content was increased with an increase in pressure because it had less active polar sites with less probability of (—OH) bond slippage with internal shear stresses resulting from an increased external hydrostatic stress. A similar phenomenon was observed for wood gels by Barkas (1940).

The hydrostatic compressibility and bulk modulus at pressures of 4000, 8000, 12000 and 16000 psi are given for ground whole corn, endosperm and germ in Tables 1, 2 and 3. The values of bulk modulus (inverse of hydrostatic compressibility) for endosperm were higher than for whole corn and for germ.

White (1966) determined the bulk modulus of gross whole corn and gross endosperm. In our experiments, however, it was found that bulk modulus of the ground whole corn and the endosperm were considerably higher than those of

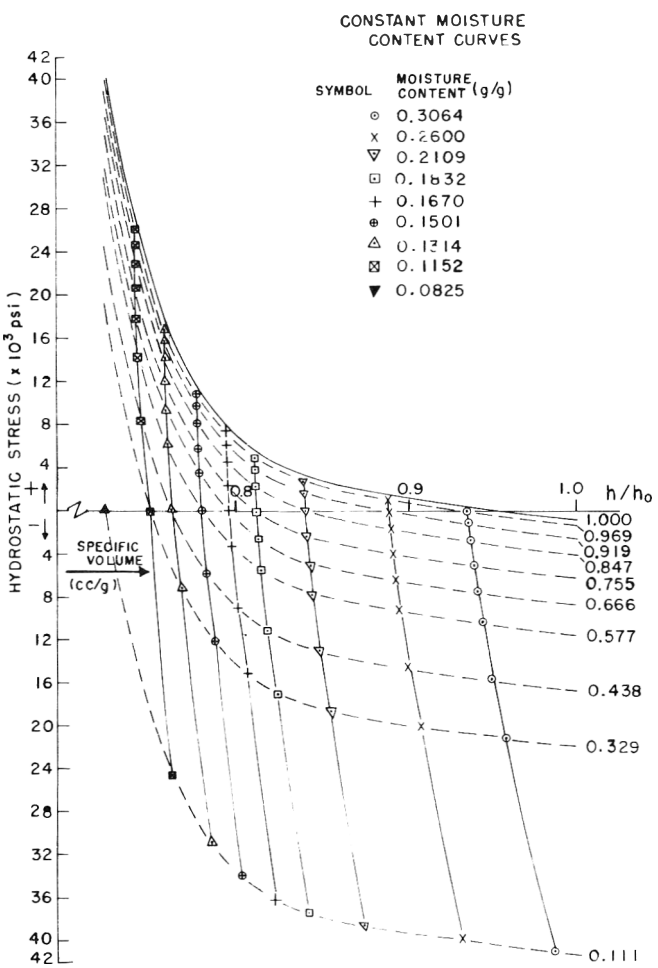

Fig. 3—Relationship between hydrostatic stress, specific volume, moisture content and relative vapor pressure for ground corn endosperm at 26° C.

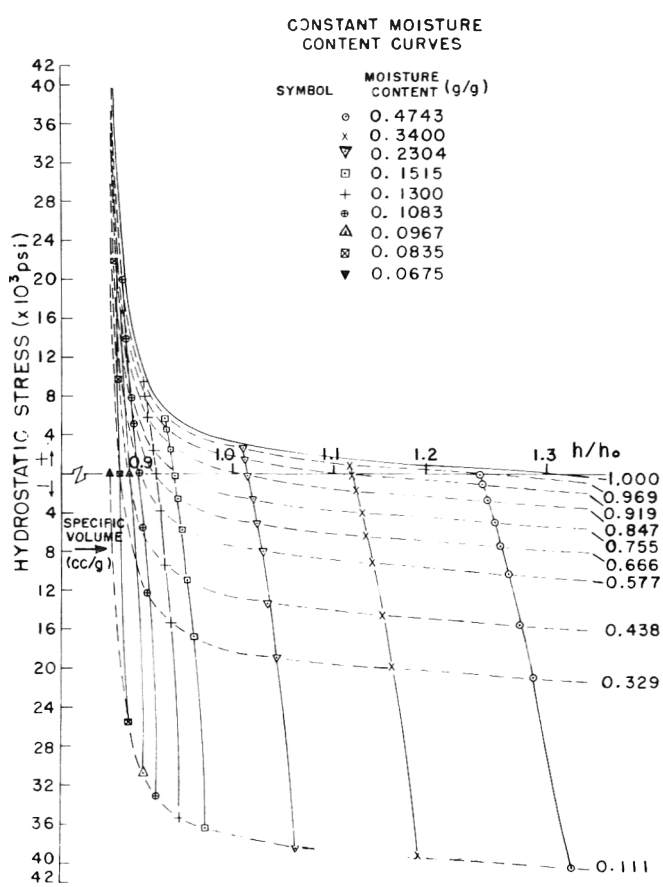

Fig. 4—Relationship between hydrostatic stress, specific volume, moisture content and relative vapor pressure for ground corn germ at 26° C.

the gross whole corn and endosperm found by White (1966). This difference was expected due to pores in the gross structure of the corn. The variation in the bulk modulus of the ground whole corn and the endosperm was less than the ground germ. A similar observation was made by Barkas (1953) on wood.

Swelling stresses

Swelling stress could be visualized as the stress which would occur if the vapor pressure was raised to the saturation point and swelling was completely restrained. The swelling stresses in the ground whole corn, endosperm and germ could be read at various conditions from the (p, $\bar{V}$, m, h) diagrams. It would be highest at the lower equilibrium moisture contents. Since at 100% relative vapor pressure the specific volume of the corn gel was not known, the (p, $\bar{V}$, m, h) diagram was established on the basis of relative vapor pressure of 97%. However, the swelling stresses were modified using equation [3] for relative vapor pressure of 100% at the two temperatures of 26°C and 50°C.

Swelling stresses at various moisture contents are shown in Figure 5 for ground corn endosperm and germ at 26°C. The decrease in swelling stress was hyperbolic in form. At 26°C for each moisture content, swelling stress for endosperm was higher than for germ up to 20.5% moisture content and vice versa for higher moisture levels. Swelling stress for ground corn germ ranged from 40750 psi at 6.75% moisture content to about 624 psi at 47.43% moisture content. For endosperm it ranged from 40750 at 8.25% to about 624 psi at 30.64% moisture content. The swelling stress in corn germ decreased faster than for corn endosperm at lower moisture contents up to 20.5%. After this, in corn endosperm, swelling stress decreased faster than in corn germ. The relationship between specific volume and swelling stress could be seen in (p, $\bar{V}$, m, h) diagram.

CONCLUSIONS

The ground whole corn, endosperm and germ were found to behave as gels. Therefore, the theory of swelling stress in gels is applicable to the swelling stresses in whole corn, endosperm and germ.

The magnitude of the swelling stresses in ground whole corn, endosperm and germ might be of the order of several thousand pounds per square inch at low equilibrium moisture contents, when subjected to increased vapor pressure.

The hydrostatic compressibility for ground whole corn, endosperm and germ increased with an increase in moisture content. This indicated that under the conditions of higher moisture contents and large magnitude of applied hydro-

Table 1–Hydrostatic compressibilities (B) and bulk moduli (K) of ground whole corn at various pressures from the (p, $\bar{V}$, m, h) diagram

Moisture content %	4000 psi		8000 psi		12000 psi		16000 psi	
	B (psi^{-1} $\times10^{-7}$)	K (psi $\times10^{6}$)	B (psi^{-1} $\times10^{-7}$)	K (psi $\times10^{6}$)	B (psi^{-1} $\times10^{-7}$)	K (psi $\times10^{6}$)	B (psi^{-1} $\times10^{-7}$)	K (psi $\times10^{6}$)
32.00	10.63	0.94	10.94	0.91	11.15	0.90	13.51	0.74
26.02	9.38	1.07	9.77	1.03	9.74	1.02	10.23	0.98
21.31	8.59	1.16	9.06	1.04	8.92	1.12	9.34	1.07
17.64	6.88	1.45	6.93	1.44	7.28	1.37	8.00	1.25
15.94	6.25	1.60	6.54	1.53	6.99	1.43	7.34	1.36
14.10	5.78	1.73	6.28	1.59	6.56	1.52	7.18	1.39
12.02	5.50	1.82	6.13	1.63	6.46	1.54	6.71	1.49
10.09	5.16	1.94	5.63	1.78	5.78	1.73	5.88	1.70

Table 2–Hydrostatic compressibilities (B) and bulk moduli (K) of ground corn endosperm at various pressures from the (p, $\bar{V}$, m, h) diagram

Moisture content %	4000 psi		8000 psi		12000 psi		16000 psi	
	B (psi^{-1} $\times10^{-7}$)	K (psi $\times10^{6}$)	B (psi^{-1} $\times10^{-7}$)	K (psi $\times10^{6}$)	B (psi^{-1} $\times10^{-7}$)	K (psi $\times10^{6}$)	B (psi^{-1} $\times10^{-7}$)	K (psi $\times10^{6}$)
30.64	8.13	1.23	8.45	1.18	8.61	1.16	8.98	1.11
26.00	6.72	1.48	6.89	1.45	7.21	1.38	8.28	1.21
21.09	5.38	1.85	5.53	1.81	6.62	1.51	7.81	1.28
18.32	5.16	1.93	5.23	1.91	6.60	1.52	6.95	1.44
16.70	5.00	2.00	5.13	1.95	6.25	1.60	6.64	1.51
15.01	4.84	2.07	5.00	2.00	6.04	1.66	6.41	1.56
13.14	4.63	2.16	4.84	2.07	–	–	–	–
11.52	4.44	2.25	4.59	2.18	4.69	2.13	4.77	2.10

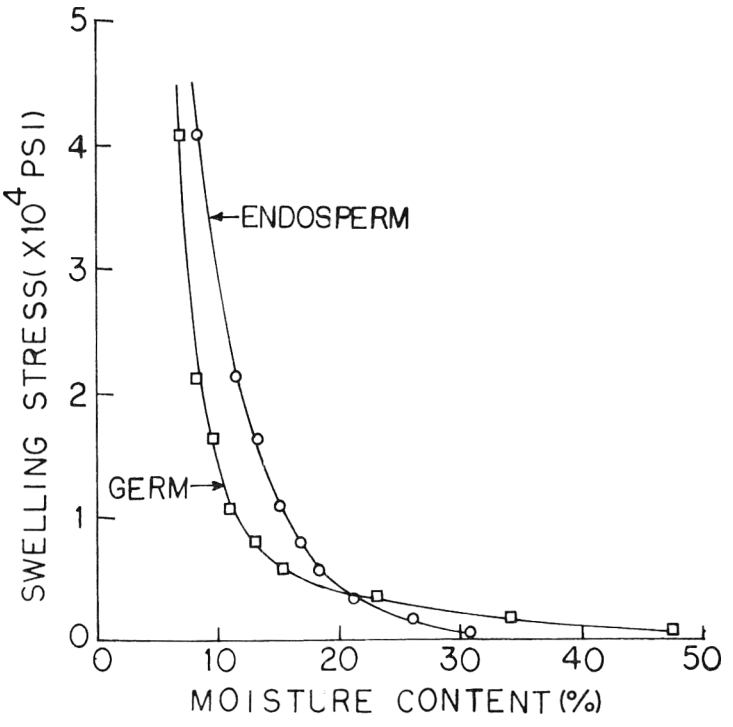

Fig. 5–Swelling stress of ground corn endosperm and germ at 26° C.

static pressures, the deformation was plastic.

SYMBOLS

- B Hydrostatic compressibility
- h Vapor pressure
- h_o Vapor pressure at saturation
- K Bulk modulus
- m Moisture content
- M Molecular weight of water
- p Hydrostatic pressure
- R_g Universal gas constant
- s_p Swelling volume increase per unit increase in moisture content at constant pressure
- T Absolute temperature
- u Specific volume of water
- v Specific volume of water vapor
- V Volume of gel
- $\bar{V}$ Specific volume of gel

Table 3–Hydrostatic compressibilities (B) and bulk moduli (K) of ground corn germ at various pressures from the (p, $\bar{V}$, m, h) diagram

	4000 psi		8000 psi		12000 psi		16000 psi	
Moisture content %	B (psi^{-1} $\times10^{-6}$)	K (psi $\times10^{5}$)	B (psi^{-1} $\times10^{-6}$)	K (psi $\times10^{5}$)	B (psi^{-1} $\times10^{-6}$)	K (psi $\times10^{5}$)	B (psi^{-1} $\times10^{-6}$)	K (psi $\times10^{5}$)
47.43	2.80	3.57	2.75	3.64	2.70	3.70	2.63	3.80
34.00	2.50	4.00	2.35	4.26	2.23	4.48	2.13	4.69
23.04	1.90	5.26	1.73	5.78	1.44	6.94	1.40	7.15
15.15	1.30	7.69	1.19	8.40	1.13	8.85	1.10	9.09
13.00	1.05	9.52	1.04	10.00	0.99	10.11	0.84	11.90
10.83	0.80	12.50	0.75	13.33	0.73	13.70	0.64	15.62
9.67	0.70	14.28	0.65	15.38	0.59	16.95	0.49	20.41

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AIR-FLUIDIZED TOASTING OF WHOLE KERNEL WHEAT—PROCESSING VARIABLES AND FUNCTIONAL PROPERTIES FOR FOOD APPLICATIONS

INTRODUCTION

EXPERIMENTS (Walker et al., 1970) have shown that the nutritional value of cereal grains fed to cattle is enhanced by pretreatment of the grain with hot air. A continuous spouting-bed grain toaster was developed (Rockwell et al., 1968) with commercial toasting costs of about $1.25 per ton, low enough to be attractive for use in producing a number of nutritious low-cost food items from toasted whole grain. Toasting grain eliminates raw flavor and substitutes a pleasant, familiar toasted flavor. Mossman et al. (1973) studied the physical, organoleptic and nutritional qualities of toasted, rolled, whole-kernel wheat. Additional processing data were needed to apply the toasting process toward several food product areas. The present investigations were planned to obtain useful data on all phases of the toasting procedure, to provide a better understanding of results obtained to date, and to extend the tests to other forms and uses of toasted wheat products. Specifically, tests were necessary to better define pretoast conditioning of the grain, to maintain closer control of the toasting and finishing steps, and to obtain quantitative evaluation of the interrelation between thermal treatment, starch modification, enzyme inactivation and product storage stability.

EXPERIMENTAL

A COMPLETE SEQUENCE of operations in this study included premoistening, tempering, toasting, cooling and finishing (debranning, cracking, milling). Calorimetric measurements were made on samples directly out of the toaster.

Raw material

Gaines (soft white) and hard red winter (HRW) wheats were used. The grain was cleaned by screening (10 mesh) to remove fines, air-blown to remove light chaff, and hand-cleaned to remove obvious remaining defects.

Premoistening and tempering

Small batches were moistened by spraying water on wheat tumbling in a bowl rotating on a horizontal axis. Large batches, or high-level moisture increases were performed by steeping wheat in a water bath held at 110°F for periods up to 2 hr. The water bath also served as a preliminary wash. Moistened wheat was tempered in closed containers held at 34°F in most tests. Accelerated tempering was performed by holding steeped, drained wheat in closed rotating containers at 110°F.

Toasting

A direct gas-fired pilot plant toaster (Midland-Ross Corp.) was modified for these tests (Fig. 1). A screen-covered tray with a 4 in. × 18 in. hinged screen bottom was charged with 1/2 lb of moistened tempered wheat (loading 1 lb/sq ft). In the toasting zone, the grain was fluidized by hot air flowing upward at 1300 ft per min. After a timed interval, the grain was quickly dropped through the trap door bottom of the tray into a receiver—either a forced-air quenching basket, or a calorimeter (a Dewar bottle containing a weighed amount of cool water at a known temperature). After 5 min equilibration in the calorimeter with stirring, the steady-state temperature was noted. The temperature reached by the grain in the toaster (mass-average temperature) was calculated from the equation:

$$t_{MA} = \frac{S_W(W_W + C)(t_f - t_W)}{S_G W_G} + t_f$$

where

- t_{MA} = mass average temp of the grain, °F
- t_f = equilibrium temp of the calorimeter, °F
- t_W = initial temp of the water, °F
- W_W = initial wt of the water, g
- C = calorimeter equivalent, g water
- W_G = net wt of grain after toasting, g (determined by difference)
- S_W = specific heat of water, cal/g/°C
- S_G = specific heat of grain, cal/g/°C, at moisture range into the calorimeter

Specific heat of wheat and calorimeter equivalent were determined calorimetrically in separate tests.

Puff index

The expansion of grain during toasting is expressed as puff index (PI), where PI = (specific vol., toasted grain, ml/g)/(specific vol., original grain, ml/g). Specific volume was determined by gently tapping a 250 ml graduate cylinder of grain on a wood surface until volume was constant, noting weight and final volume of grain.

Kernel center temperature

Holes were carefully drilled, 0.0135 in. diam, along the major axis of several typical wheat kernels, displacing only about 1% of the kernel. A butt welded 30 gauge (0.0100 in. diam) copper-constantan thermocouple was threaded through the hole and the junction was centered. Four extra drilled kernels were threaded bead-like onto the thermocouple wire on each side of the test kernel to reduce conduction effects. Fresh wheat kernels were used for each test. Temperature was recorded on a rapid-response electronic pyrometer.

Finishing

For flour, toasted grain was roller milled in a Quadramat Jr. (C.W. Brabender Inst. Co.).

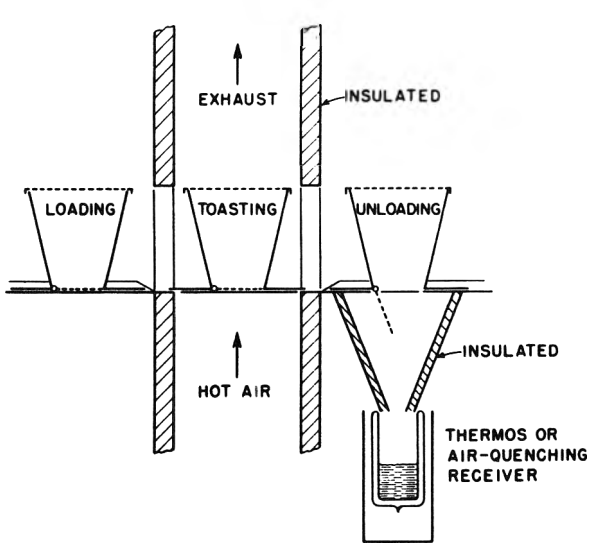

Fig. 1—Schematic of pilot plant toaster showing three positions of sample tray; collection method optional, for measurement desired.

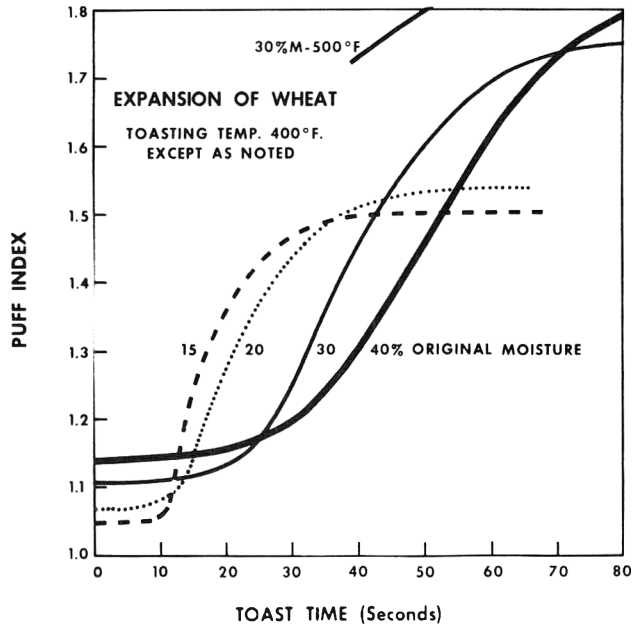

Fig. 2—Expansion (Puff Index) of Gaines wheat after toasting at 400°F (except as noted). Puff index is the ratio of specific volume of toasted moistened grain to that of the unmoistened original grain at 10–12% moisture.

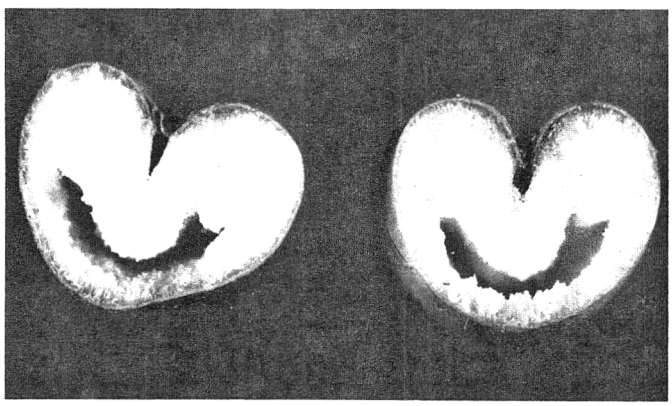

Fig. 3—Appearance of typical Gaines wheat kernel after toasting.

For whole grain debranning, a CeCoCo Debranning Machine (Central Commercial Co.) or a laboratory Rice Mill #3 (H.T. McGill Co.) were used. A coffee mill (Hobart Mfg. Co.) was used for making cracked toasted grain.

Viscosities

Slurries of pregelatinized flour are non-Newtonian (thixotropic) and carefully standardized viscosity procedures must be followed. For testing with a Brookfield Viscometer with a helipath stand (Brookfield Eng. Labs.), 20g of flour and 150g of room temperature water were slurried with a fork for 2–3 min, held for a total of exactly 5 min without additional stirring, quickly poured into the test cell and a reading taken after 1 min with spindle #2 at 60 rpm.

For visco-amylo-graph (C.W. Brabender Inst., Inc.) readings, 525g of slurry was made up with flour to 12.4% total solids and run on a standard time-temp program.

Esterase analysis

Fluorometric assays were used, employing the caprylate ester of 4-methylumbelliferone, according to the method of Jacks and Kirscher (1967).

RESULTS & DISCUSSION

Moistening and tempering

Both Gaines and HRW wheats absorbed moisture sharply to 27% in 20 min in water at 110°F, then each continued in constant but different moisture absorption rates through 2 hr total steeping time, Gaines at 0.08% per min and HRW at 0.12% per min. Optimum tempering times at 34°F were 24–48 hr; shorter times gave poor moisture penetration into the endosperm, as evidenced in more rapid moisture loss and less starch gelatization during toasting. Viscosities were also lower in flour slurries made from toasted wheat that had been tempered too long at 34°F, possibly due to enzymatic action in the moistened grain. In one test on HRW, tempering was accelerated by holding 4 hr in closed containers, at 110°F; viscosities of flour slurries were equivalent to those from wheat held 24–48 hr at 34°F.

Toasting

The wheat kernels expand during toasting, as shown in Figure 2 for Gaines wheat. Almost twofold expansion occurs, under the most favorable conditions. The overlapping sigmoidal pattern of these curves is attributed to (in sequence), the initial small volume increase of added moisture, the delayed expansion of high moisture samples because of evaporative cooling, starch granule expansion with gelatinization, and formation of fissures and voids as internal vapor pressure rises. Development of large irregular internal voids (Fig. 3) or of surface blisters complicates matters, and reduces the value of PI as a criterion of toasted quality. As suggested below, viscosity appears to be a more useful criterion.

An unexpected phenomenon was observed in t_{MA} of grain during toasting (Fig. 4 and 5). Gaines wheat initially at 14% moisture rises smoothly to approach air temperature during toasting at 300°F; however, at 400°F, grain temperature rises smoothly to only 320°F, then actually drops (after 15 sec toast time) and remains depressed for about 10 sec more before it resumes its upward climb. At 500°F, with high heat flux, the inflection is minor at 14% moisture but pronounced when initial grain moisture is high, although delayed a few seconds. This phenomenon is probably due to temporary reversal of heat flow when vigorous moisture vaporization (endothermic) commences, while temperature gradients slowly decrease and reduce heat input. This effect is not reflected in the center temperature of the endosperm (Fig. 4). Heat quantities can be estimated from Figures 4 and 5, and from the evaporation rates shown in Figure 6. At 300°F air temperature, heat flow and evaporation are balanced, at 400°F they are balanced until evaporation becomes excessive and at 500°F, heat flow is always higher than evaporation, except as follows: When initial grain moisture is high, evaporative cooling is dominant and keeps grain temperatures down until the

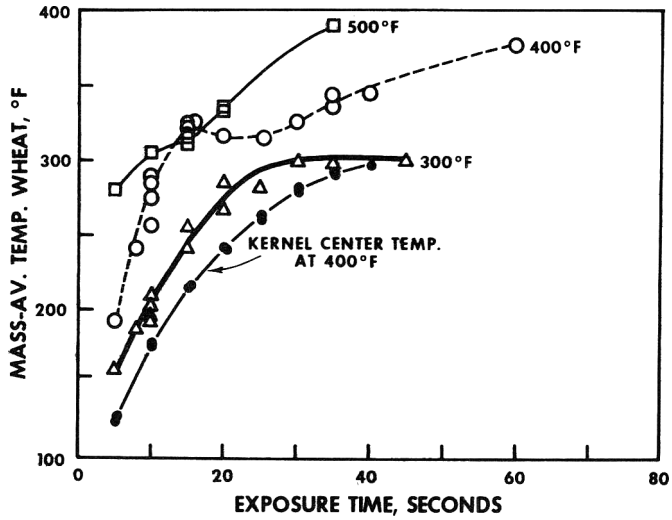

Fig. 4—Mass-average temperature of wheat (Gaines) during toasting. Initial wheat moisture, 14%.

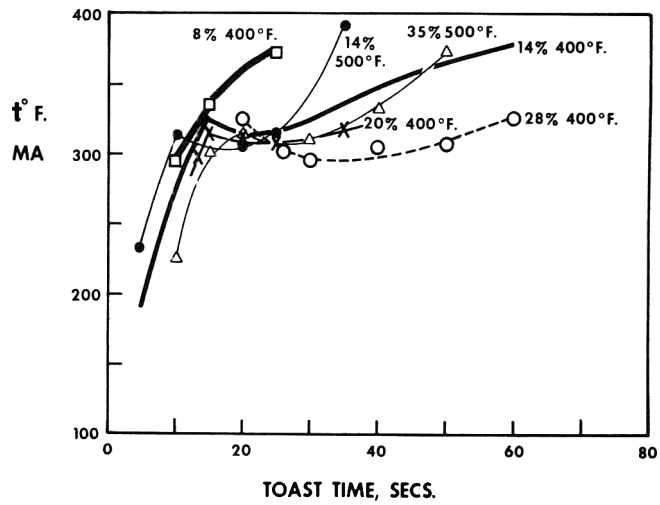

Fig. 5—Mass-average temperature of wheat (Gaines) during toasting. Effect of initial wheat moisture.

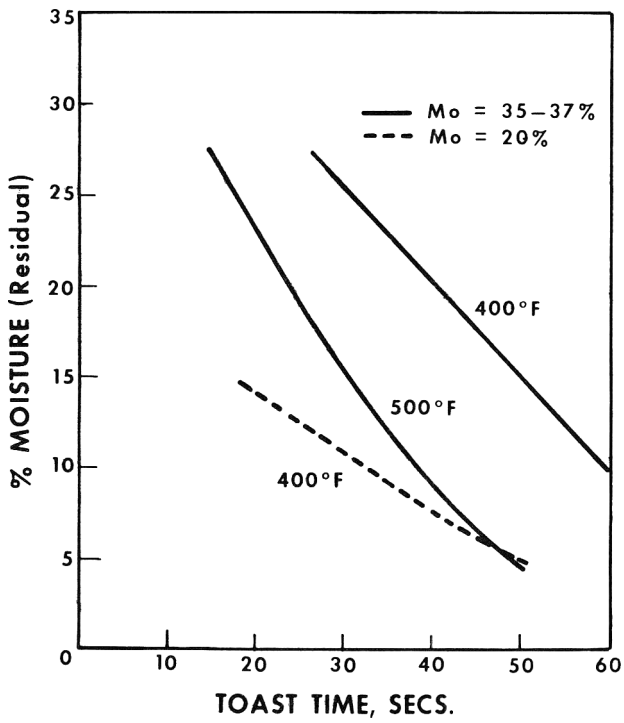

Fig. 6—Residual moisture content of Gaines wheat after toasting and air-quenching. Solid lines 35–37% initial moisture, broken lines 20%.

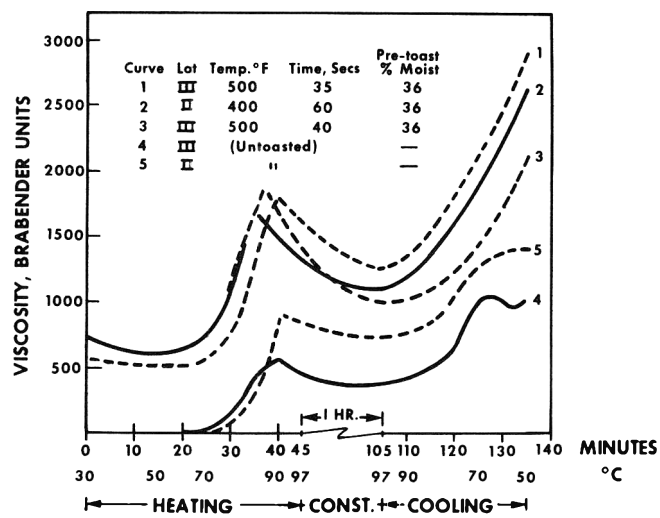

Fig. 7—Visco-amylo-graph viscosities of Gaines toasted wheat flour. Heating-cooling program as indicated.

surface dries out and evaporation slows, then heat flow dominates until internal vapor pressures build up, fissures develop, and release of internal pressure momentarily cools the product, and finally grain temperature rise resumes. The crackling sounds of the expanding grain at the time of the inflection in the curve, are audible above the roar of the toaster. The large latent heat value of water magnifies the endothermic effect. Gelatinization of starch is also endothermic, but of small magnitude. The higher the initial moisture, the longer the initial cooling is noted because the moisture "reservoir" is greater. Indirect confirmation of this theory is seen on analysis and correlation of puff index curves, flour viscosity values, endosperm appearance, and enzyme inactivation results. This could result in a product with less gelatinization with 35% M_o, than at 28% M_o or even 20% M_o, if at higher toaster temperatures evaporation is more rapid than gelatinization, or if the grain is over-toasted, as discussed below.

Enzyme inactivation

Residual esterase activity in toasted grain is shown in Table 1. Even though the bran layer is always hotter than the endosperm during toasting, the original esterase content of the bran fraction is over five times that in the milled flour fraction, and therefore more difficult to inactivate completely. It can be seen that

Table 1—Residual esterase activity in toasted wheat[a]

Toast conditions			Residual esterase, %	
Time sec	Temp °F	t_{MA} °F	Bran	Flour
0	–	–	100	00
5	400	195	2.2	4.0
10	400	275	1.6	0
20	300	275	2.7	0
15	400	323	0	0

[a] Gaines wheat, 14–15% moisture before toasting

Table 2—Effect of over-toasting on viscosity

	Initial moisture %	Toast conditions		Brookfield viscosity cp
		Temp °F	Time sec	
Gaines, C3	30.9	400	60	365
Gaines, C3	30.9	400	70	255
Gaines, C3	30.9	400	80	218
Gaines, B11	28.2	500	30	358
Gaines, B11	28.2	500	40	295
Gaines, B11	28.2	500	50	255
HRW, B1	35.5	500	15	170
HRW, B1	35.5	500	35	305
HRW, B1	35.5	500	45	275
HRW, B1	35.5	500	50	210

Table 3—Comparison of viscosities of pregelatinized flour with other similar products

Sample	Brookfield viscosity, cps
Cracker meal	10
Scarified debranned bulgur (Gaines)	10
Commercial steamed wheat flour	25
Bulgur-type preparation (Gaines)[a]	155
Toasted wheat (Gaines)[b]	350

[a] Wheat steeped to 24.5% moisture according to increasing-temperature bulgur program, except not steamed; toasted 60 sec at 400°F.
[b] Wheat steeped to 30% moisture, at 110°F, equilibrated 34 hr at 34°F; toasted 55 sec at 400°F.

Table 4—Applications for toasted wheat

Applications	Form
Batters and breaders for fish, poultry, meat, vegetables	Flour, meal
Fried specialties, snacks	Flour, extrusions
Baked specialties, snacks	Flour, meal
Gels	Flour
Thickener (sauces, soups, gravies)	Flour
Gruel	Flour, meal, grits, flakes
Rice-like, or bulgur like, casserole item	Grits, debranned kernels

total inactivation of whole grain requires a minimum toasting of 15 sec at 400°F, or more than 20 sec at 300°F, so that t_{MA} attains 275°F or higher. Residual enzyme activity affects storage stability, as well as viscosity values, as will be discussed below.

Viscosities

Walker et al. (1970) found that disappearance of birefringence of starch granules was difficult to correlate with susceptibility to digestion by β-amylase, and chose digestion to measure starch granule disruption. Since viscosity also depends on granule disruption, we used the viscosity of a cold water slurry of flour from the toasted wheat as a measure of extent of gelatinization acquired in treatment. Viscosity increases linearly with pretoasting grain moisture content, but is significantly affected by tempering, as discussed above. Overtoasting can also seriously reduce viscosity (see Table 2 and discussion of storage stability). Toasting temperatures of 300°F are not included because little or no viscosity increases result. Pyrolytic degradation occurred in most samples toasted excessively. Walker et al. (1970) found temperatures of 450–475°F permissible on 11–25% moisture wheat for 30 sec, but longer times in the toaster only increased degradation of the product. For assurance of control, optimum conditions of the toaster appear to be 400–450°F. Viscosity values tend to be higher for Gaines than for HRW.

Visco-amylo-graph (VAG) viscosity patterns on toasted and on untoasted wheat are shown in Figure 7. Initial (cold) viscosities are substantial on the toasted samples, as are peak and final set-back (50°C) viscosities. Enzyme (amylase) inactivation is believed to be responsible for the greater peak and set-back values, although it has been suggested that starch granule modification may be a factor.

Finishing

Toasted wheat can be roller milled to obtain flour yields as high as 70–75%, but the grain must be below 10% moisture for highest yields. Fiber content of the flour was reduced to as low as 0.6% from wheat originally 2.95%, ash content was halved, and fat content reduced 40%. Debranning whole grain requires premoistening toasted grain surfaces with 10% water, tempering 7 min before milling in a McGill rice mill 30 sec under the pressure of a 2-lb weight, and finally buffing 30 sec with lever arm only. The bran remains in shreds, unpulverized, and must be air separated from the debranned grain. With only a 5% debranning weight loss, fiber is reduced 30%. Cracked toasted wheat grits were readily prepared in the coffee mill with a minimum of sifting and regrinding and a minimum of fines.

Applications

Several comparisons of wheat products by viscosity are shown in Table 3. Not only does toasted wheat have the highest cold viscosity of those products shown but additional viscosity can be obtained if desired in certain applications, by further cooking (Fig. 7). We tested several applications of toasted wheat; those that show promise are shown in Table 4. The char-

acteristics needed in each of these applications can often be "made-to-order." Toasted wheat meal used as breaders for fish, poultry and meats, clings very well to damp food surfaces, and can be prepared as fine or coarse grind as needed for appearance or taste. Products with high consistencies can be made if needed for extrusions, or fried or baked specialties. Thickeners for soups, sauces, or gravies improve the suspension of solids during preparation or cooking. Gruels can be prepared instantly with boiling water. Casserole items can be formulated with wheat compatible with other quick-cooking ingredients. Specifications for items made for foreign aid program (Elder and Weisberg, 1970) can easily be met. An example, is the wheat component in Wheat-Soy-Blend (Horan, 1973), of which nearly 100 million pounds worth $7.5 million, were exported in 1971 (Anon, 1970). Cracked toasted wheat was informally evaluated as an acceptable substitute for cracked bulgur, and required less cooking in our tests.

Storage stability

Samples for storage tests were prepared early in this investigation, to run concurrently with other tests. Selection of toasting conditions for storage samples was made arbitrarily, hoping to bracket anticipated typical processing operations, while limiting the number of samples. However, subsequent toasting tests indicated that the range chosen was too broad and did not include what were found to be optimum toasting conditions (400–450°F). Detailed storage tests are to be reported in a subsequent publication (Guadagni, 1973). In general, two types of off-odors appear to develop, enzymatic and pyrolytic in origin. Enzymatically derived off-odors occur in samples of wheat toasted with combinations of high initial moisture, low toast temperature, and short toast times which leave significant concentrations of enzymes. Pyrolytically induced off-odors occur in samples in which no active enzyme system remains, but excess heat exposure has developed carbonyl compounds, alcohols and free fatty acids (Lorenz and Johnson, 1972), not to mention damage to protein and amino acids (Whistler and Paschall, 1967). Guadagni (1973) found correlation between hexanal content and off-odor development in toasted wheat. In work on drum drying (Whistler and Paschall, 1967) destruction of the starch granules formed pyrodextrins and caused rancidity to develop in the stored dry powder unless salts of orthophosphate were added. It appears that additives commonly used to stabilize cereals might be required to extend the storage life of toasted wheat products beyond 4 months.

CONCLUSION

JUDICIOUS SELECTION of conditions in all phases of preparation and toasting of whole kernel wheat is required to impart the desired properties without overheating or otherwise reducing quality. If low moisture toasted products are required, it is preferable to reduce moisture in a secondary drying step rather than increase the severity of the toasting operation. Conventional treatments for extending storage life of cereals are recommended for use in toasted wheat products.

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Reference to a company and/or product named by the Department is only for purposes of information and does not imply approval or recommendation of the product to the exclusion of others which may also be suitable.

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ENRICHMENT OF TORTILLAS WITH SOY PROTEINS BY LIME COOKING OF WHOLE RAW CORN-SOYBEAN MIXTURES

INTRODUCTION

AS IS WELL KNOWN, tortillas are a basic component of the Mexican diet. They are consumed by all social classes, and are usually eaten with most foods and meals. Tortillas perform multiple functions in eating, being used as a substitute for bread, snacks, eating utensils, and a principal component of the diet if a family is very poor. The importance of tortillas in Mexico may be appreciated by noting that average annual per capita consumption is of the order of 120 kilos.

In absolute terms, tortillas are a good calorie source but a poor protein source. The latter fact is due to two basic reasons: 1) their protein content is low, of the order of 9% on a dry basis; and 2) their protein quality is poor, having a Protein Efficiency Ratio (PER) of the order of 1.5 (Cravioto et al., 1950).

Poor protein quality in tortillas is not important if family income permits acquisition of animal protein foods such as meat, milk, eggs, fish, etc. On the other hand, a very serious problem arises if such resources are not available, and tortilla proteins become predominant in total dietary proteins. In such cases severe protein malnutrition can occur, with all its somber consequences.

Since tortillas are a universal food in Mexico, they would be the ideal vehicle for enriching diets in the country. This would also be true for most of Central America. Recognizing this fact, a number of investigations have been carried out attempting to improve tortilla protein quality: (1) Enrichment with soy flour (Cravioto et al., 1950; Cravioto and Cervantes, 1965); (2) Amino acid supplementation (Bressani, 1972); and (3) Development of Opaque II corn (Bressani, 1972). Of these three possibilities, the first two have a serious disadvantage: using the traditional, and by far the most common method for making tortillas (see "Experimental" section), it is impossible to introduce the enriching agent uniformly within the tortillas, given the impossibility of uniformly mixing a powder such as soy flour with cooked limed corn or corn dough. It should be noted that it is possible to uniformly enrich tortillas made by a newer method—limed corn flour—with soy flour or amino acids; in this case, both powders are simply mixed. Corn flour thus has a good potential for improving the Mexican diet, especially because it can also be enriched with vitamins and other protein sources such as FPC. On the other hand, all such enriching agents, including soy flour and amino acids, are fairly expensive, so that their inclusion in tortillas by either method would price them outside the economic reach of most low income groups.

Opaque II corn would represent a good solution to the problem, since its protein quality is very high. The product, however, is still in the developmental stage and will probably not become available in large quantities for a few more years.

More recently, other solutions have been proposed for enriching tortillas such as use of synthetic corn; use of pre-impregnated (with amino acids) corn; and addition of amino acids to lime-cooked corn at the time of grinding (Molina et al., 1972). It is believed, however, that these solutions would be either too costly or difficult to carry out in practice.

Considering the foregoing, a simple and inexpensive method for enriching tortillas with soy proteins was sought. The method found was one which employs whole raw soybeans as the enriching agent, and essentially consists of lime-cooking soybeans together with corn in the usual manner, and making tortillas from the cooked corn-soya mixture, also in the usual manner.

EXPERIMENTAL

THE PROPOSED enrichment method was extensively studied with the purpose of determining its adequacy and effectiveness. The following points were considered.

Method for making enriched tortillas

The traditional method for making tortillas was employed. Whole raw corn-soybean mixtures were boiled for 50 min in limewater at a temperature of 100°C, using a lime dose of 1.4–2.0% (expressed as calcium hydroxide) based on the weight of the dry mixture, after which the cooked mixture was allowed to stand overnight (10 hr) in the cooking liquor. After standing, the liquor was decanted and the cooked corn-soya mixture was washed twice with tap water. The mixture was finally ground in a stone mill into a dough, and tortillas were made from the dough in the usual manner (i.e., making thin pancakes from the dough and cooking the pancakes on a hot plate).

Proximate chemical analysis

Tortilla protein, fat and ash contents (on a dry basis) were calculated by the usual methods: micro-Kjeldahl for proteins, Soxhlet extraction for fat, and burning in a muffle furnace for ash (AOAC, 1965).

Protein quality

Two indices of protein quality were determined: Protein Efficiency Ratio (PER) and Net Protein Utilization (NPU), using casein as a reference protein (Munro and Allison, 1964; Miller, 1963). Five male weanling rats, 22–23 days old and weighing 27–28g, were used in each determination. Before initiation of feeding, all animals were standardized by starving for 24 hr. Protein levels in all tortilla diets were adjusted to 9.0%, which was the protein content of unenriched tortillas supplemented with vitamins and minerals. Tortilla diets therefore consisted of tortillas, vitamins and minerals, and an amount of cornstarch required for adjusting protein level to 9.0%. The casein diet was a standard diet with protein level adjusted to 10%.

The feeding period was 28 days in all cases. Rat weights and amount of food consumed by each rat were recorded daily during the duration of the test period. All diets were analyzed by the Kjeldahl method (AOAC, 1965) in order to determine their protein and nitrogen contents. Protein quality indices were calculated for each rat at the end of the test period as follows:

Body nitrogens were measured by sacrificing the animals, homogenizing the carcasses and analyzing the homogenates by the Kjeldahl method (AOAC, 1965). Body nitrogen at the beginning of the test period was taken equal to average body nitrogen of five of the same weanling rats (population, age and average weight) as those used in the feeding tests. Average PER and NPU values, with corresponding standard deviations, were finally calculated for each group of animals fed the same diet, based on individual values.

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Table 1—Proximate chemical analysis of tortillas enriched with soya by lime cooking of whole raw corn-soybean mixtures, dry basis

Enrichment level, % soya	% Proteins, exp	% Proteins,[a] calc	% Fat, exp	% Ash, exp
0%	9.5	–	1.33	–
8%	12.4	12.0	1.71	1.54
16%	14.7	14.4	2.35	1.77

[a] Calculated protein contents were based on an assumed protein content of 40% for soybeans and 9.5% for corn.

Table 2—Protein quality of tortillas enriched with soya by lime cooking of whole raw corn-soybean mixtures

Enrichment level, % soya	Protein Efficiency Ratio (PER)[a]	% PER to casein	PER on basis of casein PER = 2.5	Net Protein Utilization (NPU)[a]	% NPU to casein
0%	1.8 ± 0.05	59	1.5	28.0 ± 0.50	46
8%	2.5 ± 0.32	84	2.1	38.1 ± 0.60	65
16%	2.6 ± 0.22	86	2.2	41.8 ± 0.20	70
Casein	3.0 ± 0.15	100	2.5	61.0 ± 0.80	100

[a] PER and NPU values are reported as mean ± standard deviation.

Table 3—Protein quality of different products

Product	Protein Efficiency Ratio (PER)	Dietary protein level, %	Reference
Raw soybeans	1.99	9.1	FAO, 1970, p. 222
Soy flour	2.37	10.0	FAO, 1970, p. 223
Tortillas + 8% soy flour	2.27	10.0	Cravioto and Cervantes, 1965
Tortillas + 0.41% Lysine + 0.05% Tryptophane	2.42	10.0	Bressani, 1972
Opaque II corn tortillas	2.66	10.0	FAO, 1970, p. 166

Organoleptic testing

The purpose of these tests was to determine if a detectable difference existed between normal unenriched tortillas and tortillas enriched with soya by the described method. The triangle test was used, employing an expert panel of 21–25 tasters (ASTM, 1968). Results of these tests were analyzed by the usual statistical methods.

Inactivation of toxic factors in soybeans

It was considered necessary to determine if the combined treatment of boiling in limewater and cooking the dough in a hot plate when making tortillas, was sufficient to inactivate toxic factors present in raw soybeans (antitrypsins and hemagglutinins). Urease activity in soy-enriched tortillas was therefore measured as an index of inactivation of these factors (De, 1971), using Method No. 22-90 of the American Association of Cereal Chemists (1962).

Cost analysis

Costs for enrichment of tortillas by the corn-soya lime cooking method were calculated and compare with costs for enrichment with soy flour.

It should be noted that in all cases, tortillas were prepared and studied at three enrichment levels: 0% (control), 8% and 16% soya. Soy percentage in a given mixture was equal to dry weight of raw soybeans in the mixture × 100 divided by dry weight of the raw mixture.

RESULTS & DISCUSSION

Method for making enriched tortillas

Soybeans treated by the lime cooking method previously described were found to be well cooked; i.e., they were soft and could be easily ground in the stone mill. The presence of soybeans in both dough and tortillas was not visually detectable, except for a slightly darker color in samples containing 16% soya. The 8% samples were visually indistinguishable from the controls.

Proximate chemical analysis

Table 1 reports results of proximate chemical analyses of the tortillas. It may be seen that experimental data on protein contents coincided well with calculated data, based on an assumed 40% protein content for soybeans and a 9.5% protein content for corn. Fat content of tortillas also increased with increasing soya dose due to the high (approximately 20%) oil content of soybeans.

Protein quality

Results of protein quality determinations are reported in Table 2. Since it is becoming common practice to recalculate PER's on a basis of casein equal to 2.5, Table 2 also reports PER values converted to that basis. Statistical analysis of these results by application of t-tests showed that increases in PER and NPU values of the enriched samples with respect to the controls were significant. On the other hand, no significant difference was found to exist between corresponding values of the enriched (8% and 16% soya) samples.

Data in Table 2 are best studied by reference to Table 3, which presents data on protein quality of raw soybeans, tortillas enriched with soy flour and amino acids, and tortillas made from Opaque II corn. It may be seen that protein quality of tortillas enriched with lime-cooked soybeans was essentially the same as that for pure soy flour, as well as for tortillas enriched with soy flour or with amino acids. Opaque II corn tortillas apparently had slightly higher PER values. It should be noted that, although dietary protein levels in Tables 2 and 3 were different (9 and 10% respectively), the difference was sufficiently small so that valid comparisons could be made.

Since increasing the soya dose from 8% to 16% was found to produce no significant difference in protein quality, it is concluded that tortilla enrichment with 8% soya would probably be enough to obtain most, if not all, of the benefit of increased protein quality. On the other hand, it is true that tortillas with 16% soya would have the additional benefit of a higher protein content with respect to tortillas with 8% soya.

An additional point to consider is the well known fact that protein quality of a given diet is inversely related to protein level in the same diet (FAO, 1970). Thus, feeding enriched tortillas at their own protein level (i.e., 12.4% and 14.7%) instead of the 9.0% level employed in the tests, would probably result in lower PER and NPU values than those reported in Table 2. This reduction would probably be higher with the 16% than with the 8% soya tortillas, and would therefore favor enrichment at the lower level.

Finally, it might be concluded from reference to Tables 2 and 3, that toxic factors in soybeans were probably inacti-

Table 4—Results of triangle tests for detection of difference between control (unenriched) tortillas and tortillas enriched with soya by lime cooking of whole raw corn-soybean mixtures

Enrichment level, % soya	No. of tasters in test	No. of tasters who detected difference	Critical number of tasters at significance level of 95%	99%
8%	21	5	12	13
16%	25	11	13	15

Table 5—Cost of different enrichment treatments and levels for soya-enriched tortillas[a]

Type of treatment	Enrichment level, % soya	Cost per kilo tortillas, U.S. $	% Protein	PER	Cost / PER	Cost / g protein
Control	0%	0.1200	9.5	1.8	0.067	0.00126
Lime cooked whole raw corn-soybeans	8%	0.1304	12.4	2.5	0.052	0.00104
Lime cooked whole raw corn-soybeans	16%	0.1416	14.7	2.6	0.055	0.00097
Soy flour	8%	0.1504	13.9	2.3	0.065	0.00110

[a] These costs were calculated making the following assumptions: cost of raw corn, $0.08/kg; cost of raw soybeans, $0.28/kg; cost of soy flour, $0.64/kg; yield of tortillas with respect to whole raw corn-soybeans, 1.5 kg/kg; cost of unenriched tortillas, $0.1200/kg. The PER value for tortillas enriched with soy flour is taken from Cravioto and Cervantes, 1965.

vated by the lime-boiling hot plate-heating treatment. This is evident from the fact that soy enriched tortillas had higher PER values than raw soybeans, but the same PER values as pure soy flour and tortillas enriched with soy flour. If these factors had not been inactivated, enriched tortilla PER values would have probably been considerably lower than those obtained.

Organoleptic tests

Results obtained in the triangle tests are reported in Table 4. It may be seen that no significant difference was found at either level of enrichment, even at the lower (95%) significance level. On the other hand, the proportion of tasters who detected a difference between enriched and unenriched tortillas increased from 25% in the first test to 44% in the second test. This is reasonable since, as noted previously, the 16% soy tortillas were slightly darker than the controls, whereas the 8% soy tortillas were practically visually indistinguishable from the controls. It should be noted that subsequent preliminary work has shown that the presence of soybeans in tortillas is not significantly detectable below a soya level of 20%.

Inactivation of toxic factors

Urease activity was found to be negligible in all cases. These results are not surprising, in view of the high PER and NPU values obtained with soy-enriched tortillas. As noted previously, the soybean toxic factors were probably inactivated by the combined effect of two heat treatments: boiling the corn-soybean mixture in limewater and heating the corn-soya dough in a hot plate when making tortillas.

Cost analysis

Calculated cost data for enrichment by different treatments and at different levels are reported in Table 5. It may be seen that enrichment by the corn-soybean lime cooking method was undoubtedly cheaper than enrichment by addition of soy flour. Within the first method of enrichment the cost of each PER unit was cheaper at the 8% level than at the 16% soya level. On the other hand the opposite was true with the cost of each gram of protein, due to the lower cost of soya proteins with respect to corn proteins. The recommendable enrichment level would probably have to be dictated by the consumer, who would have to choose between higher-priced more proteinated tortillas or vice versa. It is believed that the choice would probably favor the lower enrichment level, due to the lower cost and given the high price elasticity of tortillas in Mexico. In any case, either enrichment level would have the benefit of the high protein quality.

CONCLUSIONS

IT HAS BEEN established that enrichment of tortillas by lime cooking of raw corn-soybean mixtures is effective, since protein quality is apparently increased to levels obtainable by enrichment with either soya flour or amino acids. This increase in protein quality is probably due to amino acid complementation between corn and soya proteins.

No significant difference in protein quality (PER and NPU) apparently exists between tortillas enriched with 8% and 16% soya when both are fed at a dietary protein level of 9.0%.

Resulting corn-soya mixtures are completely homogeneous, so that enrichment of dough and tortillas is uniform. The same method of enrichment produces a significant increase in the protein content of tortillas. The enrichment method changes neither eating habits nor food recipes, since it employs the traditional method for making tortillas. The presence of soya in tortillas so enriched is not significantly detectable by a trained panel, at least up to a soya level of 16%.

Tortilla enrichment is obtained at a minimum cost, since whole raw soybeans are employed.

Toxic factors present in raw soybeans used for enriching tortillas are apparently inactivated by the tortilla-making process.

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SLUSH EVAPORATION: A NEW METHOD FOR CONCENTRATION OF LIQUID FOODS

INTRODUCTION

PRESENT-DAY fruit juice concentration methods all suffer from drawbacks of one type or another. The products of high temperature evaporation systems tend to have poor taste and aroma characteristics. Freeze concentration processes suffer from juice loss due to entrainment with the ice mass. Reverse osmosis encounters problems of severe concentration polarization. The operating conditions and drying rates of freeze drying make it much more expensive than other approaches. These various difficulties have stimulated research looking for new processes which offer the promise of concentrating juices at a reasonable cost, and with less loss of taste, aroma and nutritive value than present-day evaporative methods.

Slush evaporation

Slush evaporation is a process which was proposed and initially examined by Chandrasekaran and King (1971, 1973). Its main features are that the feed juice is in a partly solid, partly liquid state, and that the drying mechanism is a combination of vaporization and sublimation. Although the process was proposed as a fruit juice concentration method, it should have applicability to water removal from other liquid foods and non-food liquids.

The name "slush drying" was given to this approach originally; however "slush evaporation" is used in the present work to emphasize that the product is a concentrated, rather than totally dry substance. While it is anticipated that there are ways of taking the product from a slush process to full dryness, the greater immediate interest would seem to lie in its use as a concentration process.

The physical characteristics of slush evaporation are that the feed material is kept at a temperature where 20–90% of the water present is frozen. In Figure 1, a phase diagram for apple juice is presented (Heiss and Schachinger, 1951). As can be seen by application of the lever-rule mass balance, for a specific dissolved solid (sugar) concentration the percent ice present increases as the temperature decreases. For example, at −5°C the equilibrium concentration contains 31% dissolved solids. If a feed juice with 14% dissolved solids is equilibrated at −5°C, there will be (0.31−0.14)/(0.31) = 55 wt % ice present, or (0.55)/(1−0.14) = 64% of the water will be present as ice.

Slush evaporation should have an advantage in aroma retention over air or vacuum drying from the liquid state because of the beneficial effects of solute concentration and temperature. Under evaporation or drying conditions leading to substantial aroma retention, the loss of volatiles is governed by a diffusion mechanism (Thijssen and Rulkens, 1968; Chandrasekaran and King, 1972b). As has been found by these previous investigators and others, aroma retentions increase markedly in most drying processes with increasing initial dissolved solids concentration. This is a result of diffusion coefficients for volatile solutes decreasing much more rapidly than those for water as the dissolved solids content increases. In slush evaporation, the separation of some of the water as ice leaves liquid concentrate of higher dissolved solids content. Evaporation from this liquid should then occur with improved volatiles retention. Furthermore, any water removed by sublimation should not entail much volatiles loss. Volatile compounds can be expected to remain with the concentrate upon partial freezing, rather than being incorporated into the ice crystals, a behavior which accounts for the high degree of volatiles retention which is found for freeze concentration. At the temperatures prevailing for slush evaporation diffusion within the concentrate should keep volatiles from accumulating ice crystal-concentrate interfaces, unless the solubility limit is passed for formation of a volatiles-rich oil phase (Massaldi and King, 1974).

The low temperatures of slush evaporation should also help aroma retention, since diffusivities of volatile solutes decrease more rapidly with decreasing temperature than does that of water (Chandrasekaran and King, 1972a; Thijssen, 1971).

In comparison to freeze-drying, the benefit of slush evaporation is that the drying rates will be higher. Freeze-drying rates for fruit juices and other sugar-containing substances are low because of the need for holding very low frozen-zone temperatures to get adequate solidification during drying (Bellows and King, 1972, 1973). At the much higher temperatures of slush evaporation, the mass transfer driving forces are higher because of the much higher vapor pressure of water at the operating temperature, and hence the drying rates are much higher.

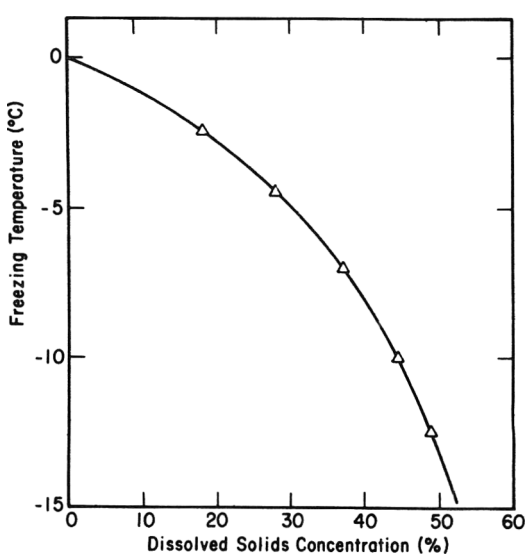

Fig. 1—Equilibrium freezing diagram for apple juice (after Heiss and Schachinger, 1951).

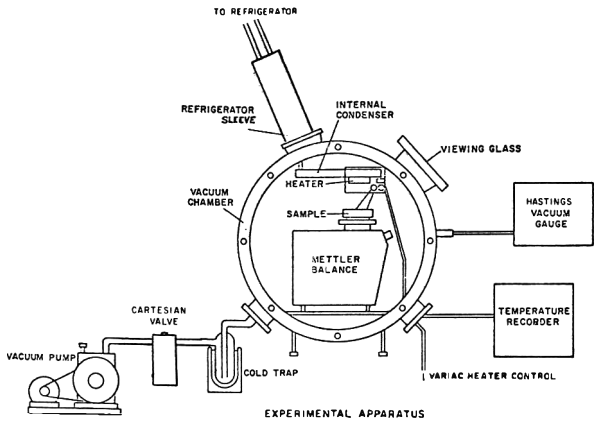

Fig. 2–Drying apparatus.

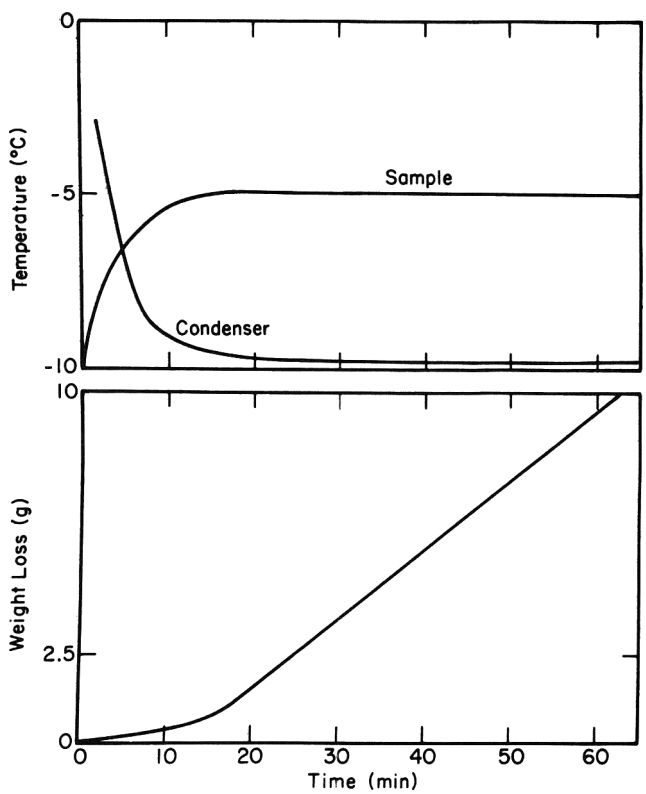

Fig. 3–Typical run conditions.

Another advantage in comparison to freeze drying is the lower refrigeration cost involved in maintaining what can be a higher condenser temperature.

In comparison to freeze concentration, slush evaporation has similarly beneficial low operating temperatures and is not subject to loss of viscous concentrate entrained with the ice during a solid-liquid phase separation.

Objectives

This study was split into two parts. The first section examined the aroma retention of slush evaporation quantitatively for apple juice under varying conditions of sample temperature, drying rate, mode of drying (overhead radiant heating or bottom conductive heating), sample thickness, initial concentration, amount of water removed and presence or absence of foam. The amount of retention should give a good indication of the product quality. The second part was a study of the amount of dissolved solids (sugar) loss due to spattering, entrainment or other causes during slush evaporation of sugar solutions. The results show the order of magnitude to be expected for such losses in larger-scale apparatus and show the directional effects of different operating variables. Dissolved solids loss is of critical economic importance for concentration of fruit juices because of the high ratio of product value to processing cost.

EXPERIMENTAL

Apparatus

The apparatus employed is shown in Figure 2. It was identical in most respects to that described by Bellows and King (1973) and by Chandrasekaran and King (1972b). The vacuum-evaporation chamber was a cylindrical steel vessel 20 in. in diameter and 20 in. long. A 9 × 13 in. flat-faced internal condenser was connected to an external refrigerator capable of supplying refrigerant to the tubes inside the condenser at −25°C. The refrigerant flow rate, and hence the condenser temperature, was controlled by connecting a variable transformer to the pump. Two heaters were used at different times. A 1150w adjustable electrical resistance heater (shown in Fig. 2) provided overhead radiant heat, while bottom conductive heat was achieved through an adjustable 55w circular heater (not shown) placed immediately below the sample holder. Sample weight was measured by a Mettler Model No. P1200 balance observed through a side viewing port. Sample and condenser temperatures were measured by two Chromel P-Constantan thermocouples connected to a two-channel recorder. The exhaust line consisted of a cold trap followed by a Manostat Corp. mercury manostat which was used to control the pressure in a range (1–3 mm Hg) such that the condenser temperature could effectively control the sample temperature. Removal of inerts was accomplished by means of a Model 1402 Welch Duoseal vacuum pump. A Hastings SP-1S thermocouple vacuum gauge was used to measure the chamber pressure.

General procedure

A 50 ml sample prechilled to about 7°C was poured into a 9.6 cm diameter petri dish, and a thermocouple was suspended in the center halfway from the bottom of the sample. The wall heights of the petri dishes were 1.2 and 4.7 cm for the aroma retention and dissolved solids loss experiments, respectively. The sample was covered and chilled to around −15°C in the freezing compartment of a household refrigerator, forming the slush. The contents were mixed to ensure uniformity. The sample was then uncovered, placed in the drying chamber, and allowed to melt partially. The vacuum and condenser refrigerant pumps were then started, with the manostat valve fully opened. After 3 min the chamber pressure had dropped sufficiently to allow the heater to be turned on and the manostat valve to be partially closed. The balance of the run was spent observing the weight losses at various times and adjusting the refrigerant flow to achieve the desired constant sample temperature. After a predetermined loss of weight from the sample, the run was terminated.

It should be noted that, although the drying rate is dependent upon the heater output, the sample temperature was controlled by the condenser temperature. Figure 3 shows typical temperature-time curves for the sample and condenser temperatures, along with a corresponding resultant plot of the sample weight loss vs. elapsed time. From this figure it may be

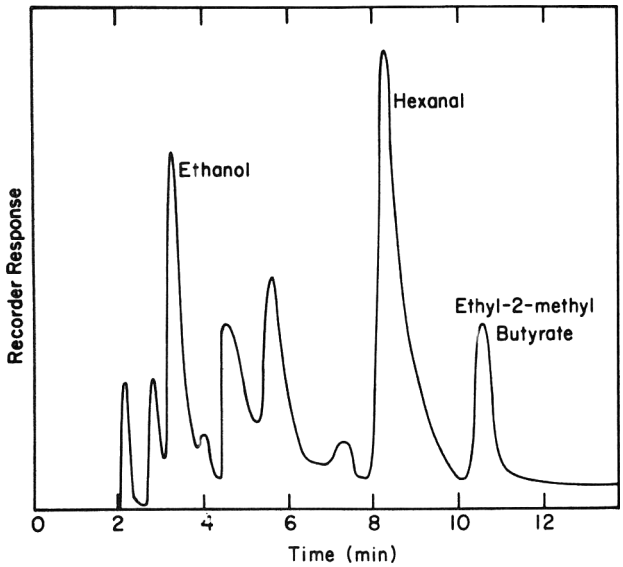

Fig. 4–Typical head space chromatogram.

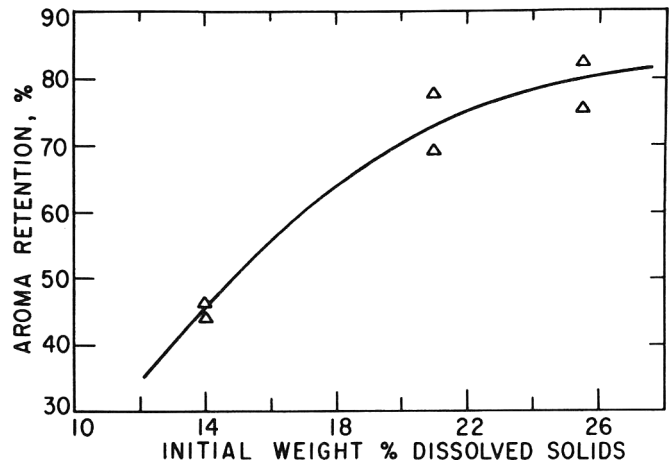

Fig. 5–Effect of initial dissolved solids content of apple juice upon volatiles retention during slush evaporation; 68% of water frozen as ice in all cases.

seen that the drying rate does not become constant until the sample temperature has risen and the condenser temperature has dropped such that their difference represents a mass transfer driving force large enough for evaporation at the desired rate. In the case shown, this point occurs about 15 min into the run.

RESULTS & DISCUSSION

Aroma retention

Apparatus and procedure. Except for the experiments examining the effect of concentration, Safeway Townhouse natural-strength apple juice, purchased locally, was used. For more concentrated solutions Tree Top, Inc. (Selah, Wash.) concentrated apple juice, also purchased locally, was diluted as required.

After the completion of a run, the sample was covered and allowed to melt completely. It was then transferred into a 250 ml Erlenmeyer flask, from which an aliquot was analyzed for dissolved solids concentration with a Technical Instrument Co. Model 94105 refractometer. Following common practice, the calibration of wt % dissolved solids for sucrose solutions was used. After dilution of the juice back to the original juice concentration, as monitored by the refractometer, the flask was covered with aluminum foil. Care was taken to make sure that the only opportunity for appreciable aroma loss occurred during the evaporation step.

A 50-ml sample of the same juice as the test sample was poured into another flask and covered; then both flasks were allowed to equilibrate at room temperature. Vapor head-space analysis with a Varian Aerograph No. 1740 gas chromatograph, equipped with a flame ionization detector, was used to measure the aroma retention. The column was 25 ft × 1/8 in. i.d., packed with 100–120 mesh Chromosorb G, impregnated with 1% methyl silicone oil SF-96(50) plus 0.05% Igepal. The column temperature was kept at 100°C, with the injector and detector temperatures at 125°C. The carrier gas was pure helium; for the detector flame purified hydrogen and breathing quality compressed air were used. 5-ml samples of the vapor space above the apple juice were injected into the chromatograph. A typical chromatogram is presented in Figure 4. The labelled peaks in Figure 4 were determined to contain the particular component indicated by means of comparison with pure-component residence times and by comparison of the chromatogram with those from a previous study (Chandrasekaran and King, 1971) using similar conditions with apple juice. The attenuation of the chromatograph was adjusted after the ethanol peak to achieve better resolution.

The degree of aroma retention was determined by measuring the areas under the three peaks labelled in Figure 4 from the chromatograms of both the concentrated and re-diluted sample and the untreated sample. For ethanol the peak area was referred to the original zero base line. Since the attenuation change caused some baseline change, the areas of the other peaks were referred to the final baseline. Peak areas of the processed juice were divided by the respective areas of the untreated sample, and an average of the ratios was taken. This result was taken as the degree of aroma retention. A linear relationship between volatile retention and peak area was postulated on the basis of experimental confirmation of linearity for head-space analyses of other volatile compounds in water and sugar solutions (Massaldi and King, 1973; Chandrasekaran and King, 1972a). Retentions did not vary greatly between the three peaks, although ethanol did tend to be somewhat higher than the average and ethyl-2-methyl butyrate somewhat lower (Lowe, 1973).

Base case. A standard run was set up to which changes in all variables were compared. Sample volume: 50 ml; Sample make-up: Natural-strength apple juice (14 wt % dissolved solids concentration); Mode of drying: Overhead radiant heating, without bottom heating; Sample temperature: −6.5°C; Degree of frozenness (defined as % of total initial water in the form of ice at the sample temperature): 68%; Sample weight loss: 10g; Drying rate: 0.195 lb/hr ft^2, (2.65 × 10^{-5} g/sec cm^2).

The variation of some of the run conditions showed either little or no effect on the degree of aroma retention. In this category fell the effects of changing the drying rate, sample thickness and the mode of heat supply. Retentions of 39–48% were found for overhead heating at drying rates of 0.1–0.3 lb/hr ft^2, and retentions of 27–45% were found for bottom heating at drying rates of 0.2–0.6 lb/hr ft^2. Retentions ranged from 35–47% for slush bed thicknesses of 0.2–0.7 cm (Lowe, 1973).

Effect of initial dissolved solids concentration. The results of this set of experimental runs are shown in Figure 5. For these runs the % of water as ice (68%) was held constant by changing the sample temperature. These results con-

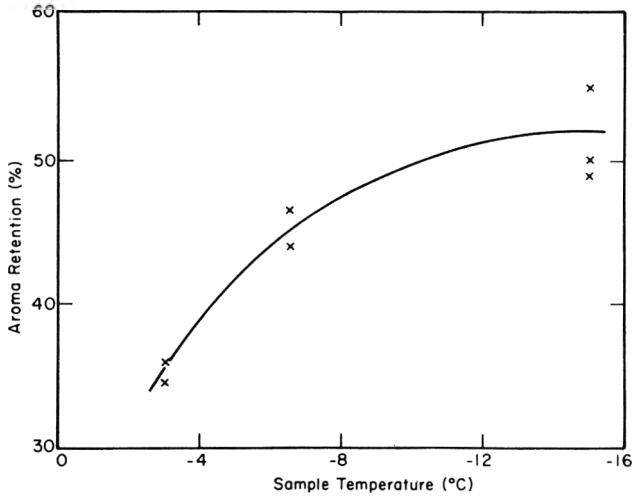

Fig. 6–Effect of sample temperature on volatiles retention during slush evaporation of apple juice (14% initial dissolved solids).

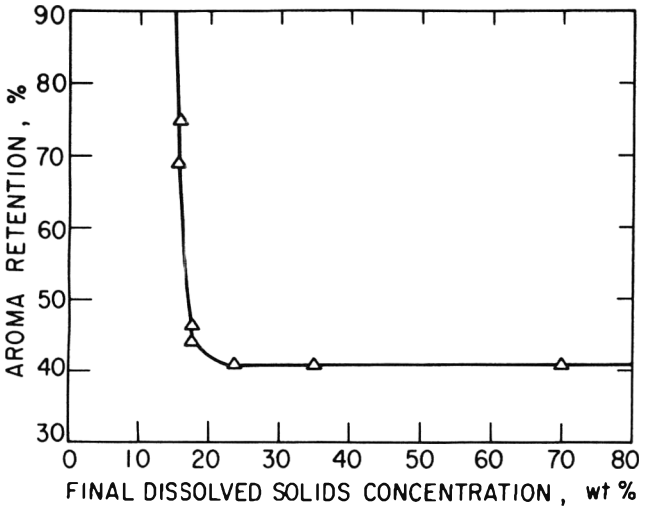

Fig. 7–Volatile aroma retention during slush evaporation of apple juice, as a function of the amount of water removed (14 wt % initial dissolved solids).

firm the findings of Chandrasekaran and King (1971); the aroma retention for a given amount of water removal (10g from 50 ml) improves markedly with higher initial dissolved solids concentrations. This behavior probably reflects the lower ratio of volatile solute diffusivity to water diffusivity at higher dissolved solids contents, as well as higher viscosities, which deter fluid motion within the slush. There may also be some direct effect of the temperature change required to hold the % water as ice constant, although the range of temperatures was not great (−6.5 to −12.5°C).

Effect of sample temperature. Figure 6 shows that the aroma retention improves as the temperature of the slush during evaporation decreases, and hence as the % of the water present as ice increases, since these two variables are interdependent. The improvement in retention is probably principally an effect of the higher % water frozen, since at lower temperatures the liquid portion of the slush has a higher dissolved solids concentration, which has been seen to give higher retention values.

Effect of presence or absence of foam. In the slush evaporation experiments of Chandrasekaran and King (1971), they observed the appearance of a stable, coarse foam over the slush bed. The amount and durability of this foam were found to increase with increasing dissolved solids concentration. So as to separate the effect of the foam upon volatiles retention from the other effects of higher initial dissolved solids contents, experiments with and without the foam were carried out in the present work. The foam was created by subcooling the juice to below −30°C and maintaining it at this temperature until the vacuum had taken effect; otherwise the foam did not form. The tendency to foam is favored by higher dissolved solids concentration and by lower slush temperature, suggesting that the appearance of the foam is promoted by higher viscosity of the juice.

Several pairs of runs with randomly selected conditions were conducted to test the effect of the foam on the drying rate and on the aroma retention. A pair of runs consisted of one sample evaporated with the foam present and one sample evaporated under the same conditions but without foam. The results of five foam-foamless sets of runs showed that the aroma retention was not significantly affected by the presence of foam and that the drying rate was reduced by 0–15% when foam was present.

Effect of amount of water removed. One of the most interesting findings of this work is given in Figure 7, which shows the amount of aroma retention for samples evaporated from an initial 14 wt % dissolved solids to different final dissolved solids concentrations. All runs were carried out at a constant temperature of −6.5°C. For this temperature the concentrate in equilibrium with ice contains about 35% dissolved solids (Fig. 1); hence all ice disappeared when overall concentrations well above that level were achieved. It is apparent that after a certain degree of concentration further aroma loss is minimal. There is however, some scatter in individual component retentions comprising the averages plotted (Lowe, 1973), so it should not be concluded that there is no additional loss at all beyond a concentration of 25% dissolved solids.

A possible explanation of the greatly reduced volatiles loss beyond an overall concentration of 25% lies in the generation of a slush that is nonhomogeneous on the macroscale during evaporation. Visual observation supports this proposal. Examination of the slush after the runs were ended showed that there was a 1 or 2 mm layer of material at the top which was higher in viscosity than the rest of the slush. This material therefore probably has a higher sugar content, resulting from preferential evaporation at the top of the slush and a lack of fluid mixing within the slush. With evaporation from this layer rich in dissolved solids, the incremental loss of volatile solutes would then be characteristic of the higher dissolved solids content of that layer (see Fig. 5), and could therefore lead to the shape of the curve shown in Figure 7.

Loss of dissolved solids

Apparatus and procedure. Because of restrictions imposed by the analytical method, the dissolved solids loss experiments were conducted using solutions modelling apple juice and orange juice instead of the natural juices. These model solutions consisted of sugars (sucrose, fructose and glucose) in the same percent and the same proportions as in the natural juices.

The preparation and concentration of the sample were identical to the procedures outlined for the aroma retention experiments, except that the sample weight was measured with the Mettler balance prior to freezing. This was done to avoid any problems resulting from evaporation prior to the start of the run. After the end of the run, the final weight was noted, and distilled water was added to the sample in a precisely controlled amount until the original weight reading

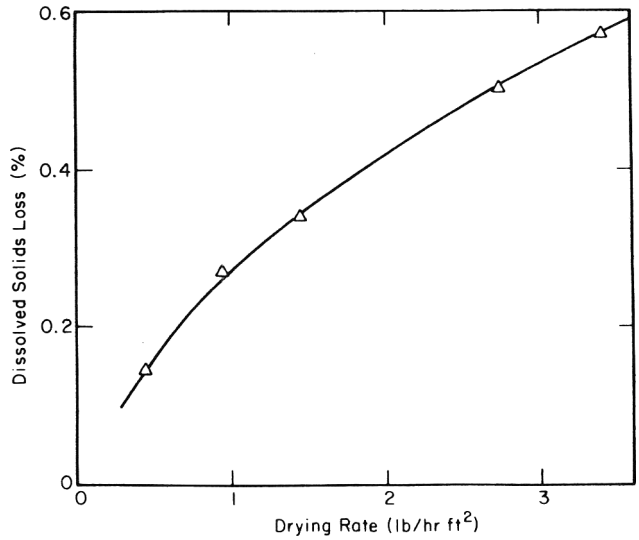

Fig. 8–Effect of drying rate upon the loss of dissolved solids during slush evaporation of 65% fructose-20% glucose-15% sucrose solution.

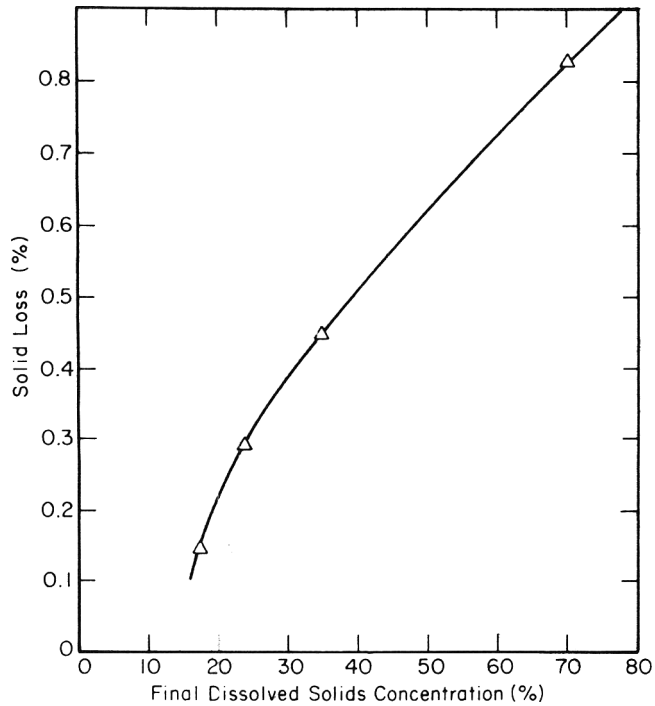

Fig. 9–Effect of amount of water removed upon the loss of dissolved solids during slush evaporation of 65% fructose-20% glucose-15% sucrose solution.

was realized on the Mettler balance. The sample was then covered and allowed to equilibrate to room temperature. An untreated sample was also covered and allowed to equilibrate to room temperature.

The amount of dissolved solids loss was determined by measuring the difference in refractive index between the processed and untreated samples. This was done with a calibrated Carl Zeiss (Jena, East Germany) Model G-1 laboratory differential interferometer with 2 cm cells. This instrument gave readings accurate to ± 0.005 wt % for concentration differences of 0.85% or less. The concentration difference gives the loss of dissolved solids due to entrainment or spattering:

$$\% \text{ loss} = (100)\,(d)/(w)$$

where d = wt % concentration difference and w = initial wt % dissolved solids.

Base case. As in the aroma retention experiment, a standard run served as the base case. Sample volume: 50 ml; Sample make-up: Model Apple Juice (65 wt % fructose, 20% glucose, 15% sucrose), 14 wt % in water; Sample temperature: −5°C; Degree of frozenness: 66%; Sample weight loss: 10g; Drying rate: 0.32 lb/hr ft² (4.3×10^{-5} g/sec cm²).

Effect of drying rate. Figure 8 shows the influence of the rate of evaporation upon dissolved solids loss for the removal of 10g of water from 50 ml of solution. At higher rates visual observation of the slush shows a greater amount of bubbling and spattering which is probably the cause of the greater loss of dissolved solids. Although the losses encountered are not great, an improved apparatus design could probably reduce the amount of loss for higher evaporation rates.

Effect of amount of water removed. Figure 9 shows losses observed for removal of 10, 20, 30 and 40g of water from the sample, at the base case drying rate, and a constant temperature of −5°C. All ice disappears before the highest concentration (70 wt%) is reached. The results show a relatively even rate of loss as more water is removed.

Effect of sample temperature. The effect of different slush temperatures is shown in Figure 10. The tendency toward

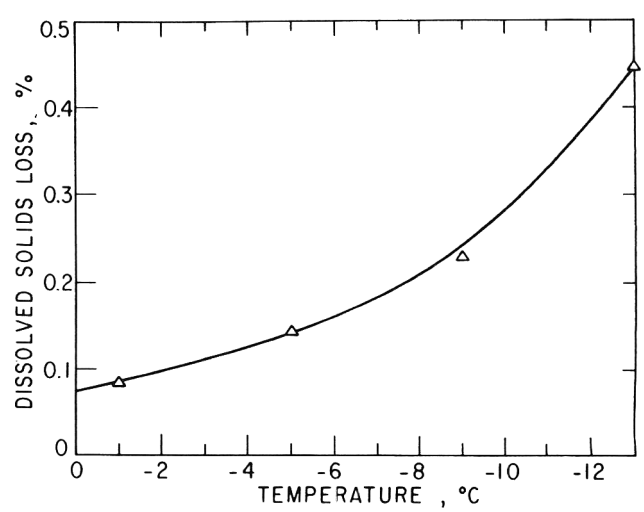

Fig. 10–Effect of specimen temperature upon the loss of dissolved solids during slush evaporation of 65% fructose-20% glucose-15% sucrose solution.

increased solids losses at lower temperatures can partly be explained by noting that, as the temperature decreases, the concentration of the liquid portion of the slush rises. For a given volumetric amount of spattering, the amount of solids losses will increase with increasing dissolved solids concentration of the liquid. This effect, with a fixed volume of spattered liquid, does not predict as large a rise in loss at lower temperatures as was observed.

Freeze-drying gives low losses if fines are properly handled, but above the collapse temperature for freeze-drying (Bellows and King, 1972, 1973) large losses are often encountered through entrainment and spattering. Apparently the losses must peak somewhere between the lower temperatures of this study and the freeze-drying collapse temperature.

Effect of mode of heat input. The most pronounced effect on the solids retention was produced by the change in heat input from overhead radiant to bottom conductive heating. At identical run conditions (including the drying rate) dehydration using bottom, conductive heat produced solids losses 15–27 times higher than those found for overhead radiant heating. This is probably due to the observed creation of a boiling mechanism with bottom heating, with consequent evolution of large bubbles through the slush surface.

Effect of initial dissolved solids concentration. Table 1 shows the results from two identical runs performed using 30% model apple juice at the same temperature, −5°C, as was used in the standard run, with 10g water removed from a 50-ml sample.

Effect of sugar make-up. A model orange juice (50 wt % sucrose, 24% glucose, 26% fructose) was compared with the model apple juice at 14 wt % initial dissolved solids content. The sample temperature was −5°C in each case, giving 66% and 72% of the water as ice for the apple juice model and the orange juice model, respectively. The losses for removal of 10g of water from 50 ml were 0.143 and 0.160% for the apple juice model and 0.215 and 0.210% for the orange juice model.

Effect of pectin. The effect of adding 0.5 wt % soluble citrus pectin to the model apple juice was tested. Experimental results show that greater solids losses result from the addition of pectin, giving 0.285% and 0.410% losses for the runs with pectin compared to 0.143% and 0.160% for those without pectin.

Table 1—Effect of initial dissolved solids content upon loss of dissolved solids

Initial Conc (wt %)	Solids loss, %
14	0.143, 0.160
30	0.660, 0.692

CONCLUSION

FROM THE AROMA retention data gathered in this work it appears that slush evaporation is a promising concentration process. Even after concentration to 70% dissolved solids, aroma retentions of over 40% are obtainable. Retentions of this amount are known to be well above acceptable consumer standards. Another favorable aspect of the findings is that the retentions were fairly well balanced from component to component.

From the aroma retention experiments it was found that the aroma losses decreased with lower temperatures, while from the dissolved solids loss results it was found that the solids loss increased with decreasing temperature. Apparently, if slush evaporation is to be used commercially an optimal temperature balancing these effects must be found.

An important question is whether an economically high enough drying rate can be achieved for slush evaporation, which will at the same time produce economically low dissolved solids losses. The drying rates obtained in the solids retention section of this work ranged from 0.2–3.2 lb/hr ft^2, and are substantially higher than those of freeze drying, which usually range from 0.05–0.2 lb/hr ft^2. Even higher slush evaporation drying rates may be obtained with improved methods of control (i.e., a larger condenser) and/or lack of concern over constancy of slush temperature.

The impact of the cost of solids losses depends upon the value of the product, and upon the cost of processing. The processing cost for high-temperature, short-time (HTST) evaporation of citrus juice without essence recovery is about 5¢ per lb of product (Oetjen, 1967). If slush evaporation is to be a viable concentration system, then the processing costs must be comparable to those for processes which produce juices of comparable quality (freeze concentration, HTST evaporation with essence recovery). The processing costs for these processes are higher than those for HTST evaporation without essence recovery. Depending upon the drying rate and the amount of water removal, the solids loss in this work with radiant heating ranged from 0.1–0.9%, which represents a potential loss of from 0.04¢ to 0.35¢ per lb of product, if the product value is 44¢/lb. When compared to the likely processing costs, these figures appear promising.

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EFFECTS OF pH ON QUALITY OF STORED TOMATO JUICE

INTRODUCTION

RECENT NEW DEVELOPMENTS in tomato processing appear to be revolutionizing the traditional product industry. The bulk storage concept involves aseptic storage of tomatoes or products in large tanks (1,000–100,000 gal each) for later processing. This affords the opportunity to extend the total processing season, cope with large seasonal supplies, and reduce container handling and storage. In such a system the more acidic a product, the more easily controlled is asepsis. The acid-break technique involves treatment of tomatoes with acid to pH 2.75 to facilitate more complete extraction of usable tomato product with reported subsequent increases in product consistency (Wagner and Miers, 1967; Miers et al., 1967; Wagner et al., 1968; and Miers et al., 1970). More recently the acid break has been suggested for incorporation into a field processing system in which tomatoes are chopped, enzymes inactivated and juice finished by a portable unit which is literally operated in the tomato fields (Schultz et al., 1971; Miers et al., 1971). Juice would then be transported to a central processing plant for further processing.

The emergence of one or more of these developments separately or in combination as accepted practices in the tomato processing industry has stimulated considerable interest in the effects of pH on quality of stored tomato products. In bulk storage, acidification of tomato juice would reduce the required heat treatment and would aid in potential spoilage control during storage. Acid-break juice could possibly be maintained at the low pH for long periods of time to aid in transport or storage of such juice. Miers et al. (1971) suggested that holding stability of juice would be increased if juice was held at the extraction pH 2.75; however, nonmicrobial quality measurements were not included. The following investigation was initiated to further evaluate selected quality attributes of tomato juice subjected to pH alterations under conditions similar to those which might be encountered in bulk storage.

EXPERIMENTAL

Sample preparation

Tomatoes (Campbell 28) used for juice were grown at a Purdue University Experimental Farm near Lafayette, Ind. Three replications of 300 lb raw fruit each were washed and chopped through a 0.375 in. screen. Chopped fruit was heated immediately to 91°C, after which juice was extracted through a 0.023 in. screen. Juice was then heated to 91°C and filled at that temperature into 303 × 406 tomato-enameled cans. Cans contained sufficient concentrated hydrochloric acid to give the product a final pH of 4.0, 3.5, 3.0, 2.5 and 2.0. Controls were prepared with no acid added. Cans were sealed immediately, inverted for 2 min and cooled in water. Equal numbers of cans from each treatment were stored at ambient temperature (18–22°C) and at 40°C up to 3 months. This segment of the work was, therefore, designed as a 6 × 4 × 2 × 3 factorial experiment.

Table 1—Effect of pH on consistency (efflux) and serum viscosity (Ostwald) of acidified and stored tomato juice. (Values are means of 24 observations)[a]

pH	Efflux (sec)	Serum viscosity (sec)
4.3	62.3	147.8
4.0	66.2	145.5
3.5	54.5	143.3
3.0	46.8	142.1
2.5	42.5	144.5
2.0	41.8	146.2

[a] Means in the same column connected by the same line are not different at the 1% level of significance.

Evaluations

Three cans of each treatment were equilibrated at room temperature for 24 hr. Contents of the three cans were combined and mixed uniformly. Consistency was then measured by the efflux method of Wagner and Miers (1967) in triplicate. A 500-ml aliquot was neutralized to pH 4.3 with 50% sodium hydroxide solution. All samples were again uniformly stirred and consistency measurements made.

Beginning at the first month of storage, serum viscosity was also determined for all treatments by use of an Ostwald viscosimeter. Neutralized juice was centrifuged at 9750 × G for 15 min. Serum (supernatant) was decanted and filtered through Whatman #1 paper, after which a 5-ml aliquot was analyzed at 25°C. The time of serum flow through the designated portion of the viscosimeter was measured in triplicate.

The remaining juice was utilized for color difference determinations on a Hunter Color Difference Meter standardized with ceramic plate #D 33C-440 (L = 26.6, a_L = 28.2, b_L = 13.7).

Further consistency studies

To further evaluate pH effects on consistency, aliquots (250 ml) of locally purchased commercial canned tomato juice were adjusted to various pH levels from 3.0 to 5.0 at 0.2 intervals. HCl (3N) and NaOH (3N) were used for pH adjustment. NaCl was added as necessary to the same samples in order to maintain a consistent ionic strength as a result of each addition. All samples were uniformly stirred and maintained at the given pH for 4 hr, after which they were neutralized to pH 4.3 and NaCl added as necessary. Samples were again uniformly stirred and evaluated for consistency by the previously described efflux method. Differences in liquid volumes added to all samples were equalized with distilled water. The entire experiment was replicated three times.

To observe the direct effects of NaCl on consistency of tomato juice, up to 7.5% NaCl was added to aliquots of juice. Juice was uniformly stirred, equilibrated for 4 hr, uniformly stirred, and evaluated by the efflux method. Three replications were again used.

All data were statistically evaluated by analysis of variance (Li, 1957).

RESULTS & DISCUSSION

WHEN TOMATO JUICE was acidified to various pH levels as low as 2.0, sealed in cans and stored for as long as 3 months, pH had a highly significant effect on consistency of the product neutralized to pH 4.3 (Table 1). As pH decreased below 4.0, consistency showed a significant decline through pH 2.0. There was also an increase in consistency when pH was adjusted to 4.0 compared to that of untreated juice. Essentially no differences were noted between consistencies of juices before and after neutralization to pH 4.3; therefore, only results of neutralized juice are reported. Neutralized juices maintained the relative pH effects on consistency even after 24 hr equilibration, indicating an irreversible phenomenon.

Serum viscosity, however, was unaffected by the pH treatments (Table 1), indicating that hydrolysis of soluble pectic components by the low pH media were not responsible for the changes in consistencies. Previous work (Bartolome, 1971) indicated that consistency of tomato products was positively correlated with serum viscosities. Serum viscosities did, however, decrease with both storage time and increasing storage temperatures

(Table 2). This indicated possible hydrolysis of pectic substances as a result of those variables.

To further evaluate possible causes of the above consistency changes, the effects of various levels of NaCl on commercial juice consistency were studied (Table 3). To adjust tomato juice to pH 2.0, about 1% concentrated HCl was added in the original experiment above. Upon neutralization of this juice back to pH 4.3 with 50% NaOH, about 0.7% NaCl was generated. According to Table 3, even the addition of 1% NaCl to tomato juice did not significantly change the consistency. Several authors have indicated that viscosity of dilute pectic substances is decreased by the addition of NaCl (Hirota, 1961; Owens et al., 1944; Deuel and Stutz, 1958). NaCl has been postulated to cause a decrease in charge on the pectin molecule. This, in turn, allows formation of parallel dimers and trimers as well as increases in molecular folding. Hydrogen bonding could also be reduced. Whittenberger and Nutting (1957, 1958) demonstrated the role of insoluble solids in tomato juice consistency, indicating that the cell wall surfaces are electrically charged, helping to maintain walls in suspension. In their work involving the addition of 0.2% NaCl and $CaCl_2$ to washed cell wall material, the electrolytes dampened the charges considerably causing decreased consistency. The whole tomato juice used in this study did not demonstrate such effects.

Further evaluation of pH effects on consistency changes were made when commercial juice was adjusted to selected pH levels from 5.0 to 3.0 and evaluated for changes. Again there was a significant pH effect (Table 4). Since electrolyte concentration and ionic strength were kept constant, it is apparent that pH alone is the causative factor of consistency differences. There appeared to be a critical region between pH 4.2 and 4.6 at which consistency was highest after the 4-hr exposure time. Tomato juices have been considered to be diluted solutions of pectic substances (Luh et al., 1954), containing less than 0.25% pectins expressed as calcium pectate. Luh et al. (1954) also observed that the majority of the pectins from tomatoes were water soluble and somewhat esterified. According to Owens et al. (1944), the viscosities of diluted pectic solutions increased to maxima at about pH 6. Baker (1948) observed that highly esterified pectins exhibited slight decreases in viscosity at pH 1.5–3. In the results above, there was little indication of increased viscosity beyond pH 4.3.

Hydrogen bonding and other associations among pectins, celluloses and/or other constituents could have been permanently disrupted as a result of the acidification. As pH was decreased, hydrogen ion concentration was greatly increased. Under such conditions partial negative charges on molecules could have been essentially neutralized or satisfied by the excess hydrogen ions instead of by hydrogen atoms on other molecules. This essentially creates a lack of intermolecular interactions, reducing apparent or functional molecular sizes and, therefore, reducing consistencies. At the same time the neutralized molecules could change shape from essentially linear to spherical as a result of the charge alterations. This could increase the possibility of the irreversible effects observed above.

Color

Lightness (Hunter color L) of acidified tomato juice decreased with pH after 3.0 (Table 5). In addition there was darkening with time at the 40°C storage temperature (Table 6). Hunter color a_L decreased significantly with pH after the pH

Table 2—Effects of storage time and temperature on serum viscosity (Ostwald) of acidified tomato juice. (Values are means of 18 observations)[a]

Time (Months)	Temperature	
	Ambient	40°C
1	159.8 a	152.6 b
2	150.3 c	132.3 d
3	148.8 c	125.7 e

[a] Means followed by the same letter are not different at the 1% level of significance.

Table 3—Effects of NaCl on consistency of tomato juice. (Values expressed are means of three replications)[a]

NaCl added (%)	Efflux (sec)
0	46.5 a
0.25	46.3 a
0.50	45.2 ab
0.75	46.1 a
1.00	45.8 ab
1.25	44.8 bc
2.50	44.7 bc
3.75	43.9 cd
5.00	42.8 de
7.50	42.6 e

[a] Means followed by the same letter are not different at the 1% level of significance.

Table 4—Effects of pH adjustment and ionic compensation with NaCl on consistency (efflux) of tomato juice neutralized to pH 4.3[a]

pH	Efflux (sec)
3.0	41.0 f
3.2	41.4 ef
3.4	41.6 def
3.6	42.1 def
3.8	42.7 def
4.0	43.8 ced
4.2	44.2 bc
4.4	49.0 a
4.6	46.7 b
4.8	46.4 b
5.0	46.1 bc

[a] Means followed by the same letter are not different at the 1% level of significance.

Table 5—Effects of pH on Hunter color L, a_L and b_L for acidified and stored tomato juice[a]

pH	L	a_L	b_L
4.3	26.20 a	24.29 c	13.14 ab
4.0	26.36 a	24.63 a	13.27 a
3.5	26.28 a	24.45 b	13.22 ab
3.0	26.04 a	24.30 c	13.11 b
2.5	25.53 b	24.02 d	12.83 c
2.0	24.42 c	23.39 c	12.36 d

[a] Means in the same column followed by the same letter are not different at the 1% level of significance.

Table 6—Effects of storage time and temperature on Hunter color L, a_L and b_L for acidified tomato juice. (Values are means of 18 observations)[a]

Time (Months)	L		a_L		b_L	
	Ambient	40°C	Ambient	40°C	Ambient	40°C
0	26.01 a	26.01 a	24.58 s	24.58 s	13.29 x	13.29 x
1	26.00 a	25.58 b	24.14 u	23.80 v	13.02 y	12.78 z
2	26.04 a	25.55 b	24.39 t	23.68 w	13.12 y	12.61 z
3	26.05 a	25.10 c	24.70 r	23.57 w	13.17 xy	12.64 z

[a] Means within each color measurement followed by the same letter are not different at the 1% level of significance.

4.0 treatment, indicating a decrease in redness. There was a similar decrease with storage time at 40°C up to 2 months, after which there was no change (Table 6). Color b_L (yellowness) was decreased by pH treatments less than 3.0 (Table 5). There was also a significant decrease in yellowness after 1 month of storage, and the 40°C storage treatment yielded lower b_L values than did ambient storage. The a/b ratios showed no changes since both a_L and b_L values decreased at about the same rate. The decreased lightness could have been caused by a type of browning reaction. Due to the presence of reducing sugars, proteins and amino acids, Maillard-type reactions could have occurred. However, such reactions decrease in rate with decreased pH (Meyer, 1960). Carmelization is enhanced by decreased pH; however, the product was heated for only a relatively short time. Ascorbic acid is known to cause browning by decomposing to furfural, which then polymerizes (Braverman, 1963). The lower the pH, the more readily this reaction can occur. The carotenoids, β-carotene and lycopene could have decomposed to cause changes in redness and yellowness.

Overall, the storage of tomato juice for periods of time of 1 month or more at reduced pH levels resulted in irreversibly decreased consistency even when pH was readjusted to 4.3. It was apparent that this effect was caused by pH changes alone rather than NaCl or electrolyte addition. There were also observed to be possible detrimental color changes with decreased pH, especially below 3.0. Further work is in progress to more specifically identify the changes and their causes.

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pH AND ACIDIC STABILITY DURING STORAGE OF ACIDIFIED AND NONACIDIFIED CANNED TOMATOES

INTRODUCTION

HIGH TOMATO pH values have been noted in surveys of several varieties (Farrow, 1963) and have been associated with a greater incidence of pack failures (Lopez et al., 1968).

Rice and Pederson (1954) found that flat-sour spoilage of canned tomatoes by *Bacillus coagulans* takes place at pH values greater than 4.3. With higher bacterial loads produced by mechanical harvesting, pH values lower than 4.3 are desirable for increasing the efficiency of heat processing and for inhibiting the growth of surviving heat resistant microorganisms. At pH 4.6 or higher, growth and toxin production by some types of *Clostridium botulinum* may occur. Under special circumstances growth and toxin production may take place at lower tomato pH (Slocum et al., 1941).

To correct for conditions of high tomato pH the U.S. Food and Drug Administration authorized the use of certain organic acids as optional ingredients in the Standards of Identity for Canned Tomatoes (Fed. Register, 1966). Lopez et al., (1968) have recommended that phosphoric acid also be included as an optional ingredient in the Standards of Identity. The addition of an acid to tomatoes adjusts the pH and provides an additional benefit of increased acid buffer which guards against subsequent pH rises. Schoenemann and Lopez (1973) reported that citric, malic, fumaric and phosphoric acids, individually, maintained a pH desirably reduced for preservative qualities without accelerating physical or chemical changes during processing.

Approximately one-third of tomato acidic constituents are salts of potassium and other cations (Davies, 1964). It has been demonstrated that neutral salts, including salts of calcium, magnesium and sodium, are effective in lowering the pH of canned tomatoes. However, the amount of acid buffer does not increase with added neutral salts. Therefore, added acids may be necessary for assuring stable pH values of canned tomatoes during storage after processing.

Objectives of this study were to evaluate pH stability of acidified tomato packs during storage for 1 yr at 24°C, to associate pH stability with tomato composition and to determine the variability of pH within the canned product caused by tomato cell walls and other structural matter.

EXPERIMENTAL

TABLETS containing acid and several salts previously described by Lopez et al. (1968), and 5% phosphoric acid solution were used for acidification of the packs investigated in this study. Salt tablets were composed of calcium and sodium chlorides. Acid tablets were composed of calcium sulfate, other substances necessary for tablet forming and for dispersion of components, and of either citric, fumaric, or malic acid. Samples acidified with phosphoric acid also contained added salt tablets. Only the tomatoes packed in California had sucrose added.

Tomatoes were packed in the VPI&SU pilot plant, and in commercial plants in Maryland, Indiana and California. All varieties packed in the pilot plant were of canning red ripeness. #303 × 406 F-enameled cans were used and heat processed for 45 min in a boiling water bath at 99°C, as recommended for whole toma-

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Table 1—Initial pH values and changes in pH of acidified and nonacidified canned tomatoes after storage for 1 yr at 24°C

	Variety					
	Roma[a]	H1350[b]	H1370[c]	H1350[a]	VF-145-7879[d]	Rutgers[a]
Additive	Initial pH and pH change					
Nothing added	4.45 – 0.01	4.37 + 0.04	4.36 + 0.01	4.21 + 0.08	e	4.23 + 0.07
Salts only[f]	4.34 + 0.03	4.25 + 0.08	4.23 + 0.02	4.19 + 0.02	4.23 + 0.09	4.13 + 0.05
Salts with citric acid	4.27 + 0.02	4.14 + 0.06	4.15 + 0.02	4.11 + 0.02	4.12 + 0.03	4.07 + 0.02
Salts with fumaric acid	4.12 + 0.07	4.10 + 0.10	4.11 + 0.01	4.16 + 0.01	4.03 + 0.01	4.03 – 0.01
Salts with malic acid	4.28 + 0.02	4.19 + 0.02	4.21 – 0.01	4.11 + 0.02	4.08 – 0.01	4.08 + 0.03
Salts with phosphoric acid	4.09 + 0.05	4.12 + 0.10	e	4.16 + 0.01	e	4.00 – 0.04
LSD @ 5% probability	0.04	0.03	0.04	0.03	0.04	0.04

[a] Packed in pilot plant
[b] Packed in Maryland commercial plant
[c] Packed in Indiana commercial plant
[d] Packed in California commercial plant
[e] Variable not included
[f] Salts were sodium and calcium chloride.

toes packed in #307 × 409 cans and smaller when processed in still retorts and water cooled (Lopez, 1969). Commercially packed tomatoes were processed in continuous cookers at 100–104.4°C for 10–12 min and air cooled. Data for this study were obtained from a total of 1182 cans.

All pH determinations were made using a Corning pH meter with expanded scale readings. Fresh pH standards were prepared from potassium acid phthalate crystals dissolved in water to 0.05M concentration. Total acidities were titrated using 0.1000N NaOH. Alcohol soluble acidities were determined by titration of acids extracted by 80% ethanol from tomatoes and values expressed on the basis of the total tomato weight before extraction. Total reducing sugars were determined on a portion of the alcohol extracts by the dinitrosalycilate procedure of Sumner and Sisler (1944). Free amino acids were determined by the ninhydrin procedure of Rosen (1957). The ninhydrin analysis was made on the alcohol extracts after removal of ammonia by vacuum distillation with the pH of the extracts adjusted to 10.

RESULTS & DISCUSSION

THE INITIAL pH values for the canned tomato samples and the changes after 1 yr storage at 24°C are shown in Table 1. Although pH differences of 0.04 were statistically significant at 0.95 probability, all pH changes were small with no increases found to be more than 0.10 pH for any additive in any variety. Without additives, high pH tomatoes did not show as great a pH rise after storage as lower pH tomatoes did. However, with acidification, high pH tomatoes showed more significant trends for pH rise after storage. It is difficult to explain these trends. A chemical breakdown of organic acids is a possibility. However, the release of carbon dioxide from acids was small since one milliequivalent (meq) acid yields about 22 ml of carbon dioxide gas which would produce a sharp drop in can vacuum. Such a change was not observed. Sheets and Lopez (1962) have shown that only small increases of carbon dioxide in container headspace take place in tomato products during storage. Losses in can vacuum are partly offset by reaction of the product with free oxygen in the can. Slight can corrosion through pinholes in enamel was noted in the samples stored for 1 yr at 24°C. Oxidative corrosion of tinplate produces tin and iron cations that are capable of neutralizing part of the tomato acids. Neutralization of acids selectively may remove the strongests acids resulting in significant pH changes.

Determinations were made of several canned tomato constituents in an attempt to relate compositional changes during storage to pH stability. Table 2 shows that few compositional trends could be associated with pH changes during storage. The Rutgers variety had higher total acidity than the Roma variety. The Rutgers variety had higher alcohol insoluble acidity (1.9 meq) as determined by the difference between total and alcohol soluble acidities. This may explain the different pH trends in the two varieties. As shown in Table 1, the significant pH increases in Roma tomatoes occurred in samples acidified to pH values less than 4.27. The Rutgers variety significantly increased in pH after storage when the initial pH was greater than 4.08. The Rutgers tomato pH decreased when the initial value was low (4.00). There opposite trends could originate from nonenzymatic changes in the pectic substances affected by pH-catalyzed reactions. Generally, low pH catalyzes de-esterification which frees acid groups in pectinic acid. This reduces solubility unless the resultant pectic molecule is fragmented. Little information is available concerning pectic fragmentation caused by moderate acid catalysis (pH 4.00) over a period of approximately 1 yr or longer. In tomatoes, the solubilization of pectic acid by molecule fragmentation could provide sufficient acidity to prevent pH rises.

In another phase of this study (Schoenemann and Lopez, 1973), heat processing #303 × 406 cans of tomatoes in boiling water at 99°C for 15 min instead of the recommended 45 min permitted a reduction of approximately 0.2 meq pectinic acidity per 100g tomato. This was not sufficient to cause significant pH changes but would affect the acid buffer capacity of the samples.

Different fractions of tomato material separated by mechanical means showed that concentration of insoluble tomato solids produced a lowering of pH within the product, as shown in Table 3. This means that within a can of tomatoes there are regions with different pH values caused by difference in concentration of insoluble solids. The data in Table 3 were derived from 10 tomato varieties with all additives used in this study. As tomato debris was compacted by centrifugation, the pH became progressively lower than the particle-free serum (separated by centrifugation). This effect may be due mainly to the pectic acidity of the tomato cell walls. It indicates that if pH electrodes were in closer proximity to the debris, the pH differential would be greater. Consequently, the settling of tomato particles may have a tendency to allow a rise in pH within the upper portion of the product in a container.

Results obtained in this study and in associated work done by the authors show that pH of acidified tomatoes is as stable during heat processing and storage as that of nonacidified tomatoes.

Table 2—Concentrations of total acidity (TA), alcohol soluble acidity (ASA), free amino acids (AA) and reducing sugars (RS) in nonacidified canned tomatoes after 1 yr storage at 24°C

Pack		Composition				
		Initial TA[a]	Final TA[b]	Final ASA[b]	Final AA	Final RS
Variety	Plant location	(meq/100g)				%
Roma	Pilot plant	5.04	5.22	4.11	2.55	4.20
Heinz 1370	Indiana	4.88	5.11	4.02	2.60	4.60
VF-145-7879	California	7.46	7.09	5.58	3.30	6.50
Rutgers	Pilot plant	6.38	6.80	4.90	2.55	5.00

[a] Initial determinations were made 3 wk after canning
[b] Final determinations were made after 24°C storage for 1 yr.

Table 3—Differences in pH between various mechanically separated fractions of canned tomatoes

Number of Cans in each determination	Canned tomato fractions		Mean pH difference[a]
	Fraction 1	Fraction 2	Fraction 1—Fraction 2
723	Drained tomato fruit	Drained juice	−0.01
36	Blended tomatoes	Centrifuged serum	−0.01
36	Compacted tomato solids	Centrifuged serum	−0.03

[a] All mean differences were significant at $P < 0.001$.

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A LABORATORY STUDY ON COUNTERCURRENT DESALTING OF PICKLES

INTRODUCTION

SALT INTRUSION lowers water quality. As a consequence, some food processors with a brine disposal problem can no longer discharge their spent brine into fresh water streams, ponds and wells, or even into sanitary sewer systems. Lowe and Durkee (1971) described a technique for recovering salt from spent processing brines, and the cost of that process is directly related to the concentration of salt in the spent brine. This research applies the technique of countercurrent leaching to the desalting of salt stock pickles to produce a spent brine of much higher salt concentration than normal.

Salt stock for manufacturers of pickle products is usually desalted by a continuous flushing or successive washings with fresh water, a process known as freshening. Typically, in the freshening the cured stock is reduced from 15–18% salt to 4% salt with at least two changes of water (Etchells et al., 1951). These washings produce a "weak brine" of 2–3% salt which must be discarded since the recovery of salt from a solution of this low a concentration is prohibitively expensive. With countercurrent leaching, however, the salt stock and water are countercurrently contacted in successive tanks, resulting in a proportionately lower volume of high concentration spent brine (13–14%), from which it may be economically feasible to recover salt.

This paper briefly reviews the principles and basic equations used in solving countercurrent leaching problems, and then shows how these can be applied to pickle salt stock freshening. The experimental data confirm that the assumptions necessary to make the calculations are a reasonable approximation of the actual process.

Calculation of number of stages

The countercurrent leaching process can be represented as shown in Figure 1. From McCabe and Smith (1956, p. 760), since changes in density and viscosity of salt solutions are not large enough to affect the calculation, the following equation applies for the number of stages required to achieve a given separation for all cases where $C \neq W$:

$$N_{theoretical} = \frac{\log\left(\frac{y_b - x_b}{y_a - x_a}\right)}{\log\left(\frac{y_b - y_a}{y_b - x_a}\right)} \qquad [1]$$

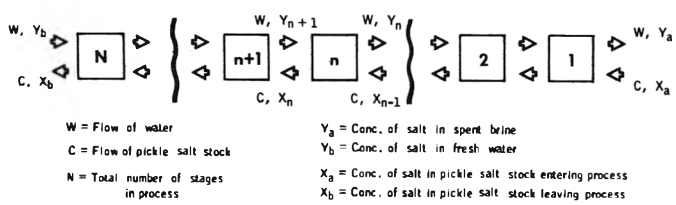

Fig. 1—Schematic diagram of countercurrent desalting process.

However, the special case where C = W involves a simpler equation:

$$N_{theoretical} = \frac{y_b - y_a}{y_b - x_b} = \frac{x_b - x_a}{y_b - x_b} \qquad [2]$$

These equations, along with an overall salt balance,

$$Cx_b + Wy_a = Cx_a + Wy_b$$

or

$$x_b = x_a - \frac{W}{C}(y_a - y_b) \qquad [3]$$

can be used to calculate the number of stages required to achieve a given salt concentration in either the spent brine or the desalted pickle stock.

Calculation of efficiency

Equations [2] and [3] will give the actual number of stages required for a separation only if an infinite length of time were allowed in each stage for salt equilibration. For a finite time in each stage the actual number of stages can be calculated if some assumptions are made about the conditions of leaching:

(1) The salt stock pickles are long circular cylinders of uniform dimensions that can be geometrically described with an equivalent radius:

$$a = (m/\rho\pi L)^{1/2}.$$

(2) Each stage is well-stirred; i.e. the resistance to transfer of salt is inside the pickle and not in the solution.

(3) The diffusion coefficient of salt in the pickle does not depend on the salt concentration.

With these assumptions salt leaching can be described by the following diffusion equation:

$$\frac{\delta c}{\delta t} = D\left(\frac{1}{r}\frac{\delta}{\delta r}\, r\, \frac{\delta c}{\delta r}\right) \qquad [4]$$

where c = concentration, r = cylindrical coordinate in a cylinder of radius a, t = time and D = diffusion coefficient (Crank, 1956, p. 62). The solution to this equation for a well-stirred solution of limited volume is given by Crank (1956, p. 70) in terms of M_t, the total amount of solute leaving the cylinder after time t, as a fraction of the corresponding amount M_∞ at equilibrium:

$$\frac{M_t}{M_\infty} = 1 - \sum_{n=1}^{\infty} \frac{4\alpha(1+\alpha)}{4 + 4\alpha + \alpha^2 q_n^2} \exp\left(\frac{-Dq_n^2 t}{a^2}\right). \qquad [5]$$

The q_n's are the positive nonzero roots of

$$\alpha q_n J_0(q_n) + 2 J_1(q_n) = 0. \qquad [6]$$

J_0 and J_1 are Bessel functions, and α = the ratio of the volume

of the solution to that of the cylinder. Equation [5] is shown graphically in Figure 2.

McCabe and Smith (1956, p. 769) show that the Murphree stage efficiency for leaching is as follows:

$$\eta = \frac{M_t}{M_\infty} \qquad [7]$$

They also state that when W/C is nearly unity, the average overall efficiency is nearly equal to the Murphree stage efficiency, and thus

$$N_{actual} = \frac{N_{theoretical}}{\eta} \qquad [8]$$

N_{actual} is the number of stages required to achieve a given salt concentration in the spent brine or in the desalted pickle for a finite residence time in each stage.

Method for measuring effective diffusion coefficient

The effective diffusion coefficient, D, for salt in salt stock must be determined in order to use Equation [5]. This is accomplished by experimentally determining the salt concentration in the leaching solution as a function of time at a given temperature. By substituting these concentrations and times into Equation [5], D can be determined. Using the value of D, Equation [5] can be applied to countercurrent leaching conditions. The method and calculations for measuring D, first described by Carman and Haul (1954), were applied to salt stock pickles by Pflug et al. (1967). The conditions and results for pickle stock used in this work are shown in Table 1.

Example calculation

The following example calculation illustrates how this method can be used to calculate the actual number of stages to produce a desired salt concentration in the spent brine. Suppose the following conditions are specified:

W/C = 1.0, y_b = 0% NaCl, x_a = 16.8% NaCl,
a^2 = 1.3 cm² (1–1-1/8" max diam)
t = 8 hr, N_{actual} = 6 stages, temp = 120°F

Since W = C, equation [2] can be used, and the equation can be combined with Equations [3] and [8] to give:

$$y_a = \frac{y_b + \eta\, N_{act}\, x_a}{(1 + \eta\, N_{act})}$$

From Table 1, D = 1.5 × 10^{-5} cm²/sec at 120°F. ∴ $(Dt/a^2)^{1/2}$ = 0.576.
Using Figure 2 and α = W/C = 1.0, η = 0.970.

$$y_a = \frac{0 + (0.970)\,(6)\,(0.168)}{(1 + 0.970 \times 6)} = 0.143$$

Thus the brine leaving the leaching process would be 14.3% NaCl, and from Equation [3] the finished salt pickles contain 2.5% NaCl.

EXPERIMENTAL

Measurement of effective diffusion coefficient

In this work the effective diffusion coefficient of salt stock pickles was measured using α = 9, i.e., nine times as much water as salt stock. This gave an effective diffusion coefficient averaged over a wide range of salt concentrations in the salt stock pickles (3–17%).

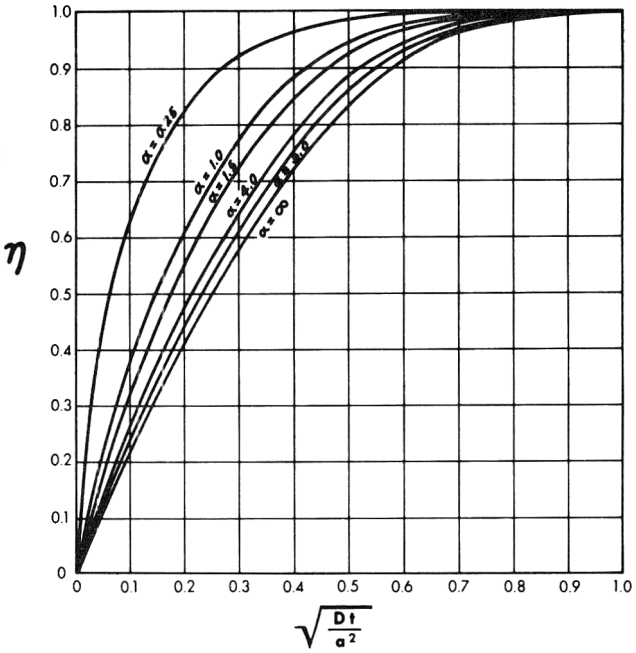

Fig. 2–Solution of diffusion equation for a cylinder in a stirred solution of limited volume (Crank, 1956, p. 72).

Approximately 300g of salt stock pickles were put into a battery jar (14.5 cm × 30 cm) with nine times their weight of distilled water. The jar was covered with a watch glass, and air was bubbled through the solution for agitation. A 3-ml sample of brine was withdrawn every hour for 8 hr. Losses due to evaporation and sampling were small and considered negligible. The chloride in the brine and salt stock pickle samples was measured by an Aminco-Cotlove titrator (American Instrument Co., Inc., Silver Springs, Md., Catalog No. 4-4420B). Chloride in salt stock samples was measured by grinding with an equal weight of water in a blender.

Using Figure 2 $(Dt/a^2)^{1/2}$ was determined for each $\eta = M_t/M_\infty$, and the slope of the line Dt/a^2 vs. t was used to determine D. The averages of two or three replications of experimentally measured values of D used in this work are shown in Table 1.

Experimental measurement of countercurrent leaching

To verify the applicability of the assumptions about salt stock freshening made in the above calculation, a countercurrent leaching experiment was done. Seven 150 mm × 75 mm crystallizing dishes were used as leaching vessels. The schedule for brine transfer is shown in Table 2 where the vessels are labeled A through G. Each vessel held 500 ± 5g of 1–1-1/8 in. max diam salt stock and 500 ml of leaching brine, which

Table 1–Diffusion coefficients of salt from pickle stock

Max diam (in.)	Temp (°F)	D × 10^5 (cm²/sec)	a^2 [a] (cm²)
1-1/2–1-3/4	78	0.53	3.5
1-5/16–1-1/2	77	0.94	2.4
1–1-1/8	77	1.1	1.3
1–1-1/8 (Perforated)[b]	77	0.89	1.3
1–1-1/8	120	1.5	1.3

[a] a^2 = the square of an equivalent radius determined by the formula $a^2 = (m/\rho\pi L)$, where m = weight of a salt stock pickle; ρ = density of a salt stock pickle, assumed to be the same as a salt-water solution; and L = length of a salt stock pickle.
[b] 0.072 in. pin, 1/2 in. deep on 1/2 in. centers.

Table 2—Schedule of brine transfer for six stages

Time (hr)	0	8	16	24	32	40	48	56	64	...	104
Add salt stock to vessels	A, B, C	D	E	F	G	A	B	C	D	...	B
Take spent brine from vessels					F	G	A	B	C	...	A
Transfer brine from vessels		A to D	D to E B to D A to B	E to F D to E C to D B to C A to B	E to G D to F C to E B to D A to C	F to A E to G D to F C to E B to D	G to B F to A E to G D to F C to E	A to C G to B F to A E to G D to F	B to D A to C G to B F to A E to G	...	G to B F to A E to G D to F C to E
Add water to vessels	A, B, C	A	A	A	B	C	D	E	F	...	D
Take freshened salt stock from vessels					A	B	C	D	E	...	C

was distilled water initially. The vessels were covered with watch glasses and held at 120°F. The freshened salt stock and spent brine removed after each 8-hr period were analyzed for chloride by the method described above.

RESULTS & DISCUSSION

THE CHLORIDE ANALYSES for the countercurrent leaching experiment are shown in Table 3. Steady-state operation for this procedure was found to occur in about 48 hr, the spent brine salt concentration being in the range of 13.2–13.6%, and the freshened salt stock 2.0–2.8%. These concentrations agree within 10–20% of the results obtained in the above calculation, which used the experimental conditions as an example.

The calculated concentrations of salt in the spent brine and freshened salt stock are suitable for examining the effect of different numbers of stages, residence times and temperatures. Table 4 summarizes the results of calculations for a number of conditions which might be considered for freshening salt stock pickles.

A number of combinations of stages and residence times can be used to produce a spent brine of much higher concentration than the present "weak brine." Most of the conditions used in the calculation are realistic, and countercurrent leaching appears to be a practical desalting technique. Total desalting time is 24–48 hr, which is comparable to the time presently used for successive or continuous washing. The much higher salt concentration of the spent brine should make salt recovery economically feasible, or if the salt is not recovered, the volume of brine for disposal would be considerably reduced.

The diffusion coefficient, D, increases with temperature. All other process variables being equal, increasing the leaching temperature increases the rate of salt removal. In countercurrent leaching however, the change in external salt concentration as the pickles move from stage to stage overshadows the effect of any increase in temperature. This situation has also been observed in conventional freshening as pointed out by Switzer et al. (1939) where they state that the slight increase in freshening rate for temperature above 80°F is not worth the increased cost.

The results of Table 4 show that the increased salt removal resulting from using a higher temperature in countercurrent leaching is indeed slight, and thus probably not worth the additional expense. In most cases the efficiency (η) is high, and thus any increase in the diffusion coefficient would not markedly affect the salt removal. Only in those cases where the efficiency is low (large size pickle or short desalting time in each stage) would a temperature increase provide a notable change in the concentration of salt in the spent brine and desalted pickle stock.

Table 3—Pickle stock desalting experiments

Time (hr)	y_a Spent brine leaving stage #1 (% NaCl)	x_b Desalted pickle stock leaving stage #6 (% NaCl)	
32	14.7	1.2	Experimental conditions
40	13.7	1.9	N = 6 stages
48	13.5	2.0	t = 8 hr
56	13.6	2.1	temp = 120° F
64	13.5	2.2	W/C = 1.0
72	13.4	2.8	x_a = 16.7% NaCl
80	13.4	2.1	
88	13.3	2.8	
96	13.2	2.7	
104	13.2	2.4	

Table 4—Calculation of desalting conditions[a]

N_{actual}	t (hr)	Max diam (in.)	Temp (°F)	y_a (%)	x_b (%)	η
6	8	1–1-1/8	77	14.4	2.6	0.943
6	8	1–1-1/8	120	14.5	2.5	0.971
6	6	1–1-1/8	77	14.3	2.7	0.906
6	4	1–1-1/8	77	14.2	2.8	0.835
4	8	1–1-1/8	77	13.4	3.6	0.943
2	8	1–1-1/8	77	11.1	5.9	0.943
8	6	1–1-1/8	77	14.9	2.1	0.906
6	8	1-5/16–1-1/2	77	14.1	2.9	0.820
4	8	1-1/2–1-3/4	77	12.2	4.8	0.629
6	8	1-1/2–1-3/4	77	13.4	3.6	0.629

[a] W/C = 1.0, y_b = 0, x_a = 17.0

Both the data of Pflug et al. (1967) and that in Table 1 show that D decreases as the size of the pickle salt stock increases. Pflug et al. (1967) suggest that this decrease in D may be due to a denser skin in the larger salt stock. Comparisons between values of D as measured by Pflug et al. (1967) and those in Table 1 show differences of 20–30%. The two sets of data indicate that variations in D for salt stock from different seasons and locations are not large, and it is possible to measure a reliable value of D for each salt stock size.

Perforation of the salt stock did not change the effective diffusion coefficient (Table 1), and thus had no effect on the desalting process.

Although the present study showed that countercurrent leaching is technically possible for freshening salt stock, the optimum number of stages can be determined only after considering the overall economics and production needs of a given plant. The present study gives a basis for making a pilot plant scale test, which can then be used for determining the economic, quality and scheduling aspects of the process.

NOMENCLATURE

Symbol	Description	Dimensions
a	Equivalent radius of salt stock pickle, $a = m/\rho\pi L$	length
C	Flow of salt stock	mass/time
c	Salt conc in salt stock pickle	mass/(length)^3
D	Effective diffusion coefficient	(length)^2/time
J_0 & J_1	Bessel functions	
L	Length of salt stock pickle	length
m	Mass of salt stock pickle	mass
M_t	The amount of salt having left salt stock after time t	mass
M_∞	The amount of salt having left salt stock at equilibrium	mass
N	Total number of stages in desalting process	
q_n	The positive nonzero roots of $\alpha q_n J_0(q_n) + 2J_1(q_n) = 0$	
r	Radial distance for salt stock pickle	length
t	Time of desalting in each stage	time
W	Flow of brine	mass/time
x_a	Concentration of salt in salt stock pickles entering countercurrent desalting	% NaCl by wt
x_b	Concentration of salt in salt stock pickles leaving countercurrent desalting	% NaCl by wt
x_n	Concentration of salt in salt stock pickles at stage N in countercurrent desalting	% NaCl by wt
y_a	Concentration of salt in spent brine leaving countercurrent desalting	% NaCl by wt
y_b	Concentration of salt in fresh water entering countercurrent desalting	% NaCl by wt
y_n	Concentration of salt in brine at stage N in countercurrent desalting	% NaCl by wt
α	The ratio of volume of brine to volume of salt stock in countercurrent desalting: $\alpha = W/C$	
ρ	Density of salt solution	mass/(length)^3
η	Murphree stage efficiency for countercurrent leaching; $\eta = M_t/M_\infty$	

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YIELD, PROXIMATE COMPOSITION AND MINERAL ELEMENT CONTENT OF THREE CULTIVARS OF RAW AND ROASTED PEANUTS

INTRODUCTION

PEANUTS and peanut products are important sources of protein and other essential nutrients in the American diet. The per capita civilian consumption of peanuts in 1971 was 6.0 pounds (USDA, ERS, 1972). Peanut products are distributed in large quantities in the commodity food programs of the U.S. Dept. of Agriculture. Peanuts may also be useful in diets in which intake of one or more nutrients, such as sodium or potassium, must be controlled.

Data on the nutrient content of peanuts, raw and roasted, are scattered. Hoffpauir (1953) and Woodroof (1969) summarized many of the early investigations on the composition and properties of raw peanuts. Eheart et al. (1955) reported a 5-yr study on the chemical composition of different varieties of raw peanuts grown in Virginia. The proximate composition of roasted Virginia and Spanish peanuts was reported by Smith et al. (1962). Sekhon et al. (1970) studied the proximate composition of six varieties of peanuts, both raw and roasted, grown in India. Hoffpauir (1953) reviewed compositional changes associated with roasting for such components of the peanut as moisture, B-vitamins, amino acids and sugars.

Data on content of mineral elements in raw peanuts were summarized by Hoffpauir (1953) and Woodroof (1969). In both instances, a major source of their data was the work of Freeman et al. which was published in 1954. Walker and Hymowitz (1972) analyzed raw peanuts from 16 cultivars of the 1971 crop from various states for content of 10 mineral elements. Few other data are available on mineral element content of peanuts, either raw or roasted, and those few are primarily from foreign sources.

This study was undertaken to update information on yields, proximate composition, and mineral element content of raw and roasted Virginia-type peanuts, and to measure changes which occur with roasting.

METHODS & MATERIALS

REPRESENTATIVE SAMPLES of three cultivars (Virginia-70, North Carolina-2 and Florigant) of Virginia-type peanuts were obtained from the Tidewater Research Station, Holland, Va. Replicate, unshelled 100-g samples of each variety were roasted in an electric oven at 177°C for 35 min. Matching 100-g raw samples and the roasted samples were shelled and the shells and kernels (including skins) were weighed separately to determine yield.

The raw and roasted kernels with skins were ground and stored at −21°C until analyzed. Moisture, crude fiber, fat, nitrogen and total ash were estimated according to the methods recommended by AOAC (1970). Samples of approximately 3g were ashed by chemical methods by using nitric and perchloric acid (Engel et al., 1967) and were analyzed for Ca, Mg, Na, K, Fe, Cu, Zn and Mn by the procedures described for the Perkin-Elmer atomic absorption spectrophotometer. Phosphorus was determined from an aliquot of the diluted ash by the colorimetric method of Fiske and Subbarow (1925).

The experimental design employed was a 3 × 2 × 2 factorial, with three cultivars, two physical states (raw and roasted) and two lots of each variety-physical state combination. For proximate composition and mineral element analyses, each observation was the mean of two determinations. Analyses of variance were made on the completed data to test for differences among varieties, physical states and lots. A further analysis was made by subdividing the sums of squares for the main effects and interactions

Table 1—Yield and proximate composition of three cultivars of raw and roasted peanuts[a]

	Yield					Proximate composition[b]			
Cultivar and physical state	Whole nut (g)	kernels[c] (%)	Shells %	Moisture %	Crude fiber %	Fat %	Nitrogen %	Protein (N × 5.46) %	Ash %
Raw:									
Virginia-70	100.0	74.8	24.3	5.5 ± 0.13 a	4.8 ± 0.02 a	47.7 ± 0.40 b	4.41 ± 0.07 a	24.1 ± 0.37 a	2.36 ± 0.02 a
North Carolina-2	100.0	75.5	24.0	8.3 ± 0.25 c	5.0 ± 0.07 a	45.1 ± 0.35 a	4.43 ± 0.03 a	24.2 ± 0.17 a	2.85 ± 0.05 b
Florigant	100.0	75.3	24.8	6.4 ± 0.10 e	4.8 ± 0.11 a	48.1 ± 0.42 b	4.75 ± 0.08 b	25.9 ± 0.42 b	2.39 ± 0.01 a
Roasted:									
Virginia-70	93.2	75.9	24.2	2.4 ± 0.11 xy	5.2 ± 0.19 x	49.7 ± 0.05 y	4.60 ± 0.15 x	25.1 ± 0.82 x	3.48 ± 0.03 y
North Carolina-2	91.2	76.1	24.1	2.3 ± 0.06 x	5.5 ± 0.16 y	47.2 ± 0.91 x	4.72 ± 0.10 x	25.8 ± 0.52 x	3.47 ± 0.24 y
Florigant	91.4	74.9	24.8	2.7 ± 0.03 y	5.2 ± 0.03 x	49.4 ± 0.12 y	4.96 ± 0.10 y	27.1 ± 0.55 y	3.23 ± 0.01 x
Overall mean:[d]									
Raw	100.0	75.2	24.4	6.7	4.9	47.0	4.53	24.7	2.54
Roasted	91.9	75.6	24.4	2.5	5.4	48.8	4.76	26.0	3.39

[a] Different letters in the same data column indicate significant differences ($P < 0.05$).
[b] Means and standard errors are given.
[c] No significant differences for percent of kernels were noted among cultivars or physical states.
[d] All differences between raw and roasted peanuts in proximate composition were significant ($P < 0.01$).

(Snedecor and Cochran, 1967), to determine which of the means were significantly different from each other. A separate analysis of variance was made on the pooled data to test for significance of differences between raw and roasted peanuts.

RESULTS & DISCUSSION

Yield and proximate composition

The yield of kernels averaged about 75% and the shells represented about 25% (Table 1) of the raw and roasted samples. No significant differences were observed for yield of kernels in relation to cultivar or physical state (raw or roasted). Woodroof (1969) reported average yields of kernels to be 80% for Spanish and 74% for Runner peanuts. The findings of this study are in good agreement with Woodroof's values.

The proximate composition of the samples is also shown in Table 1. The data summarized there are for the mean and standard error of the mean for each of the three cultivars, before and after peanuts were roasted. As indicated by analysis of variance on pooled data, raw and roasted peanuts differed significantly for all proximate components. Analyses of variance also showed significant differences among cultivars for moisture, fat, nitrogen and ash in raw peanuts, and for all proximate components in roasted peanuts. A few differences were noted in nutrient content between lots of a cultivar. These differences between lots, although statistically significant, were not of practical importance, as none of them exceeded 8% of the lower value, and several of the differences were less than 1%.

NC-2 raw peanuts were lower in fat and higher in moisture and ash content than were the other two cultivars. Nitrogen values were significantly higher in Florigant than in the other two cultivars, but there were some overlapping values. None of the three cultivars was consistently the highest or lowest in content of the various proximate components.

Differences in moisture content among the different lots of raw peanuts were greatly reduced by roasting. Differences in moisture content and crude fiber in roasted peanuts do not appear to be of practical importance. The pattern of differences for values on the dry basis was similar to that shown for the data in Table 2.

The mineral values of the cultivars tested are within the range reported for peanuts by Freeman et al. (1954) and agree fairly well with the values reported by Watt and Merrill (1963). Walker and Hymowitz (1972), whose samples included two (NC-2 and Florigant) of the cultivars also included in this research, reported average values that were in reasonable agreement with values found in this study for content of magnesium, potassium, copper and manganese, but

Table 2–Mineral element content (mg/100g) of three cultivars of raw and roasted peanuts[a,b]

Cultivar and physical state	Calcium	Magnesium	Phosphorus	Sodium	Potassium	Iron	Copper	Zinc	Manganese
Raw:									
Virginia-70	74.2 ± 0.46 a	174.4 ± 2.31 b	414.9 ± 7.98 a	6.98 ± 0.08 c	634.0 ± 2.02 c	1.43 ± 0.03 a	1.14 ± 0.05 a	6.13 ± 0.05 a	1.75 ± 0.01 a
North Carolina-2	85.8 ± 0.34 b	182.8 ± 1.59 c	429.0 ± 3.11 b	6.16 ± 0.07 b	618.2 ± 2.13 a	1.76 ± 0.02 c	1.27 ± 0.01 b	6.15 ± 0.03 a	1.77 ± 0.01 a
Florigant	87.8 ± 1.98 c	165.2 ± 0.42 a	470.3 ± 0.76 c	5.79 ± 0.08 a	626.6 ± 1.31 b	1.58 ± 0.01 b	1.26 ± 0.01 b	6.09 ± 0.06 a	2.01 ± 0.03
Roasted:									
Virginia-70	80.5 ± 0.42 x	180.0 ± 0.68 x	510.4 ± 1.60 y	6.18 ± 0.07 z	657.6 ± 0.99 y	1.54 ± 0.08 x	1.25 ± 0.02 x	6.69 ± 0.03 x	2.08 ± 0.03 y
North Carolina-2	91.7 ± 0.25 z	195.9 ± 2.10 y	501.1 ± 0.55 x	4.97 ± 0.03 x	647.1 ± 1.05 x	1.79 ± 0.02 z	1.30 ± 0.02 x	6.56 ± 0.02 y	1.94 ± 0.03 x
Florigant	91.3 ± 0.75 y	178.8 ± 0.27 x	538.2 ± 0.99 z	5.53 ± 0.10 y	647.8 ± 1.05 x	1.70 ± 0.01 y	1.36 ± 0.02 y	6.64 ± 0.02 xy	2.17 ± 0.02 y
Overall mean:[c]									
Raw	82.6	174.1	438.1	6.31	626.3	1.59	1.22	6.12	1.84
Roasted	87.8	184.9	516.6	5.56	650.8	1.68	1.30	6.63	2.06

[a] Different letters in the same data column indicate significant differences ($P < 0.05$).
[b] Means and standard errors are given.
[c] Differences between raw and roasted peanuts are significant ($P < 0.01$).

Table 3–Retentions of nutrients (percent) on three cultivars of roasted peanuts

Variety	Moisture	Crude fiber	Fat	Protein	Ash	Calcium	Magnesium	Phosphorus	Sodium	Potassium	Iron	Copper	Zinc	Manganese
Virginia-70	41.6	102.3	98.5	98.6	139.4	102.5	97.5	116.4	83.8	98.0	102.3	103.9	103.2	112.2
North Carolina-2	25.0	102.6	96.0	98.0	111.6	98.3	98.5	107.4	74.1	96.2	94.0	93.8	97.8	100.4
Florigant	38.6	100.0	93.3	94.8	122.5	94.6	98.4	104.0	86.8	94.0	97.5	97.4	99.4	98.0
Overall mean	35.0	101.6	96.0	97.1	124.5	98.5	98.2	109.2	81.6	96.1	97.9	98.4	100.1	103.5

were higher in calcium and iron and lower in phosphorus and zinc than values in the present study.

Retentions of nutrients with roasting

Retention values for nutrients in peanuts were calculated, using the following formula:

$$\frac{\text{batch wt of kernels before roasting} \times \text{nutrient content/100g roasted kernels} \times 100}{\text{Batch wt of kernels after roasting} \times \text{nutrient content/100g raw kernels}}$$

These retentions are listed in Table 3.

If a nutrient has been neither inactivated, volatilized, nor transferred to a processing by-product such as expressed oil, drippings, or cooking water, retention values should center around 100%. Because of error in analytical systems, some values could be expected to be higher, and some lower.

Retentions of nutrients for the roasted peanuts in this study were generally well within a range that could be attributed to analytical error, 90–110%. Moisture retentions were low, about 35%, indicating the extent to which moisture was volatilized from the raw peanuts with roasting. Ash retentions were high for all cultivars. Retentions for phosphorus and manganese in the cultivar VA-70 were also high. The reason for these high retentions could not be determined. Recovery values for phosphorus and manganese averaged 99% and 101%, respectively. Agreement among replicate values for these elements and for ash was good.

Sodium retentions were low for all cultivars, ranging from 74–87%. Reasons for these unusual retentions could not be established, but it can be speculated that sodium was lost to the shell of the peanut when roasted. Analyses were not done on the shells, so this possibility cannot be substantiated.

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INDIVIDUAL HEAT TRANSFER MODES IN BAND OVEN BISCUIT BAKING

INTRODUCTION

IN CONVENTIONAL heating operations, heat may be transferred to food products by the general mechanisms of conduction, convection, or radiation. Conduction refers to the direct transfer of heat energy between adjacent molecules within a stationary system; convection, which includes free and forced convection, refers to direct transfer of heat energy between adjacent molecules in different phases, as between a solid surface and a convecting fluid; radiation refers to the remote transfer of heat between emitting and absorbing masses via electromagnetic radiation in the infrared wavelengths.

It is the intent of this paper to examine the major effects of these individual heat transfer modes in biscuit baking, and to present a mathematical model which can be used to account for heat transferred by the individual heat transfer modes in band oven baking applications.

In band oven baking it is convenient to identify five separate modes of conventional heat transfer to the product: conduction from support medium, forced convection, free convection, radiation from refractory surfaces and radiation from flames. For indirect fired ovens, conduction, forced convection and radiation from refractory surfaces are most important, while for direct fired ovens, conduction, free convection and radiation from both refractories and flame may be important.

The transfer of heat by each individual mode may be expressed mathematically in terms of a temperature driving force, a transfer area and a transfer coefficient as given below. Discussions and derivations of some or all of these expressions may be found in Rohsenow and Choi (1961), Eckert and Drake (1959), Perry (1963), Charm (1971) and Matz (1960; 1972), among others.

The expressions for conduction, forced convection, and free convection, respectively, may be written as follows:

$$q_C = k \frac{A_b}{x} (T_B - T_b) \qquad [1]$$

$$q_F = h_F A_s (T_a - T_s) \qquad [2]$$

$$q_f = h_f A_s (T_a - T_s) \qquad [3]$$

The expressions for radiation from refractory surfaces and directly from flames, respectively, may be written as follows:

$$q_{Rr} = F_{sr} A_s \sigma (T_r^4 - T_s^4) \qquad [4]$$

$$q_{Rf} = F_{sf} A_s \sigma (T_f^4 - T_s^4) \qquad [5]$$

in which the coefficients are given by:

$$F_{sr} = \frac{1}{\frac{1}{\bar{F}_{sr}} + (\frac{1}{\epsilon_s} - 1) + \frac{A_s}{A_r}(\frac{1}{\epsilon_r} - 1)} \qquad [6]$$

$$F_{sr} = \frac{1}{\frac{1}{\bar{F}_{sf}} + (\frac{1}{\epsilon_s} - 1) + \frac{A_s}{A_f}(\frac{1}{\epsilon_f} - 1)} \qquad [7]$$

Identifications of the individual terms in these expressions are given in the Nomenclature section.

If we consider the baking of biscuits in an indirect fired oven with the biscuits supported directly on a band through which no significant air flows, then the important mechanisms of heat transfer to the biscuits include conduction from the band to the biscuit bottom, radiation from oven refractory surfaces to biscuit top and sides, and forced convection to biscuit top and sides. In this case the equation for transfer of heat to the product may be written using expressions [1], [2] and [4]:

$$q = q_C + q_F + q_{Rr} = k\frac{A_b}{x}(T_B - T_b) + h_F A_s (T_a - T_s) + \epsilon_s A_s \sigma (T_r^4 - T_s^4). \qquad [8]$$

In equation [8] the term for radiation is written in a simplified form made possible by assuming that both the refractory emissivity ϵ_r and the geometry factor $\bar{F}_{sr}$ are about unity, assumptions which were valid in the present study. The expression for total heat transferred must equal an expression for total heat absorbed. For most products both the gross temperature rise of the product (sensible heat increase) and the latent heat of vaporization of baked out moisture must be considered, while such factors as heats of fusion of fats, heats of reaction and solution, and heats of vaporization of volatiles other than water can usually be ignored. Thus we may write the total heat absorbed as in equation [9]:

$$q = \frac{\bar{W}\,\bar{C}_P\,(\bar{T} - T_i)}{\Delta t} + \frac{L\,\lambda_v}{\Delta t}. \qquad [9]$$

Identifications of the terms in this expression are given in the Nomenclature section.

MATERIALS & METHODS

EQUATIONS [8] and [9] were applied to the baking of biscuits in an indirect fired band oven process. The biscuit dough used was an unproofed chemically leavened dough; it was sheeted to about 5/8 in. thickness and cut to about a 2-in. diam piece yielding a baked biscuit of about 1.25 in. height, except for conduction trials utilizing a hot plate in which case the dough was sheeted to 3/8 in. thickness and cut to about a 4-in. diam piece yielding a baked biscuit of about 1.0 in. height. The band oven which was used utilizes burning of the gas in a chamber remote from the baking chamber with the heated air convected into the baking chamber through recirculation dampers. The oven features three separately controlled oven zones and was used with a 2-ft wide tight weave mesh band. Process and product temperatures were monitored continuously using type J iron-constantan thermocouples which were connected to a monitoring computer with teletype and punched tape output. Several thermocouples were located in permanent positions in each oven zone to monitor air and refractory temperatures. The measurement of oven band and biscuit temperatures utilized a number of thermocouples which traveled through the oven with the band and product so as to monitor these temperatures continuously as they changed through the oven.

Before applying the complete equations to the band oven process, an attempt was made to isolate the individual modes of heat transfer in separate experiments using lab scale equipment so as to determine values for the heat transfer constants k, h_F and ϵ_s, and to identify the

Table 1—Average biscuit bottom thermal conductivity k as a function of plate temperature and depth into biscuit (18 min. bake time)

Trial no.	Avg plate T, °F	Biscuit T, °F 2 mm (9 min)	k at 2 mm	Biscuit T, °F 5 mm (9 min)	k at 5 mm
1	319	200	0.044	183	0.097
2	353	205	0.039	187	0.086
3	380	219	0.040	196	0.087
4	407	227	0.034	201	0.075

major contributions to biscuit baking which these three modes of heat transfer make. The values determined for these constants using lab scale equipment are able to be extrapolated to band oven situations if no large temperature changes (e.g., to affect ϵ_s) or air velocity changes (to affect h_F) are involved in going from one to the other.

To determine a value for the thermal conductivity k of the biscuit bottom and to determine the role of conduction in the baking process, individual biscuits were heated on a hot plate to measure conduction rates from support medium to biscuit. Temperatures were monitored by using two thermocouples on the surface of the hot plate, two inside the biscuit at a distance 2 mm from the plate surface, and two inside the biscuit at a distance 5 mm from the plate surface.

For the other two modes of heat transfer considered in this model, a small laboratory electric oven was used to study the separate effects of radiation and forced convection when isolated from each other and from the effects of conduction, and to obtain values of the coefficients ϵ_s and h_F. The oven consisted of a small cavity fitted with a glass surfaced electric heating element and a fan located near the top. Biscuits were placed in the cavity in full view of the heating element on a support which minimized transfer of heat to the biscuit bottom by conduction or free convection. The biscuits were heated in the oven with the fan off to simulate purely radiative transfer, and with the fan on to simulate transfer by both radiation and forced convection.

The constants k, h_F and ϵ_s were calculated from these test results by using appropriate terms in equations [8] and [9]. For the radiation calculation using the test oven with fan off, correction was made for effects of free convection using dimensional equations as found in Perry (1963).

RESULTS

FOR TESTING the separate effects of conductive heat transfer, several biscuits were heated from the bottom only using a hot plate at each of four different hot plate settings. It was observed that bottom heating from the hot plate is sufficient to give a biscuit its normal oven spring but is not sufficient to set the structure in the raised biscuit. Approximately the bottom one-fourth of the biscuit is baked to a normal extent by conduction from the plate. The maximum biscuit heights obtained varied from 1.02–1.14 in. which represented a small but significant increase in height compared with the 1.0 in. standard for this biscuit when conventionally baked.

Using the conduction portion of the heat transfer model, average values of the thermal conductivity k were calculated for each plate temperature and for depths of 2 mm and 5 mm into the biscuit bottom. In Table 1 data are given for four single trials which show thermal conductivities of about 0.04 Btu/hr ft°F for the 2 mm bottom crust layer and about 0.09 Btu/hr ft°F for the 5 mm layer with some indication of decreased thermal conductivity with increased plate temperature. The value of k likely decreases during the heating period due to the effects of the heating process which create good insulating crust or crumb structures from relatively good conducting dense dough material. This is indicated by the increase in k from 0.04 to 0.09 in going from 2 to 5 mm into the dough piece. The value of k likely increases continuously from some value less than 0.04 near the interface between the biscuit and the plate to a value of about 0.20 Btu/hr ft°F or so within the raw dough. Because the outer 2 mm of crust material appeared relatively uniform, taking the average value of 0.04 Btu/hr ft°F for the entire outer 2 mm layer is probably a reasonable assumption. Use of expressions for overall heat transfer coefficients as found, for example, in Perry (1963) indicated that conduction can be viewed as being dominated by the 0.04 value using the foregoing assumption and the fact that values for k are considerably larger for the subsequent interior layers. The value 0.041, which is the average of the first three values in Table 1, was used in the subsequent calculations of this work.

In testing the separate effects of radiation and forced convection, the small test oven was run at two different heating element settings at each of three different fan settings. At a given setting of the oven heating element, the total heat transferred to a test biscuit was about the same whether or not the fan was on, but the character of the heating was significantly different. With the fan off, heat is transferred mostly by radiation and the resulting biscuits were baked to a near normal extent except that the bottoms were gummy and underbaked

Table 2—Biscuit emissivities and forced convection heat transfer coefficients in test oven

Trial no.	Heating element setting	Fan setting	T_r, °F	T_a, °F	q[a] Btu per hr	q_{Rr}[b] Btu per hr	q_F[c] Btu per hr	q_f[d] Btu per hr	ϵ_s	h_F[e] Btu/-hr ft² °F	Biscuit surface browning
1	510	0	510	325	67.0	55.6	0	11.4	0.73	—	dark brown
2	510	low	357	329	70.4	23.0	47.4	—	—	6.2	light brown
3	510	high	313	295	68.4	14.5	53.9	—	—	10.0	pale
4	435	0	435	300	56.0	47.6	0	8.4	0.97	—	light brown
5	435	low	295	267	50.6	11.6	39.0	—	—	10.7	pale
6	435	high	257	240	53.1	7.6	45.5	—	—	15.7	very pale

[a] Heat absorbed as latent heat and sensible heat, equation [9]
[b] Using $\epsilon_s = 0.85$ except for trials 1 and 4 for which ϵ_s is calculated from q_{Rr} = q less about 15% correction for free convection
[c] Assuming $q_F = q - q_{Rr}$ for trials 2, 3, 5, 6 and $q_F = 0$ for 1 and 4
[d] Estimated using equations in Perry (1963) and Rohsenow and Choi (1961)
[e] Using $h_F = q_F/A_s(T_a - T_s)$

due to the lack of conductive transfer to the biscuit bottom; the top crusts were found to have a normal level of crust browning in these cases. With fan on, radiative transfer decreased and forced convection increased, resulting in biscuits which were similar in overall degree of doneness but with significantly decreased crust browning.

Numerical results for the six individual trials are given in Table 2. Using equation [9] the total heat absorbed by the test biscuits was calculated using temperature and moisture data. For trials 1 and 4 with fan off, this quantity, less a small correction for effects of free convection, was set equal to the expression for radiative heat transfer in equation [8]. The free convection correction utilized the dimensional correlations given by Perry (1963) and amounted to about 15% of total heat transferred. This calculation gave values for the biscuit surface emissivity of 0.73 at the high temperature setting and 0.97 at the low temperature. These values indicate an average emissivity for biscuit surfaces of about 0.85 in this temperature range, indicating that biscuits absorb and emit nearly as a black body in the thermal wavelengths. The value of 0.85 was used for ϵ_s in subsequent calculations in this work.

For the variables with the convection fan on at low and high speed, the measured emitter temperature dropped considerably which greatly decreases the contribution of radiative heat transfer according to equation [8] due to the fourth power dependency on the emitter temperature T_r. Setting the forced convective heat transfer term q_F equal to $q - q_{Rr}$ for these variables, heat transfer coefficients ranging from 6.2–15.7 Btu/hr ft^2°F were calculated for transfer from the moving air to the biscuit, with an average of about 8 at the low velocity and 13 at the high velocity.

In these trials the extent of the decreases in T_r as fan speed was increased may have been exaggerated due to the difficulty in measuring the surface temperature of the radiant emitter for this apparatus which involved the use of type J thermocouples secured to the emitter surface. This would cause a concomitant increase in the estimated values of h_F and q_F for these trials. Indeed the values of h_F would appear somewhat high in view of the fact that average air velocities were only about 100–180 ft/min at the low fan setting and about 250–270 ft/min at the high fan setting.

The dependence of biscuit crust browning on the relative quantities of q_{Rr} and q_F as well as on the overall oven temperature setting can be seen from the data of Table 2. With high temperature setting and high q_{Rr} biscuit browning is extensive, while decreases in q_{Rr} with constant total q cause large decreases in biscuit browning at both temperatures. The data suggest that browning occurs most extensively on surfaces receiving considerable radiant heat transfer from radiant sources at high temperatures (greater than about 350°F), and that the particular mode of the heat transfer is more important than the total heat transferred to the product.

Table 3–Complete heat balance for baking of biscuits in an indirect fired band oven. Basis: heat transferred to a single biscuit

	Contribution to q in Btu/hr	Percent of total q
Heat absorbed		
by biscuit = q =	68.0	100
as sensible heat	32.1	47.2
as latent heat	35.9	52.8
Heat transferred		
to biscuit = q =	68.0	100
by radiation q_{Rr}	29.3	43.1
by convection q_F	25.0	36.8
by conduction q_C	13.7	20.1

The complete heat transfer model as expressed by equations [8] and [9] was applied to the continuous baking of biscuits in an indirect fired band oven using the previously calculated values for k and ϵ_s. Using continuous computer monitoring of a wide range of process temperatures, each term of equations [8] and [9] was calculated for this application. The total heat transfer q was set equal to the sum of latent and sensible heat uptakes, q_{Rr} and q_C were calculated directly from equation [8], and q_F was calculated using $q_F = q - (q_{Rr} + q_C)$. This approach was used so that the latter equation could be solved for the convective heat transfer coefficient h_F. In Table 3 results are given for a band oven baking trial which indicates that approximately 50% of the heat absorbed by the baking biscuits was sensible heat with about 50% of the heat absorbed as the latent heat of vaporization of water. Of the total heat transferred to the biscuit, about 20% was transferred by conduction from the band to the biscuit bottom according to the model, about 37% was transferred by convection, and about 43% by radiation. This distribution indicates significant contributions from all three major heat transfer modes.

The foregoing results were obtained using oven recirculation damper settings which were normally used for this product: "open" recirculation dampers in all three lower sections (below the band) and in the second and third upper sections (above the band), and "closed" in the first upper section. Air flow to the baking chamber is considerable in this oven whether the recirculation dampers are in their nominally "closed" or "open" positions with overall average velocities near the band of about 240 ft/min with all upper dampers "closed" and 380 ft/min with all upper dampers "open." Several other damper setting combinations were tested to calculate the effect of these settings on the theoretical heat transfer profile. Constants used in the terms q_{Rr}, q_F and q_C were those used in the foregoing experiment except that the convection coefficient h_F, calculated to be 2.88 Btu/hr ft^2°F in that experiment, was normalized to the average air velocity. A Reynolds number analysis indicated laminar air flow at all damper settings so that a dependency of h_F on average velocity to the 0.5 power was used.

A schematic representation of the oven recirculation dampers and exhaust dampers, and an example of their settings is given in Figure 1. In Table 4 calculated results are given for this experiment for single trials using each of six damper setting combinations. For each trial, the six tabulated designations for recirculation damper settings refer to settings above and below the band in the first, second and third oven zones, respectively, while the three designations for exhaust dampers refer to settings for the entire respective oven zone. For all variables the nominal oven temperature settings were kept the same at 375°F in sections 1 and 2 and 400°F in section 3.

The results indicate that, for these temperature settings, changes in the recirculation dampers significantly changed the total heat transferred to the product, but for the most part had little effect on the proportions of heat transferred by the various heat transfer mechanisms according to the theoretical model. Variable 1 had settings the same as for the data of Table 3 and had a total heat transfer and transfer profile which was very similar to it. With all dampers open (variable 2), total heat transfer was about the same and the transfer profile and biscuit parameters such as crust browning and biscuit height remained essentially unchanged. Leaving all upper dampers open but closing all lower dampers (variable 3) left total heat transferred to the product essentially unchanged and decreased conductive heat transfer by a small amount. Closing both upper and lower recirculation dampers (variable 4) decreased total heat transfer about 20% which was sufficient to yield biscuits which were considerably less brown and had somewhat less oven spring than the other five variables of the ex-

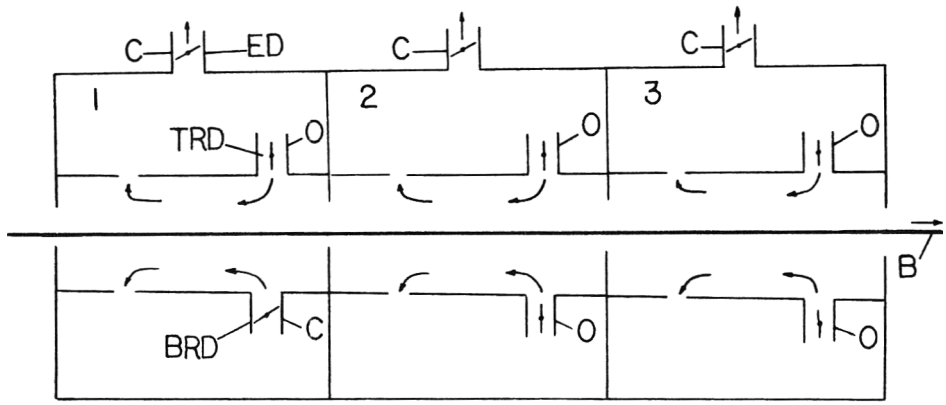

Fig. 1—Schematic representation of band oven damper settings and air flows. 1, 2, 3—Oven sections; TRD—top recirculation dampers; BRD—bottom recirculation dampers; ED—exhaust dampers; B—band; C—nominally closed position; O—nominally open position.

periment; this change caused a decrease in proportion of heat transferred by forced convection and with it a large decrease in moisture bakeout. In variable 5 the upper dampers were kept closed and the lower dampers were opened; these settings also produced decreased total heat transfer, but total heat input was sufficient to yield normal crust color and oven spring. In variable 6 the recirculation dampers were set the same as in variable 1, but the exhause dampers, which were kept in their "closed" position in all previous runs, were changed to their fully open position; this change was found to have no effect on product characteristics, total heat transfer, or heat transfer profile, but did cause a 20% increase in fuel consumption.

These results indicate that the heat transfer profile is relatively stable for this oven at a given set of oven temperature settings. This is due in part to the fact that the forced convection coefficient has only a half power dependence on air velocity in the model so that large changes in air velocity only translate to modest changes in the convective heat transfer coefficient. In addition, increases in upper zone air velocity tend to increase both the ceiling temperature T_r and the air temperature T_a so that both radiation and convection increase. Changes in the lower zone air flow can modify the proportion of heat transferred by conduction slightly by changing the band temperature, but upper zone temperatures tend to blunt these effects somewhat. In order to significantly change the heat transfer profile to the product one would probably have to change specific temperature settings along with specific damper settings.

If the problem of a direct fired oven is considered it is necessary to consider free convection instead of forced convection, and to take account of direct radiation from flame to product in addition to radiation from refractory surfaces. The free convection contribution can be dealt with by using equations for the free convection heat transfer coefficient h_f in Perry (1963) or Rohsenow and Choi (1961) among others.

The term for direct radiation from flame to product can be of considerable importance depending upon the thickness of the flames which are in direct view of the product, and the flame emissivity ϵ_f. If we consider an application such as an oven band full of biscuits in an oven which has small flames spaced a few feet apart, we might take the view factor $\bar{F}_{sf}$ equal to about 0.1, ϵ_s nearly unity, A_s/A_f equal to about 10, and for flames whose depths are only an inch or less, ϵ_f would be of the order 0.01 using Hottel and Broughton (1932) and Sherman (1934). These values would give a value of F_{sf} equal to about 0.001. From equations [5], then, we would have

$$q_{Rf} = 0.001\ A_s\ \sigma\ (T_f^4 - T_s^4).$$

Table 4—Band oven heat transfer profiles for biscuit baking as a function of damper settings in an indirect fired band oven

Trial no.	Recirculation damper settings[a]	Exhaust damper settings[a]	Percent radiation (q_{Rr})	Percent forced convection (q_F)	Percent conduction (q_C)	Total q Btu/hr	Moisture bakeout %
1	C O O O O O	C C C	43.9	35.1	21.0	65.2	7.40
2	O O O O O O	C C C	45.9	34.7	19.4	68.9	9.77
3	O O O C C C	C C C	45.9	35.4	18.7	70.1	8.31
4	C C C C C C	C C C	47.4	29.3	23.3	53.7	6.26
5	C C C O O O	C C C	44.4	32.9	22.7	58.7	7.48
6	C O O O O O	O O O	45.1	35.3	19.6	69.2	8.46

[a] C designates nominally "Closed" position; O designates the most open position.

Using $A_s = 0.0601$ ft^2 for one biscuit as used in the previous calculations and using $T_f = 2000°F = 2460°R$ and $T_s = 212°F = 672°R$ we get a value for q_{Rf} of about 4 Btu/hr transferred to one biscuit. This figure would be about 8 Btu/hr if T_f were 2500°F. These values compare with the total of 60–70 Btu/hr transferred to biscuits in the foregoing examples and values of around 30 Btu/hr for radiant heat transferred from refractory surfaces. Thus it would appear that direct flame radiation would play a relatively minor roll in terms of total heat transferred in a direct fired oven, with the major radiation coming from refractory surfaces which are in turn heated by convection and radiation from the flames. However, because of the high temperature of the flame, the direct flame radiation may assume more importance than indicated by its total heat transfer rate, especially in terms of browning reactions, and hence would probably have to be considered in the overall model.

DISCUSSION

THE OVERALL RESULTS of this analysis indicate that the most important effects of the individual modes of heat transfer, at least for biscuit baking applications, may be the effect of oven spring by conduction, browning effects by radiation and drying effects by convection.

The results using a hot plate to "bake" biscuits by conduction only indicated that normal biscuit heights could be obtained by supplying as little as half the total heat which is normal for baking this product if the heat is supplied by conduction. Biscuits which had no browning and very little moisture bakeout nevertheless had normal or greater than normal volumes. All heat transfer modes tend to simultaneously expand the gas in gas cells which increases volume, while drying and denaturing the dough mass which tends to set the dough and limit increase in volume. Apparently conduction from the support medium tends to act more in expanding than in setting the dough due to the gradual transfer of heat up through the entire volume of the dough, whereas convection and radiation may function more to limit volume increase due to rapid transfer of heat to the exposed biscuit surfaces which would tend to set the overall structure and limit oven spring.

The most important effect of convection, especially forced convection, may be moisture removal. Convecting air tends to increase water evaporation by removing the stagnant moisture boundary layer at the evaporating surface of the product as the rate determining step in the moisture diffusion and evaporation process. The convecting air continually presents a fresh low moisture air mass to the product surface to increase tendency of moisture to migrate outward. In Table 4 the one variable which had significantly lower proportion of heat transferred by forced convection (variable 4) also had a greatly decreased moisture bakeout.

Radiation, as indicated by the data of Tables 3 and 4, accounts for a large percentage of total heat transfer and is therefore likely important in all baking mechanisms. Its most important role, however, may be in surface color development. Browning reactions generally require high temperatures on exposed product surfaces and these are more likely provided by radiation than by convection because less simultaneous evaporative cooling will likely be associated with radiation than convection. The results in Table 2 indicate a considerable decrease in biscuit surface browning as the proportion of heat transferred by radiation decreases.

CONCLUSION

THESE RESULTS suggest that for many band oven applications it may be desirable to control the amount of heat supplied to the product by the individual modes of heat transfer rather than control only the total heat supplied to the product, and that a mathematical heat transfer model can be useful in estimating the magnitude of heat transfer contribution from each individual heat transfer mode.

NOMENCLATURE

A_b Area of product bottom in contact with support medium, ft^2

A_f Area of radiating flame surface, ft^2

A_r Area of radiating refractory surface, ft^2

A_s Surface area of product exposed to convection or radiation, ft^2

$\bar{C}_p$ Average heat capacity of product, Btu/lb°F

$\bar{F}_{sf}$, $\bar{F}_{sr}$ Geometry factors: fraction of radiation from product intercepted by flame or refractory surface, respectively, dimensionless

F_{sf}, F_{sr} Overall coefficients for radiative heat transfer, dimensionless

h_F, h_f Heat transfer coefficients for forced and free convection, respectively, Btu/hr ft^2°F

k Thermal conductivity of product near surface of support medium, Btu/hr ft°F

L Moisture mass loss during baking, lb

q Total heat transferred to or absorbed by product, Btu/hr

q_C Heat transferred by conduction, Btu/hr

q_F, q_f Heat transferred by forced and free convection, respectively, Btu/hr

q_{Rf}, q_{Rr} Heat transferred by radiation from flame and refractory surfaces, respectively, Btu/hr

$\bar{T}$ Average final product temperature, °F

T_a Air temperature, °F

T_B, T_b Temperature of support medium and product surface in contact with support medium, respectively, °F

T_f Flame temperature, °F

T_i Initial product temperature, °F

T_r Temperature of radiating refractory surface, °F

T_s Temperature of product surface, °F

Δt Elapsed time, hours

$\bar{W}$ Average mass of product through baking, lb

x Distance through product surface for which thermal conductivity is equal to k, ft

ϵ_f, ϵ_r Gray body emissivity of flame and refractory surfaces, respectively, dimensionless

ϵ_s Gray body emissivity of product surface, dimensionless

σ Stefan-Boltzman constant, 0.1713×10^{-8} Btu/hr ft^2°R^4

λ_v Latent heat of vaporization of water, 972 Btu/lb

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PREDICTING AN EQUILIBRIUM STATE VALUE FROM TRANSIENT STATE DATA

INTRODUCTION

VALUES of many biological, chemical, or physical factors in sample material change with time when reactive potential is applied to these factors. If this potential is time invariable, the rates of change in these values are progressively reduced and values of the factors finally reach constant. These final values are called the values at equilibrium state or equilibrium values. For example, when dehydrated food is exposed to humid air, it adsorbs moisture from the air. The amount of moisture adsorbed by the food reaches a plateau after long exposure. This is usually called the equilibrium moisture content of the food.

Equilibrium values of various factors are frequently required for kinetical analyses of various processes. For example, a number of survivors in a biological population at an equilibrium state is required for analyzing its growth or death dynamics (Meadows et al., 1972). Equilibrium moisture is needed for determining mass diffusivity of sample material or for predicting the rate of moisture sorption (Chen and Johnson, 1969; Chu and Hustrulid, 1968; Crank, 1964; Del Valle and Nickerson, 1967; Hamdy and Barre, 1969; Satterfield et al., 1971). An equilibrium reaction rate is required for analyzing the mechanisms of enzymic reactions (Reiner, 1959). The maximum rates of photosyntheses are used for evaluating various treatments on plant growth (Devlin, 1969). Mature body weights are used for analyzing animal growth processes (Maynard and Loosli, 1969).

The determination of an equilibrium value is time consuming when transient state values slowly converge to an equilibrium state and this determination is almost impossible when sample material deteriorates biologically, chemically and/or physically during experimentation. In the present investigation, a mathematical procedure was developed for the fast determination of equilibrium values by using transient state data.

DEVELOPMENT OF FORMULAE

A NUMBER of transient and equilibrium state data for various biological, chemical, or physical processes were collected from published literature and from experimental data obtained in our laboratory. The careful examination of these data revealed the following relationship. A major portion of the following curve may be approximated with one or more straight line segments: The curve, which is obtained by plotting the common logarithms of differences between transient and equilibrium state values of a factor against values of an independent variable, Figure 1.

The present procedure was developed by applying the observed relationship on transient and equilibrium state data. For computational simplicity, we assume that transient state values, y, of a factor in sample material are given at uniform intervals of an independent variable, t.

The following equations are obtained from any three successive y values in a straight line segment on the curve.

$$|y_{i-1} - y_e| = b \cdot 10^{-t_{i-1}/S} \quad [1]$$

$$|y_i - y_e| = b \cdot 10^{-t_i/S} \quad [2]$$

$$|y_{i+1} - y_e| = b \cdot 10^{-t_{i+1}/S} \quad [3]$$

All symbols used are defined in the nomenclature. In the above equations, b, s and y_e are unknown. Among these unknowns, y_e represents an equilibrium state value of y. This value may be estimated by solving the above equations simultaneously.

$$S = D/\left\{\log_{10}[(y_i - y_{i-1})/(y_{i+1} - y_i)]\right\} \quad [4a]$$

$$b = (y_i - y_{i-1})/[10^{-t_{i-1}/S}(10^{-D/S} - 1)] \quad [4b]$$

$$y_e = y_i - b \cdot 10^{-t_i/S} \quad [4c]$$

APPLICATION OF FORMULAE

IT IS MOST LIKELY that transient state y values are not determined at uniform intervals of t. In this case, y values for evaluating an equilibrium value by the proposed procedure are obtained as follows:

(1) Plot y values against t values on regular graph paper with square grids.
(2) Find a curve to represent accurately points plotted on the paper through the application of a least square method or through visual inspection.
(3) Obtain y values from this curve at uniform intervals of t.

A y_e value may be determined entering these y values into Equations 4a, b and c. Trial calculations reveal that the above stated procedure should be applied to y values even when they are determined at uniform intervals of t since each y value determined has experimental or other errors.

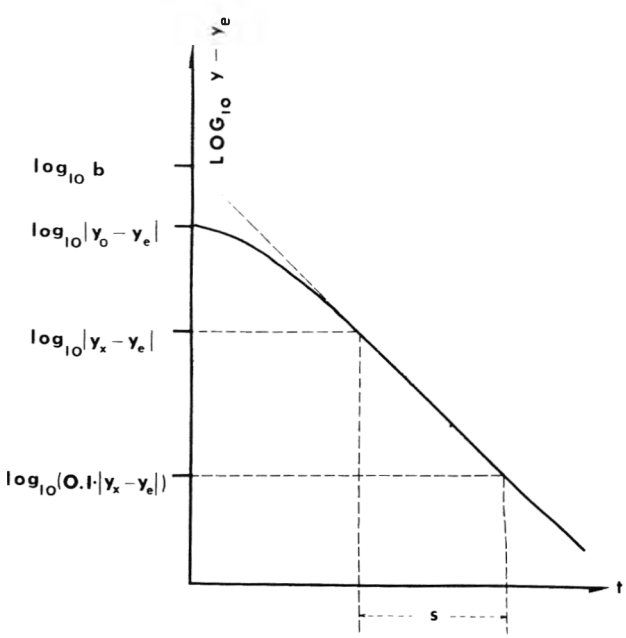

Fig. 1—Hypothetical semilogarithmic curve of transient state data.

Table 1–Comparison of equilibrium values determined experimentally and those predicted

Item	D	R/Rmax	s	t_{i+1}	t_e	y_e Predicted	y_e Experimental	% error[a]	References
Isothermal moisture sorption									
Cream of rice	2.5 hr	0.228	23.3 hr	12.5 hr	35.0 hr	26.3% DB[d]	26.6% DB	1.2(%)	Hayakawa (1972)
Cream of rice	2.5 hr	0.186	18.9 hr	10.0 hr	25.0 hr	15.9% DB	15.6% DB	–2.0	Hayakawa (1972)
Nonfat milk powder	2.0 hr	0.222	15.6 hr	12.0 hr	25.0 hr	28.0% DB	27.5% DB	–1.8	Hayakawa (1972)
Nonfat milk powder	1.0 hr	0.205	6.3 hr	8.0 hr	20.0 hr	13.8% DB	13.9% DB	1.0	Hayakawa (1972)
Wheat flour	60.0 min	0.222	199 min	280 min	2200 min	12.9% DB	13.0% DB	0.5	Udani et al. (1968)
Wheat flour	60.0 min	0.212	542 min	460 min	2200 min	19.1% DB	19.0% DB	–0.4	Udani et al. (1968)
Wheat starch	25.0 hr	0.218	96.3 hr	75.0 hr	250 hr	10.5% DB	10.5% DB	0	Bosin and Easthouse (1970)
Wheat starch	25.0 hr	0.146	118 hr	75.0 hr	250 hr	15.5% DB	14.9% DB	–3.5	Bosin and Easthouse (1970)
Nonisothermal moisture transfer									
Fish	10.0 hr	0.176	21.5 hr	20.0 hr	60.0 hr	54.8% DB	54.5% DB	–0.6	Chen and Johnson (1969)
White sucker	0.5 hr	0.198	2.1 hr	7.0 hr	12.0 hr	22.1% DB	21.5% DB	–2.8	Lantz and Iredale (1971)
Mass extruction									
Plastic film	2.0 day	0.302	6.1 day	8.0 day	16.0 day	3.3 ppm	3.2 ppm	–4.1	Giacin and Wong (1973)
Plastic film	2.0 day	0.172	5.8 day	8.0 day	16.0 day	11.1 ppm	11.2 ppm	0.2	Giacin and Wong (1973)
Enzymic hydrolysis									
Palmioleo oleostearin	4.0 day	0.148	15.0 day	16.0 day	32.0 day	89.2%	91.0%	2.0	Acker and Wiese (1972)
Trioleim	4.0 day	0.217	19.3 day	12.0 day	32.0 day	91.5%	88.4%	–3.5	Acker and Weise (1972)
Others									
Human blood cell[b]	10.0 mm Hg	0.275	39.7 mm Hg	50.0 mm Hg	100 mm Hg	101.4%	100%	–1.4	Kiesow et al. (1973)
2nd human kidney transplants[c]	2.0 mo	0.192	7.8 mo	6.0 mo	12.0 mo	30.6%	31.5%	–2.2	Opelz et al. (1973)
2nd human kidney transplants	2.0 mo	0.136	4.7 mo	6.0 mo	12.0 mo	30.4%	29.0%	–4.8	Opelz et al. (1973)

[a] % error = 100 × [(experimental y_e – predicted y_e)/experimental y_e].
[b] Oxygenation of cells
[c] Survival ratios after transplanted
[d] Dry basis

Equations 4a, b and c were applied to a number of transient state data available in order to obtain additional conditions which should be satisfied for accurate prediction of y_e values. Equilibrium values predicted by Equations 4a, b and c were carefully examined and compared with those obtained experimentally. This comparison revealed that the following four requirements should be satisfied in order to predict accurately y_e value.

(1) There should be the following relationship among values to be used for predicting y_e values

$$(y_i - y_{i-1})/(y_{i+1} - y_i) \neq 1 \qquad [5]$$

(2) A value of D should be less than one-third of an s value, which is estimated by Equation 4a.

(3) There should be at least two and one-half significant digits in each of these two differences: $y_i - y_{i-1}$ and $y_{i+1} - y_i$. (In this paper, two and one-half significant digits signify that the least significant digit is rounded to the nearest 0, 5, or 10.)

(4) Transient state y values should be collected until the following condition is satisfied.

$$(y_{i+1} - y_i) \cdot D \simeq 0.2 \cdot R_{max} \qquad [6]$$

Equation 5 represents nonlinearity of three successive data points plotted with Cartesian coordinates. The rate of change in y values, R, becomes maximum when t is close to zero. This rate gradually approaches zero with an increase in t values after it reaches maximum. Therefore, there is no ambiguity in finding y values which satisfy Equation 6.

The developed procedure was applied to transient state values of various factors for estimating their equilibrium values. Table 1 shows typical results on this esti-

Table 2–Transient state data used in sample problem–Moisture adsorption by nonfat milk powder

Time (hr)	Moisture (% dry basis)	Rate of moisture Rate (% hr)	Ratio
0	2.29	3.6	1.00
2	9.49	2.4	0.67
4	14.15	1.6	0.44
6	17.25	1.2	0.33
8	19.55	1.1	0.31
10	21.70	0.8	0.22
12	23.30		

mation. The errors of equilibrium values predicted by the developed procedure are less than 5%.

Sample calculations

A sample problem is presented for predicting y_e values using the proposed procedure.

Dehydrated nonfat milk powder purchased at a local market was exposed to 75.5% RH 23°C air. Moisture sorption data determined are shown in Figure 2. Transient state moisture contents of the sample were recorded from the curve shown in this figure at 2-hr intervals (Table 2). A ratio of the current and maximum rates of changes in the moisture contents became approximately equal to 0.2 at 12 hr of exposure. Therefore, the last three sorption data in Table 2 were used to predict an equilibrium moisture content of sample material by using Equations 4a, b and c. We noted that there were three significant digits in the differences between each of two adjacent moisture data selected. Therefore, an s value was estimated by equation 4a.

$$S = 2.00/\left\{\log_{10}[(21.70 - 19.55)/(23.30 - 21.70)]\right\}$$
$$\simeq 15.59 \text{ (hr)}$$

Since the present D value, 2.0, was less than one-third of this s value estimated, we continued our calculations.

$$b = (21.7 - 19.5)/[10^{-8/15.59}(10^{-2/15.59} - 1)]$$
$$\simeq -27.40 \text{ (hr)}$$

$$y_e = 21.7 - (-27.40) \cdot 10^{-10/15.59} \simeq 27.95\% \text{ dry basis}$$

An equilibrium value determined experimentally is equal to 27.5% dry basis.

DISCUSSION & CONCLUSION

THERE ARE several procedures published for quickly determining equilibrium moisture contents of dehydrated food (Bosin and Easthouse, 1970; Lendrock and Proctor, 1951). These procedures estimate accurately the moisture contents. However, they require special instrumentation and they are not applicable for determining equilibrium values of other factors. The proposed procedure needs no special equipment and is applicable to predict equilibrium values of various biological, chemical and physical factors.

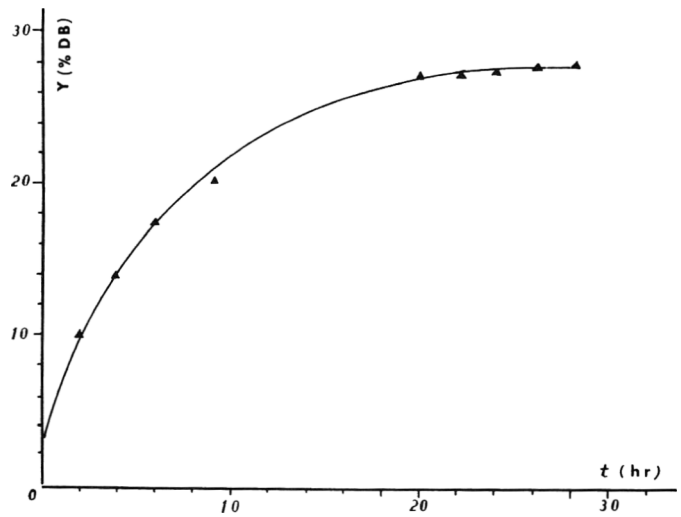

Fig. 2—Transient state sorption data of nonfat milk powder exposed to 75.5% RH, 23° C air.

As shown in Table 1, transient state data were collected up to those t values, which were equal to or less than 50% of t_e values in order to satisfy the requirements. In other words, the proposed procedure determines y_e values with less than 50% the time effort when compared with conventional procedures for determining these values.

According to trial calculations, the proposed procedure predicts y_e values even when the slope value, s, of a semilogarithmic curve changes within three successive y values selected for this prediction.

We tend to select a small D value for accurately estimating a y_e value. However, the selection of a too small D value leads to a poor estimation of equilibrium values since this selection likely results in the following situations:

(1) Equation 5 is satisfied by y values selected.
(2) There are less than two and one-half significant digits in the differences of y values given in Equation 4a.

Therefore, a proper D value should be carefully selected for each set of transient state data.

There are most likely errors in y values determined through experimentation. A formula, Equation 7, for estimating an error of a y_e value, which was predicted by using these y values, was derived through the calculation of a total differential (Schelkunoff, 1965).

$$|\Delta y_e| < |\Delta y_i| + |b| \cdot \eta \cdot 10^{-t_i/s} \cdot \left\{1 + 2t_i/D + \frac{2 \cdot |b|}{D \cdot |y_{i+1} - y_i|} \cdot |t_i \cdot 10^{-t_i/s} - t_{i-1} \cdot 10^{-t_{i-1}/s}|\right\} \quad [7]$$

where

$$\eta = 2 \cdot |\Delta y_i| / |y_{i+1} - y_i| \quad [7a]$$

An example for using Equation 7 is given below. An error in a y_e value owing to rounding the least significant digit was estimated by using data shown in Table 2. Moisture contents of this problem were obtained by rounding the fourth digits after the decimals to the nearest 0 or 10. Therefore, we estimated as follows:

$$|\Delta y_e| < 0.005 + 27.40 \times \frac{1}{160} \times 10^{-10/15.59}\left\{1 + \frac{2 \times 10}{2} + \frac{2 \times 27.4}{2 \times (23.30 - 21.70)} \times |10 \times 10^{-10/15.59} - 8 \times 10^{-8/15.59}|\right\} \simeq 0.55\% \text{ dry basis}$$

According to the previous calculation, a difference between the experimental and predicted y_e values is 0.5% dry basis. The round off is a major reason for this difference.

The proposed procedure was developed by assuming that a major portion of a semilogarithmic curve consists of one or more straight lines. This assumption is satisfied by the transient state data for many biological, chemical and physical processes. However, data should be carefully examined for the applicability of the proposed procedure. This examination is accompoished by calculating a rate of change in y values at each of the uniform t intervals. If this rate decreases progressively with increase in t values, the proposed procedure may be used to estimate a y_e value.

In conclusion, a procedure was developed for predicting an

equilibrium value from transient state data, which satisfies several requirements. This procedure predicts equilibrium values fairly accurately according to computational experiments which were conducted by using various data available.

NOMENCLATURE

b Constant estimated by Equation 4b. This constant represents an intercept coefficient of a line segment of a semilogarithmic curve (Fig. 1).

D Uniform intervals of t, at which y values are estimated.

i Arbitrary integer

R Rate of changes in y values for unit change in t value. This rate represents a slope of a curve, which is obtained by plotting $t \sim y$ data in Cartesian coordinates.

s Constant estimated by Equation 4c. This constant represents a value of t, which is required for a line segment of a semilogarithmic curve to traverse one log cycle (Fig. 1).

t Value of independent variable

y Value of dependent variable

η Value estimated by Equation 7a

Δ Error of a value, which is represented by a symbol placed after Δ

Subscripts

e Equilibrium state

max Maximum value

i−1, i, i+1. These subscripts represent the ith, i + 1st, and 1 + 2nd values of t or y, respectively.

x Arbitrary value

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EVALUATION OF THERMAL PROCESSES FOR CONDUCTION HEATING FOODS IN PEAR-SHAPED CONTAINERS

INTRODUCTION

MODERN PROCESS calculation theory is based on rate processses encompassing the whole container volume which necessitates a complete understanding of the heat transfer for foods heating by conduction. Stumbo (1948) suggested that the location of greatest probability of survival of a thermal process was not the point of greatest temperature lag, and developed a graphical procedure (1949) for integrating the probabilities of bacterial survival at all locations in the container. Hicks (1951), Gillespy (1951) and Stumbo (1953) developed mathematical methods for process evaluation that incorporated this concept of integrated lethality throughout the container.

Recent literature on food process evaluation contains several examples of computer applications. Teixeira et al. (1969) presented a computer program for process evaluation based on a finite difference approximation of the heat conduction equation, which featured considerable flexibility. Processes having nonuniform initial spore and temperature distributions could be evaluated. Similarly, the final viable spore distribution could be determined; this method supported the theory of Stumbo (1948).

There has been some interset in utilizing the synergistic effect obtained from consecutive combined thermal and irradiation processes. For this, each individual method of process evaluation must be able to accept and produce nonuniform spore distributions. Purohit et al. (1971) evaluated the thermal portion of processes with a numerical solution similar to that of Teixeira et al. (1969).

The previously mentioned literature is pertinent only to foods processed in cylindrical containers. A very limited amount of literature discusses process calculations for foods processed in rectangular, oval or pear-shaped containers. Although Manson et al. (1970) applied the concept of integrating the lethal effects throughout rectangular containers, foods processed in oval or pear-shaped containers have been restricted to process evaluation by the single point lethality concept. A theoretical and experimental analysis of food processed in oval containers was presented by Iwata (1940). The heat conduction equation was transferred to elliptical coordinates (z plane) and an analytical solution was developed for the center point temperature history.

Schack et al. (1959) reported on the experimental determination of the temperature histories for 21 different points in a pear-shaped container of ham. The F_o values of the process at the points of temperature measurement were determined by the formula method of Ball (1928) and the method of Patashnik (1953). The results indicated that process times derived by traditional methods (single point lethality concept) may provide a lethal treatment that is considerably excessive, hence, resulting in an overcooked product. Schack et al. (1959) also concluded that the integrated lethality concept of Stumbo would be applicable to this type of process.

Thus, this investigation was designed to develop a theoretical model capable of predicting the temperature histories at any number of points (hence, integrated lethality) in a conduction heating food processed in a pear-shaped container, and to develop a procedure whereby these processes could be evaluated utilizing an existing method originally designed for foods in cylindrical containers.

EXPERIMENTAL

Theoretical considerations

Heat transfer in foods heating primarily by conduction may be approximated by the following equation:

$$\frac{1}{\alpha}\frac{\partial T}{\partial t} = \frac{\partial^2 T}{\partial x^2} + \frac{\partial^2 T}{\partial y^2} + \frac{\partial^2 T}{\partial z^2} \qquad (1)$$

Figure 1 illustrates two situations that may occur when finite difference equations are employed to solve equation (1) for geometries having nonregular curved boundaries. These situations arise whenever the boundary intersects the grid system at places other than nodes.

In both cases the partial element occurs in the negative x-direction and Taylor Series approximations around node i,j yield the following finite difference equation for the second derivative with respect to x:

$$\left.\frac{\partial^2 T}{\partial x^2}\right|_{i,j} = \left[\frac{T_{i+1,j} - T_{i,j}}{1+E} + \frac{T_{i-1,j} - T_{i,j}}{(1+E)E}\right]\frac{2}{(\Delta x)^2} \qquad (2)$$

The upper diagram of Figure 1 illustrates the case where the partial element occurs in the positive y-direction. A Taylor Series approximation yields the following finite difference equation for the second derivative with respect to y (Ozisik, 1968):

$$\left.\frac{\partial^2 T}{\partial y^2}\right|_{i,j} = \left[\frac{T_{i,j+1} - T_{i,j}}{1+F} + \frac{T_{i,j-1} - T_{i,j}}{(1+F)F}\right]\frac{2}{(\Delta y)^2} \qquad (3)$$

The lower diagram of Figure 1 illustrates the case where the partial element occurs in the negative y-direction. Similarly, the following equation can be derived for this situation:

$$\left.\frac{\partial^2 T}{\partial y^2}\right|_{i,j} = \left[\frac{T_{i,j+1} - T_{i,j}}{(1+F)F} + \frac{T_{i,j-1} - T_{i,j}}{1+F}\right]\frac{2}{(\Delta y)^2} \qquad (4)$$

For all conditions E and F are defined as proportionality factors having values between zero and one.

The rate of bacterial destruction as a function of temperature has been expressed by the following equation (Ball, 1928):

$$R = \log^{-1}\frac{T - Tx}{z} \qquad (5)$$

where R = destructive rate (min^{-1}); T = temperature (°F); Tx = reference temperature (250°F for most sterilization processes); and z = in-

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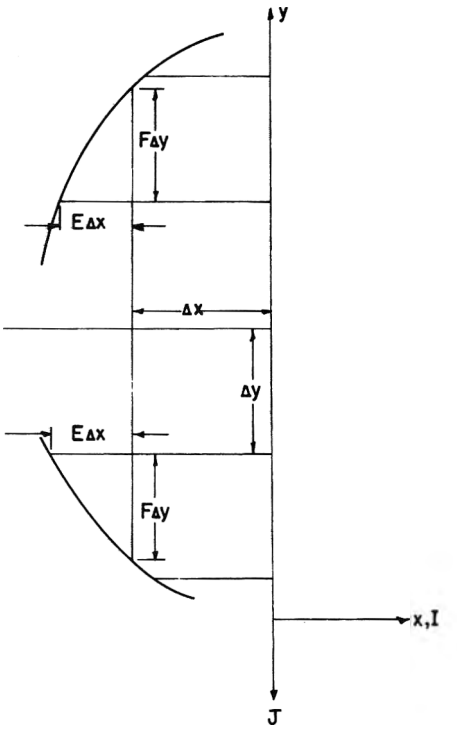

Fig. 1–Grid system for curved boundary.

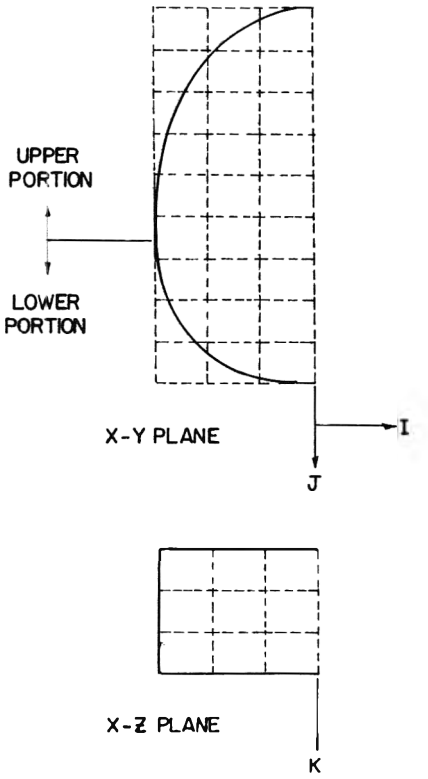

Fig. 2–Grid system for pear-shaped container.

verse slope of thermal destruction curve–numerically equal to the number of Fahrenheit degrees (F°) required for the thermal destruction curve to traverse one log cycle.

Integration of the lethal rate equation over a period of time for a given point yields the F value of heat at that point.

$$F_\lambda = \int_0^t \log^{-1} \frac{T - Tx}{z}\, dt \qquad (6)$$

where F_λ = the equivalent, in minutes at some given reference temperature (usually 250°F) of all heat considered; and t = time (min).

The final concentration of viable spores (or first order reactant), at this given point, can be determined from the following equation (Stumbo, 1948):

$$F_\lambda = Dr\,(\log a - \log b) \qquad (7)$$

where a = initial concentration (previous to heat treatment) per unit volume; b = final concentration per unit volume; and Dr = time, in minutes at reference temperature, for 90% reduction. (A reference temperature of 250°F was employed in this study.) Hence the absolute effect can be determined by multiplying the final concentration at a point by the volume associated with that point.

For large values of time, t, under conditions of negligible surface thermal resistance, transient dimensionless temperature may be expressed by:

$$T = je^{-(G\pi^2 \alpha t/L^2)} \qquad (8)$$

where T = dimensionless temperature; j = lag factor; G = geometry index (Smith, 1966); α = thermal diffusivity; and L = characteristic length–shortest distance over which the greatest temperature gradient exists during transience (Smith, 1966). The f_h value has been previously defined by several authors as the reciprocal of the slope of the semilog temperature-difference versus time plot. Hence the value G can be determined from the semilog temperature-difference versus time relationship.

$$G = \left[\frac{2.303\, L^2}{\pi^2\, \alpha f_h}\right] \qquad (9)$$

The geometry index of a finite cylinder (diameter greater than height) in terms of its significant dimensions (Smith, 1966) may be represented by:

$$G = \left[\frac{0.586}{a^2} + \frac{0.25}{b^2}\right] L^2 \qquad (10)$$

where a = radius; b = half height; and L = characteristic length (equal to b). Hence the dimensions of a cylinder for a given G and characteristic length can be determined from equation 10.

Development of model

A stable reverse sweep (alternating-direction) explicit technique for integrating finite difference equations was presented by Allada and Quon (1966). Application of this technique, utilizing the appropriate equations (1) to (4), to a grid configuration similar to Figure 2 yields the following difference equation for integration in the forward direction:

$$T^{n+1}_{i,j,k} = Vl\left[T^n_{i,j,k} + P/E\,(T^{n+1}_{i-1,j,k}) + P\,(T^n_{i+1,j,k} - T^n_{i,j,k}) + Ql\,(T^{n+1}_{i,j-1,k}) + Q2\,(T^n_{i,j+1,k} - T^n_{i,j,k}) + S\,(T^{n+1}_{i,j,k-1} - T^n_{i,j,k} + T^n_{i,j,k+1})\right] \qquad (11)$$

where $P = 2\alpha\,\Delta t/(\Delta x)^2\,(1 + E)$; $Q = 2\alpha\,\Delta t/(\Delta y)^2\,(1 + F)$; $S = \alpha\,\Delta t/(\Delta z)^2$; n = superscript denoting time level; and i,j,k = subscripts denoting grid locations.

At any node in the upper portion of the object in Figure 2:

$$V1 = \frac{1}{1 + P/E + Q/F + S} \tag{12}$$

$$Q1 = Q/F \tag{13}$$

$$Q2 = Q \tag{14}$$

At any node in the lower portion of the object in Figure 2:

$$V1 = \frac{1}{1 + P/E + Q + S} \tag{15}$$

$$Q1 = Q \tag{16}$$

$$Q2 = Q/F \tag{17}$$

A similar derivation yields the following difference equation for integration in the reverse direction:

$$T^{n+1}_{i,j,k} = V2\left[T^{n}_{i,j,k} + P/E\,(T^{n}_{i-1,j,k} - T^{n}_{i,j,k}) + P\,(T^{n+1}_{i+1,j,k}) + Q1\,(T^{n}_{i,j-1,k} - T^{n}_{i,j,k}) + Q2\,(T^{n+1}_{i,j-1,k}) + S\,(T^{n}_{i,j,k-1} - T^{n}_{i,j,k} + T^{n+1}_{i,j,k+1})\right] \tag{18}$$

At any node in the upper portion of the object in Figure 2:

$$V2 = \frac{1}{1 + P + Q + S} \tag{19}$$

$$Q1 = Q/F \tag{20}$$

$$Q2 = Q \tag{21}$$

At any node in the lower portion of the object in Figure 2:

$$V2 = \frac{1}{1 + P + Q/F + S} \tag{22}$$

$$Q1 = Q \tag{23}$$

$$Q2 = Q/F \tag{24}$$

Complete derivation of equations (11) through (24) are beyond the scope of this paper—only a brief description of methods can be given. A Fortran IV computer program was written utilizing the appropriate combinations of equations (11) through (24) as demanded by the location of the particular nodes. Since symmetry exists in the x and z directions only one quarter of the object was encompassed.

It was noted that all nodes more than one Δ in from the surface boundary the values of the proportioning factors, E and F, were unity. Thus, in addition to the normal boundary conditions, it was necessary to input a coding and the corresponding proportioning factors for the first nodes inside the surface boundary of the x-y plane. These data were acquired by tracing the surface boundary, assigning a grid network, as in Figure 2, and, noting the node and increasing the corresponding proportioning factors as in Figure 1. The coding and proportioning factors were determined for the following geometries:

(i)	6.06 diameter cylinder	
(ii)	3.88 × 5.73 pear-shaped container	
(iii)	5.25 × 7.62 pear-shaped container	Inside base
(iv)	6.20 × 9.15 pear-shaped container	dimensions
(v)	7.38 × 10.55 pear-shaped container	(in.)

The temperature dependent lethal rates (actually any first order kinetic) were determined by applying the Improved General Method of Stumbo (1973) to each node in the container. At each time step the lethal rate was determined as a function of temperature (equation 5). The area under the curve was found by multiplying the lethal rate by the time step Δt and summing them for each node location thus yielding an F value (sterilization F) for each node. The final concentration (per unit volume) at each node was determined from equation (7). The integrated lethal effect was determined by multiplying the final concentration times the volume of the container associated with it and summing throughout the container. This was easily applied in all areas except near the curved boundary where the volume elements have varied shapes. The destructive effects in these areas were evaluated by averaging the final concentrations of all the adjacent nodes and multiplying this average by the difference between the total volume of the container and total of the regular shaped containers. Since the total volume of the container was required for prediction of the integrated destructive effects, the areas of x-y traces of the container studied were determined by planimeter (eight replicates).

RESULTS & DISCUSSION

THE ACCURACY of the numerical model was investigated by comparison with results obtained from analytical solutions for finite cylinders. The appropriate node codings and proportioning factors for 603 diameter cylinders of five different heights were employed as boundary conditions for the numerical model. Radial and axial temperature distributions were calculated for various elapsed times following imposition of a 90°F step change on the surface temperature.

The temperature distributions from the model and the analytical solution (Hayakawa, 1964) compared favorably for all five cylinders. The maximum deviation occurred at the geometrical center. In the taller three cylinders, this deviation ranged from 0.03–0.05F° or less than 0.1% of the 90F° step change. An error of 0.2% was noted for distributions of the 6.06 × 2.38 cylinder which was the case giving the least agreement of the five studied.

Center point temperature histories were also generated using the numerical model. The f_h value of each heating curve was determined by a least squared semilog regression analysis of the straight line portion; lag factors (j_h) were also determined. Theoretical f_h and j_h values were calculated from equations of Olson and Jackson (1942). Table 1 lists the comparisons of f_h values and j_h factors. It is noted that the differences in the f_h values are generally greater in the flatter containers. Since the regression analysis program (Manson, 1971) measures the line of best fit (not the asymptote or tangent) the differences observed for the two methods are more than likely due to the method of measuring the f_h and j_h values of the computer solution. Thus, these differences were not considered significant.

Similarly, lethality predictions for cylindrical container processes, as calculated by the numerical model, were compared with those determined by other methods. Table 2 contains a comparison of the lethality predictions obtained by the computer model (three dimensional) with those obtained by the method of Teixeira et al. (1969). It is noted that as with the temperature history comparisons above, the greater differences occur in the examples calculated for the flatter cylinders.

Table 1—Computer model compared with analytical solution—f_h values and j_h factors

Container size (cylinders)	Analytical solution f_h	Analytical solution j_h	Computer model f_h	Computer model j_h
6.06 X 1.50	32.1	1.91	33.0	1.82
6.60 X 2.38	67.8	2.04	68.5	2.04
6.06 X 3.50	112.1	2.04	112.2	2.04
6.06 X 4.38	140.6	2.04	140.9	2.04
6.06 X 4.82	152.6	2.04	152.9	2.04

A similar trend is noted in the comparisons with the modified Stumbo method (Table 3). Excellent agreement is observed in the taller two cylinders (D1, D2, E1, E2). Examples A2, B2 and C2 (for Dr = 3.2, z = 14F°) do not indicate any great difference between the methods. For a Dr = 1.0 and z = 8F° (cases A1, B1, C1), the differences appear to be quite significant but these results are distorted since the sensitivity of the kinetics is far greater than would ever be experienced in practical applications. In examples A1 and B1, the differences between the methods are less than the effect of one-half a minute change in process time. A comparison of the survivors calculated by the method of Teixeira et al. (1969) (Table 2) with the results in Table 3 illustrates the sensitivity of the kinetics, since for most practical applications the methods of Teixeira et al. (1969) and Jen et al. (1971) have been considered to compare satisfactorily.

Process calculations for pear-shaped containers

Thermal process calculations (with the numerical model) were made for several different sizes of pear-shaped containers. A mesh spacing of 0.25 in. and a time increment, Δt, of 1.0 min were found to be the most efficient. The additional computation time required by a smaller time step as mesh spacing was not justified by improved accuracy. A thermal diffusivity of 0.0143 in^2/min (Stumbo, 1973) was assumed as a typical example. Comparison between F_s (integrated) and the corresponding F_c (cold spot single point) lethalities are shown in Table 4. It is noted that integrated F_s values exceed F_c values (by more than 50%) for several of the sample problems, confirming the hypothesis of Schack et al. (1959).

Several of the examples in Table 4 illustrate the evaluation of the temperature dependent degradation of a nutrient or organoleptic quality factor (Dr = 154; z = 46), which could not have been accomplished utilizing the "single point" concept. Since the three dimensional numerical model (applied to cylindrical geometry with appropriate approximations) was able to predict temperature histories, lethalities and nutrient retentions that concurred with previously accepted methods (for two dimension geometry) it is inferred that the model can predict the same for pear-shaped geometries. The model can be extended to perform similar calculations for almost any shape. In addition, since the logarithmic destruction of bacteria is analagous to a first order kinetic reaction, any temperature dependant reaction or degradation that follows first order kinetics could be evaluated. In fact, the model can be easily modified to integrate the effects of any reaction providing the kinetics are expressed algebraicly as a function of time and temperature.

Table 2–Computer model compared with model of Teixeira et al. (1969)–Lethality predictions

	Predicted probable no. of survivors	
Code	Computer model	Teixeira model
A1	0.0000046	0.000015
A2	0.031	0.047
B1	0.047	0.051
B2	0.20	0.22
C1	0.0095	0.011
C2	0.015	0.016
D1	0.0015	0.0015
D2	0.0019	0.0019
E1	0.17	0.18
E2	0.046	0.045

Table 3–Computer model compared with modified method of Stumbo (1973)–Lethality predictions

	Computer model		Modified Stumbo			
			Theoretical		Empirical	
Code	F_s	Survivors	F_s	Survivors	F_s	Survivors
A1	9.29	0.0000046	9.79	0.0000016	9.03	0.0000083
A2	17.5	0.031	18.0	0.020	17.17	0.039
B1	5.29	0.047	6.15	0.0064	5.87	0.012
B2	14.9	0.20	16.1	0.081	15.7	0.11
C1	5.98	0.0095	6.56	0.0025	6.53	0.0026
C2	18.5	0.015	19.5	0.0074	19.4	0.0077
D1	6.78	0.0015	6.88	0.0012	6.81	0.0014
D2	21.4	0.0019	21.4	0.0018	21.3	0.0020
E1	4.67	0.19	4.66	0.20	4.62	0.22
E2	16.9	0.045	16.8	0.051	16.7	0.055

Table 4–Sample evaluation of foods processed in pear-shaped containers

	Values of parameters								
Container size	T_r[a]	T_I[b]	a	B[c]	D_r	z	F_s	F_c	% Retention
400 X 512 X 114	235	40	1000	45	0.2	14	1.63	1.38	–
400 X 512 X 114	235	40	1000	50	1.0	14	4.36	2.87	–
400 X 512 X 114	235	40	100	60	154	46	–	–	75.6
506 X 710 X 301	250	160	100	105	1.0	8	2.90	1.69	–
506 X 710 X 301	240	40	100	120	154	46	–	–	59.2
606 X 904 X 312	250	130	100	200	154	46	–	–	17.3
606 X 904 X 312	250	130	100	200	3.2	14	25.0	21.0	–
506 X 710 X 403	240	40	100	240	154	46	–	–	30.6
506 X 710 X 403	250	160	100	170	3.2	14	15.5	10.4	–
506 X 710 X 403	250	160	100	170	1.0	8	4.27	2.54	–
506 X 710 X 403	250	160	100	170	154	46	–	–	33.0

[a] T_r = retort temperature, °F
[b] T_I = initial product temperature, °F
[c] B = process time, min.

Geometry index of a pear-shaped container

"Cold spot" heating curves were generated for 22 different pear-shaped containers by the transient temperature computer model developed as described earlier. A thermal diffusivity of 0.0143 was assumed as was an initial temperature of 40°F. The surface temperatures were increased by a 200F° step change. The temperatures were truncated at four decimal places.

The semilog regression program was employed to determine the f_h value of the linear portion of each heating curve. Approximately 20 temperatures of each time-temperature history was included in the analysis over the temperature range from 237–238.5°F. This small range was considered in order to reduce the effects of truncation error. The statistical variance ratio of each curve fit was greater than 10^5, indicating a confidence level above 0.99.

A geometry index, G, was calculated for each of several pear-shaped containers from equation (9). The geometry index and corresponding characteristic heat transfer length for most common sizes of pear-shaped containers are listed in Table 5. (The characteristic length of each of these containers equaled the inside half-height.) The geometry indices and characteristic heat transfer lengths can be employed, with equation (9), to convert heat penetration data from one pear-shaped container size to another.

Thermal process evaluations for "equivalent cylinders"

Twenty theoretical process evaluations (for five different pear-shaped containers) were made, first, with the numerical method, and secondly, by applying the modified Stumbo method of Jen et al. (1971) to corresponding "equivalent cylinders." Dimensions of any equivalent cylinder were determined by arbitrarily choosing the height to equal the characteristic length of the pear-shaped container and solving for the radius using equation (10) (and using the G value of the pear-shaped container). The corresponding f_h value was calculated by the following equation (Olson and Jackson, 1942).

$$f_h = \frac{0.398}{(1/a^2 + 0.427/b^2)\alpha} \quad (25)$$

Heating and cooling j factors (for the equivalent cylinder) were estimated by the following equations (Olson and Jackson, 1942).

$$\log j_h = 0.3096\,[1 - 8.6w\,(e^{-1.8 \cdot 4w} + 2.5q\,e^{-23qw})] \quad (26)$$

$$j_c = 1.27 + (j_h - 1.27)\frac{T_1 - T_w}{T_1 - T_2} \quad (27)$$

Table 5—Geometry index G values for several pear-shaped containers

Container size	Characteristic length (in.)	Geometry index
400 X 512 X 114	0.75	.305
400 X 512 X 208	1.06	.363
400 X 512 X 212	1.19	.404
400 X 512 X 214	1.25	.421
400 X 512 X 304	1.44	.479
506 X 710 X 214	1.25	.339
506 X 710 X 301	1.35	.356
506 X 710 X 308	1.56	.394
506 X 710 X 314	1.75	.434
506 X 710 X 401	1.85	.456
506 X 710 X 408	2.06	.507
606 X 904 X 214	1.25	.313
606 X 904 X 304	1.44	.334
606 X 904 X 308	1.56	.351
606 X 904 X 312	1.69	.370
606 X 904 X 408	2.06	.432
606 X 904 X 412	2.19	.456
709 X 1011 X 400	1.81	.345
709 X 1011 X 404	1.94	.362
709 X 1011 X 408	2.06	.377
709 X 1011 X 412	2.19	.394
709 X 1011 X 504	2.44	.430

Table 6—Pear-shaped model compared to equivalent cylinder—Process calculation for a thermal diffusivity of 0.0143

Run[a] code	Computer model F_s	Computer model Probable survival	Computer model % Retention	Modified Stumbo (equivalent cylinder) F_s	Modified Stumbo (equivalent cylinder) Probable survival	Modified Stumbo (equivalent cylinder) % Retention
2	1.63	0.0000067		1.77	0.0000016	
4	4.36	0.044		4.33	0.047	
15						
5	290	0.12		3.27	0.053	
12			59.2			62.3
8			17.3			21.8
9	25.0	0.0000016		26.3	0.0000055	
11			30.6			32.5
17	15.5	0.0015		15.4	0.0015	
18	4.27	0.0054		4.31	0.0049	
19			23.0			22.9

[a] The calculations were made based on the processes described by Manson (1971)

Table 7—Pear-shaped model compared to equivalent cylinder—Process calculations for a thermal diffusivity of 0.0120

Run[a] code	Computer model F_s	Computer model Probable survival	Computer model % Retention	Modified Stumbo (equivalent cylinder) F_s	Modified Stumbo (equivalent cylinder) Probable survival	Modified Stumbo (equivalent cylinder) % Retention
3	5.35	0.00045		5.02	0.00095	
13			63.4			68.0
14			65.1			66.0
20			36.3			37.6
10	9.95	0.043		9.92	0.049	
16	7.54	0.0000029		7.69	0.0000020	
1	3.67	0.022		3.50	0.031	
6	14.5	0.0030		13.7	0.0051	
7	5.93	0.00012		5.57	0.00026	

[a] The calculations were made based on the processes described by Manson (1971)

where T_1 = temperature of cooling medium; T_w = temperature 0.1 inch from wall before cooling; T_2 = initial temperature before cooling; $q = b^2/a^2$; a = radius; b = half height; p = log cycles from origin to point where tangent is measured; $w = p/(2.344q + 1)$.

Process variable values were chosen in the ranges that one would expect to encounter in most applications. However, extreme values of certain highly sensitive parameters were employed in many instances to ensure the relationships developed were valid over the complete range of interest. The results of 20 processes are given in Tables 6 and 7.

It is noted that the values of F_s, probable number of survivors and percent retention (nutrient, Dr = 154, z = 46), determined by application of the method of Jen et al. (1971) to an "equivalent cylinder," compare favorably with those values determined for the corresponding pear-shaped container using the numerical model.

It is anticipated that the inherent errors of numerical techniques (truncation and round-off) contribute to the slight differences noted in the solutions. Closer agreement is observed among the results illustrated in Table 7. This is probably a result of the effect on the truncation errors in the numerical solution caused by the different values for thermal diffusivity. Truncation errors in finite difference solutions for transient heat conduction are a function of grid size, time increment and thermal diffusivity (Ozisik, 1968); the grid size and time increment were the same for all solutions. The choice of values for these parameters is very critical. However, for practical applications the agreement between these methods is comparable to that noted for thermal process evaluation methods as reported by Stumbo (1973), Teixeira et al. (1969), Hayakawa (1969) and Jen et al. (1971).

Significance and applications

Thermal processes for conduction heating foods in the various pear-shaped containers listed in Table 5 can be practically evaluated by applying the "equivalent cylinder" concept, as previously described. The thermal diffusivity can be determined from heat penetration data from the required pear-shaped container (solving equation 9), or, it can be determined from data collected from a cylindrical container. Heat penetration data can not only be converted from one size to another of the same shaped containers but from one shape to another (e.g., cylindrical to pear-shaped) utilizing equations (9), (10) and (25).

The numerical model is valid for evaluating foods processed in pear-shaped containers and could be modified to handle most any other shaped container.

The same techniques utilized in this model could be used to simulate mass transfer and coupled phenomena (e.g., drying) in anomalous shapes; verification would be required.

CONCLUSIONS

1. Transient temperatures in a conduction heating pear-shaped object can be predicted by a numerical model.
2. Bacterial lethality and other first order kinetics rate processes for thermally conductive foods processed in pear-shaped containers can be determined by a numerical model.
3. In many cases single point lethality calculations do not adequately evaluate processes for foods in pear-shaped containers.
4. Bacterial lethality and nutrient retention for thermally conductive foods processed in pear-shaped containers can be determined by application of a modification of Stumbo's method (Jen et al., 1971) to an equivalent cylinder.

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CHARACTERIZATION OF MECHANICALLY DEBONED HOT AND COLD MUTTON CARCASSES

INTRODUCTION

MUTTON CARCASSES are particularly vulnerable to high labor costs because boneless mutton sells at a lower price than most other boneless red meats and the amount of labor required per unit of meat obtained is greater. Recent engineering advancements have made it possible to obtain mechanically deboned mutton. The process is similar to that used for mechanically deboned poultry (Food Engineering, 1970).

Increased interest has been shown in mechanically deboned poultry meat for use in emulsified products (Froning, 1970). Froning et al. (1971) and Dimick et al. (1972) have reported on the organoleptic qualities of mechanically deboned poultry meat and Grunden et al. (1972) have outlined its physical and chemical characteristics. A microbiological evaluation of mechanically deboned poultry meat has also been made (Ostovar et al., 1971). Mutton carcasses differ in many respects from poultry. No skin is present on the carcass; the bones are much larger and harder; and mutton fat is more highly saturated. Therefore, mechanically deboned mutton would be expected to have different properties than mechanically deboned poultry. To date, no published information on mechanically deboned mutton is available. Mechanical deboning has the potential of saving all the lean, red meat that is produced. With hand boning some lean red meat adhering to the bones ends up as a by-product. Mechanically deboned mutton should result in an increased volume of meat in addition to a saving in labor.

This study was undertaken to characterize the chemical, physical and organoleptic properties of mechanically deboned mutton and to compare these properties to properties of hand-boned mutton.

EXPERIMENTAL

TWO SEPARATE TRIALS were conducted. In the first trial, 24 good and utility grade mutton were divided into six lots of four carcasses each. Each carcass, which had aged 7 days at −1°C, was split lengthwise and one side was ground through a 1.9 cm plate of a bone grinder. The four sides were mixed together and mechanically deboned using an AUX 70 Beehive deboner with 0.635 mm diam holes in the cylinder.

The four remaining sides from each of the six lots were hand boned and all lean was physically separated from the bone by hand. The total bone weight of the four sides was divided by the total weight from the four sides to obtain percent bone in hand-boned sides. Lean and fat from the four hand-boned sides from each lot was ground together, mixed in a commercial food mixer, reground and subsamples (approximately 3 kg) were homogenized prior to chemical and microbiological analysis.

Subsamples of the meat from each of the six lots of mechanically deboned meat were also homogenized prior to analysis. Care was taken to keep the meat from the hand-boned sides and the machine-boned sides at the same temperature throughout all procedures so that bacterial counts would be more comparable. During machine boning and physical separation of the bone, meat from both sides had a similar temperature rise. Equipment which came in contact with the hand-boned and machine-boned meat was cleaned with a commercial sanitizing agent to insure that bacterial counts would not be affected by variable counts on the equipment.

In the second trial 10 good and utility grade mutton were divided into five lots of two carcasses each. Each carcass was split lengthwise at approximately 20 min postmortem and procedures identical to those used in trial 1 were followed. The only difference was that two carcasses were in each lot instead of four.

All mutton carcasses in trials 1 and 2 were from 7-yr-old Western whitefaced ewes and all carcasses in trials 1 and 2 weighed between 20 and 27 kg. Each lot of four carcasses in trial 1 and each lot of two carcasses in trial 2 were slaughtered in the University abattoir on different days to allow time for data collection.

Boneless composite samples from each lot of mutton sides were analyzed for fat, protein, moisture and ash by standard AOAC methods (1970). Calcium, used as an indicator of the amount of bone contamination in the meat, was determined by atomic absorption spectrophotometry as outlined by the Perkin-Elmer Corp. (1964). Hydroxyproline was determined according to the method of Woessner (1961). Total bacterial counts were determined as outlined by Ockerman (1970).

Hand-boned and machine-boned meat from each lot of mutton carcasses in trial 1 were made into bologna. Bologna was made with hand-boned or mechanically deboned mutton plus cure, bologna seasoning and ice water. No binder, beef or pork was added. Emulsion stability (Townsend et al., 1968) was reported as volume of components released in ml per 100g of emulsion. Bologna shrinkage, expressed as the difference between fresh stuffed weight and chilled weight 24 hr after cooking divided by fresh stuffed emulsion weight was recorded. Color of the cooked bologna was determined as ppm of hematin (Hornsey, 1956). Subjective color and texture scores for bologna were assigned using a one to five scale. Five equaled the brightest and most desirable color. Bologna lacking air, fat and jelly pockets was scored one, while bologna with the least desirable texture (exhibited extensive air, fat and/or jelly pockets) was scored five. Fat stability in bologna at 3 and 30 days after processing was determined using 2-Thiobarbituric acid values (TBA) as described by Witte et al. (1970).

Six triangle difference tests were employed using 30 different staff members and students from the College of Agriculture for each of the six tests. Panel members were instructed to pick the different sample according to differences they detected in flavor or texture. They were not aware that color differences might be present and members of the panel could not detect

Table 1—Composition, yield and bacterial content of hand- and mechanically-deboned mutton carcasses[a]

	Cold boned (N=6 lots)		Hot boned (n=5 lots)	
	Hand	Machine	Hand	Machine
Temperature, °C				
Original	−1	−1	34	34
Out of boner	–	11	–	30
Bone discarded, %	18.33a	23.81b	17.36a	17.98a
Fat, %	19.11a	19.70a	18.13a	19.78a
Protein, %	19.61a	19.11a	17.98a	16.00a
Dry matter, %	40.42a	40.97a	37.86a	37.68a
Ash, %	0.98a	1.42b	0.89a	1.01a
Calcium, %	0.02a	0.19c	0.02a	0.09b
Hydroxyproline, %	0.35a	0.30a,b	0.34a	0.21b
Yield, %	81.67a	76.19b	82.64a	82.02a
Bacteria/g of meat	318000a	434000a	17600b	20190b

[a] Means on the same line followed by different letters are significantly different ($P < 0.05$).

Table 2–Characteristics of bologna made from hand- and machine-boned mutton[a]

Item	Hand boned	Machine boned
TBA (3 days)	0.14a	0.16a
TBA (30 days)	0.16a	0.21a
Emulsion stability	3.58a	1.52b
Total pigments	152.98a	202.75b
Subjective color[b]	3.16a	4.33b
Subjective texture[c]	3.16a	2.66a
Processing shrink, %	11.58a	9.13a

[a] Means on the same line followed by different letters are significantly different ($P < 0.05$).
[b] Scores ranged from one to five with five being the brightest most desirable color.
[c] Scores ranged from one to five with one being the most desirable texture.

bologna color differences while the panel was in progress. Bologna from each of the six lots of chilled mutton carcasses was tested after it had been made 3 days and again after 30 days. The only difference in the samples served was that one was made with hand-boned meat and two were made with machine-boned meat or vice versa. The number of correct identifications of the odd sample was compared to a probability table to determine the statistical significance of differences (Roessler et al., 1948).

Differences in bologna characteristics from hand- and machine-boned meat and differences in means for cold and hot, hand- and machine-boned meat were determined by least squares analysis (Steel and Torrie, 1960).

RESULTS & DISCUSSION

AVERAGE TEMPERATURES of the meat during the deboning process were recorded (Table 1). Original internal temperature of the cold, machine-boned mutton carcasses was −1°C, while internal temperature of the hot, machine-boned carcasses was 34°C. Temperature of the hot, machine-boned carcasses decreased from 34 to 30°C during grinding and boning. Chilled carcasses increased in temperature from −1 to 11°C during machine boning. It is evident that machine-boned meat must be chilled or processed immediately if bacterial counts are to be kept low.

Grinding and deboning procedures, including machine settings, were identical for carcasses machine boned hot or cold. Bone discarded was higher for carcasses machine boned cold than for carcasses machine boned hot. Therefore, machine boning of hot carcasses resulted in a greater yield of meat than machine boning of chilled carcasses. The hand-boned carcasses had all visible lean and fat removed from the bone. This would not be economical under commercial operations where some lean is discarded with the bone. Therefore, percent lean and bone discarded under commercial hand boning operations would be higher than the 18% listed here.

Fat, protein and dry matter figures were not statistically different for the four boning procedures (Table 1). However, there was a tendency for meat from the hot-boned carcasses to contain less dry matter or more moisture. The highest ash content was found in meat from carcasses machine boned cold.

Calcium content of hand-boned mutton carcasses at 0.02% was slightly higher than the average figure of 0.01% reported by Watt and Merrill (1963) for red meat (Table 1). The higher value of 0.02% calcium may be a result of bone particles from the closely trimmed bones contaminating the meat. When percent calcium was determined on bone free lean meat analyses also showed that lean contained an average of 0.01% calcium. Mutton machine boned cold contained more calcium and ash (more bone particles) than mutton machine boned hot even though the meat yield from hot-boned carcasses was higher (76.19 vs. 82.02%). Hydroxyproline content of meat from carcasses machine boned hot tended to be lower than hydroxyproline content of meat from carcasses machine boned cold. Carcasses hand boned hot or cold had the highest hydroxyproline contents. A reduction of connective tissue as a result of machine boning has been consistent throughout other tests (Field and Riley, 1972) with red meat and it agrees with information available on deboned poultry meat (Satterlee et al., 1971).

Bacterial counts per g of meat immediately after boning were lower in hot-boned than in cold-boned carcasses. This result would be expected since bacterial growth on the hot carcasses did not have time to occur. The lack of statistically significant ($P < 0.05$) differences in meat from hand- or machine-boned carcasses points out that initial bacterial contamination of equipment and temperature differences are responsible for the rise in bacterial counts of mechanically deboned meat in some plants. The rise in counts when compared to hand boned meat is not due to mechanical deboning as such.

TBA values were slightly, but not significantly, higher in machine-boned than in hand-boned meat (Table 2). Values increased with storage time. Emulsion stability favored the machine-boned product. This would be expected because of the lower connective tissue content of machine-boned meat. Higher total pigments in machine-boned mutton may also reflect lower connective tissue scores since connective tissue does not contain meat pigments. The higher total pigment concentration in bologna from mechanically deboned meat indicated that some pigments from bone marrow are also being forced into mechanically deboned meat. Subjective color scores for bologna from machine-boned meat confirmed that more pigments were present than in bologna made from hand-boned meat. Subjective texture scores and percent shrink on processing were not significantly different but both favored machine-boned bologna.

Untrained panel members used a triangle test to see if the bologna sample with a different flavor or texture could be detected (Table 3). Differences between bologna made with machine-boned mutton and bologna made with hand-boned

Table 3–Triangle test results for bologna made with hand-boned mutton and mutton machine boned cold

Time tested	No. of judgments	No. correct	No. preferring	
			Hand boned	Machine boned
3 days	179	120[a]	77	43
30 days	180	127[a]	76	51

[a] Significant differentiation ($P < 0.01$)

mutton were detected by each of the six panels of 30 judgments each. The overall summary in Table 3 reflects the results for each panel at 3 and 30 days after processing. Differences were not much more pronounced after 30 days storage than they were 3 days after the bologna was made. At both time periods, bologna made with hand-boned meat was preferred to that made with machine boned meat. It is possible that the texture of the bologna made with hand-boned meat was more similar to that which the panel members were accustomed to. The panel may have preferred bologna from hand-boned meat for this reason alone.

Bologna made with 100% machine-boned meat was not objectionable. However, it is evident that bologna made with 100% machine-boned meat is different organoleptically than that made with hand-boned meat. Froning et al. (1971) have shown that frankfurters containing 15% mechanically deboned turkey meat were comparable to all hand-boned meat frankfurters. It is probable that at least 15% mechanically deboned red meat could be incorporated into an emulsified product without any detrimental effect from a flavor or texture standpoint.

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INFLUENCE OF YIELD ON CALCIUM CONTENT OF MECHANICALLY DEBONED LAMB AND MUTTON

INTRODUCTION

THE HIGH labor cost for hand boning low grade lamb and mutton carcasses, lamb breasts and lamb necks makes these items particularly well suited for mechanical deboners. In recent years it has become feasible to separate meat from the bones utilizing mechanical deboners. Mechanically deboned poultry and fish products are becoming increasingly popular and a limited amount of research on these items is available (Grunden et al., 1972; Dimick et al., 1972; Crawford et al., 1972; Froning et al., 1971; Ostovar et al., 1971). Mechanically deboned red meat is not USDA approved and no research on mechanically deboned red meat has been published. However, some manufacturers in the USA are producing mechanically deboned red meat for export and red meat processors in other countries are beginning to utilize mechanical deboners (A.R. McFarland, personal communication).

One of the major factors involved in product acceptance of mechanically deboned meat is the amount of microscopic bone particles remaining in the meat. The amount of bone contamination can be estimated by determining the amount of calcium present. According to Watt and Merrill, (1963) the calcium content of meat is very low (0.01%) and the amount is relatively constant. Therefore, any calcium increase in mechanically deboned meat over that found in fat and lean is an indicator of increased bone particles. Some factors which affect the amount of calcium (bone particles) in mechanically deboned meat are the size of grinder plate through which the meat and bone is ground prior to deboning, the proportion of bone to meat in the original product and design of the deboning equipment.

The objective of this study was to determine what effect increasing the yield (weight of mechanically deboned meat ÷ weight of bone plus meat) would have on calcium content of mechanically deboned meat and to determine if palatability differences exist for mechanically deboned meat of variable yield and calcium content. Several different lamb cuts were surveyed to determine which ones might be the most acceptable for mechanical deboners. Calcium content of deboned lean meat emerging from different sections of the deboner cylinder was also obtained to ascertain homogeneity of the deboned meat.

EXPERIMENTAL

GOOD AND UTILITY grade mutton carcasses and lamb carcass parts from prime, choice and good grade carcasses were obtained from the University of Wyoming Meat Laboratory and machine deboned using a Beehive AUX 70 deboner. The same cylinder with 0.635 mm diam holes and the same auger were used to mechanically debone all lamb and mutton so that variation in calcium would reflect differences in yield of machine-boned meat. One side of each mutton carcass and approximately 20% of the lamb cuts from each 100 kg batch were physically separated by hand in order to determine the amount of bone in the product which was mechanically deboned. Meat and bone from each group of carcasses or cuts was ground through a 12.7 mm plate of a bone grinder and mixed prior to deboning. Yield of deboned meat from similar groups of carcasses or cuts was increased by tightening the ring valve. Analysis of mechanically deboned meat coming through different sections of the deboner cylinder was conducted to determine the amount of variability in composition of deboned meat from the same cylinder.

The mechanically deboned meat from each

Table 1—Calcium moisture and fat content of mechanically deboned meat of variable yield

Product deboned	USDA grade	Yield[a]	Calcium, %[b]	Moisture, %[b]	Fat, %[b]	Bone, %[c]
Mutton carcasses	utility	52%	0.09	73.77	8.62	21.27
Mutton carcasses	good	70%	0.20	64.20	17.10	18.30
Mutton carcasses	good	84%	0.27	56.22	24.93	17.19
Lamb necks	choice + prime	32%	0.21	64.47	15.76	28.60
Lamb necks	choice + prime	69%	0.37	55.41	25.39	26.37
Lamb shoulders	good	65%	0.18	67.13	13.56	22.14
Lamb shoulders	good	84%	0.26	66.13	14.72	22.04
Lamb breasts	choice + prime	75%	0.11	47.55	35.52	11.11
Lamb breasts	choice + prime	87%	0.13	45.23	37.26	10.89
Lamb legs	good	74%	0.21	66.95	11.53	17.77
Lamb legs	good	84%	0.28	66.17	12.68	17.89

[a] All products were ground through a 12.7 mm plate and boned with a 0.635 mm cylinder. Yield = weight of mechanically deboned meat ÷ weight of bone plus meat.
[b] Data are expressed on a fresh weight basis.
[c] Physically separated bone based upon one side of each of the carcasses and upon a random 20% of the lamb cuts

group of carcasses or cuts was mixed thoroughly prior to sampling. Fat, protein and moisture were run in duplicate using standard AOAC methods (1970). Hydroxyproline was determined by the method of Woessner (1961). Calcium was determined by atomic absorption spectrophotometry as outlined by the Perkin-Elmer Corp. (1964). Because of a limited supply of lamb and mutton and because the deboned lamb and mutton was not USDA approved, economic considerations allowed only one 100 kg batch of each group of carcasses or cuts of a specific yield to be mechanically deboned.

Triangle difference tests were conducted to compare bologna made from sides of mechanically deboned mutton to bologna made from sides of hand-boned mutton. The deboned meat for the triangle difference tests came from cylinders with 0.635, 0.787 or 1.016 mm diameter holes. Formulas and processing procedures for hand- and machine-boned bologna were identical. Hand- or machine-boned mutton was used as the only meat item added to the formula for some batches of bologna. For other batches of bologna 50–90% of the hand- or machine-boned mutton was replaced with lean beef and with pork fat. Three percent nonfat dried milk was added to all bologna formulas. All batches of bologna, after processing, contained 25 ± 2% fat by analysis. Untrained staff members and students from the College of Agriculture were used as panel members for the triangle tests. 33 different panel members were asked to pick the different bologna sample. Three coded samples were served on the same plate. The only difference in the samples served was that one sample was made with hand-boned meat and two were made with mechanically deboned meat or vice versa. The number of correct identifications of the odd sample was compared to a probability table to determine the statistical significance of differences (Roessler et al., 1948). Panel members were also asked to indicate which sample they preferred, that is, the different sample or the identical samples.

RESULTS & DISCUSSION

CALCIUM, moisture and fat content of mechanically deboned lamb and mutton is given in Table 1. Calcium and fat content of the mechanically deboned lean was extremely low when the ring valve of the Beehive deboner was set to obtain 52% of the mutton carcass weight as boneless lean. When the ring valve was tightened and a 70% yield of boneless meat from mutton carcasses was obtained, calcium content increased from 0.09–0.20%. A third group of mutton carcasses was run with the ring valve tightened to give what was considered a maximum yield of 84%. The 16% bone discarded was slightly less than the 17.19% bone discarded when bones from the opposite sides were carefully cleaned of all lean and fat by physical separation. Therefore, some bone particles entered the mechanically deboned meat. More fat (17.10% fat for 70% yield vs. 24.93% fat for 84% yield) also ended up in the deboned mutton when the ring valve was tightened and the yield increased.

Trimmed lamb necks from two different lots were mechanically deboned and the results are shown in Table 1. Mechanically deboned lean (32% yield) contained 0.21% calcium. Since carefully cleaned fresh neck bones made up 28.60% of the neck weight, 39.40% of the necks other than bone was discarded. When the ring valve was tightened to save more lean (69% yield) the calcium content of the deboned meat rose to 0.37% of the product. Moisture decreased and fat percentage increased when the yield of mechanically deboned meat increased. When boneless lean from lamb necks with a yield of 69% was compared to boneless lean from mutton carcasses with a 70% yield the lean from lamb necks had a much higher calcium content. A higher percentage of bone in the lamb necks prior to deboning is partially responsible for this difference. However, maturity of bone and type of bone (long bones vs. vertebrae) also affect calcium content of the deboned meat product. Grunden and MacNeil (1973) stated that deboned meat from the more mature types of poultry had higher bone solids than the younger counterparts. They stated that the higher degree of calcification of more mature bones caused more fragmentation when passing through the deboner, resulting in an increased level of bone particles.

Lamb breasts had the lowest percentage of bone of any of the products studied. Mechanical deboned meat from breasts also had much lower calcium percentages than other cuts with comparable yields of boneless meat. Low calcium content may be related to type of hard tissue in breasts. The high percentage of cartilage found in lamb breasts does not fragment as much as bone during deboning. Therefore, fine particles of cartilage are not as likely to be found in mechanically deboned meat. It is clear that lamb breasts, which have a low economic value, are ideally suited for the mechanical deboning process (Table 1).

Low grading lamb legs and shoulders are not as well suited for mechanical deboning as breasts because they are easier to hand bone than lamb breasts. These good grade lamb cuts yielded boneless lean which had calcium contents similar to the calcium contents of mechanically deboned lean from good grade mutton carcasses. The maximum yield of 84% meat which was obtained for mutton carcasses, lamb shoulders and lamb legs is higher than can be obtained with hand boning. If mechanically deboned meat from these high yields is USDA approved, a substantial increase in boneless meat available for sale could result.

Analyses of the mechanically deboned meat which was extruded from different portions of the cylinder are given in Table 2. The third of the cylinder next to the deboning machine yielded the highest percentage of fat and the lowest percentage of calcium and hydroxyproline. The reverse was true for the third of the cylinder next to the discharge ports. This finding has significance for deboning machine manufacturers, some of which are making the holes in the third of the cylinder next to the discharge ports smaller so that less bone and connective tissue can escape. The data in Table 2 clearly indicate that mechanically deboned meat must be mixed after deboning if it is to be homogenous.

Evaluation of bologna made from machine-boned and hand-boned mutton

Table 2—Analysis of mechanically deboned meat from different portions of a cylinder with 0.635 mm holes

	Cylinder divided into thirds[d]		
	Next to machine %	Middle %	Next to discharge ports %
Mutton carcasses[a]			
Fat	36.35	25.55	19.12
Protein	12.42	16.41	18.54
Moisture	48.77	55.11	59.49
Calcium	0.08	0.08	0.09
Hydroxyproline	0.12	0.15	0.30
Lamb necks[b]			
Calcium	0.20	0.20	0.24
Lamb necks[c]			
Calcium	0.28	0.32	0.52

[a] Yield of mechanically deboned meat was 80.5%. Whole mutton carcasses at 20 min postmortem were ground through a 19.0 mm plate prior to deboning.
[b] Yield of mechanically deboned meat was 32%. Necks were ground through a 12.7 mm plate prior to deboning.
[c] Yield of mechanically deboned meat was 69%. Necks were ground through a 12.7 mm plate prior to deboning.
[d] All data are expressed on a fresh weight basis. Each mean is the average for samples which were run in triplicate.

Table 3–Triangle test evaluation of bologna made from machine-boned and hand-boned mutton carcasses

Amount of machine-boned meat in bologna	Size of holes in cylinder (mm)	Calcium content of machine boned meat[a]	No. of judgments	No. correct	No. preferring	
					Machine boned	Hand boned
100%	1.016	0.48%	None (obvious grit)			
50%			55	26*	9	17
10%			60	23	10	13
100%	0.787	0.21%	None (obvious grit)			
50%			33	13	8	5
100%	0.635	0.25%	33	17*	5	12
50%			33	12	7	5

[a] Data are expressed on a fresh weight basis.
* Significant discrimination between bologna from hand-boned meat and bologna containing machine-boned meat at 5% level.

carcasses is shown in Table 3. When bologna was made with meat obtained from a cylinder with 1.016 mm holes, it contained bone particles which could easily be detected by biting into a slice of bologna. Therefore bologna, made from 100% deboned meat containing 0.48% calcium, was not subjected to a triangle test. Mechanically deboned meat from the same lot was mixed with beef lean and pork fat so that mechanically deboned mutton or hand-boned mutton made up 50% or 10% of the meat source. Results of the triangle test showed that panel members could detect the different sample when bologna made with 50% machine-boned meat from the 1.016 mm hole cylinder was compared to bologna made with hand-boned meat. In addition, bologna made with hand-boned meat was preferred 17 out of 26 times. Bologna containing 10% machine-boned mutton was not significantly different than bologna made with 10% hand-boned mutton. Cylinders with 1.016 mm holes are being used commercially for mechanically deboned poultry. This test indicates that they cannot be used for mechanically deboned mutton unless the mechanically deboned mutton is diluted more than half with hand-boned meat.

Bologna made with 100% machine-boned mutton from the 0.787 mm cylinder also contained bone which was readily detectable even though the calcium content of the meat was less than half that of the meat from the 1.016 mm cylinder. Bologna made with 50% mechanically deboned mutton from the same source was not significantly different ($P < 0.05$) when compared to that made with 50% hand-boned mutton. The lack of difference for bologna made with 50% mechanically deboned meat from the 0.787 mm hole cylinder when compared to a significant difference for bologna made with 50% mechanically deboned meat from the 1.016 mm cylinder is probably related to the larger sized bone particles which passed through the larger cylinder holes. Microscopic examination of the deboned meat indicated that some bone particles as large as the holes in the cylinders are found in mechanically deboned meat.

A third cylinder with 0.635 mm holes was also used to obtain mechanically deboned mutton (Table 3). Bologna made with 100% machine-boned mutton from this source did not have bone particles which could be detected by organoleptic evaluation. Therefore, bologna made with 100% mechanically deboned meat from the 0.635 mm cylinder was compared to bologna made from hand-boned mutton. 17 of the 33 judges could detect the difference. 12 of the 17 who could detect a difference preferred bologna from hand-boned mutton. When bologna was made with 50% machine-boned meat from the 0.635 mm cylinder, no significant difference between this bologna and that made with 50% hand-boned bologna could be detected. It is apparent that bologna containing some mechanically deboned red meat can be produced. From a palatability standpoint, cylinders with 1.016 and 0.787 mm holes appear to be less acceptable for deboning mutton than cylinders with 0.635 mm holes.

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EFFECTS OF SOY CURD ON THE ACCEPTABILITY AND CHARACTERISTICS OF BEEF PATTIES

INTRODUCTION

THE UTILIZATION of soybeans as human food is not new. However, methods are needed to improve the palatability of soybean products for wider acceptance as an excellent and cheap source of protein. A good way to achieve this would be to use soy curd (Japanese tofu) as an extender in ground beef patties. The use of soy curd in fresh meat products has not been exploited. Thus, it is within this context that this study was conducted.

In much of Asia soy curd is widely used either as a deep fat fried food, or as an ingredient in soups. The tofu is also eaten directly after dipping small cubes in soy sauce. The manufacture and use of soy curd and related products has been adequately reviewed by Watanabe (1969). No references have been found in which soy curd has been combined with or used as a meat extender except for a report from The Japan Protein Industry Co., Ltd., Tokyo, where soy curd was used for fish meat ham, sausages and hamburger (Anonymous, undated).

EXPERIMENTAL

Preparation of soy milk

The rapid hydration, hot grind method of Wilkens et al. (1967) was used in making soy milk. Clark soybeans were soaked in water at 60°C using a ratio of 1:9 (beans:water) w/w until the beans had doubled their weight. The soak water was decanted and the beans were washed. The beans were ground for 5 min (3 min at low, 1 min at medium, 1 min at high speed) with boiling water (1:9 = dry beans:water, w/w) to inactivate lipoxidase enzymes during grinding. Filtration was done immediately after grinding through a Buchner funnel under vacuum equipped with 7-in. standard Agway-GLF milk filtering pads with one layer of coarse pad on top of a fine pad. The filtrate which constitutes soy milk was then boiled for about 3 min.

Preparation of soy curd

While the soy milk was still hot (above 75°C), 0.4% of $CaSO_4$ based on the dry beans weight was stirred in. A white precipitate of Ca proteinate quickly formed. When a clear separation of curd and whey occurred, the curd was transferred to a perforated wooden box lined with 4–5 layers of cheese cloth. The whey was allowed to drain off. The warm curd, wrapped in cheese cloth was then subjected to compression in a hydraulic press and maintained at the required pressure for 5 min. Curds were prepared under three different pressures, namely 300 psi, 600 psi and 1100 psi and were then stored at 5°C.

Preparation of meat used

Freshly ground chuck beef was obtained from the Meat Division of the Dept. of Animal Science, Cornell University. The meat was mixed carefully to give a homogenous sample consisting of about 60% moisture, 21% fat and 18% protein.

Preparation of patties

Patties 1/4-in. thick were made with 0%, 5%, 10%, 20%, 75% and 100% (w/w) soy curd-meat combinations using 300 psi, 600 psi and 1100 psi soy curd. Patties of each type were subjected to a number of tests as outlined in Table 1.

Red food color, McCormick & Co., Inc., Baltimore, (2 drops in 5 ml water) was added to the coarsely blended soy products of patties 1 and 2 before formation, whereas only soy products of patty 2 were flavored with Steero Instant Beef Bouillon, American Kitchen Products Co., New Jersey, (3g/100g soy). All the patties were quickly frozen after formation to −20°C and then stored at 0°C.

Taste panel

Groups of 10 panelists selected from laboratory personnel were used for difference testing. Patties were thawed, broiled evenly by oven broiling for 3–4 min on each side and served to panelists while warm. Doneness was determined subjectively from the extent of external browning and patty firmness.

Proximate analysis

The samples for proximate analysis were subjected to freeze drying for 4 days to stabilize the samples and to determine moistures and total solids. Samples were quickly ground in a dry powder milk blender (Braum, Frankfort) and stored in dry bottles at 0°C.

The Wilfarth-Gunning Kjeldahl Method (AOAC. 1960) was used to determine nitrogen. Crude protein was calculated as 6.25 times Kjeldahl nitrogen. The amount of protein extracted was expressed as a percentage of the dry and the wet sample weight.

Lipids were determined by ether extraction using the AOAC (1960) official method. Following extraction with diethyl ether for 72 hr in a soxhlet extraction apparatus, the samples were dried in a vacuum oven for 24 hr before weighing and calculating the percentage of fat.

After ether extraction the thimbles with their dried samples were placed in a 550°C oven for 24 hr. The completely ashed samples were cooled to room temperature in a desiccator before they were weighed and the percentage of ash calculated.

Gross energy of each sample was determined using an Oxygen Bomb Calorimeter (Parr Instrument Co., Inc., Ill.).

Drip and cooking losses

Samples were weighed before and after dripping and cooking operations. The frozen patties were allowed to drip freely on paper towels until they had fully reached room temperature before they were weighed and broiled. Broiling was done evenly for 3–4 min on both sides at a constant temperature. The patties were allowed to cool to room temperature before weighing. Percentage of drip, cooking and total losses were then calculated.

Table 1—Types of treatments and tests performed on the patties

Patties	Treatments of soy products before patty formation	Tests used on the patties
1	Coarsely blended and color added	Taste panel
2	Coarsely blended and color + flavor added	Taste panel
3	Coarsely blended only	Proximate analysis
4	Coarsely blended only	Texture, drip and cooking losses, juiciness

Juiciness determination

Cooked patties were sampled for juiciness determination by removing two cylindrical samples from each patty with a No. 5 cork borer. Each cylinder was subjected to a constant pressure of 10,000 lb for 1 min between two small sheets of aluminum foil and again between two filter papers. The residue was weighed and the juice absorbed by the filter paper (an estimate of juiciness) was calculated as percentage of sample weight before pressing.

Texture-tenderness determination

A puncture test employing the Instron texture machine (Model TTCM, Instron Engineering Corp., Mass.) was used. A single flat surface contact type punch with 1/8-in. diam and with uniform cross section throughout its length was used as it expressed good textural differences between the patties. Each cooked patty was punched 8–10 times. The forces required for the punch to puncture the patties were automatically recorded on a chart. These data were then analyzed to reflect the tenderness differences.

RESULTS & DISCUSSION

Taste panel

The concentrations of soy curd added to beef patties were found to influence the acceptability of curd-beef patties. Figure 1 shows the percent of panelists who accepted the soy patties at different levels of concentration. It was found that the flavoring of soy curd with 3% beef bouillon completely altered the acceptability of soy curd beef patties. In Figure 1 the slope of the acceptability curves shows an improvement of panel acceptability of soy patties as pressure was increased on the curd from 300 psi, to 600 psi, and to 1100 psi. Soy curd-beef patties made with 1100 psi curd with color and flavor added were accepted at levels as high as 75% curd. Easy detectability of flavored curd did not significantly alter the high acceptability of the patties.

The panelists were tested for their sensitivity to detect the difference between different soy concentration levels in the patties. The results are plotted in Figure 2. When color was added to mask visible differences between patties, the panel found no significant differences at the 5% significance level between patties of up to 26% soy concentration for 1100 psi curd, and up to 10% for flavored 600 psi and 1100 psi curd.

The taste panel also indicated the degree of difference between each soy concentration level and these differences were plotted in Figure 3. As the pressure on the curd increased or as the soy was flavored, the degree of difference became lower even for those at higher soy concentration levels. This low degree of difference might have contributed to the high acceptance of curd-beef patties although their differences were easily detected by the panel.

300 psi curd-beef patties

Soy curd subjected to 300 psi had composition and physical properties very similar to ground meat except that its percent fat and ash were slightly higher (Fig. 4). This higher fat and higher ash was due to the use of full fat soybeans and to the addition of $CaSO_4$ to the soy milk to precipitate the protein. The higher fat content was reflected in a corresponding increase in energy since the protein content remained relatively constant. As the concentration of soy was increased, the total losses from drip and cooking decreased (Fig. 5). This stabilizing ability of soy protein confirms similar observations reported by Rock et al. (1966), Pearson et al. (1965), Circle and Johnson (1958) and Inklaar and Fortuin (1969), who worked with soy flour concentrates and isolates in whipped toppings, frankfurters, sausages, bologna and frozen desserts. At the 10% level of curd, water retention in the patties seemed to be highest. This was reflected in larger amounts of juice expressed, in increased tenderness and in a reduction of cooking loss. With a confidence belt of 8 at the significance level of 5%, it was found that the degree of difference indicated by the taste panel had high correlations with texture, juiciness, percent fat and percent water content. The percentage total losses correlated very well with tenderness, juiciness, percent cooking loss, percent protein, percent ether extract and percent moisture content of the uncooked patties (Table 2).

600 psi curd-beef patties

At a pressure of 600 psi the soy curd resulted in a 7% reduction of moisture

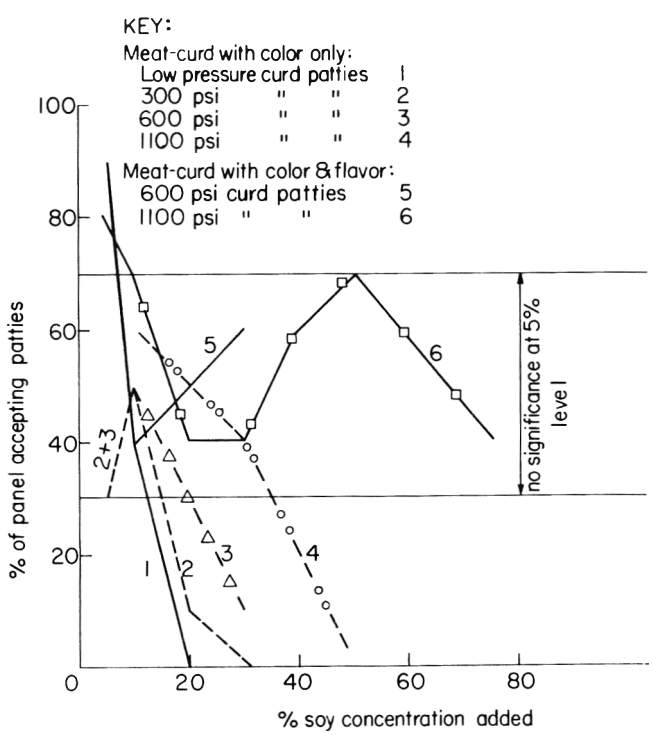

Fig. 1–Taste panel acceptance curves.

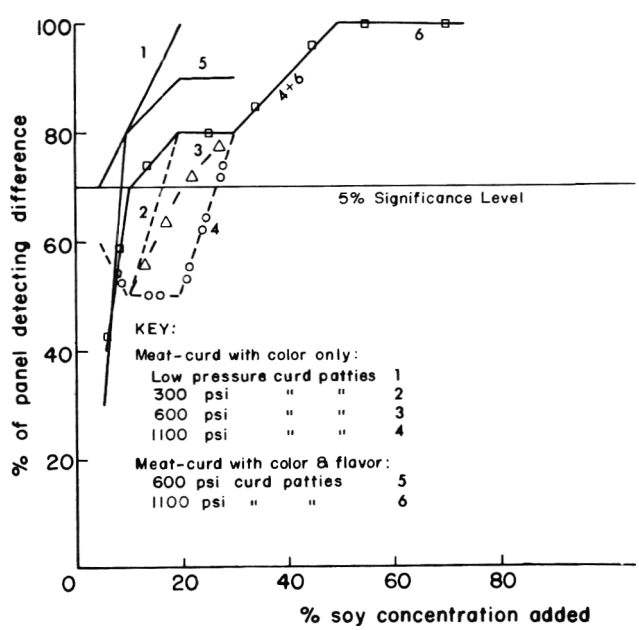

Fig. 2–Number of taste panelists detecting a difference among soy concentration levels.

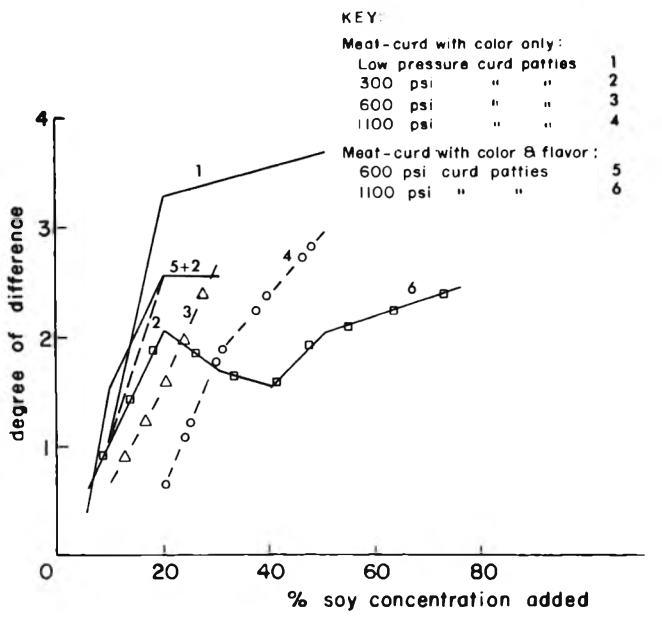

Fig. 3–Degree of difference among soy concentration levels in patties as detected by taste panel.

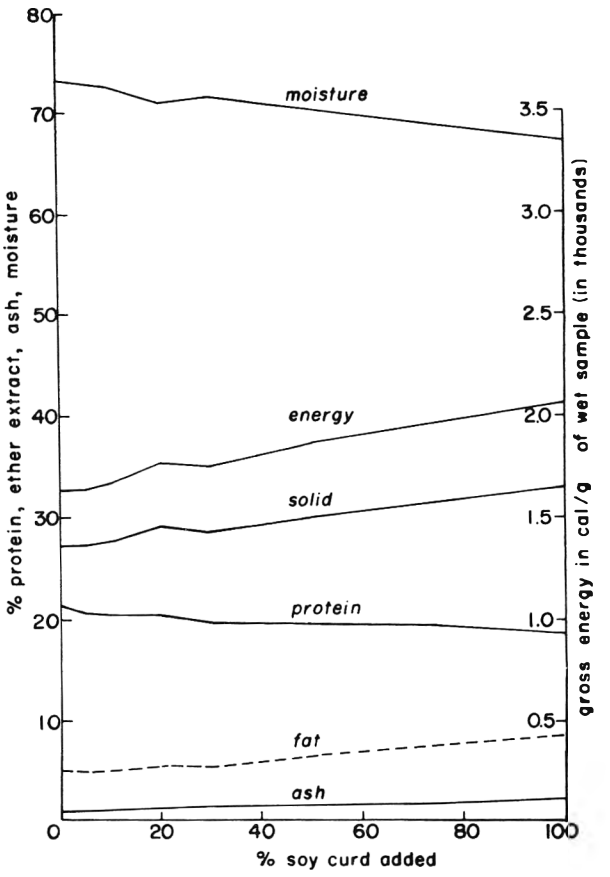

Fig. 4–Composition of 300 psi curd-beef patties.

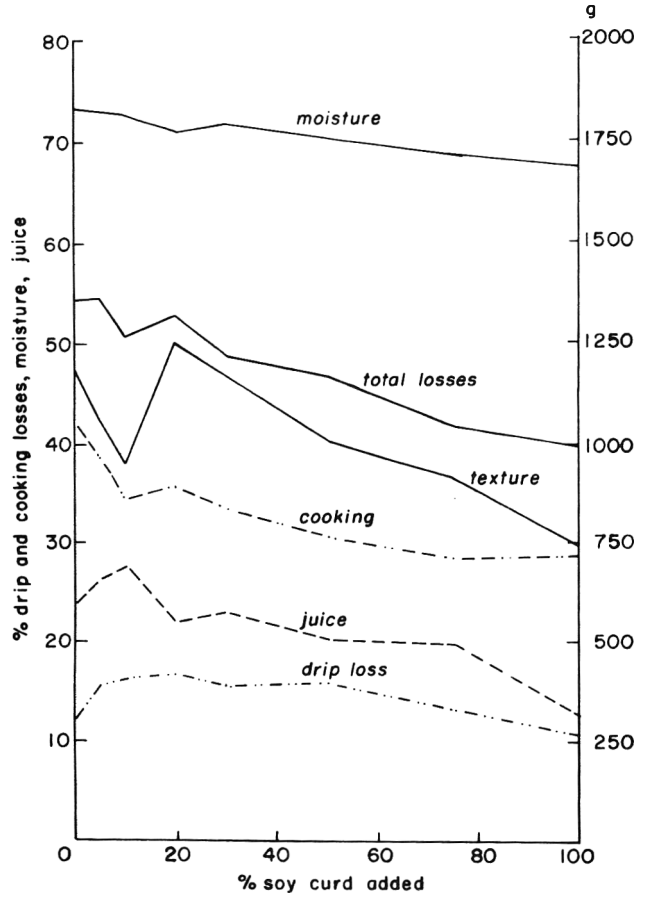

Fig. 5–Physical properties of 300 psi curd-beef patties.

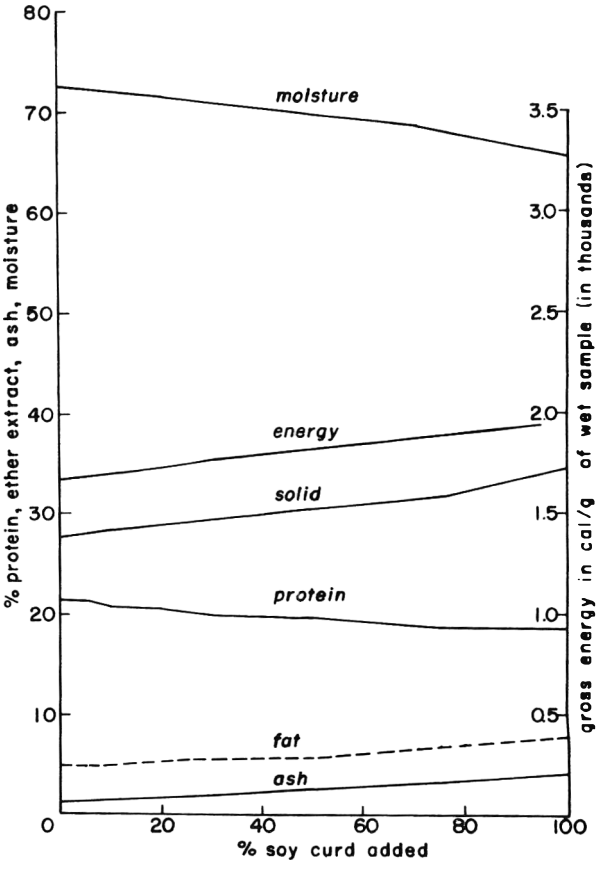

Fig. 6–Composition of 600 psi curd-beef patties.

Table 2–Correlation coefficient matrix for 300 psi soy curd-meat patties

Item	Taste panel degree of diff.	Texture	% Juice	% Drip loss	% Cooking loss	% Total loss	% Protein	% EE	% H_2O	% Ash	Gross energy
Taste panel degree of diff	1	.98	−.97	.14	−.17	−.12	−.49	.83	−.80	.81	.83
Texture		1	.59	.56	.68	.80	.77	−.79	.70	−.76	−.70
% Juice			1	.65	.68	.83	.81	−.93	.93	−.92	−.92
% Drip				1	.19	.56	.36	−.67	.51	−.53	−.52
% Cooking loss					1	.92	.93	−.81	.88	−.89	−.88
% Total loss						1	.93	−.95	.95	−.97	−.96
% Protein							1	−.89	.92	−.94	−.92
% EE								1	−.98	.98	.98
% H_2O									1	−.99	−1.0
% Ash										1	.99
Gross energy											1

content when mixed with uncooked ground beef. Thus, the addition of curd resulted in an increase in percentage solids in the curd-beef patties. As the result of this lower moisture content in the curd, the percentage of ether extract and ash increased (Fig. 6). The higher percentage of ether extract, i.e., fat, contributed to a higher caloric value. The increased amount of ash in the curd came from the $CaSO_4$ used for protein precipitation. The protein content of the patties remained quite constant with the addition of soy curd. The rapid decrease in texture resistance value at high curd concentrations (Fig. 7) was due to the tendency of the patty to crumble rather than due to increased tenderness of the soy patties since much of the water necessary for binding had been pressed out and thus the patties at these high soy concentrations became relatively dry. The juiciness peak appeared at 5% curd level where drip and cooking losses were lowest. But

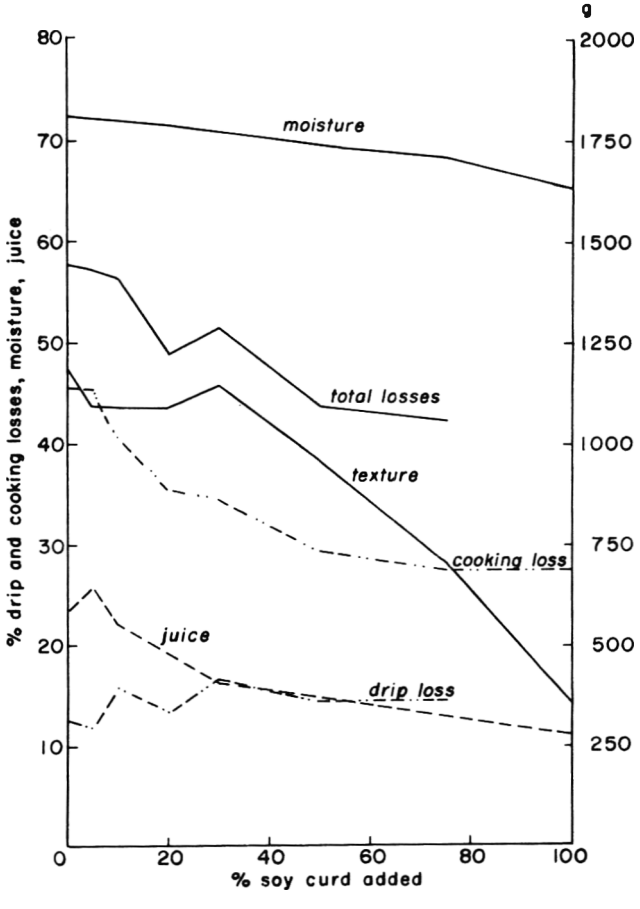

Fig. 7–Physical properties of 600 psi curd-beef patties.

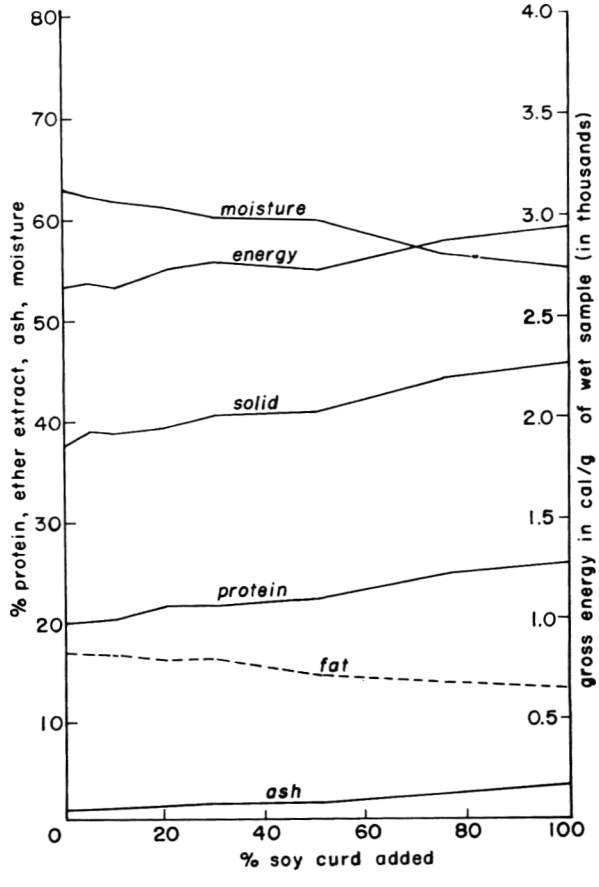

Fig. 8–Composition of 1100 psi curd-beef patties.

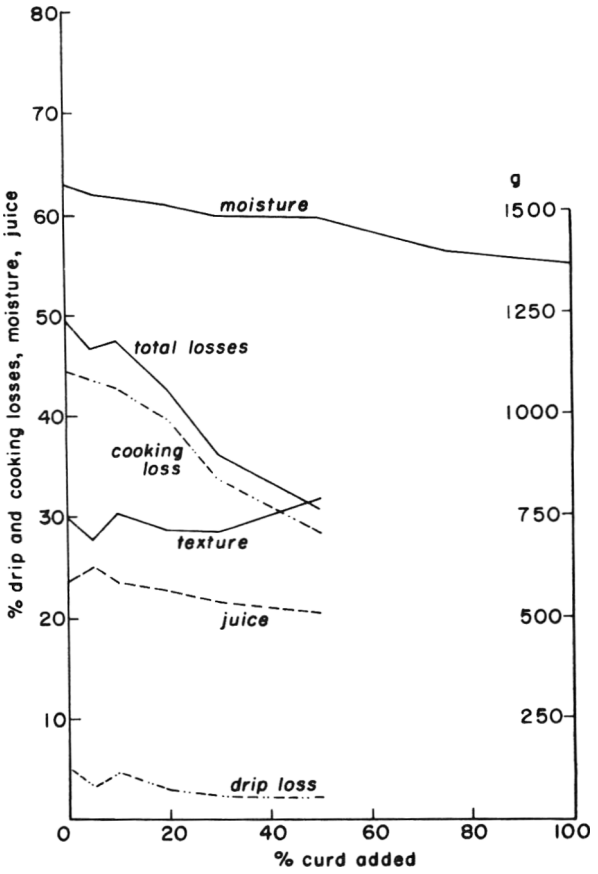

Fig. 9–Physical properties of 1100 psi curd-beef patties.

according to taste panel results (Fig. 1) panelists would easily accept these patties up to 20% curd content without the addition of flavor.

On correlating the different variables with the taste panel's sensitivity to detect differences in the patties, it was found that the taste panel scores had a high negative correlation to juiciness, percentage of protein, and percentage of moisture as was also the case when 300 psi curd was used. The functional role of soy protein as a stabilizer and water retainer seems to be evident. It is interesting to note that the percentage of protein correlated highly with the percentage of cooking loss but not with the percentage of drip loss. This may suggest that during broiling the heat was high enough to alter the physical properties of protein such as its ability to bind water and fat, and/or denature the protein. Hence, cooking loss was accelerated during the broiling process. Taste panel results of patties with color only and with color and flavor added were quite well correlated except that the addition of flavor sharply altered the taste panel's response on textural aspects of the patties. Tenderness correlated highly with the percent juiciness, percent drip and cooking losses, percent protein, percent extract and percent moisture.

1100 psi curd-beef patties

Soy curd subjected to 1100 psi was firm and contained a lower moisture and fat content than ground beef (Fig. 8). In contrast to curds that were subjected to 300 psi and 600 psi, the percentage of fat here was slightly lower. This might be the result of pressure which may have been high enough to express some of the fat along with water from the curd. With the expression of water and some fat, the protein content increased by 6.2% over the 100% meat patty.

Figure 9 shows the highest degree of tenderness for 1100 psi curd-beef patties was at 5% curd concentration. At this curd level, the percentage of total losses from dripping and cooking was low and the percentage of juiciness was high. High concentrations of curd made the patties drier, less tender and therefore, less juicy in spite of a significant drop in losses from dripping and cooking. Patties could not be made with 75% or more 1100 psi curd because of the associated dryness and crumbling.

The taste panel did not easily detect the presence of 1100 psi curd in beef patties and failed to detect the different curd concentrations added. This conclusion of the panel was independent of the textural characteristics of the patties regardless of flavor addition but correlated highly with juiciness, percent protein, percent fat and percent moisture of the patties.

Percentage of ether extract had a correlation of –0.78 with texture, 0.77 with juiciness and 0.8 with total losses. This correlation of percent ether extract with total losses was largely due to high cooking loss and not so much to drip loss. Since percent ether extract was low in 1100 psi curd, its contribution to gross energy was also low. An increase in gross energy thus came from the combustion of protein which had a correlation of 0.98 with gross energy value.

Comparative study of soy patties

Juiciness had a high positive correlation with percentage of moisture in the soy-beef patties and it seems to depress the panel's sensitivity to differences in curd concentration levels.

In general, the sensitivity of the taste panel to the degree of difference between soy concentrations in each set of meat-soy comparisons indicated that texture and juiciness had a tremendous impact on the panel's judgment. The textural differences between 300 psi, 600 psi and 1100 psi curd-beef patties were also detected by the panel. The optimum meat-soy curd combination was found to be at 5% soy level with regard to tenderness, juiciness, drip loss and cooking loss.

Since soybeans contain a very small amount of carbohydrates, its caloric value comes mainly from fat and protein. The increase in caloric value in 300 psi and 1100 psi curd-beef patties were due to their high fat and protein contents, respectively. Thus, these high protein and fat contents in the soy curd-beef patties may provide an acceptable and cheap energy source in the worldwide problem of protein-calorie malnutrition.

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SPECIFICITY OF LIPOLYSIS DURING DRY SAUSAGE RIPENING

INTRODUCTION

LIPID forms the major fraction in dry sausages, also known as fermented sausages. The distinctive flavors of these products were found to be related, at least in part, to the hydrolytic and oxidative changes occurring in the lipid fraction during ripening (Maillet and Henry, 1960; Alford et al., 1971; Nurmi and Niinivaara, 1964). Hydrolytic changes, involving liberation of glycerol ester bound fatty acids, and here referred to as "lipolysis," are due mainly to bacterial lipase activity (Cantoni et al., 1967a; Gervasini and Caserio, 1969; Lubienicki v. Schelhorn, 1972), although muscle and adipose tissue lipases may also be active (Wallach, 1968). Furthermore, bacterial metabolism is an important, if not major cause, of oxidative changes in the unsaturated fatty acids, resulting in the production of lipid peroxides and carbonyl compounds (Wahlroos and Niinivaara, 1969; Alford et al., 1971). Lipid peroxides, formed by nonenzymatic oxidation, can be further metabolized by bacteria to carbonyl compounds and fatty acids (Smith and Alford, 1969; Cerise et al., 1973). Although Alford et al. (1971) found no correlation between lipase production and the oxidative activities of microorganisms, Cantoni et al. (1967b), Cerise et al. (1973) and Coretti (1965) suggest, that peroxides and carbonyls in dry sausage are formed from free fatty acids (FFA), liberated by lipolysis. If so, selective hydrolysis of unsaturated fatty acids may be a factor, controlling further oxidation and, thus, flavor. In recent years, an extensive literature has accumulated demonstrating lipolysis during dry sausage ripening. Maillet and Henry (1960) stated that FFA content may reach values up to 5–7% in the finished product. Such high values were reported for Eastern European very dry sausages, with long ripening periods (Stanculescu et al., 1970) as well as for dry sausages, with shorter ripening periods (Wurziger and Ristow, 1966). Values between 1 and 5% can be calculated from data reported by Ten Cate (1960); Terplan (1969); Duda (1966); Mihalyi and Körmendy (1967); Cantoni et al. (1967a) and Wahlroos and Niinivaara (1969).

Evidence indicating selective liberation of unsaturated fatty acids has been reported occasionally (Duda, 1966; Wahlroos and Niinivaara, 1969). In these studies, however, determination of total FFA is by titration of a lipid extract while individual FFA are identified by gas chromatography after extraction of FFA alkali salts with aqueous or ethanolic alkali. This method can cause artifactual generation of FFA by hydrolysis of esters (Meinertz, 1971; Laurell, 1957), while alkaline extracts are selectively enriched in short-chain and unsaturated fatty acids (Stoll, 1972; Demeyer and Hoozee, unpublished results). In this paper, we report a systematic study of lipolysis during dry sausage ripening, using a combination of thin-layer and gas chromatography to quantitate individual lipid classes, as recently described by Christie et al. (1970).

Table 1—Composition of sausage mixture

Ingredient	Amount
Cooled, deboned and chopped pork	3 kg
Cooled, deboned and chopped beef	4 kg
Cooled and chopped lard	3 kg
Salt (NaCl)	255g
Coloring salts (KNO_3 + $NaNO_2$)	20g
Sugars[a]	70g
Soy protein concentrate	80g
Smoke concentrate	10g
Spices	35g
Polyphosphates	10g

[a] A commercial preparation, containing oligosaccharides with small amounts of ascorbic acid, glucono-delta-lactone and salt was used. In batch A, 5g of glucose was added to 7g of the preparation.

EXPERIMENTAL

Preparation of sausage

Separated by a period of 2 months, two batches of 10 sausages each were prepared by a local butcher, according to usual practice. A mixture of ingredients (Table 1) is homogenized in a cutter. The resulting emulsion is filled into artificial casings using a hand-operated filler, and the sausages transferred into hermetically closed pans where they are kept under partial vacuum (water pump) at 23°C for 5 (batch A) or 3 days (batch B). They are then hung in a conditioning cabin at 22°C (RH 90–95%) until the eighth day and at 19°C (RH 85–90%) until the twelfth day. During this period, an overnight treatment with cold smoke is carried out. At last, they are transferred to the drying chamber, where they are kept at 18°C and 75% RH until ready for consumption (after 40–50 days). Sausage diameter was 70 mm (batch A) and 90 mm (batch B).

Sampling procedure

At different stages of the ripening process, a sausage was transported to the laboratory. After removing the casing, it was mixed three times in a commercial meat grinder and the mixture used immediately for analysis or stored in air tight bottles at −20°C for periods up to 2 wk.

Analytical methods

Samples were analyzed for dry matter, crude protein (Kjeldahl method) and crude fat (ether extract) according to standard methods of the E.E.G. (Europese Economische Gemeenschap), currently in use in this laboratory (Anonymous, 1971, 1972).

Quantitative determination of lipid classes

A 10-g sample was homogenized for 1 min in a Virtis Apparatus (Gardiner, N.Y.) with 20 ml of a chloroform/methanol mixture (2/1 v/v). The homogenate was filtered on a Büchner filter and the residue with Whatman No. 1 filter paper, again homogenized for 1 min with 20 ml of the same mixture and filtered. Filtrates were pooled and dried over anhydrous Na_2SO_4. 1 ml of extract was spotted on 20 × 20 cm glass plates, coated with Kieselgel G (Merck, Darmstadt, Germany) in 0.5 mm thick layers and the plates developed with hexane-diethylether-acetic acid (75/25/2 v/v/v). Lipid classes were detected under UV after spraying with a 0.1% w/v solution of 2.4-dichlorofluorescein in ethanol and identified by comparison with standard mixtures. (Nu-Check – Prep. Lab., Minn., U.S.A.) containing cholesterol oleate, triolein, oleic acid and lecithin (Standard no. 18-5-A) and monopalmitin, dipalmitin, tripalmitin and methyl palmitate (Standard no. 16-0-A). Triglycerides, free fatty acids (FFA), diglycerides, monoglycerides and polar lipids were scraped from the plate into ampules (with a constricted neck for sealing) containing an appropriate amount of heptadecanoic acid (Margaric Acid, E. Merck, Darmstadt, Germany) as internal

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Table 2—Crude fat content and total fatty acids in dry sausages of batch A (g/100g of dry matter)

	Stage of ripening (days)								Mean value
	0	5	8	12	19	27	33	40	± S.E.
Crude fat	63.2	60.5	63.2	68.9	65.8	64.1	63.7	67.1	64.5 ± 2.9
Total fatty acids	51.1	56.7	54.6	49.4	53.7	51.2	46.4	58.1	52.6 ± 1.3

standard. Preliminary experiments showed that the proportion of this acid in sausage lipids was always less than 0.5% (Stoll, 1972). Esterification was carried out with methanolic H_2SO_4 (230/2 v/v). The sealed ampules containing about 5 ml of this reagent were heated in a boiling water bath for 1 hr. Methylesters were extracted three times with petroleum ether; pooled extracts were washed with H_2O (three times) and evaporated to dryness under N_2 at 30°C. The residue was dissolved in an appropriate amount of hexane and analyzed by gas liquid chromatography as described by Demeyer and Henderickx (1967), but using Chromosorb W AW DMCS high performance (Hewlett-Packard, U.S.A.) as support. Fatty acid composition was determined directly from the proportionalities of the peak areas, calculated by triangulation. The weight of each individual fatty acid, present in each class, was calculated from the amount of IS added as:

$$A = \frac{a}{X_a} \cdot X_A$$

where: a = mg of IS added; A = mg of fatty acid present; X_a, X_A = peak area of fatty acid (X_A) or IS (X_a). The total amount of fatty acids present in each class, was obtained by the addition of the individual amounts.

Determination of carbonyl compounds

Total aldehydes were determined on approximately 2g of sample, using the benzidine reaction as described by Tóth (1970). The amount of aldehydes present was calculated from the absorbance measured, using the molar absorption of the heptanal reaction product as 1,370 (Holm et al., 1957) and an average molecular weight of 91. Indeed, Langner (1972), Halverson (1972) and Cantoni et al. (1967a) showed that mainly aliphatic saturated aldehydes are present in dry sausage, and from the compounds listed by Langner (1972) an average molecular weight of 91 can be calculated. For batch A, total carbonyl compounds were also isolated as 2,4-dinitrophenylhydrazones (DNP-hydrazones) on a celite column, impregnated with DNP-hydrazine (Schwartz et al., 1963) as described by Hansen and Keeney (1970). The sample (10g) was mixed in a mortar with celite 545 to a dry powdery paste and extracted with 200 ml of carbonyl free hexane (Hornstein and Crow, 1962) in a reciprocating shaker (24 hr). The mixture was filtered onto the column, carbonyl-free hexane added and the effluent collected (500 ml; 2–3 ml/min). The same volume of effluent was collected from a blank column without sample. From the absorbance of the hexane effluent, corrected for the blank, and measured at 340 mμ, the concentration of carbonyl compounds was calculated, using a mean molar absorption of 22,500 (Jones et al., 1956).

RESULTS & DISCUSSION

FIGURE 1 SHOWS a 15–20% increase in dry matter content during the ripening process. Average values for crude protein content in dry matter were 27.8 ± 1.0% (mean ± S.E., 40 determinations on 8 different stages in the ripening process) for batch A and 34.6 ± 0.2% for batch B (35 determinations on 7 different stages). Crude fat content was determined for batch A, and found to be higher than the total fatty acid content as determined on the lipid extract using margaric acid as the internal standard (Table 2). This discrepancy is obviously, at least partly due to the presence of glycerol and lipids containing no fatty acids such as cholesterol in the crude fat. The up and down variation in both crude fat and total fatty acids occurring during ripening may indicate stick-to-stick variation due to incomplete mixing during manufacture. The validity of the method used to quantitate fatty acids in lipid classes is illustrated by data presented in Table 3. The total fatty acids, determined on the lipid extract, were recovered by addition of fatty acids determined in lipid classes. To correct for variations in this recovery, fatty acid distribution in lipid classes, is expressed as % of the total obtained by addition. These data, for various stages of the ripening process, are presented in Figure 2.

It is clear that a continuous decrease in triglyceride fatty acids during ripening occurs, with corresponding increases in FFA, diglycerides and, less prominent, monoglycerides. Polar lipid fatty acids only decrease later in the ripening process. At the end of this process, FFA rep-

Table 3—Recovery of total fatty acids in lipid classes, as determined at various stages of ripening

Stage of ripening (days) Batch		I Total fatty acids determined on lipid extract (g/100g dry matter)		II Total fatty acids recovered in lipid classes (% of I)	
A	B	Batch A	Batch B	Batch A	Batch B
0	0	51.1	40.8	87.3	99.9
5	3	56.7	36.9	90.0	107.1
8	8	54.6	44.4	95.2	98.6
12	13	49.4	41.0	94.3	107.0
19	16	53.7	44.9	97.2	101.9
27	37	51.2	53.7	99.0	93.6
33	50	46.4	45.6	92.9	107.1
40		58.1		92.3	
		52.6 ± 1.3[a]	43.9 ± 6.3[a]	93.5 ± 4.2[a]	102.1 ± 6.2[a]

[a] Mean value ± S.E.

Table 4—Average fatty acid composition of lipid classes in dry sausage (%)[a]

Fatty Acid[b]	Triglycerides		Diglycerides		Monoglycerides	
	Batch A	Batch B	Batch A	Batch B	Batch A	Batch B
16:0	25.8	24.9	29.5	25.3	32.2	28.0
18:0	14.2	13.0	10.2	9.5	12.7	12.4
18:1	45.9	48.4	46.4	48.9	44.3	48.6
18:2	9.6	7.5	9.2	8.4	7.0	6.6

[a] Only major fatty acids are shown

[b] Shorthand notation gives number of carbon atoms:number of double bonds.

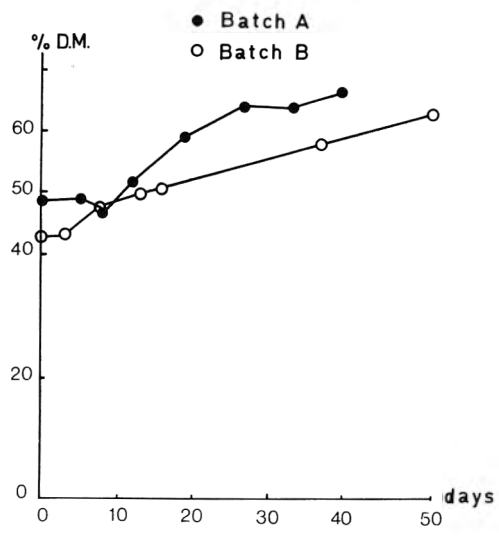

Fig. 1–Increase in dry matter content (% DM) with time.

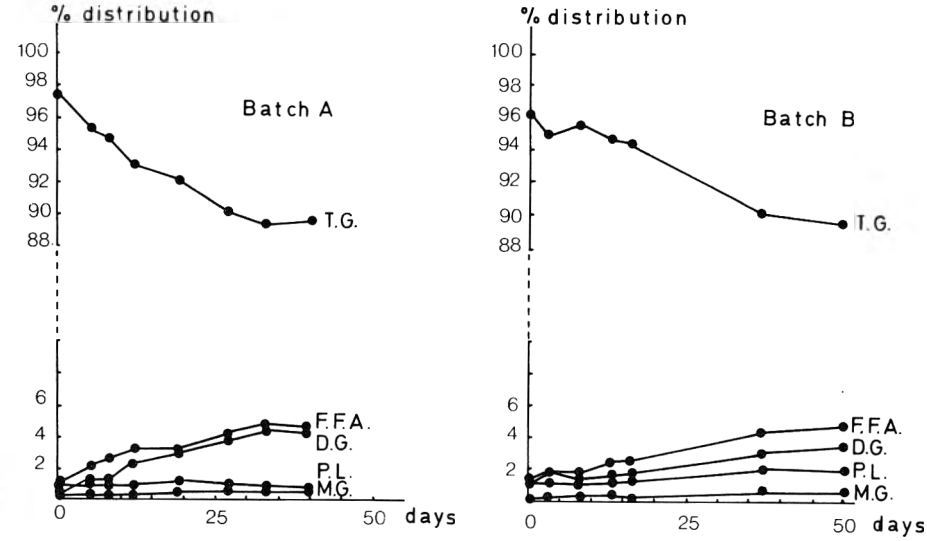

Fig. 2–Changes in fatty acid distribution over lipid classes (%) during the ripening process: M.G. (monoglycerides), D.G. (diglycerides), T.G. (triglycerides), F.F.A. (free fatty acids) and P.L. (polar lipids).

resent approximately 5% of the total fatty acids or less than 2% of the sausage weight. No difference was observed between the two batches, although micrococci counts tended to be higher in batch A (De Ketelaere et al., 1974). Micrococci are generally accepted to be the major group of microorganisms responsible for lipolysis in dry sausage (Cantoni et al., 1967a; Nurmi and Niinivaara, 1964), but evidence is available for production of lipases by lactobacilli (Coretti, 1965; Oterholm et al., 1968). Lipases are known to hydrolyze preferentially the outer fatty acids of a triglyceride molecule (Alford et al., 1971). The accumulation of diglycerides, together with FFA, suggests that preferentially one of the outer positions is attacked. It is known, that pig fat triglycerides show a particular fatty acid distribution pattern, most of the stearic acid (ca. 60%) being present at position 1, palmitic acid (60–80%) at position 2 and octadecenoic acids (50–60%) at position 3 (Brockerhoff, 1966). If the lipases specifically attack position 1 or position 3 bound acids, the fastest rate of lipolysis should be observed, either for stearic acid or for octadecenoic acids.

The percent distribution over the lipid classes of total palmitic, stearic, oleic and linoleic acid present was calculated for all samples, and showed that linoleic acid was liberated into the FFA fraction at a faster rate than all other acids. Rate of lipolysis decreased in the order linoleic > oleic > stearic > palmitic acid (Fig. 3).

As the molecular weights of these acids differ only slightly these results clearly indicate specificity of lipolysis for

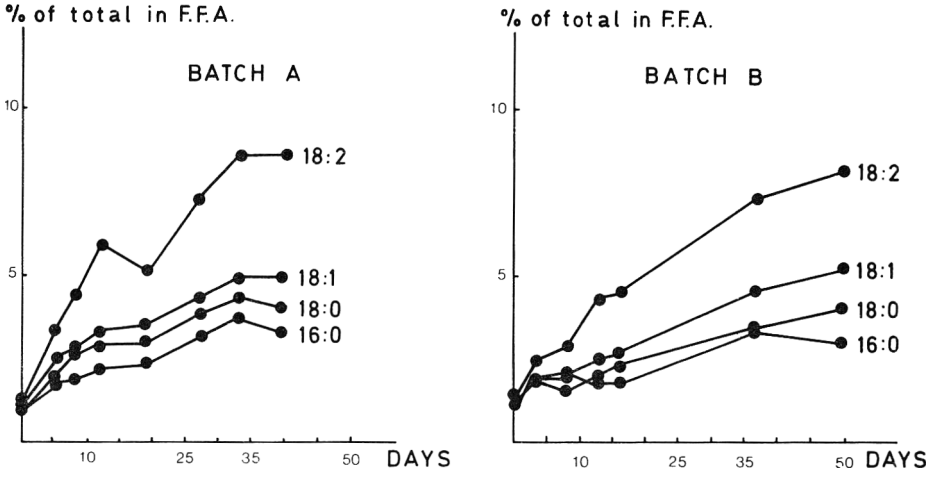

Fig. 3–% of total palmitic (16:0), stearic (18:0), oleic (18:1) and linoleic (18:2) acid present in FFA.

position 3 of the triglycerides. The difference observed for linoleic and oleic acids may be related to a specificity for fatty acid structure, as both positional and structural specificity are known to occur in microbial lipases (Alford et al., 1971). Diglycerides and monoglycerides are enriched in palmitic acid, as shown by their mean fatty acid composition, calculated over the whole ripening period (Table 4).

The evolution of the carbonyl compounds during the ripening process is illustrated in Figure 4. For batch A, results obtained using the benzidine reaction are lower than those obtained with DNP-hydrazine, in the first week of the ripening period, indicating the presence of a large proportion of ketone compounds. Later in the ripening process, similar amounts were found using both methods. In both batches, carbonyls increased during the first week of ripening, decreased after smoking and again increased to final values of about 300 μmoles/100g of dry matter or about 150 μmoles/ 100g of sausage. This is somewhat lower than values reported by Langner (1972): 16.7–143.4 mg/100g of sausage or 200–1,400 μmoles/100g of sausage. The initial increase is probably due to compounds formed in carbohydrate fermentation, most intensive in this period (De Ketelaere et al., 1974), while the increase in the last stages may be due to further metabolism of lipid peroxides, as suggested by Cerise et al. (1973). The decrease, observed after smoking, was also reported by Langner et al. (1970).

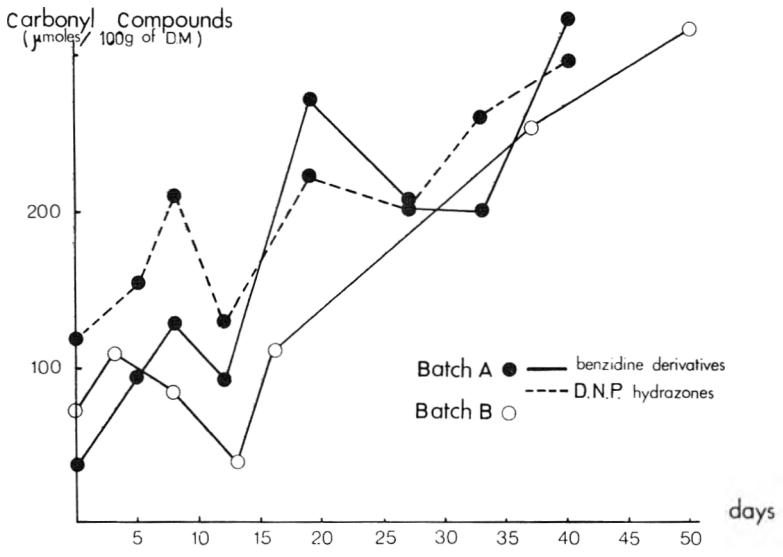

Fig. 4—Changes in carbonyl concentration during ripening.

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STOICHIOMETRY OF CARBOHYDRATE FERMENTATION DURING DRY SAUSAGE RIPENING

INTRODUCTION

DENATURATION of salt soluble proteins during ripening of dry sausage is brought about by a decrease in pH due to the production of lactic acid by bacterial metabolism of carbohydrates added or present in the meat (Ten Cate, 1960; Tändler, 1963). According to Ten Cate (1960) lactic acid is mainly formed following the stoichiometry of homofermentation: $C_6H_{12}O_6 \rightarrow 2CH_3CHOHCOOH$. This stoichiometry can be used to calculate the amount of carbohydrates required, to produce sufficient lactic acid for the desired decrease in pH. Amounts obtained by such calculation were always lower than actual amounts of carbohydrates needed (Andersen and Ten Cate, 1965). The authors explained this discrepancy, arguing that part of the carbohydrate was metabolized following heterofermentative pathways to products such as acetic acid or ethanol. Indeed, the presence of ethanol in dry sausage was reported by Pezacki and Szostak (1962), whereas Halverson (1973) recently reported the presence of acetic acid and other volatile fatty acids. Coretti and Scheper (1966) also pointed out the importance of heterofermentation and drew attention to the work of Pezacki and Szostak (1962), who reported the presence of pyruvic acid, in concentrations similar to those of lactic acid, as an end-product of bacterial metabolism in dry sausage (see also Pezacki, 1970). These acids were formed, mainly from substrates other than carbohydrates, as their concentration was not affected by the addition of sucrose to the sausage mixture prepared from meat containing very little glycogen. In contrast to these findings, radioactivity of added U-^{14}C glucose, was mainly recovered in lactic acid, whereas only minor amounts appeared in pyruvate (Fiszer, 1970). Also, Andersen and Ten Cate (1965) rightly pointed out that pyruvate is an intermediate in carbohydrate metabolism and only rarely accumulates as an end-product in bacterial fermentations. Pezacki and Jarozewski (1963) observed CO_2 production and O_2 consumption during dry sausage ripening, and, from further results, obtained with 1-6-^{14}C glucose (Pezacki and Fiszer, 1966), it was concluded that oxidative dissimilation of carbohydrates and other compounds can occur during the last stages of ripening.

In view of the complexity and discrepancy of the available data, the experiments described in this paper were carried out to evaluate the stoichiometric relationship between the disappearance of carbohydrates and the production of organic acids during dry sausage ripening.

Table 1—Composition of sausage mixture (expt 2 and 3)

Ingredient	Amount
Cooled, deboned and chopped beef	75 kg
Cooled, deboned and chopped pork	25 kg
Cooled and chopped lard	40 kg
Cooled and chopped pork-rind	8 kg
Salt (NaCl)	4.3 kg
Coloring salts ($NaNO_2$ + KNO_3)[a]	50g
Sugar mixture[b]	6 kg
Smoke concentrate	150g
Monosodium glutamate	150g
Pepper	450g
Starter culture[c]	75g

[a] Added with NaCl: 36g of KNO_3 + 14g of $NaNO_2$
[b] 4.5 kg in expt 3
[c] Only in expt 3, equivalent to 10^{10} cells/kg and 68g of glucose

EXPERIMENTAL

Preparation of sausages

Three experiments were carried out, involving three batches of sausages. In expt 1, batch B described in the preceding paper (Demeyer et al., 1974) and prepared by a local butcher was used. In expt 2 and 3, two batches were prepared simultaneously in a local factory. The composition of the sausage mixture is given in Table 1 and differed by the addition in expt 3 of a starter culture, containing lactobacilli and micrococci (Duploferment 66, Rudolf Müller and Co, Giessen, Germany), and 1% less of a "sugar mixture" was added. The sugar mixture was prepared in the factory, by mixing three commercial products, and contained, as analyzed in our laboratory 52.8% of total carbohydrates, 25.5% crude protein, 6.3% ash and 6.0% H_2O. Thin-layer chromatography (Lato et al., 1969) showed the presence of lactose and glucose only. The sausage mixture is filled into semi-synthetic casings using a vacuum filling machine (Alexander Werke F.K.V. 300, Remscheid, Germany). The sausages (approx. 3.5 kg each, diam 105 mm) were then transferred to a conditioning chamber for 6 days. During this period, temperature was gradually lowered from 22°C to 18°C and RH from 95% to 85%, while cold smoke was applied 3 hr daily. They were then transferred to a drying chamber, where they were kept for a further 30 days at 16°C and 85% RH, until ready for consumption.

Analyses were also carried out on nine different brands of dry sausages (numbered 1 to 9) as obtained from various shops.

Sampling procedure

At different stages of the ripening process, a sausage was transported to the laboratory (expt 1) or a sample of approx. 500g was removed, making a transverse cut (expt 2 and 3). The cut surface of the remaining sausage was sealed off by immersion in liquid gelatin, after each sampling. Samples were treated as described previously (Demeyer et al., 1974).

Analytical methods

Samples were analyzed for dry matter, crude protein (Kjeldahl method) and crude fat (ether extract) according to E.E.G. standard methods, currently in use in this laboratory (Anonymous, 1971, 1972). Using a Radiometer 22 apparatus (Radiometer, Copenhagen) with expanded scale, pH was measured by careful insertion of pointed electrodes in the sample (casing removed). Protein, soluble in buffer of low ionic strength (Sarcoplasmic protein) and in buffer of high ionic strength (myofibrillar protein) was determined according to Mihalyi and Körmendy (1967), but samples were centrifuged (10 min at 3.000 × G) and not filtered.

For the determination of total carbohydrates, lactic acid and pyruvic acid, 5g of sample was homogenized with 50 ml of 0.6N $HClO_4$ (approx. 60°C) in a Virtis homogenizer (Gardiner, N.Y.) for 3 min at max speed. The mixture was centrifuged (10 min at 7.000 × G), the residue washed with 0.6N $HClO_4$, filtered, the filtrate added to the supernatant and made to volume (100 ml). Aliquots were used for the determination of lactic acid by micro-diffusion analysis in Conway discs (4 hr at 37°C) (Conway, 1957), determination of pyruvic acid as total α-keto acids (Umbreit et al., 1959) and determination of total carbohydrates using the anthrone reagent as described by Herbert et al. (1971). Using the latter method and glucose as standard, an average recovery of 99.8% was obtained for glycogen, dextrines, starch, sucrose and maltose. Although the anthrone method is known to be less sensitive for lactose (recovery obtained = 82%), comparison with the phenol

Table 2—Recoveries of volatile fatty acids added to sausage

	Acetic acid	Propionic acid	Butyric acid
μmoles present	155.9	1.4	1.0
μmoles added	35.3	13.4	17.4
Total	191.2	14.8	18.4
Total μmoles recovered	196.6	17.8	18.3

method (Herbert et al., 1971) on samples containing lactose showed that differences were within the experimental error.

Volatile fatty acids (VFA) were isolated by steam-distillation: 5g of sample were mixed with 10 ml of H_2O and 3g of $MgSO_4 \cdot 7H_2O$ in a Virtis homogenizer. The mixture was transferred to a Markham Still and 5 ml of 85% H_3PO_4 (A.R. Merck, Darmstadt, Germany) added. The outlet of the condensor was immersed in 10 ml NaOH 0.1N containing phenolphtaleine and 200 ml of condensate was collected. The alkaline distillate was evaporated under reduced pressure in a rotary evaporator and the dry salts dissolved in 2.5 ml of 10% H_3PO_4. The VFA were separated as free acids by gas-liquid chromatography using 5 μl of solution and an F&M 700 apparatus (Hewlett-Packard, Brussels) equipped with a flame ionization detector, as described in earlier work from this laboratory (Van Nevel et al., 1969). Quantitation was carried out by comparison of sample peak heights with peak heights of standard mixtures, also subjected to steam distillation and injected at regular intervals between samples. Table 2 shows recoveries obtained for known amounts of VFA added to a sausage sample. Carbonyl compounds were determined as saturated aldehydes (mean M.W. 91) using the benzidine reagent as described earlier (Demeyer et al., 1974).

Quantitative determination of bacteria

In expt 1, before grinding the sausage, a slice was removed with a knife. The sample was weighed, homogenized (1 min) and diluted tenfold in a Waring Blendor, (14,000 rpm) using a solution containing 0.1% peptone, 0.85% NaCl and 0.04% agar. Inoculation, incubation and counting of bacteria was carried out using the ringed-plates technique described by Van der Heyde (1963, 1964). Lactobacilli were incubated anaerobically on Rogosa SL agar and Micrococci aerobically on S 110 agar (Difco).

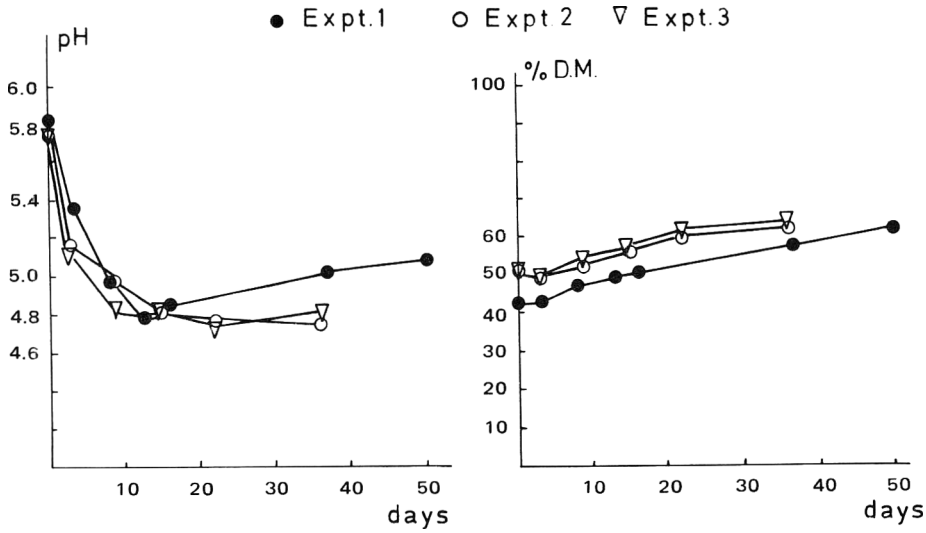

Fig. 1—Changes in pH and dry matter (D.M.) during dry sausage ripening.

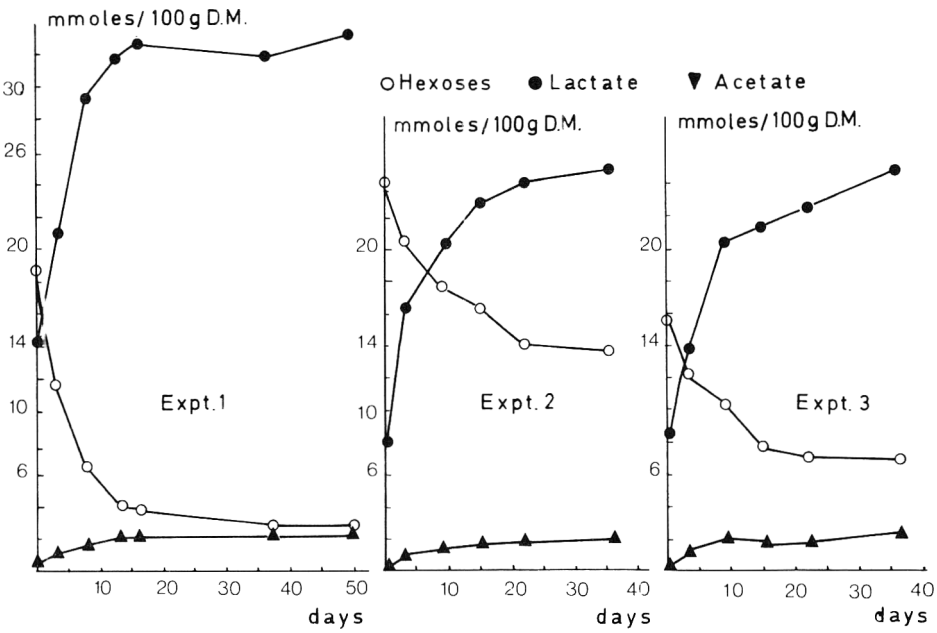

Fig. 2—Changes in concentration of hexoses, lactate and acetate during dry sausage ripening.

RESULTS & DISCUSSION

FIGURE 1 SHOWS that in all experiments, dry matter content increased to approximately 60% during the ripening process. Values of pH dropped from an initial value of about 5.8 to approximately 4.8 during the first 15 days of ripening, and changed little afterwards, except for expt 1 where an increase was observed. The drop in pH coincides with an accumulation of lactic acid and the disappearance of carbohydrates (Fig. 2), both of these processes being nearly completed after 15 days of ripening. Together with lactic acid, smaller amounts of acetic acid are formed (Fig. 2) and very small, but significant amounts of propionic and butyric acids (10–20 μmoles/100g dry matter). No α-keto acids could be detected by the method used, whereas total carbonyl concentration never exceeded 0.5 mmole/100g dry matter. The percentage of total crude protein, soluble as "myofibrillar protein," decreased from approximately 45% to 25% during the first 15 days of ripening, whereas "sarcoplasmic protein" decreased from approximately 18% to 5% after 35 days of ripening. The presence of a starter culture in expt 3 did not produce significant changes for any of the characteristics measured (Fig. 1, 2 and 3).

From the amounts of carbohydrates, expressed as mmoles of hexose, and the amounts of lactate and acetate produced, fermentation balances can be calculated, according to the reactions:

$$C_6H_{12}O_6 \rightarrow 2CH_3CHOHCOOH$$

$$\text{and } C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 2CO_2 + 8H$$

It is clear from these reactions, that for

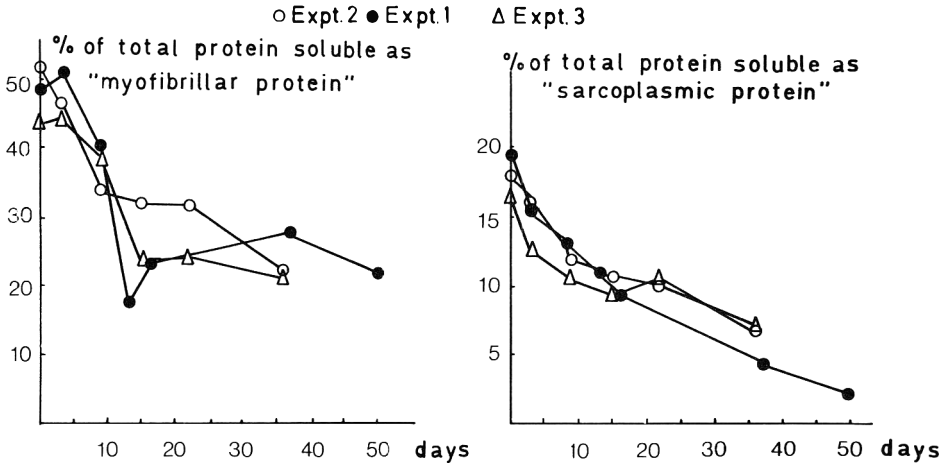

Fig. 3–Changes in protein solubility during dry sausage ripening.

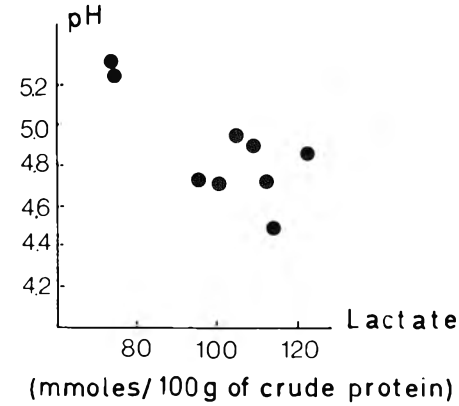

Fig. 5–Relationship between pH and lactate concentration (data from Table 4).

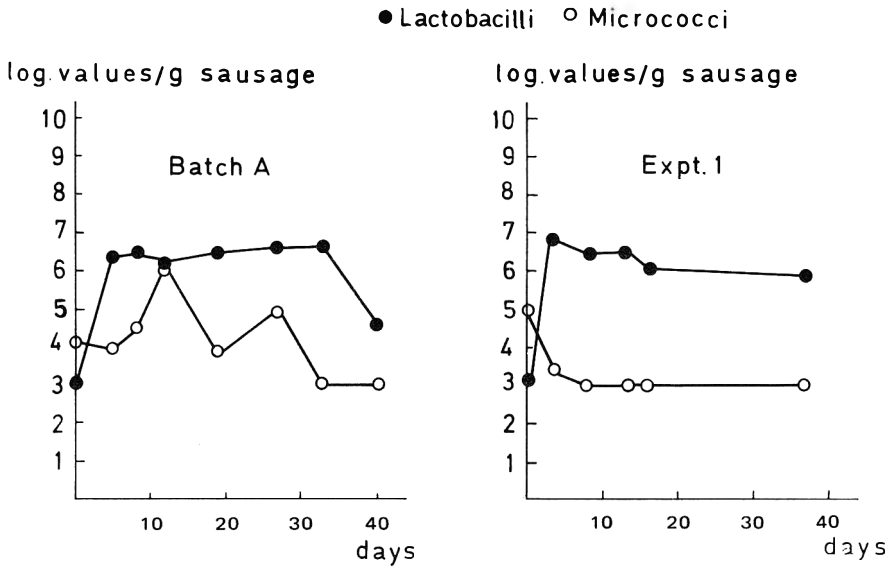

Fig. 4–Counts of lactobacilli (●) and micrococci (○) at different stages of ripening.

each mole of hexose disappearing, two moles of lactate and/or acetate should be formed. The theoretical amounts of these acids, calculated from hexose metabolized, are compared to the amounts actually found for the different periods of the ripening process, as well as for the whole period, in Table 3.

It can be seen that for the whole period, in expt 2 and 3, the amounts of lactate and acetate found correspond to the amounts calculated, indicating that all hexose metabolized was anaerobically converted to lactate and acetate, the former being the major end-product. For the period 15–36 days (Table 3), the amounts of lactate + acetate found differ significantly from the amounts calculated. This may indicate lactate production from substrates other than carbohydrates (expt 3) or further metabolism of lactate formed (expt 2). However, these differences are within experimental error when the whole period is considered. In expt 1, lactate and acetate found can only account for about 2/3 of all hexose metabolized, indicating that other end-products were formed. The small amounts of propionate, butyrate and carbonyl compounds formed cannot explain this discrepancy. However, regeneration of reduced cofactors in anaerobic carbohydrate fermentation may produce other reduced compounds such as ethanol and other low molecular weight alcohols, not determined in these experiments. In view of the magnitude of the discrepancy, and the low concentration of ethanol reported elsewhere (Pezacki and Szostak, 1962), a more likely explanation may be related to the initial presence of more oxygen in the sausages of expt 1, as compared to expt 2 and 3. Indeed, whereas sausages were vacuum filled in the latter experiments, they were not in the former. A higher oxygen concentration may induce a complete oxidation of part of the carbohydrate, with production of CO_2 and H_2O. Such oxidative dissimilation of carbohydrates has been suggested for the last stages of ripening by Pezacki and Fiszer (1966). However, as is clear from Table 3, the discrepancy between end-products found and substrate metabolized, is most prominent for the first 3 days of ripening. In all experiments, fermentation balance discrepancies were observed for the last period of ripening (Table 3), but the amounts involved are of minor importance, compared to the first two periods.

Although very early in the ripening period lactobacilli become the predominant flora of dry sausages, ripening under the conditions described, the number of micrococci initially present is comparable to the number of lactobacilli (Reuter et al., 1968). Micrococci may contribute to complete oxidation of carbohydrate during the first days of the ripening period. In expt 1, micrococci and lactobacilli were enumerated and comparable numbers were only observed for the first sample (Fig. 4). Numbers of micrococci tended to be higher, however, in samples obtained from batch A, described in the preceding paper (Demeyer et al., 1974), ripened under similar conditions as batch B (expt 1) and for which preliminary results on carbohydrate metabolism indicated even more prominent fermentation balance discrepancies (Fig. 4). Although the stoichiometry clearly indicates a different pattern of carbohydrate metabolism in expt 1, compared to expt 2 and 3,

Table 3—Fermentation balances, calculated at various stages of dry sausage ripening

Period (days)	Hexose fermented[a]			Lactate + Acetate formed[a]		
	Expt 1	Expt 2	Expt 3	Expt 1	Expt 2	Expt 3
0–3	7.17	3 83	3.56	7.82(14.34)[b]	9.07(7.66)	6.01(7.12)
3–15		4 30	4.56		7.17(8.60)	8.33(9.12)
3–16	7.68			13.12(15.36)		
15–36		2 38	0.75		2.42(4.76)	3.88(1.50)
16–50	0.84			0.61(1.68)		
0–36		10 51	8.87		18.66(21.02)	18.22(17.74)
0–50	15.69			21.55(31.38)		

[a] All results expressed as mmoles/100g of dry matter
[b] Numbers in parentheses are theoretical values calculated from glucose fermented.

Table 4—Composition of dry sausage

	Brand									
	1	2	3	4	5	6	7	8	9	Mean ± S.E.
Dry matter (D.M.) (%)	58.6	62.2	73.1	64.0	70.2	65.6	65.0	66.4	61.6	65.1 ± 1.5
Protein (% D.M.)	29.7	27.5	25.4	26.7	31.0	28.2	30.0	27.9	27.0	28.1 ± 0.6
Fat (% D.M.)	60.0	—	66.8	61.2	61.1	60.6	60.3	56.5	62.1	61.0 ± 1.0
pH	4.86	4.70	5.23	4.72	5.31	4.94	4.90	4.72	4.48	4.87 ± 0.09
% of protein as										
Myofibrillar	35.4	18.8	23.4	20.2	31.5	26.1	17.1	17.0	27.8	24.1 ± 2.2
Sarcoplasmic	8.9	7.1	8.6	7.1	9.5	6.2	7.5	9.4	8.1	8.0 ± 0.4
Organic Acids										
Lactate[a]	36.4	27.7	18.8	25.4	22.7	29.3	32.7	31.2	30.8	28.3 ± 1.8
Acetate[a]	4.2	1.7	1.8	2.1	3.1	3.4	1.4	1.8	2.4	2.4 ± 0.3
Butyrate[b]	21.0	22.7	9.8	9.5	13.4	8.2	4.2	13.1	19.1	14.5 ± 2.1
Propionate[b]	17.2	6.2	7.8	4.6	45.3	5.9	6.8	8.4	3.9	11.7 ± 4.4
Carbonyl compounds[b]	222	360	253	246	213	416	796	345	162	334 ± 64
Hexoses[a]	7.8	10.6	20.0	7.5	1.9	1.6	6.9	21.3	5.7	9.3 ± 2.4

[a] mmoles/100g of D.M.
[b] μmoles/100g of D.M.

the absolute amounts of lactic and acetic acid formed in all experiments are similar (Table 3).

Also, the final concentrations of these acids, as well as other characteristics measured are similar to the mean values calculated for nine samples obtained commercially (Table 4). Individual values of pH for these samples were found to be inversely related to the concentration of lactic acid, expressed per 100g of crude protein, as suggested by Andersen and Ten Cate (1965) (Fig. 5).

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CHANGES IN NONPROTEIN NITROGEN COMPOUNDS DURING DRY SAUSAGE RIPENING

INTRODUCTION

IT IS well known, that the concentration of water soluble nitrogen compounds in dry sausage increases during ripening and can reach values up to 25% of the total nitrogen (Maillet and Henry, 1960; Niinivaara et al., 1961; Mihalyi and Körmendy, 1967). The composition and concentration of several groups of these compounds, such as free amino-acids, peptides, nucleotides and nucleosides determine to a large extent the final aroma of dry sausage (Dahl, 1970).

The availability of automated analysis has recently intensified research into free amino acid production in dry sausage (Reuter and Langner, 1968; Langner, 1969) which is at least partly due to bacterial protease activity (Pohja and Niinivaara, 1966; Sajber et al., 1971). Also, Cantoni et al. (1967) stated that the major nucleotide present initially in sausage is inosinic acid (IMP) formed by deamination of adenylic acid, soon after rigor mortis. During ripening, phosphomonoesterase and nucleosidase activity produce inosine nucleoside and hypoxanthine respectively from inosinic acid. Langner (1972) determined ammonia on 12 different brands of dry sausage, whereas Niinivaara et al. (1961), Körmendy and Gantner (1962) and Stanculescu et al. (1970) report values for total free α-amino acid nitrogen (α-NH_2-N) in dry sausages.

We are not aware, however, of any work describing the quantitative contribution of different compounds to the total nonprotein-nitrogen fraction (NPN fraction) at various stages of dry sausage ripening.

In this paper, we report changes in different groups of NPN compounds during dry sausage ripening as influenced by the presence of a "starter culture." The NPN compounds studied include ammonia, free amino acids, peptides, nucleotides, nucleosides and amines.

EXPERIMENTAL

Preparation of sausages

Two batches of sausages, referred to as expt 2 and 3 respectively, were used. The preparation, composition, change in dry matter content (D.M.), pH, concentration of carbohydrates and concentration of carbohydrate metabolism products of these sausages are reported in an accompanying paper (De Ketelaere et al., 1973).

Sampling procedure

The samples used for analysis were those obtained as described by De Ketelaere et al. (1974).

Analytical methods

Determination of total NPN and individual NPN fractions. Different NPN extraction methods (ethanol 80% v/v, trichloroacetic acid 10% w/v, $ZnSO_4$ 10% w/v treated with an equal volume of 0.5N NaOH, and $HClO_4$ 0.6N) were compared. As it was found that $HClO_4$ 0.6N extracted the highest amount of total N (Table 1) it was used in further experiments.

5g of sample were homogenized in 25 ml of 0.6N $HClO_4$ as described earlier (De Ketelaere et al., 1974). After filtration, neutralization

Table 1—Comparison of different NPN extraction methods (mg N/g sausage extracted)

Extraction agent used	EtOH 80%	TCA 10%	$HClO_4$ 0.6N	$ZnSO_4$ 10%
Nitrogen recovered				
Total N (NPN)	5.40	6.09	6.33	4.34
NH_3	0.55	0.67	0.69	0.62
Free α-NH_2-N	2.46	2.19	2.19	2.50

Table 2—Concentration of NPN compounds at various stages of ripening (mg N/100g) of dry matter

	Expt 2 Stage of ripening (days)						Expt 3 Stage of ripening (days)					
	0	3	9	15	22	36	0	3	9	15	22	36
NH_3	24	30	40	58	62	76	25	27	43	61	57	73
Free α-NH_2-N	141	188	204	234	243	255	155	200	225	230	255	302
Peptide bound α-NH_2-N	161	195	209	152	147	145	225	235	204	168	171	113
Nucleot.-N	34	33	15	13	12	12	37	21	17	16	13	14
Nucleos.-N	33	41	54	78	83	83	31	42	51	75	89	89
Total NPN												
Determined	537	775	790	789	803	820	544	706	805	802	806	889
Calculated[a]	494	615	660	664	677	704	600	670	683	683	727	730
% Recovery	92.2	79.3	83.5	84.1	84.3	82.0	110.2	94.9	84.8	85.1	90.1	82.1

[a] Obtained by addition, after correction of α-NH_2-N for 25% non-α-NH_2-N.

Table 3—Concentration changes of NPN compounds at various stages of the ripening process (mg N/100g dry matter)

	Expt 2 Period (days)				Expt 3 Period (days)			
	0–3	3–15	15–36	0–36	0–3	3–15	15–36	0–36
NH_3	6	28	18	52	2	34	12	48
Free α-NH_2-N	47	46	21	114	45	30	72	147
Peptide bound α-NH_2-N	34	−43	−7	−16	10	−67	−55	−112
Nucleot.-N	−1	−20	−1	−22	−16	−5	−2	−23
Nucleos.-N	8	37	5	50	11	33	14	58

with KOH 30% w/v, filtration and dilution to known volume, total NPN was determined by the micro-Kjeldahl method [as described in the standard methods of E.E.G. (Europese Economische Gemeenschap)] (Anonymous, 1972). Samples of the extract were used for determinations of NH_3 (1 ml) (Conway, 1962), total free α-NH_2-N (1 ml) using leucine as standard (Rosen, 1957), total peptide bound α-NH_2-N after acid hydrolysis (24 hr) and correction for free α-NH_2-N (Weidner and Eggum, 1966), total nucleotides (1 ml) expressed as IMP and total nucleosides, expressed as hypoxanthine (Macy et al., 1970).

Automated analysis of free amino acids and amines. Free amino-acids and weak amines were extracted from a separate sausage sample with picric acid (Stein and Moore, 1954) and quantitated using a standard Technicon "Auto Analyzer" and norleucine as Internal Standard (Spackman et al., 1958). Part of the highly basic amines were extracted from a third sample of sausage (Hill et al., 1970) and separated using a standard Technicon "Auto Analyzer" as described by Vandekerckhove and Henderickx (1973).

Table 4—Concentrations of free amino acids[a] at three stages of ripening (mg α-NH_2-N/100g dry matter)

	Expt 2 Stage of ripening (days)			Expt 3 Stage of ripening (days)		
Free amino acids	0	15	36	0	15	36
Asp	0.74	1.90	5.30	0	3.79	7.25
Thr	0.82	3.30	25.20	0	4.95	6.94
Ser	1.73	5.50	9.10	0	7.20	9.85
Glu	19.00	7.20	5.60	25.40	24.30	18.30
Pro	0	3.40	5.50	0	5.72	6.45
Gly	3.00	6.15	8.80	0	7.65	14.20
Ala	10.20	20.90	25.20	3.92	23.10	22.30
Val	1.44	6.35	8.85	0	7.41	11.95
Met	0.56	2.72	3.84	1.60	3.38	5.26
Ileu	1.60	3.74	5.45	2.24	4.50	8.10
Leu	1.06	11.50	13.30	8.30	13.20	17.60
Phe	0.93	4.50	5.25	2.98	5.00	7.15
Lys	2.07	4.67	6.35	2.30	3.94	6.46
His	0.73	1.46	0.01	2.77	2.20	0
Tyr	0.77	0	0	0	0	0.30
γ-N BA[b]	0	2.72	4.07	1.22	7.89	12.50
Orn[c]	1.16	0	0	1.98	0	0.83

[a] Shorthand notation
[b] γ-amino butyric acid (γ-amino-N calculated as α-amino-N)
[c] Ornithine

RESULTS and DISCUSSION

TABLE 2 shows the concentration of the different NPN compounds investigated, expressed as mg N/100g of dry matter at various stages of the ripening process. It can be seen that the major NPN fraction present is peptide bound α-amino-N at the start, whereas free α-amino-acids (free α-NH_2-N) dominate at the end of the ripening period. Addition of individual values for each stage results in values lower than the total NPN determined. This discrepancy is obviously related to differences in color and color intensity of the ninhydrin reaction products between different amino acids, the presence of non-α-NH_2-N in the free amino acids (approximately 25% of total amino acid N) and the expression of all nucleotides as IMP and of all nucleosides as hypoxanthine. Indeed, besides nucleoside monophosphates, di- and triphosphates may be present, whereas nucleosides are present besides hypoxanthine. However, addition of all components, after correction of α-NH_2-N values for the presence of 25% non-α-NH_2-N, results in an average recovery of 91.3 ± 4.2% (expt 3) and 83.6 ± 1.8% (expt 2) of determined NPN (Table 2). From data in Table 2, the concentration changes (as mg N/100g dry matter) for the different compounds at various stages of the ripening process were calculated and presented in Table 3.

These data show that during the first 3 days of ripening, the rate of free α-NH_2-N production is maximal and exceeds the rates of NH_3 production and peptide production from proteins. During this period intensive carbohydrate metabolism and bacterial growth also takes place (De Ketelaere et al., 1974). In the following periods the rate of ammonia production increases, but remains inferior to the rate of free α-NH_2-N production, whereas the concentration of peptide bound α-NH_2-N decreases. These results indicate, that free amino acids are produced at a faster rate than ammonia and peptides: % free α-NH_2-N in total NPN increases from ca 35% to 50% at the end of the ripening period. This is in contrast to results reported by Langner (1969) indicating an initial fast production rate for ammonia, whereas production of free amino acids only starts after an "initiation period." However, Niinivaara et al. (1961) and Körmendy and Gantner (1962) also observed the fastest rate of free amino acid production during the first 3 days of ripening. The final values obtained for NH_3 are within the range reported by Langner (1972) for 12 commercial sausages (16–103 mg NH_3/100g sausage) and are comparable to data reported by Stanculescu et al. (1970) (approx 60 mg/100g sausage) and by Körmendy and Gantner (1962) (approx 80 mg/100g sausage). Values for free α-NH_2-N are somewhat lower than those reported by Stanculescu et al. (1970): approx 600 mg/100g sausage.

Nucleotides decrease in concentration, whereas nucleosides and bases increase in concentration. The lack of stoichiometry between nucleotide disappearance and nucleoside formation, is probably related

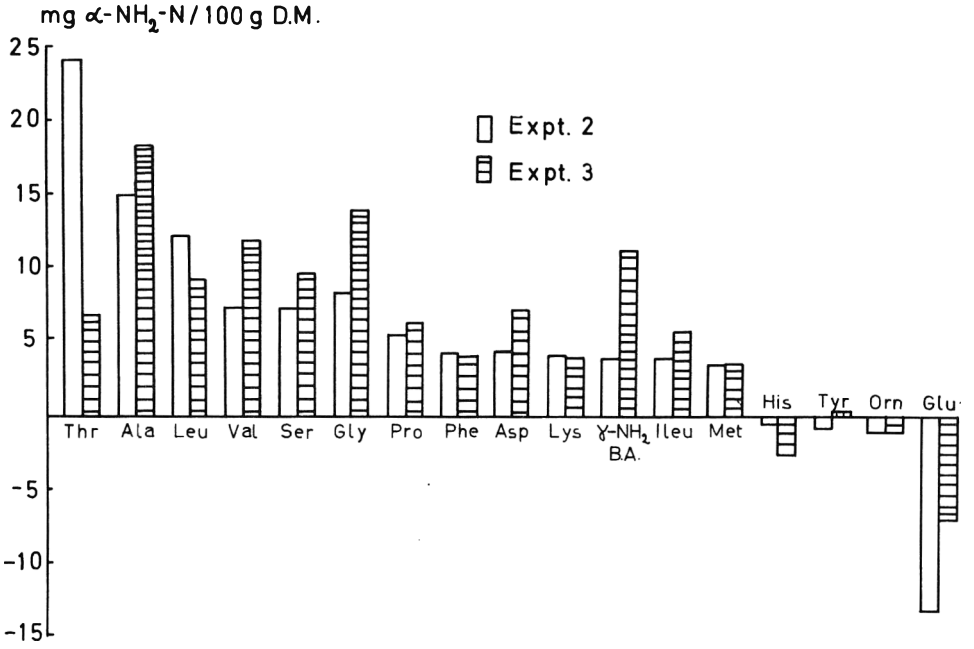

Fig. 1—Concentration changes of individual free amino acids between final and initial stage of ripening (mg α-NH_2-N/100g dry matter).

to the expression of results as IMP and hypoxanthine, as explained earlier. The presence of a starter culture (expt 3) produced no striking differences, except for a higher final concentration of free α-NH_2-N in expt 3, coupled to a lower concentration of peptide bound α-NH_2-N These findings may suggest a higher exopeptidase activity in expt 3 or may be related to a higher initial peptide concentration in the same experiment (Table 2). In both experiments, the most significant increase was observed for free α-NH_2-N (total free amino acids). In order to determine the individual amino acids responsible for the increase, amino acid analyses were carried out on samples obtained after 0, 15 and 36 days of ripening. The results are presented in Table 4. They show that glutamic acid is the predominant amino acid in the initial samples, because of its presence as an additive. The second predominant free amino acid initially present is alanine, confirming data reported by Niinivaara et al. (1961), Stanculescu et al. (1970) and Langner (1969).

Concentration changes for individual amino acids were calculated between final and initial samples, and presented in Figure 1. They show that the major amino acids responsible for the increase in total free α-NH_2-N are alanine, leucine, valine, serine, glycine and proline (increase larger than 5 mg γ-NH_2-N/100g dry matter), followed by phenylalanine, aspartic acid, lysine, α-amino butyrate, isoleucine and methionine (increase smaller than 5 mg α-NH_2-N/100g dry matter). Threonine shows the largest increase in expt 2, but not in expt 3. For most amino acids, increases observed are larger in expt 3, confirming the data obtained for total free α-N. Final concentrations for free amino acids are within the range of values reported by Langner (1972). These results are partly in agreement with data presented by Reuter and Langner (1968), Niinivaara et al. (1961), Körmendy and Gantner (1962) and Stanculescu et al. (1970) as these authors also observed the most prominent concentration increase for alanine and leucine.

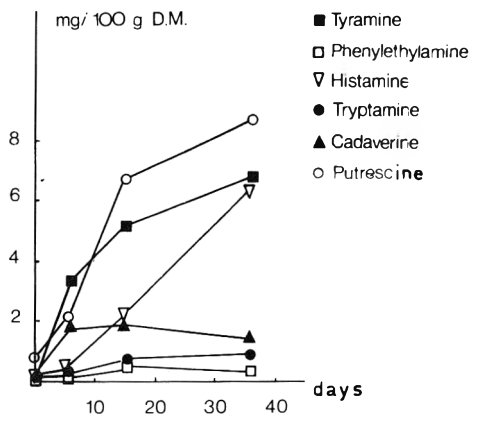

Fig. 2—Concentration of some amines at various stages of ripening in expt 3 (mg/100g dry matter.

In both experiments, a considerable part of the added glutamate disappears, and is at least partly decarboxylated to γ-amino-butyric acid, confirming results obtained by Langner (1972). Because of these results the use of glutamate as a flavor additive in dry sausage may be questioned (Langner, 1972). Other amino acids may be decarboxylated during dry sausage ripening, as indicated by the disappearance of histidine, tyrosine and ornithine. The decarboxylation products of these amino acids are histamine, tyramine and putrescine, respectively. Here, our results are in contrast to earlier findings of Langner (1969) and Reuter and Langner (1968), who also reported an increase for these three amino acids. However, tyrosine disappearance was reported by Niinivaara et al. (1961), as well as the formation of tyramine. Maillet and Henry (1960) reported the presence of histamine, whereas Langner (1972) even suggests production of cadaverine by decarboxylation of lysine.

Analysis of highly basic amines was carried out on samples obtained from expt 3. Although only very small amounts were detected, the concentration of histamine, tyramine and putrescine was increased at least tenfold, the rate of increase being maximal, during the first 3 days of ripening (Fig. 2).

The results are in line with the decrease in the concentration of histidine, tyrosine and ornithine observed in our experiments. Cadaverine, a decarboxylation product of lysine, was also detected in significant amounts.

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THE ROLE OF LIGHT AND SURFACE BACTERIA IN THE COLOR STABILITY OF PREPACKAGED BEEF

INTRODUCTION

THE STABILITY of the red meat pigment protein, oxymyoglobin, has been extensively studied in model systems. The effects of storage time, storage temperature, ionic strength, light and other forms of electromagnetic radiation upon the hemeprotein in a model system are well understood. Confusion rapidly develops when one then discusses the stability of the myoglobin molecule in intact meat. Pigment stability in intact meat involves new factors, such as O_2 penetration, bacterial growth, fat rancidity, the presence of flavin compounds and O_2 permeability of packaging film, all of which contribute to the stability of the MbO_2 molecule. Whether bacteria or light, or both, were contributing factors in the discoloration of fresh intact meat has been debated in the literature since the early 1950's. Ramsbottom et al. (1951) stated that fluorescent lamps, both cool and soft white, cause no significant discoloration of fresh beef but do cause discoloration of cured meats. This same observation was stated by Rikert et al. (1957). Kraft and Ayres (1954) using a soft white fluorescent lamp of 50 and 150 ft-c found it to be unimportant in influencing discoloration of fresh packaged beef.

Research performed in recent years using instrument techniques to follow color deterioration has shown that light may indeed affect color. Marriott et al. (1967) found that meat stored at −1°C under direct illumination deteriorated at a faster rate than did meat stored in the dark. These authors felt that the significant color change was the direct result of light enhancing bacterial growth and, in turn, the color. Leward et al. (1971) demonstrated that fresh beef stored at 0°C and inoculated with large numbers of bacteria (10^8 per cm^2) caused a depletion of surface O_2 and thereby caused the rapid conversion of surface MbO_2 to MetMb, resulting in meat discoloration. Lentz (1971) showed that meat stored at freezer temperatures and under cool white fluorescent light (150–200 ft-c) discolored more rapidly than did similar samples stored in the dark.

Solberg and Franke (1971) using selected filters (420–633 nm) on light sources illuminated intact meat samples at both 1° and 5°C and found statistically significant color changes resulting from the illumination. These authors felt that the influence of light on the enhancement of autoxidation rate was small enough to be of little commercial importance. Setser et al. (1973) exposed intact beef samples to illumination at wavelengths varying from 405–685 nm. The authors found that ultraviolet (254 nm) and low wavelength visible light (405–577 nm) caused a significant increase in MetMb production over similar samples exposed to light of 630–685 nm wavelength.

Questions which become apparent after reviewing the literature are: (1) Under controlled storage temperature and light intensity, do different types of lighting systems significantly affect meat color? (2) If alteration in color stability occurs, is it due to light, bacteria or a combination of both? (3) Is part of the color change that occurs during storage due to a partial destruction of pigment proteins by either inherent or bacterial enzymes? The following data shed some light on the importance of various factors in the color stability of red meat in the display case.

METHODS & MATERIALS

Meat samples

All meat samples used in this investigation were taken from post-rigor bovine semitendinosus muscle. The muscle was cut parallel to the fiber direction into 1.5 cm tall by 1.8 cm diameter cores and placed into 1.8 cm diameter plexiglass rings. The meat sample plus ring was then covered and sealed in PVC film (Reynolds Plastics, Richmond, Va.). The meat samples were placed in a 5°C cold room either in a dark light-tight chamber or under a specific light source. Samples were removed from the light-tight chamber and from under the lights only when necessary for analysis.

Reflectance spectrophotometry

The entire visible spectrum, as well as specific reflected absorbance at 580 nm, was obtained on a Hitachi-Perkin Elmer Model 139 reflectance spectrophotometer. To obtain a specific reflectance spectrum or reflected absorbance value, the PVC film covered meat sample and ring assembly were inserted into a stainless steel reflectance cell holder and then onto the diffuse reflectance bowl of the spectrophotometer. Immediately after taking the reading the sample was returned to the 5°C cold room.

To remove the effect of light scattering in both the spectra and 580 nm readings, K/S values were calculated for each reading at each wavelength and used in further calculations. K/S values were calculated according to the procedure of Dean and Ball (1960).

Calculation of autoxidation rate constants

The rate constant describing the loss of MbO_2 and red color in each muscle was calculated using a modification of the procedure described by Snyder and Ayres (1961). The modifications used were as follows: (1) the % MbO_2 remaining at each time period was obtained using the K/S (580 nm) value instead of the 580 nm absorbance value. (2) The value for 0% MbO_2 was obtained by spreading a 1% aqueous solution of $K_3Fe(CN)_6$ carefully over the face of the sample core and then measuring the reflected absorbance at 580 nm which was also converted to its K/S (580) value.

Bacterial analyses

Total bacteria count on the surface of the core samples from one muscle sample was determined on alternating days, starting with day zero. On alternate days, duplicate cores were unwrapped and swabbed with a dry sterile swab which was subsequently dispersed in 9 ml of sterile phosphate buffered saline solution (Collins and Lyne, 1970). Serial dilutions were made in the phosphate buffered saline solution for final analysis of total count on a cooked meat-polypeptone agar.

The cooked meat-polypeptone agar consisted of (1) the clear cooked fluid from the boiling of 500g of lean beef in 1 liter of distilled water; (2) 10g of BBL polypeptone; (3) 5g of NaCl; (4) adjusted to pH 7.4; and finally, (5) 15g of agar. All inoculated plates were incubated at 5°C for 72 hr prior to calculating total count. This medium was used to ensure as similar as possible growth conditions between the environment on the meat surface and in the petri dish.

Polyacrylamide gel electrophoresis

The pigments in the top 2 mm of selected core samples were analyzed by polyacrylamide gel electrophoresis. After reflectance data were obtained on the core, the core was frozen in dry ice and the top 2 mm was removed and macerated in a glass pestle tissue homogenizer with an equal weight of cold distilled water. The emacerated tissue was centrifuged at 10,000 × G for 30 min. The clear supernatant fluid was then treated with one crystal of $K_3Fe(CN)_6$ to convert all pigments to the met form.

Electrophoresis was used to determine if prolonged storage of the meat samples had led to a partial destruction of the pigment molecules (myoglobin and hemoglobin) by proteo-

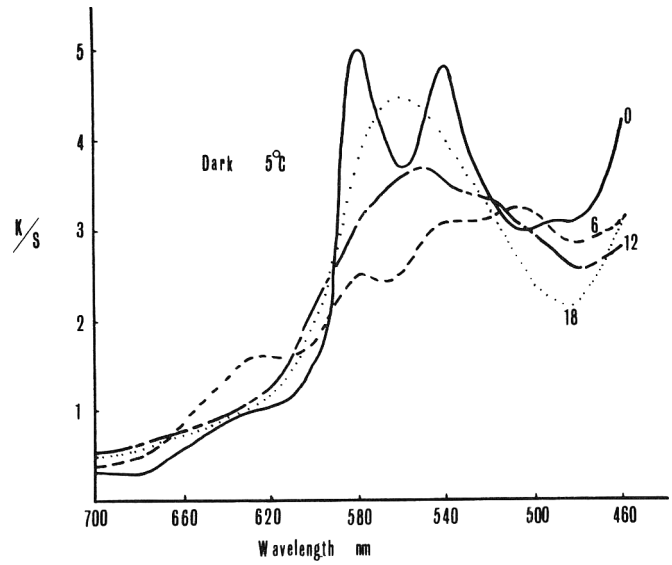

Fig. 1–Reflectance spectra, in K/S terms, of intact beef stored in the dark and at 5° C for 0, 6, 12 and 18 days.

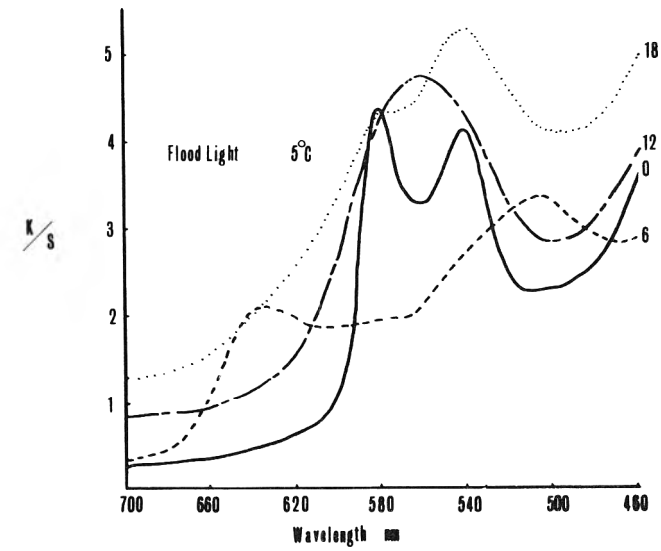

Fig. 2–Reflectance spectra of beef stored under a cool flood (incandescent) light-250 ft-c, at 5° C for 0, 6, 12 and 18 days.

lytic enzymes. The electrophoretic mobility of only heme proteins and free heme was determined, using the benzedine staining technique previously described by Haut et al. (1962).

RESULTS & DISCUSSION

A SURVEY of the local food markets indicated that the new stores, having the most up-to-date lighting facilities, have light intensities in their meat display cases which range from a low of 105 ft-c to a high of 260 ft-c. This study utilized a light intensity of 250 ft-c in order to see what effects these high light intensities had on the color stability of fresh intact beef.

The light sources used in this study were pink and soft white fluorescent, 150 watt cool flood and 100 watt incandescent. Red fluorescent was tried initially, but its inability to emit light of 250 ft-c intensity eliminated it from this study. The soft white source emitted light primarily in the region of 500–590 nm, the pink fluorescent light in the 540–565 nm region, and the incandescent lamps in the 800–1000 nm region of the spectrum.

Recording the reflectance spectra of each sample core from day zero through the 18th day allowed us to follow the appearance and disappearance of the various forms of myoglobin. Figures 1, 2 and 3 give reflectance spectra in terms of K/S units, for samples stored in the dark, under 150 watt cool flood incandescent and under soft warm fluorescent light. As shown in Figure 1, at day zero the surface pigment consists almost entirely of MbO_2. After 6 days storage in the dark, MetMb and MbO_2 are the predominant forms present. At 12 days storage, reduced myoglobin (Mb) is the predominant form present along with some residual MetMb. After 18 days storage, all of the pigment is in the reduced Mb form.

Figure 2 illustrates the rapid conversion of all MbO_2 found at day zero to MetMb in just 6 days. At the end of 12 days storage under the floodlight (250 ft-c), all MetMb had been converted to reduced Mb. Further storage indicated that some of the Mb was being oxygenated to yield some MbO_2.

Figure 3 illustrates the rapid loss of MbO_2 under the soft fluorescent light. Continued storage under this light (up to 18 days) converts the pigment to primarily MetMb with only minor amounts of MbO_2 present.

In trials where the meat was stored in the dark or under the incandescent flood, as soon as the bacterial load on the surface of the meat became sufficiently high (in excess of 10 million organisms per

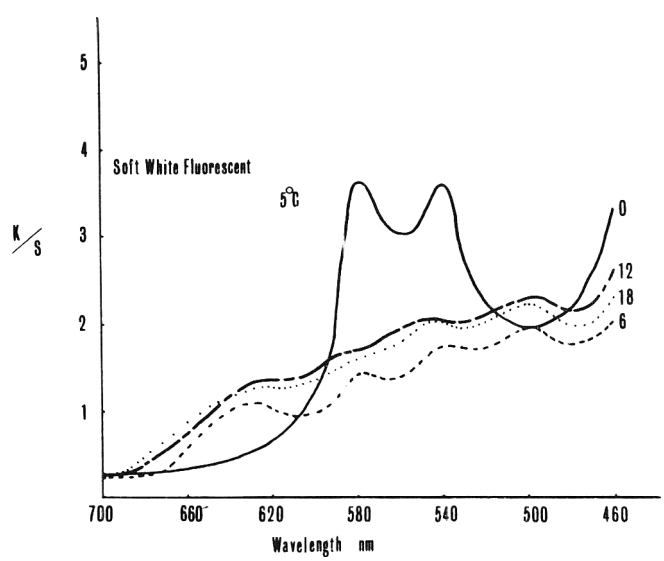

Fig. 3–Reflectance spectra of beef stored at 5° C, under soft white fluorescent light for 0, 6, 12 and 18 oays.

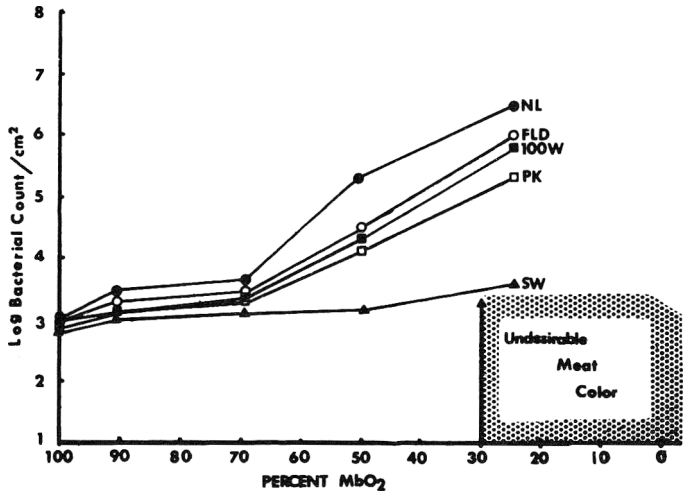

Fig. 4–The relationship of surface bacterial numbers to the % MbO_2 remaining on the meat surface, when stored under various light conditions. NL–no light (dark), FLD–incandescent flood, 100W–100 watt incandescent, PK–pink fluorescent, SW–soft white fluorescent.

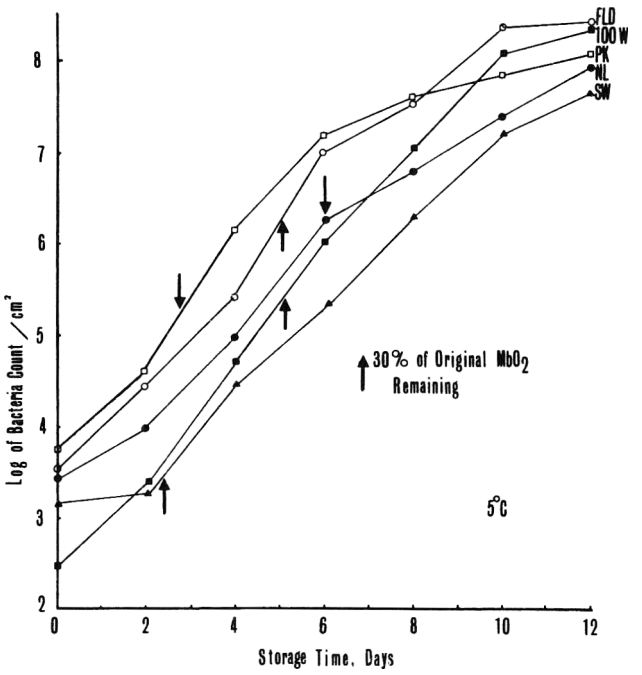

Fig. 5–Bacterial growth curves for beef stored at 5°C under various lighting conditions. NL–no light (dark), FLD–incandescent flood, 100W–100 watt incandescent, PK–pink fluorescent, SW–soft white fluorescent.

cm^2), the demand for O_2 on the meat surface became so great that all MbO_2 was deoxygenated to form reduced Mb. When the meat samples were stored under the soft white fluorescent light, no reduced Mb was formed, even after the bacterial load exceeded 10^7 organisms per cm^2. Since MetMb was the predominant myoglobin form present, and only a small amount of MbO_2 was present, the lack of O_2 on the surface had little effect on the major surface pigment, MetMb.

Figure 4 illustrates the relationship between bacterial growth and the loss of MbO_2 from the meat surface. A vertical arrow shown at 30% MetMb indicates the point at which the meat obtains an undesirable color (Daun et al., 1971). It can easily be seen on this figure that at the point where the meat turns light brown in color (30% remaining MbO_2) that the bacterial population can vary significantly. In the case of meat stored in the dark, the organisms are over one million per cm^2, whereas meat stored under soft white fluorescent light had only slightly more than 3,000 organisms per cm^2. Storage under incandescent and pink fluorescent lights allowed time for the bacterial load to reach 100,000 before the color had deteriorated.

The question now arises as to the effect of bacteria on the loss of MbO_2 and the formation of an undesirable brown color (MetMb) in meat. It is these authors' view that the primary factor causing the rapid loss of MbO_2 from the meat surface is the type of light, and the difference seen in bacterial load on the meat surface at time of discoloration is a function of the time it takes the MbO_2 to be converted to MetMb. Possibly, this can better be seen in Figure 5. Under soft white fluorescent light the color has deteriorated in only 2¼ days, whereas storage in the dark requires 6 days for color deterioration. The less severe lights (incandescent flood, 100 watt incandescent as well as pink fluorescent) require longer times for oxidation of the initial 70% of the surface MbO_2, thereby allowing time for the number of surface bacteria to become very great. The low wavelength light emitted by the soft white fluorescent lamps causes the autoxidation of the MbO_2 very rapidly, not allowing sufficient time for the growth of large numbers of surface bacteria.

Table 1 lists the autoxidation rate constants which describe the loss of MbO_2 from the surface of meat stored under various lighting conditions. The presence of light (250 ft-c intensity) definitely does enhance the autoxidation of the surface MbO_2. The effect of light of this intensity seemingly has a greater effect than does light of lower intensities (Solberg and Franke, 1971). Solberg and Franke (1971), using light of specified wavelengths and intensities varying from 46–200 ft-c, found light in the 500–600

Table 1–Autoxidation rate constant for beef exposed to light at 5°C

Light source	Intensity (ft-c)	Autoxidation rate constant (Hr^{-1})
No light	–	3.25×10^{-3}
Cool flood incandescent (150 watt)	250	5.16×10^{-3}
Incandescent (100 watt)	250	5.46×10^{-3}
Pink fluorescent (40 watt)	250	5.54×10^{-3}
Soft white fluorescent (40 watt)	250	8.20×10^{-3}

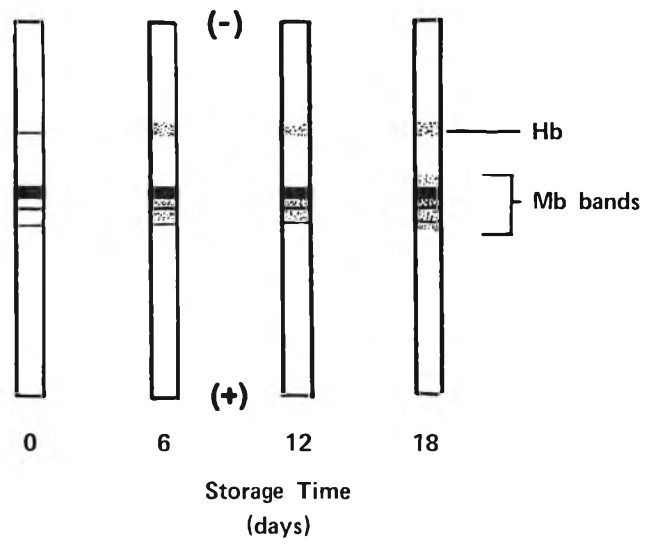

Fig. 6–Drawings of polyacrylamide gels with $K_3Fe(CN)_6$ oxidized pigment proteins (Mb and Hb) from the surface of beef samples stored at 5° C under incandescent light.

nm region had a small but significant increase in the autoxidation of the surface MbO_2. Setser et al. (1973) showed that visible light in the region of the spectrum from 474–600 nm enhanced the discoloration of intact beef muscle. The MbO_2 molecule absorbs light in 410 to 580 nm region of the spectrum, and that absorption of light energy could enhance the conversion of MbO_2 to MetMb.

The initial discoloration of the meat samples, from red (MbO_2) to brown (MetMb), when stored under lamps emitting low wavelength visible light, i.e., soft white fluorescent, is seemingly due almost entirely to the light. When meat is stored under less severe light (incandescent lamps), seemingly the light still has an effect but reduced in its severity.

Prolonged storage of the meat, under less severe lighting, will cause a reduction of the MetMb to form reduced Mb. This occurs after 10 days storage at 5°C and is probably entirely due to the lack of O_2 on the meat surface. The lack of surface O_2 is caused by the large numbers of bacteria (10 million organisms per cm^2) consuming most of the available surface O_2, thereby establishing a reducing atmosphere at the surface (Robach and Costilow, 1961; Leward et al., 1971). This does not occur when the meat is stored under soft white fluorescent light, very possibly because the low wavelength visible light will oxidize reduced Mb as fast as it is formed. It should be noted that once the MetMb was reduced to Mb and bacterial numbers exceeded 10^6 organisms per cm^2, the meat was unfit for consumption.

Finally, we were interested in seeing if during both short time and prolonged storage of meat, the pigment molecules (Mb and Hb) were structurally altered by either inherent or bacterial proteases. Figure 6 shows the drawings of polyacrylamide gels containing $K_3Fe(CN)_6$ oxidized pigment from fresh meat as well as meat stored as long as 18 days. As soon as 6 days storage, the MetHb and MetMb bands become slightly diffuse, but the diffusion does not seem to increase upon further storage. At no storage time was free heme noted on any gel, which would have indicated that the MetMb and MetHb had been destroyed.

CONCLUSION

BOTH incandescent and fluorescent lamps of 250 ft-c intensities enhance the destruction of surface MbO_2 of beef. The soft white fluorescent seemingly is most detrimental, incandescent being least detrimental. Prolonged storage under less severe lighting causes the meat to turn purple with the surface pigment being entirely reduced Mb. Very little, if any, proteolytic destruction of the surface pigment occurs during 5°C storage, even with storage up to 18 days and surface bacteria counts in excess of 10^8 organisms per cm^2.

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CONTINUOUS MICROWAVE STERILIZATION OF MEAT IN FLEXIBLE POUCHES

INTRODUCTION

EARLIER WORK by Kenyon et al. (1971) described the concept of microwave sterilization and the continuous microwave processor which was designed to carry out the experiments needed to verify the concept.

The current effort is aimed at progressing from the conceptual to the practical stage and has as its goal the establishment of processing parameters for a number of foods, principally beef, poultry and pork for use in military rations. This phase of the work was devoted to developing (1) a means for determining temperature distribution with the product; (2) a method for integrating time/temperature relationships in foods processed by microwave energy; and (3) a reliable flexible container as well as understanding the causes of package failure in the continuous microwave processor.

EXPERIMENTAL

Process

The process involves exposing food, packaged in sealed flexible pouches, to microwave energy on a continuous basis to achieve sterilization. Since temperatures on the order of 121°C or greater are obtained within the pouch during microwave processing, internal pouch vapor pressures, corresponding to temperature will exceed the rupture point of flexible pouches. An external air counter pressure in the range of 30–45 psig is therefore provided to maintain pouch integrity during heating. Thus, the equipment required five essential features:

1. An airlock for delivery of pouches on a semi-automatic basis.
2. An air pressurization system.
3. A variable speed conveyor to carry the pouches through the pressurized system.
4. A source of microwave energy.
5. A cooling section permitting a constant waterflow to reduce internal pouch temperatures below 100°C and pressure to 0 psig.

Equipment

The continuous microwave processor and its auxiliary equipment is that described by Kenyon et al. (1971) with the following modifications:

1. The aluminum sections on each end have been replaced by a similar design of welded steel having a design strength of 75 psig. Each section has a 45 psig relief valve and a rupture disc which will fail at 50 psig, as added safety features.
2. An automatic pressurizing system has also been added for ease of pressurization to a pre-determined over-pressure and for quick pressure release at termination of the heating cycle.
3. The conveyor belt is made of silicone rubber-coated fiberglass which has minimal stretch and is transparent to microwave energy.
4. All auxiliary equipment, including belt drive motor, speed control and viewing lights have been located outside the pressurized system.

A view of the processor (Litton Model C-10S-2 Manufactured by Litton Industries, Atherton Div, Minneapolis, MN) from the operating side is presented in Figure 1. The center section, with the doors opened, is the microwave cavity. The four waveguide outputs are shown, and their corresponding magnetrons and power supplies are below and behind an access panel. The epoxy impregnated fiberglass pipe which runs through the center of the cavity was added to the original machine to permit pressurization. The inlet section on the left side of the machine with its airlock is for semi-continuous delivery of pouches. The vertical cooling and discharge section is located at the right.

A typical process series, showing the approximate pouch positions during a run, is depicted in Figure 2. A constant load of three pouches is maintained in the microwave cavity section of the pipe by integrating pouch delivery intervals with belt speed resulting in a spacing of approximately 1m between pouches.

Product

Beef top rounds, USDA choice, were dry-heat roasted at 176.7°C to a center temperature of 60.0 ± 5.0°C. These were cooled overnight at 5.0°C and then sliced into 1/2 cm slices. Rectangular pieces, 5.2 × 14.6 cm weighing 50.0 ± 5.0g were cut from the center of each slice. Two of these were used per package. Unthickened gravy, made from roast beef drippings was used to adjust the weight of each pouch to 120.0 ± 0.2g. A minimum of six pouches were used per run.

Dummy load

The equipment used in this study is designed to simulate continuous operation and is

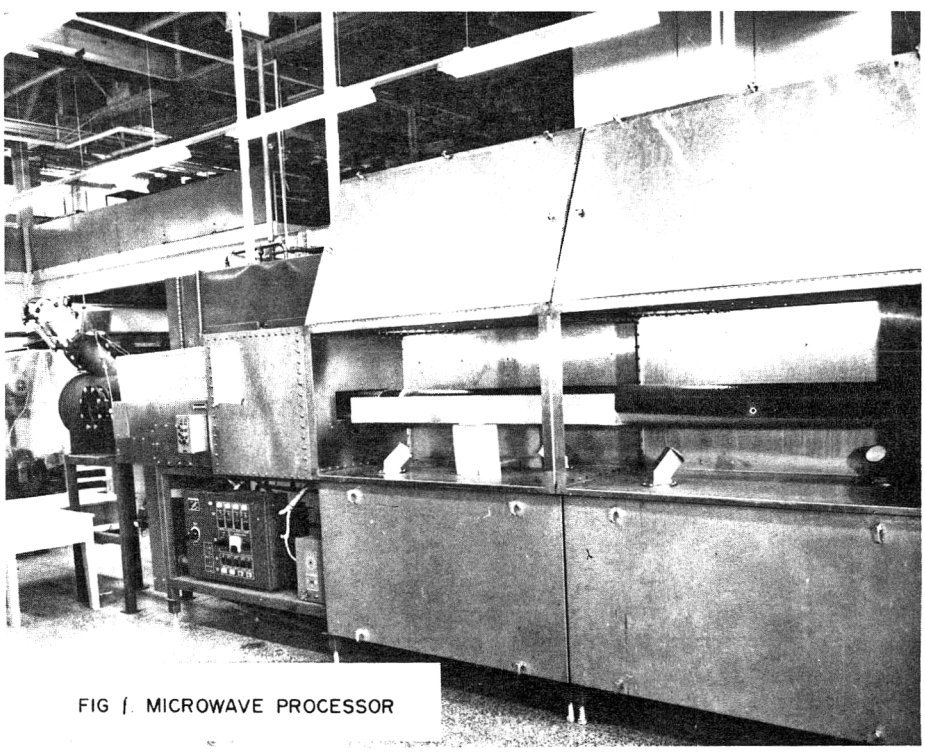

Fig. 1—Microwave processor.

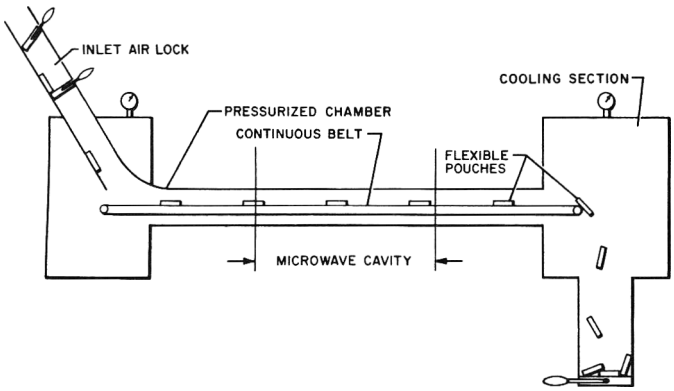

Fig. 2–Schematic of flexible processing.

limited as to the number of pouches which may be processed at one time. Therefore, no test pouches may be discarded. In order to assure that each test pouch received equal treatment, it became necessary to use a dummy load of three pouches to precede and follow the test product. This expedient assured that there was a full and uniform load whenever test pouches were entering or leaving the microwave cavity. Bentonite suspension (10% Bentonite–90% water) 120g per pouch was used as a dummy load to precede and follow pouches of beef.

Uniformity of distribution of energy, under continuous operation in a microwave cavity is dependent, to a large extent, on equal distribution of the load. In commercial practice, the first and last few pouches of product traversing the cavity would receive a process treatment different than the main body of the run and would probably be discarded.

Packaging material

A laminate (Continental Can Co., Wellesley, MA 02181) consisting of nonoriented polypropylene, 3.0 mils (Hercules Chemical Co.) with 0.05 mil mylar (duPont) was the most successful material used in this study. The material was fabricated into 7.0 × 17.5 cm pouches in our laboratory.

Insulation

Six layers of insulating paper ("Kim-Pak"–Kimberly Clark Co.) were double-wrapped around each pouch, resulting in an all-around wrap of twelve layers in order to reduce convective heat loss to the ambient temperature air used to pressurize the microwave processor.

Temperature distribution measurement

Paper thermometers (Paper Thermometer Co., 10 Stagg Drive, Natick, MA 01760) ranging from 98.9 to 148.9°C in increments of 5.0°C, having a sensitivity of ± 5°C and an accuracy of ± 1% of stated temperature, were sealed in various arrays inside polypropylene envelopes. The resulting thermogram covered the rectangular area of the slices of beef. These were placed between two slices of beef per pouch.

Time/temperature integration

Aliquots (0.5 ml) of an aqueous solution of dextrose (10.0%) and tryptone (4.0%) were heated in 1.5 × 4.0 cm polypropylene pouches in a Barnstead autoclave. The jacket temperature of the autoclave was raised to 132°C at the beginning of each cycle and the steam turned on for a period of 1 min to bring the chamber temperature to 121°C. Pressure was then adjusted to hold the chamber temperature to 121°C for the duration of each heating interval. The autoclave chamber pressure was released in 30 sec following each heating interval. Containers of the solution were heated for 3.0 min and then for 5.0 through 25.0 min in increments of 5.0 min, quickly removed from the autoclave and cooled in running tap water. The pouches were then opened and the contents transferred to "Klett" colorimeter tubes. Distilled water was added to bring the volume to 5.0 ml. The colorimeter was calibrated against a 400–450 μ blue filter using similarly diluted, unheated solution. Values of increasing optical density obtained from the colorimeter were multiplied by a factor of 10 to compensate for the tenfold dilution of the test solution, and plotted on semi-logarithmic paper to obtain a

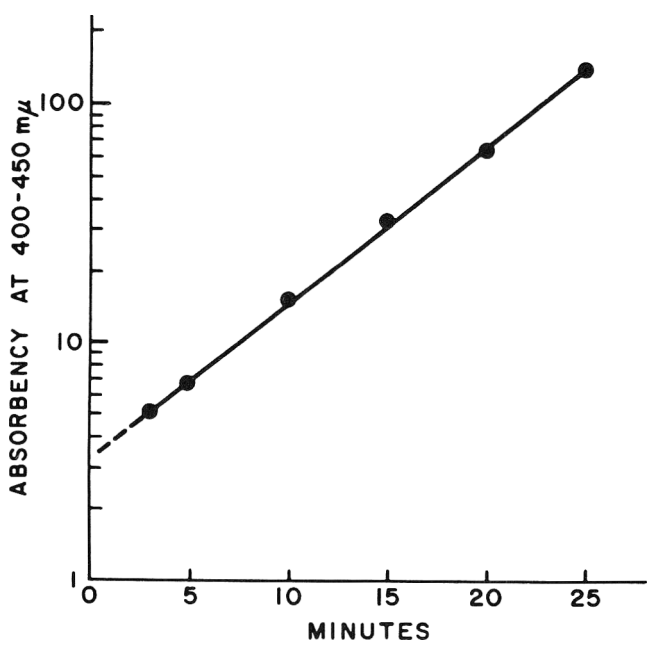

Fig. 3–Effect of heating a 0.5-ml aliquot of an aqueous solution of dextrose (10%) and tryptone (4%) at 121°C for various lengths of time on absorbency.

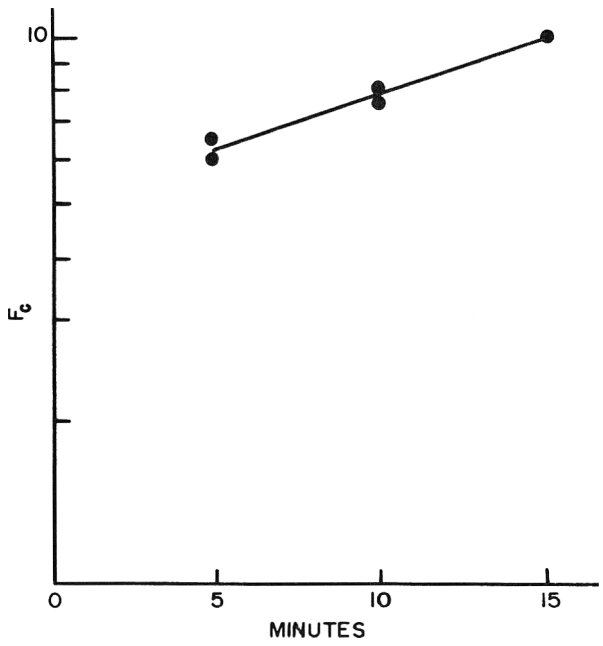

Fig. 4–Effect of post Rf exposure holding time on F_c values.

standard curve of the change in the solution's color intensity at 121°C with time.

Similarly, pouches containing 0.5 ml aliquots of the above solution were placed at the geometric center of the pouches of meat prior to processing with microwave energy. The post process magnitude of the solution's change in optical density is plotted on the standard curve (Fig. 3) for its corresponding time/temperature value. The value in this work is defined as F_c "the F value of all lethal heat received by the geometric center of a container of food during process" (Stumbo, 1965).

Product cooling

Cooling of product in the microwave processor was accomplished by allowing pouches to collect in the cooling section. They were then held at operating pressure for 5 min. Air pressure was then slowly exhausted for an additional 15 minutes to allow chamber pressure to equilibrate to ambient and the product temperature to reach 100°C or less. F_c values increased during this period (Fig. 4). An alternate approach was the use of insulated pouches held at operating pressure, following exposure to microwaves for various periods of time, and then cooled to less than 100°C by flooding the cooling section with cold tap water for a period of 2-1/2 min. A continuous flow of cooling water was obtained by opening the drain valve located near the top of the cooling chamber when the surface of the cooling water had risen above the discharge outlet. The air pressure within the processor, maintained during cooling, was rapidly reduced at the end of the cycle, simultaneously blowing out the cooling water.

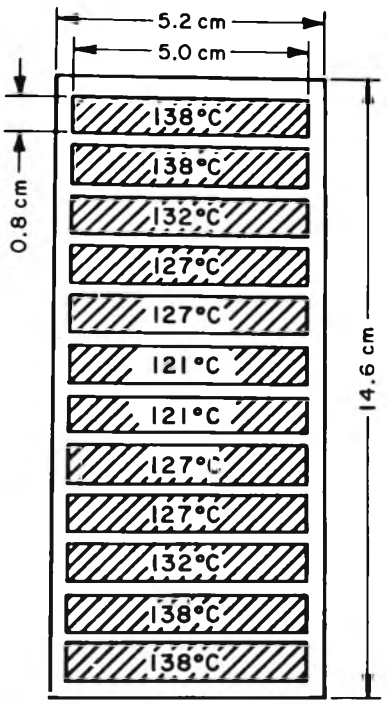

Fig. 5—Paper thermogram showing temperature distribution between two slices of beef 1/2 cm thick.

RESULTS & DISCUSSION

A KNOWLEDGE of the temperature distribution in a container of food receiving a thermal process is essential to the design of an adequate sterilization treatment. Paper thermometers were used to determine the nature of heat distribution obtained in foods heated by microwave energy (Fig. 5). Since paper thermometers are adversely affected by moisture, they were sealed in polypropylene/polyester pouches and placed between the two slices of beef inside each pouch. The paper thermometers turn black only if rated temperatures have been reached or exceeded. Therefore, a thermogram-like representation of the temperature distribution within the product was obtained. It became evident that in spite of the rapid heat rise in the product with application of microwave energy, the thermal pattern was essentially the same as that obtained by conventional thermal processing, i.e., the slowest heating point was at or near the geometric center of the product (Fig. 5), which confirmed earlier work by Copson (1962) and Decareau (1970). Watanabe and Tape (1969) observed that heating was nonuniform when wieners were processed at 2450 MHz and they also observed a slight improvement in heating uniformity when wieners were wrapped in moist paper towels. We observed that there was significant convective heat loss from the package to the unheated pressurized air which was being used to override vapor pressure build-up within the pouches during microwave processing. While the degree of heat loss was not directly determined, indirect evidence of its magnitude was obtained by wrapping the pouches in insulating material. It was determined that the reduction of temperature differential from package surface to ambient by use of insulation, was followed by a significant reduction in temperature differential from edge to center of the product. Prior to use of insulation the latter differential was at least 30.0°C. Following its use this was reduced to 5.0°C or less at the end of the process. That suppression of subsurface temperature rise occurs, when a temperature differential exists between substances being heated and ambient has been demonstrated by Stoll (1971). Stoll's results, expressed mathematically, agree with our empirical findings obtained with microwave heating at 2450 MHz.

Distribution of microwave energy is nonuniform in either a stationary cavity or one through which a product moves. However, moving product through a heterogeneous microwave field exposes the pouch to a greater uniformity of energy distribution than occurs in a stationary cavity. In addition, the use of insulation in conjunction with the above results in a nearly uniform thermal gradient within the product and increases the efficacy of the microwave process.

Prior to the use of insulating overwrap, the rate of belt speed required to obtain a product center temperature of 121°C was 2.5 ft per min (fpm). The use of the insulating overwrap enabled us to increase the rate up to threefold and still achieve sterilizing temperatures in the center of the product.

At this time, no reliable packaging material was available, nor was there an understanding of the causes of package failure. Testing of packaging materials was therefore pursued. It was found that a laminate of high density polypropylene and mylar eliminated the severest problems, those of pinhole development and weakening of the material due to temperature and pressure-induced stretching. This limited packaging problems in terms of this study, to seam failure, and the determination of processing conditions which would eliminate this cause of package failure. It was found that seam failure was a function of inadequate overpressure and that adequacy of overpressure was a function of initial product temperature and rate of product throughput (Table 1). Using six packages of beef per condition,

Table 1—Effect of overpressure, initial temperature and rate of throughput on container reliability[a]

Feet per minute	Initial temp 65.6°C Overpressure (psig)				Initial temp 76.7°C Overpressure (psig)			
	25	30	35	40	25	30	35	40
4.5	–	–	–	–[c]	–	–	–	–
5.0	–	–	–	+	–	–	–	–
5.5	+[b]	+[b]	–	+	–	–	–[c]	+
6.0	+[b]	+	+	+	–	+	+	+
6.5	+	+	+	+	+[b]	+	+	+
7.0	+	+	+	+	+	+	+	+

[a] (–) Package failed; (+) Package did not fail
[b] Packaging material distorted due to pressure within package exceeding overpressure
[c] Occasional package failure

Table 2—Effect of temperature differential among containers processed in the continuous microwave processor on F_c

Pkg no.	Product @ 76.7°C[a]—Dummy load @ 20°C[b] Belt speed (fpm)				Product @ 76.7°C[a]—Dummy load @ 76.7°C[b] Belt speed (fpm)			
	5.5	6.0	6.5	7.0	5.5	6.0	6.5	7.0
1	16.25	11.25	7.5	4.5	18.75	13.75	9.5	6.75
2	18.25	13.0	8.0	6.5	18.75	13.75	9.5	6.75
3	18.75	17.5	11.25	7.5	18.5	13.75	9.75	6.75
4	19.0	14.75	14.5	8.0	18.5	13.0	9.5	6.5
5	18.5	14.25	9.0	5.0	18.75	13.5	9.0	6.75
6	17.25	11.25	8.0	4.5	18.75	13.75	9.5	6.75

[a] Product preheated to 76.7°C to simulate hot fill
[b] Pouches of bentonite (10%) used to precede and follow pouches of beef as a dummy load

it was found that at the initial temperature of 65°C, a belt speed of 6.5 fpm was needed to prevent ruptured seams at an overpressure of 25 psig. At 30 psig, a rate of 6.0 fpm or faster prevents seam failure and 35–40 psig, 5.0 fpm or faster, results in package integrity. Similarly, a higher initial temperature requires an increase in the rate of throughput and/or greater overpressure to assure package reliability.

While Decareau (1970), Hamid et al. (1969), Jeppson (1964), Jackson (1947) and Watanabe and Tape (1969), demonstrated that sterilizing and pasteurizing temperatures can be readily achieved by application of microwave energy in foods, a means of integrating time with temperature in microwave processing was not available. Since calculation of a food preservation process by thermal means depends on the determination of time/temperature relationships (F), efforts to develop a means of integrating time/temperature in products processed by microwave energy were carried on concurrently with the above. (Time/temperature relationship at the slowest heating point in a product receiving a thermal process equal to 1 min at 121°C.) While establishing time/temperature conditions required for sterility, is easily accomplished in a conventional retort by means of thermocouples, this method does not lend itself to a continuous system where the product must be valved in and out of a pressurized, chamber approximately 30 ft long. In addition, the thermocouple wire would be heated by microwave energy giving false readings. As a result of the above considerations, it was decided that a chemical means of integrating time with temperature would provide the most suitable solution to the problem. Prior work with paper thermometers yielded data which indicated that the slowest heating point was the center of the product and that center temperatures on the order of 121°C could be achieved. In addition, the paper thermometers indicated that the edge temperatures of the product were considerably higher than the center as a result of exposure to microwave energy. It was assumed therefore that the process at the edges would be greater than at the center.

Since we had no means of determining time/temperature integration other than the above we made the following assumptions:

1. During microwave exposure the rates of increase of "F" at the edges is more rapid than the center.
2. During post microwave exposure holding time, although the temperatures of the insulated product tend to equilibrate, the surface remains somewhat hotter than the center and the F values at the edges will always be greater.

Although the paper thermometers did not provide adequate sensitivity and were not capable of integrating time/temperature, their use did provide the rationale for placing some sort of chemical integrating device at the slowest heating location of the mass. With these as considerations, a colorimetric method of integrating time/temperature was developed. Utilizing the Maillard (1913) reaction, and measuring the increase in browning of a sugar/protein solution with time at 121°C, a standard curve was established for this temperature, colorimetrically, which yielded a straight line on semi-logarithmic paper (Fig. 3). Any point along the curve represents an integrated time/temperature condition, or F. The temperature of 121°C was selected to establish the standard curve because this is the reference temperature for establishing a thermal process for commercial sterility. When pouches containing 0.5 ml aliquots of the solution are located in the center of the product and subjected to microwave heating, a degree of browning occurs in proportion to the thermal energy received at its location within the product (F_c). This change in color, measured optically, yields a numerical (colorimetric) value somewhere on the ordinate of the standard curve. A horizontal line drawn from this point on the ordinate will intercept the slope of the curve at some point. A vertical line drawn from the intercept to the abscissa will yield the F_c value directly, without recourse to calculations. With this means, we were able to relate the microwave sterilization process to conventional thermal processing. That browning reactions are a rate phenomena related to temperature has been frequently reported. Willets et al. (1958) used photometric means of measuring the rate of nonenzymatic browning with increasing pH, subsequent to heating at 114°C for 20 min. Lento et al. (1960) used photometric methods to demonstrate the time/temperature dependency of the lineality of browning reactions of a number of carbonyls at 100°C. While prior work has been done to demonstrate the lineality of some nonenzymatic browning reactions, to our knowledge the use of the browning reaction as a tool for integration of time/temperature relationships has heretofor not been used.

Incorporating this technique into our studies, it was noted that a rather wide divergence of F_c values were obtained in each of the six packages of meat from a run, and that the product at or near the beginning and end of each run had a lower F_c value than in the pouches which were centrally located in a series. This was repeatable at any rate of speed at which the product was processed (Table 2). The fact that the pouches of product were pre-heated to simulate a "hot fill" while the bentonite dummy load which preceded and followed the pouches of product, were held at ambient temperatures, suggested that microwave energy was being attracted to a greater degree by the dummy pouches which were cooler than the product pouches.

In order to verify this concept, the dummy load was pre-heated to the same temperature and otherwise treated the same as the beef prior to insertion in the microwave processor. The result was elimination of the difference in F_c from package-to-package of beef (Table 2). While additional work is required to gain further understanding of this phenomenon, the above appears to provide an empirical solution to the problem of vari-

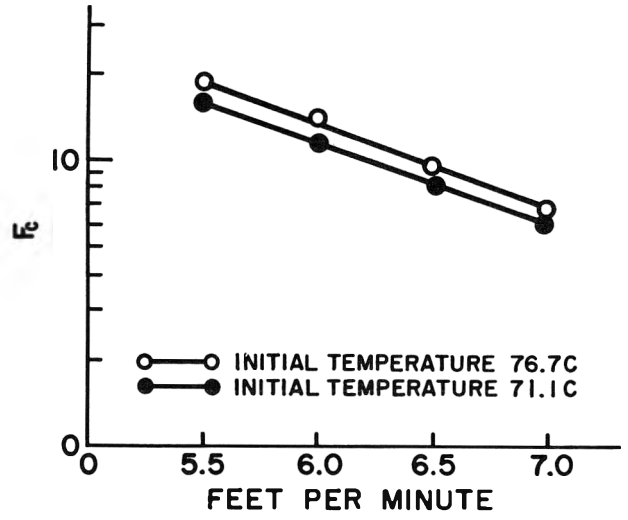

Fig. 6–Effect of initial product temperature on F_c.

Table 3–Effect of Rf energy exposure time on F_c

Rf energy exposure time (min)	F_c Initial temp 71.1°C	F_c Initial temp 76.7°C
1.39	6.0	6.7
1.49	8.0	9.5
1.62	11.2	13.2
1.76	15.5	18.7

ability of the sterilization process from container to container.

Additional variables that had a significant effect on F_c as determined in this study, were rate of throughput (Fig. 6), initial product temperature (Fig. 6) radio frequency (rf) exposure time (Table 3) and method of cooling (Fig. 4). When F_c Was plotted on the ordinate on semilogarithmic paper against rate on the was plotted on the ordinate on semilogarithmic paper against rate on the abscissa, it was found that, as expected, the F_c values increased as rate of throughput decreased and it appears that final temperatures achieved in the product as well as F_c are functions of the initial temperature (Fig. 6). It further appears that these phenomena are constants and should be useful in predicting process requirements to achieve product sterility.

Method of cooling and length of time the insulated product is held under operating pressure, prior to cooling with water, has a significant effect on F_c values (Fig. 4). The points on the curve represent two separate runs of six containers. F_c values from container-to-container within a run did not vary, but there was a variation from run-to-run of about 1/2 min in F_c at 5 and 10 min. No difference in F_c was obtained for the 15-min holding cycles. The variations obtained between runs were probably due to slight procedural differences that are bound to occur when manual operation is involved.

That F_c values within the insulation wrapped pouches increase with holding time is not surprising since the function of the insulation was to minimize heat losses and therefore reduce the temperature differential between the product and the pressurized, ambient temperature air, while allowing temperature equilibration throughout the product. Flooding the cooling chamber with cold tap water reduces the product temperature to below 100°C in less than 2-1/2 min.

Product temperatures in excess of 100°C in flexible packages can only be obtained in a pressurized system. It is clear (1) in a system that is pressurized by ambient temperature air, thermal loss due to the temperature differential between product surface and air is significant; that (2) some means, such as heated air or insulation is needed to minimize this differential; and that (3) the use of insulation results in the retention of microwave induced thermal energy without the cost of additional energy inputs. This makes insulation an attractive means of complementing the process. For commercial application, the pouches may be fed through the microwave cavity between two closely fitted parallel insulated belts, thus eliminating the need for individually wrapping each pouch.

The mylar-polypropylene laminate used in this study is an inadequate gas barrier for long term storage and a metallic-laminate overwrap would therefore be required after processing to insure product stability.

The foregoing demonstrates the feasibility of a microwave induced thermal process for the preservation of foods. While the purpose of this study was basically, to comprehend processing parameters, observations of beef receiving a sterilizing process by microwave energy indicated that an excellent product could be obtained. Studies comparing quality of meat items receiving a conventional thermal process vs. microwave processing will be carried out in our laboratory.

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EFFECT OF FRYING AND OTHER COOKING CONDITIONS ON NITROSOPYRROLIDINE FORMATION IN BACON

INTRODUCTION

RECENT SURVEYS have demonstrated that the presence of nitrosamines in very low concentrations in various types of cured meat products occurs in a random manner (Fazio et al., 1971a; Sen, 1972; Wasserman et al., 1972; Crosby et al., 1972; USDA, 1972). In bacon, however, nitrosopyrrolidine (NO-Pyr) has been found and confirmed in a very high percentage of fried, ready-to-eat samples (Crosby et al., 1972; Sen et al., 1973; Fazio et al., 1973). This is the first instance of the consistent occurrence of a nitrosamine in a food product. Since approximately 1.5 billion pounds of bacon are consumed annually, the presence of a nitrosamine—even in low concentrations—is a matter for concern.

The origin of NO-Pyr in bacon is still unknown; however, it has been suggested that it could be produced by the decarboxylation of nitrosoproline, which is present in the tissues (Lijinsky and Epstein, 1970). We are reporting the effect of various cooking methods and frying temperatures on the formation of NO-Pyr in bacon and on a possible mechanism for the formation of NO-Pyr from a precursor in bacon.

EXPERIMENTAL

Decarboxylation of nitrosoproline

Powdered nitrosoproline (0.05g, 0.347 mmoles) was added to a 100 ml single neck round-bottom flask containing 25 ml silicone oil. The flask was heated at various temperatures with stirring for 10 min in a constant temperature oil bath. After cooling, the silicone oil reaction mixture was extracted in a 125 ml separatory funnel three times with 30 ml H_2O using a 3 min shake, and the extracts were combined in a 250 ml separatory funnel. The water extracts were in turn extracted three times with 100 ml CH_2Cl_2 with gentle shaking for 3 min and the CH_2Cl_2 layers combined. The CH_2Cl_2 extracts were washed by shaking for 1 min with 50 ml 6N HCl followed by 50 ml 5N NaOH then concentrated to 1 ml using a Kuderna-Danish apparatus equipped with a three-section Snyder column. Nitrosopyrrolidine was then determined by gas-liquid chromatography.

To test the efficiency of the extraction procedure 5 mg of NO-Pyr was added to 25 ml of silicone oil and carried through the entire process. A final recovery of 50% was achieved.

Commercial survey

One-pound packages of commercial bacon were purchased in local retail markets. The bacon was fried until medium-well done in a preheated Presto teflon coated electric frying pan Model PC04AT, requiring 5–6 min at a calibrated thermostat setting of 176.7°C. The bacon slices were turned at intervals to ensure even cooking. The drippings and a portion of the uncooked bacon were saved for subsequent nitrosamine analyses.

Cooking methods

In studying the various methods of cooking, bacon was fried for 6 min in a preheated frying pan at 176.7°C or for 9 min starting with a cold pan set at 176.7°C. Bacon was also broiled for 4.5 min on a rack set 5–6 in. below the heat source (ca 305°C) or baked for 13 min at 204°C in a shallow tray placed in the center of a preheated Tappan Visualite electric oven Model EOKLV-31-3C. Using a Westinghouse "baconer" Model HBB202A, bacon was draped over a teflon-coated heating element and cooked for 7 min with the thermostat dial set between "crisp" and "more crisp" (ca 241°C). Microwave cooking of the bacon was done with a Litton Industries microwave oven Model 550 for 1 min in a rectangular cardboard tray. In all cases bacon was cooked until medium well done.

Analytical procedures

The bacon samples were analyzed for the following nitrosamines: dimethylnitrosamine, methylethylnitrosamine and diethylnitrosamine, nitrosopiperidine, nitrosopyrrolidine and nitrosomorpholine using a modification of the method of Fazio et al. (1971b) for the multidetection of volatile nitrosamines. Basic alumina (Camag, Brockman No. 1) washed with 5 to 15% CH_2Cl_2 in hexane and a CH_2Cl_2 elution solvent was used instead of silica gel in the column chromatographic clean-up procedure. The average recovery of nitrosopyrrolidine in an aliquot of the same sample with 20 ppb added was 70% for both the fried bacon and the fat drippings.

Gas-liquid chromatography

The nitrosamines were quantitated by gas-liquid chromatography using a Varian-Aerograph Model 1740-1 modified for use as an alkali flame ionization detector with a potassium chloride coated coil (Howard et al, 1970). The sample was separated on a 10 ft × 1/8 in. o.d. stainless steel column packed with 13% Carbowax 20M-TPA on 60–80 mesh Gas Chrom P equipped for on-column injection. The flow conditions used were: helium 50-, hydrogen 58- and air 200 ml/min. Hydrogen flow and, to a lesser extent, air flow were adjusted slightly from time to time in order to maintain desired detector sensitivity. Electrometer range used was 10^{-12} amp/mv injector port and detector temperatures were 185 and 220°C, respectively; column temperature was programmed from 105 to 200°C at 4°/min for the determination of the six nitrosamines. For the model system experiment involving the formation of NO-Pyr from NO-Pro an isothermal temperature of 170°C was used.

GLC-mass spectrometric analysis

For confirmation of the identity of the nitrosamines a Varian-Aerograph Model 1740-1 gas-chromatograph equipped with a 5 ft × 1/8 in. o.d. stainless steel column packed with 3% GC SE-30 on 100–120 mesh Varaport 30 was connected to a DuPont Model 21-492 mass spectrometer. The gas flow rates used were: helium 38-, hydrogen 40-, and air 400 ml/min. The temperatures used were: column-programmed from 100 to 170°C at 6°/min; detector, 200°C; and injector port, 190°C. The column effluent was split 1:1 with one-half going into a flame ionization detector and the other half passed via an inlet line heated at 190°C into the mass spectrometer operated in the peak matching mode adjusted to a resolution of 1 in 12,000 as described by Dooley et al. (1973). The mass spectra were obtained at an ionizing voltage of 70 ev and an ion source temperature of 150°C. The mass-to-charge ratio (m/e of 100.06366) for NO-Pyr was determined using the m/e 99.99361 reference peak of perfluorokerosene by measuring the difference in m/e. The signal was recorded on both an oscilloscope and a recording oscillograph.

All samples having a gas chromatography alkali flame ionization detector response with the same retention time as an authentic sample of NO-Pyr were checked by mass spectrometry. In general, 10 ppb NO-Pyr or greater could be confirmed using this procedure. Where there was no positive confirmation due to an insufficient concentration of nitrosamine or the presence of interfering background components, values are presented as apparent NO-Pyr and are used for comparative purposes only, with the understanding that the material may not be NO-Pyr.

RESULTS & DISCUSSION

THE RESULTS of analyzing bacon from six processors for the six volatile nitrosamines are shown in Table 1. Only NO-Pyr was found in confirmable concentrations in bacon fried at 176.7°C and its drippings. Other workers (Sen et al., 1973; Fazio et al., 1973) have found a major portion of the NO-Pyr in the cooked-out fat. Our observation, and that of Fazio et al. (1973), that no NO-Pyr is

present in uncooked bacon suggests that this compound is formed during the frying stage.

The fact that the rate of nitrosation is greater for proline than pyrrolidine (Mirvish, 1973) suggests that nitrosoproline (NO-Pro) is formed first followed by decarboxylation during frying to yield NO-Pyr. To determine the conditions under which decarboxylation does occur, and establish the possibility of NO-Pro as a precursor of NO-Pyr, NO-Pro was heated for 10 min in silicone oil at different temperatures. The results, in Figure 1, indicate that the maximum production of NO-Pyr occurs at 185°C, which is approximately the temperature recommended for frying bacon. Little or no NO-Pyr formed at 100°C even when heating was carried out for 20 min. Reducing the heating time to 5 min at 185°C still produced more than 2g NO-Pyr/mole NO-Pro. This model system study indicates that the conditions of frying would be important if NO-Pro is a precursor for NO-Pyr in bacon.

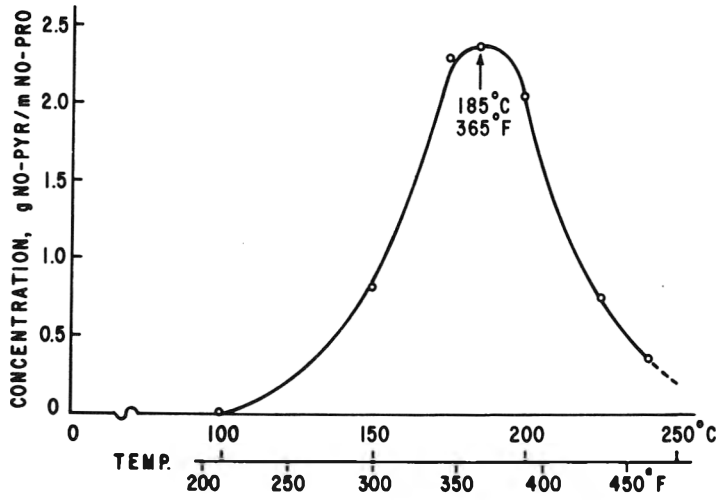

Fig. 1–Decarboxylation of nitrosoproline (NO-Pro) to nitrosopyrrolidine (NO-Pyr) in a model system.

The correlation of time and temperature of frying with the amount of NO-Pyr formed in bacon is shown in Table 2. Bacon from two bellies was obtained from a normal production run of a local meat processor to minimize differences due to composition or processing conditions. Samples from one belly, immediately after production, were fried at various time and temperature combinations until they were medium well done, or they were fried for 10 min at each of several temperatures. When bacon was fried to a visually determined degree of medium well done, increasing the temperature from 99–204°C required times varying from 105 to 4 min, respectively. No NO-Pyr was formed at 99°C, but higher temperatures produced increasing concentrations, reaching a level of 17 μg/kg (ppb) at 204°C. Bacon fried for 10 min at 99°C was still raw and contained no NO-Pyr. Increasing the temperature, at a constant exposure of 10 min resulted in darker, crisper bacon with formation of increasing concentrations of NO-Pyr. The bacon fried at either 99° or 204°C for 10 min would be unacceptable to most people on the basis of sensory quality.

The effect of the age of the bacon was also investigated by repeating the tests with the bacon from the second belly stored at 1.7°C for 2 wk. Although the concentrations of NO-Pyr formed after 2 wk storage appear to be somewhat lower than the amounts found in fresh bacon, it is not possible at this time to draw any conclusions from the effect of storage.

Even though bacon is usually prepared by pan frying, other methods of cooking have been recommended. A number of processors include directions on the package for baking or broiling bacon. The results of an investigation of the effects of various cooking methods on NO-Pyr formation in bacon prepared until medium well done are shown in Table 3. Our standard frying procedure again produced high yields of NO-Pyr; essentially none was formed with microwave cooking. This may be the result of the very short exposure time of bacon to heat in the microwave oven. Our data confirmed the results recently reported by Herring (1973). Baking, broiling and the "baconer" produced variable quantities of NO-Pyr in the bacon. However, it appears that the "baconer" may produce slightly

Table 1–Nitrosopyrrolidine (NO-Pyr) found in commercial bacon

Samples	Residual $NaNO_2$ (ppm)	NO-Pyr, ppb (Uncorr)[a] Raw	Fried	Drippings
A	89	0	11	16
B	96	0	13	39
C	97	0	11	24
D	39	0	19	23
E	53	0	29	19
F	–	0	38	32

[a] Confirmed by M.S.

Table 3–Effect of cooking methods on nitrosopyrrolidine (NO-Pyr) formation in bacon

Cooking method	NO-Pyr, ppb (Uncorr) Sample 1	2	3
Raw	0	0	–
Fried (cold pan)	9	17[a]	11[a]
Fried (hot pan)	5	20[a]	19[a]
Bake	35[a]	13[a]	12[a]
Broil	12[a]	12	14[a]
Baconer	9	7[a]	16[a]
Microwave	2	0	3

[a] Confirmed by M.S.

Table 2–Effect of frying conditions on nitrosopyrrolidine (NO-Pyr) formation in bacon

Frying conditions Temperature (°C)	(°F)	Time (min)	Degree	NO-Pyr, ppb (uncorr) Storage time–wk @ 1.7°C 0	2
99	210	105	Med. well	0	0
135	275	30	Med. well	8	5
176.7	350	6	Med. well	10[a]	6
204	400	4	Med. well	17[a]	7
99	210	10	Raw	0	0
135	275	10	V. light	0	0
176.7	350	10	Well	15	7
204	400	10	Burned	19[a]	16[a]

[a] Confirmed by M.S.

less NO-Pyr as shown in samples 1 and 2.

It is important to note that the 35 ppb value reported for the first baked bacon sample was confirmed by high resolution mass spectrometry. However, the mass spectrometer response for NO-Pyr, on a semiquantitative basis, was not as great as expected for this concentration of nitrosamine. It is possible that the gas chromatograph was responding to an additional component in the bacon sample which eluted at the same time as NO-Pyr. This points up the need for specific confirmation procedures to avoid erroneous interpretations of nitrosamine detection data alone.

(Precautions should be exercised in the handling of nitrosamines, since they are potential carcinogens.)

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RELATIONSHIP OF AGE TO GENERAL COMPOSITION, SKIN THICKNESS, PHOSPHOLIPID CONTENT AND ENZYMATIC ACTIVITY IN TURKEYS

INTRODUCTION

THE PURPOSE of lipids in muscle is manifold. Certain lipids contribute to the structural integrity of muscle cell membranes (White et al., 1968), and others provide an energy reserve. They also have insulating value. Lipids are involved in the organoleptic properties of meat, including "mouth feel" and juiciness; in meat animals, they contribute directly to the grade or quality measure of carcasses. Thus, the presence of various types and amounts of lipids affects flavor, texture, color, nutritive value and deteriorative potential of meat.

Turkey muscles normally contain 0.8–3.5% lipid (Acosta et al., 1966; Neudoerffer and Lea, 1968), but processors may increase this amount by injecting certain chemicals known to produce carcasses of improved eating quality. Several interesting relationships are worth noting here. First, the normal lipid content of muscle tissue seems dependent on age of the bird (Acosta et al., 1966; Wangen and Skala, 1968; Wangen et al., 1971) and type of muscle tissue (Acosta et al., 1966; Wangen and Skala, 1968; Marion, 1970; and Wangen et al., 1971). Second, diet influences the degree of finish in turkeys (Neudoerffer and Lea, 1968), and the degree of finish affects the yield of edible meat (Essary et al., 1968). The fatty acid composition of the ration, moreover, is known to affect the fatty acid composition of adipose tissue and skin (Mickelberry et al., 1966; Osborn et al., 1969; and Carlson et al., 1969), but composition of the ration has little effect on the fatty acid distribution in muscle tissue (Marion and Woodroof, 1966; Chung et al., 1967; Neudoerffer and Lea, 1968). Long-chained polyunsaturated fatty acids normally are found in turkey muscle in substantial quantities (Fishwick, 1968; Wangen et al., 1972). Their presence is important because of their nutritional role and potential for deterioration.

The purpose of this research was to study the lipid composition and lipid depositing potential of selected muscles in the turkey as estimated by a study of certain enzyme systems.

EXPERIMENTAL

Muscle sample

The muscle samples used in this experiment were obtained from 32 Large White (Nicholas) male turkeys grown at the Iowa State University Poultry Center. They were grown in confinement and allowed access ad libitum to a commercial ration.

Processing method

Beginning at 12 wk of age and ending at 24 wk, eight turkeys were selected at random at 4-wk intervals for processing. The turkeys were exsanguinated by severing the jugular vein and carotid artery without previous stunning. Shortly after death, samples of Pectoralis major, Biceps femoris, Gastrocnemius and wing muscles were excised and prepared for histological examination. The muscle strips were placed in liquid nitrogen within 5 min of the start of exsanguination. Similar muscles from the opposite side of the carcass were excised, frozen and kept for additional analyses.

Proximate analyses

Muscle samples were freed of skin and adhering lipid deposits and passed through a food grinder with plate perforations of 4 mm. Weighed portions of the ground samples were dried under vacuum at 90° ± 4°C for 24 hr for moisture determination. Nitrogen content was determined by micro-kjeldahl and protein content was calculated from the resulting data.

Lipid extraction

Samples (20g) of ground muscle were twice extracted with cold chloroform-methanol (2:1) by using the procedure of Folch et al. (1957). The two extracts were combined, washed with 0.03M $MgCl_2$, evaporated under reduced pressure, and adjusted to 25 ml volume with chloroform. Lipid extraction was done at 4°C. Lipid content of meat samples was determined by drying aliquots of lipid extract to a constant weight at 80°C.

Phospholipid determination

50 μliters of the total lipid extract were transferred to a test tube, from which solvent was evaporated with a stream of air. The dried extract was digested and phosphorus was determined by using the method of Chen et al. (1956). Phospholipid content was determined by multiplying the phosphorus content by 25.

Histological techniques

Fresh muscle tissue was cut with a razor blade to the approximate size for histological examination. Care was taken so that the blade touched no more than one muscle of any bird. After freezing, the samples, along with some ice flakes to prevent dehydration, were placed in a precooled (liquid N_2) plastic bag, and held at −25°C until analyses were run. Samples were mounted on crystal posts and sectioned at 20μ in a −20°C cyrostat. Sections were transferred to a precooled watch glass containing 2–3 drops of precooled incubation media. The sections froze upon contact, but thawed readily when incubation started. Incubation for the enzymes ranged from 15–60 min at 37°C in a moistened atmosphere. Incubation media were prepared after the techniques of Barka and Anderson (1963), but only 5 mg/ml of 6-phosphogluconate was prepared because it represents a saturated solution (Nachlas et al., 1958). A 0.1M phosphate buffer, adjusted to pH 7.0, was used, and 0.1M cyanide inhibitor was included in the incubating media. Enzymes studied in this experiment included β-hydroxybutyrate dehydrogenase (BHBD), glucose-6-phosphate dehydrogenase (GPD), 6-phosphogluconate dehydrogenase (PGD) and lactate dehydrogenase (LD). To test the incubating media, pork muscle was used as a control. Results are reported as percentages of enzyme-positive fibers.

Enzyme analyses

Approximately 3–5g of muscle tissue were used to prepare mitochondria according to the procedure by Lundquist and Kiessling (1967). A tris-phosphate buffer (pH 7.5) was mixed with the muscle tissue in a Potter-Elvehjem homogenizer (Kontes). Muscle mitochondria were obtained by differential centrifugation (10 min at 1000G and at 10,000G for 20 min). The precipitate which occurred at 5°C was saved. Mitochondria were frozen to lyse the walls and then incubated in a Biological Oxygen Monitor at 37°C. The mitochondrial fraction, plus buffer as a control, was added to one cell while buffer, mitochondria and substrate were added to the other cell.

About 3g of muscle tissue with 0.25M sucrose were minced in a Potter-Elvehjem homogenizer. The contents were centrifuged at 1000 × G for 15 min to remove unbroken cells and other fragments. After this separation, the supernatant was used to determine the activity of LD and GPD. GPD activity was determined in the Biological Oxygen Monitor at 37°C using the reaction medium described by Dawson and Romanul (1964). The reaction mixture used for lactate dehydrogenase also was prepared after the procedure of Dawson and Romanul (1964), but its activity was measured spectrophotometrically at 340 nm, at 26°C. The amount of protein was determined by the Biuret technique of Torten and Whitaker (1964).

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Statistical analysis

Least-squares analysis was used to estimate age, muscle type and other effects according to the procedures of Snedecor and Cochran (1967). In addition, Duncan's Multiple Range Test (Steel and Torrie, 1960) was used.

RESULTS

THE AVERAGE WEIGHTS of turkeys used in this experiment are shown in Table 1. Skin thickness at two locations increased with increasing age (Table 2) with the most rapid increase occurring after the turkeys were 20 wk old. Thickness values appear small in magnitude because moderately stretched skin was measured with a spring-loaded micrometer. There is an implication, nevertheless, that fatness (finish) of the turkey can be estimated by measuring skin thickness.

Proximate analyses of the tissues studied are reported in Table 3; see Table 4 for statistical analyses. The results show that concentrations of lipid, protein and moisture were significantly influenced by muscle type. Moreover, lipid and moisture content changed significantly with age, while protein content remained constant.

Lipid concentration was significantly affected by age and muscle type. At 24 wk of age, lipid contents of the Biceps femoris and Gastrocnemius were not significantly different, but their lipid contents differed significantly from those of the Pectoralis major and wing muscles. The Pectoralis major and wing muscles were similar in lipid content, and both had significantly lower lipid concentrations than the Biceps femoris and Gastrocnemius.

Protein content did not change with age, but was affected by muscle type (Table 4). The Pectoralis major contained the greatest concentration of protein, followed by wing muscles. Protein levels of Biceps femoris and Gastrocnemius were not significantly different, and they were consistent with increasing age.

A significant effect of age and muscle type on moisture levels was shown by the analysis of variance (Table 4). Among muscles, Pectoralis major had the lowest moisture levels (Table 3). Moisture in wing muscles was significantly greater than that of the Pectoralis major, but was comparable to the moisture levels of the Gastrocnemius and Biceps femoris.

The distribution of phospholipids in turkey muscles at various ages is shown in Table 5. Most observations were within the range of 600–700 mg/100g tissue, except for turkeys at 24 wk, at which time breast and wing samples had phospholipid values as low as 475 mg/100g tissue. Age and tissue effects were statistically significant (Table 6). There was considerable variation, however, from one age period to another and from one muscle to another. When expressed as a

Table 1—Weight of tom turkeys at various ages

Age (wk)	Weight (kg)
12	5.51 ± 0.18[a]
16	8.60 ± 0.32
20	9.98 ± 0.38
24	13.03 ± 0.30

[a] Mean and standard error based on eight turkeys in each age group.

Table 2—Skin thickness (mm) of turkeys at various ages

Age (wk)	Location	
	Beard	1 in. from beard[a]
12	0.019b	0.034b
16	0.038b	0.051b
20	0.048b	0.092b
24	1.096c	2.479c

[a] Toward proximal end of sternum. Means followed by the same letter do not differ significantly at the 0.01 level.

Table 3—Proximate analysis of muscles of turkeys at various ages[a]

Muscle type	Age (wk) 12	16	20	24
			Lipid (%)	
Pectoralis major	0.87f	1.00e	0.91f	1.11d
Biceps femoris	1.54d	1.42d	1.69c	1.83b
Gastrocnemius	1.64c	1.60d	1.94a	1.84b
Wing[b]	1.24d	0.95ef	0.88f	0.89f
			Protein (%)	
Pectoralis major	24.18a	23.87a	24.13a	23.78a
Biceps femoris	19.96c	20.19c	19.61c	19.78c
Gastrocnemius	19.86c	19.84c	19.04c	19.33c
Wing[b]	21.91b	22.31b	22.05b	22.16b
			Moisture (%)	
Pectoralis major	73.77e	73.33e	73.37e	74.22de
Biceps femoris	77.09a	76.09abc	76.38ab	76.44ab
Gastrocnemius	76.82ab	77.03a	76.85ab	76.97ab
Wing[b]	76.00bc	75.10cd	75.35c	76.03bc

[a] Means based on eight turkeys in each age group. Means followed by the same letter do not differ significantly at the 0.05 level.
[b] Wing represents a composite of muscles of the upper wing.

Table 4—Analysis of variance of proximate analysis data

Source of variation	df	Mean Squares Lipid	Protein	Moisture
Age (A)	3	0.166**	0.804	2.504*
Muscle type (M)	3	5.390**	138.107**	66.654**
AM	9	0.202**	0.484	0.714
Error	112	0.053	0.682	0.471

* Significant at 0.05 level
** Significant at 0.01 level

Table 5—Phospholipid content of muscles of turkeys at different ages[a]

Muscle type	Age (wk) 12	16	20	24
			Phospholipid (mg/100g tissue)	
Pectoralis major	672.92	714.96	615.16	474.50
Biceps femoris	654.03	672.61	662.70	691.10
Gastrocnemius	670.28	657.16	629.50	640.00
Wing[b]	606.82	640.63	621.61	475.00
			Phospholipid (% of total lipid)	
Pectoralis major	66.88a	62.53ab	58.11abc	42.89efg
Biceps femoris	52.99bcde	55.50bcd	45.50defg	39.44fg
Gastrocnemius	48.60cdef	46.98defg	37.78fg	36.82g
Wing[b]	53.61bcde	63.61ab	59.95ab	53.91bcde

[a] Means based on eight turkeys in each age group. Means followed by the same letter do not differ significantly at the 0.05 level.
[b] Wing represents a composite of muscles of the upper wing.

percentage of total lipid, phospholipids showed significant age, tissue type and interaction effects (Table 6). With either method of expressing phospholipid concentration, this parameter was negatively related to age of turkey. Biceps femoris and Gastrocnemius phospholipid contents were not significantly different at different ages, nor were breast and wing phospholipids except at 12 wk. Wing and Pectoralis major had the greatest concentrations of phospholipids.

Means for LD and BHBD enzyme responses for various muscles at different ages are presented in Table 7. Considering the data, basic tissue differences were suggested in that enzyme responses in Pectoralis major were smaller with one exception than those in Biceps femoris and Gastrocnemius. Enzyme responses for Biceps femoris and Gastrocnemius were similar, or at least not significantly different, within the age periods studied (Table 8). The largest change in enzymatic activity involved LD at 24 wk of age.

GPD and PGD were detected only on occasion in the muscles used, and when detected, the response was weak. These enzymes have been detected in pork muscles by Bodwell et al. (1965) and in pigeon Pectoralis red fibers by George and Berger (1966). Both are key enzymes in the pentose phosphate pathway, which is known to function in the generation of reduced nicotinamide adenine dinucleotide phosphate (NADPH) for lipogenesis.

Table 6—Analysis of variance for phospholipid data

Source of variation	df	Mean squares: Total Phospholipid	Mean squares: Phospholipid (%)
Total phospholipid			
Age (A)	3	99995.50**	1036.26**
Muscle type (M)	3	345713.80**	1634.34**
AM	9	6860.54	206.48**
Error	112	6792.14	58.89

** Significant at 0.01 level

Table 7—Average enzymatic activity and proportion of fibers positive for enzymes in muscles of turkeys at different ages[a]

Muscle type	Age (wk) 12	14	20	24
	LD activity (moles NADPH/min/mg protein)[b]			
Pectoralis major	–	128.4b	102.0b	98.09b
Biceps femoris	–	95.3b	113.0b	1240.00a
Gastrocnemius	–	76.0b	116.0b	1211.00a
	BHBD Activity ($\mu l/0_2$/hour/10 mg mitochondrial protein)[b]			
Pectoralis major	1.74de	1.72e	2.28bcde	1.99cde
Biceps femoris	2.65abc	2.61abcd	3.04ab	2.46abcce
Gastrocnemius	2.16cde	2.67abc	3.21a	2.66abc
	Fibers positive for LD (%)[b]			
Pectoralis major	–	–	–	–
Biceps femoris	47.62a	40.39a	51.75a	52.18a
Gastrocnemius	43.10a	48.39a	49.44a	48.41a
	Fibers positive for BHBD (%)[b]			
Pectoralis major	–	–	–	–
Biceps femoris	30.45a	42.72a	44.33a	45.83a
Gastrocnemius	31.57a	43.11a	42.27a	44.71a

[a] Means based on eight turkeys in each age group. Means followed by the same letter do not differ significantly at the 0.05 level.

[b] Abbreviations: LD—lactate dehydrogenase; NADPH—nicotinamide adenine dinucleotide phosphate (reduced); BHBD—β-hydroxybutyrate dehydrogenase.

Table 8—Analysis of variance of data on enzymatic activity and on proportion of fibers positive for enzymes

Source of variation	df	Mean squares	df	Mean squares
		LD[a] activity		BHBD[a] activity
Age (A)	2	3343402.00**	3	1.97
Error a	21	312900.60	28	0.83
Muscle type (M)	2	1472676.00*	2	6.08**
AM	6	1137360.00	6	0.29
Error b	63	393353.60	56	0.35
		Positive LD[a] fibers		Positive BHBD[a] fibers
Age (A)	3	364.89	3	27.79
Error a	28	242.02	28	520.45
Muscle (M)	1	375.10	1	1289.16
AM	3	222.92	3	674.15
Error b	28	250.95	28	405.70

[a] Abbreviations: LD—lactate dehydrogenase; BHBD—β-hydroxybutyrate dehydrogenase.

* Significant at 0.05 level

** Significant at 0.01 level

DISCUSSION

IN THESE EXPERIMENTS involving turkeys from 12–24 wk old, the results indicate that several major correlated changes occur in muscle lipids. First, the turkey seems to have more "finish" after 20 wk of age. The results of this study show that skin thickness, an estimate of fatness or finish, increases at a rapid rate as the turkey approaches market age. Although no proximate analyses were done on the skin, deposited lipid probably is the major cause of increased skin thickness with increasing age. Marion et al. (1970) reported that skin of yearling female turkeys averaged 38.5% lipid. If skin thickness is used to predict body lipids, it is necessary to take measurements at a common location, such as the neck-pectoral junction where substantial lipid is deposited.

Lipid content in individual leg muscles increased with age, whereas in breast and wing muscles, lipid content remained relatively constant. These data support previous reports (Acosta et al., 1966; Wangen and Skala, 1968; Wangen et al., 1971) in both the amount and variation in lipid content.

Phospholipid content of muscle tissue appears similar to that previously reported (Neudoerffer and Lea, 1968; Marion, 1970). An age effect was observed, however, variation among muscle groups was greater in turkeys at 24 wk than at earlier ages. When expressed as a percentage of total lipid, phospholipids present were negatively related to age. Pectoralis major maintained a relatively

high concentration of phospholipid when the latter was expressed as a percentage of total lipid.

Since the quantity of lipid varied between muscles and with age, enzyme analyses may be related to the variable physiological need for lipid by muscles. Obviously, physiological activity varies among thigh, leg and breast muscles of the turkey. Lipid content, moreover, seems to be positively correlated to a muscle's physiological activity.

Two enzymes, GPD and PGD, are known to function for the generation of reduced nicotinamide adenine dinucleotide phosphate for lipogenesis. These enzymes were analyzed in turkey muscles, but no consistent response was detected. Although they have been reported in pigeon Pectoralis fibers (George and Berger, 1966), the domestic turkey muscle fibers show at best low concentrations and (or) low activity for these enzymes.

LD and BHBD responses were measurable in the muscles listed in Table 7. Both enzymes in the leg muscles increased with increasing age of turkey, showing changes between 20 and 24 wk. The enzymatic changes occurred in the same age period that lipid content changed. Histochemical enzyme responses were not significantly affected by age, however, the increase in enzyme activity suggests that the latter may be independent of the number of fibers positive for that enzyme.

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EFFECT OF ESSENTIAL MINERALS ON CADMIUM TOXICITY. A Review

INTRODUCTION

CADMIUM is a toxic element that has no known biological function. Cadmium has chemical properties similar to those of zinc and the two elements are usually found together under geological conditions. With growing industrial use of zinc and cadmium the potential for greater human exposure is increased. Many aspects of cadmium toxicity have recently been reviewed (Friberg et al., 1971, 1973; Vallee and Ulmer, 1972).

In order to evaluate the hazards of cadmium to man, it is important to consider the relation of dose to given responses and the time required for the response to appear. The actual dose level of cadmium is probably the single most important factor. The response may be modified by the route of administration, the chemical form of cadmium, and the age, sex and species of animal. The nutritional status and dietary levels of several essential nutrients, including minerals, can greatly alter the animal's response to cadmium.

Dietary cadmium

In the U.S., man is exposed to cadmium daily via air, food and water. The "average" intake from these three sources is 0.02, 50 and 10 μg cadmium/day, respectively. If a person elects to smoke 20 cigarettes per day, the intake of cadmium is significantly increased, by approximately 20 μg/day. The amount of cadmium that actually enters the body is generally considered to be greater for inhaled cadmium, compared with ingested cadmium, provided the particle size is sufficiently small. In the present paper, the primary concern will be with dietary cadmium.

The Joint FAO/WHO Expert Committee on Food Additives (1972) has recently published "provisional tolerable weekly intake for man" of three toxic metals, including cadmium. The values, which are expressed on a weekly basis because of the long biological half-life of cadmium, are 400–500 μg/person/week and 6.7–8.3 μg/kg body weight/week. Expressed on a daily basis the corresponding values are 57–71 μg and 1–1.2 μg, respectively.

The concentrations of cadmium in foods by twelve classes have been determined by the Food & Drug Administration for several years. Foods were collected in five geographic areas of the U.S., cooked by a dietitian according to local custom and assayed by food class composite. For samples collected between June, 1968 and April, 1970, the concentrations in most foods ranged between 0.01 and 0.03 μg/g (Corneliussen, 1972). Cadmium could not be detected in many samples. The highest concentrations for individual composites, 0.08 and 0.07 μg/g, occurred in potatoes and leafy vegetables, respectively.

An estimate of total daily intake was calculated from the concentrations found in the food classes (Duggan and Corneliussen, 1972). Grains and cereals contributed the largest amount of cadmium, 14 μg. Intermediate amounts of cadmium, 4–7 μg, were contributed by dairy products; meat, fish and poultry; potatoes; leafy vegetables; fruits; and beverages. Only 1 or 2 μg of cadmium were contributed by legume vegetables; root vegetables; garden fruits; oils, fats and shortening; and sugars and adjuncts.

A few specific foods have long been known to contain amounts of cadmium that are higher than the above values. These include oysters, clams, liver and kidney. The only one of these foods included in the diet composites studied by FDA was liver.

DISCUSSION

Comparative responses between man and experimental animals

Some of the complications encountered in designing appropriate animal models for toxicity data pertinent to man are summarized in Table 1. Comparisons of food intake are calculated on the basis of a dry fiber-free diet, which is typical of experimental diets. The dietary intake for the adult rat per unit body weight is approximately six times that of man (Subcommittee on Laboratory Animal Nutri-

Table 1—Comparisons between man and the rat; considerations pertinent to interpretation of cadmium toxicity data

	Rat	Man
Food intake, g dry fiber-free diet/kg body wt/day (adult)	50[a]	8.5
Cd intake		
μg/day	–	50
μg/kg diets,[b]	14	83
Toxicity, μg/kg diet	5,000 +	–
Biological half-life, yr.	–	16–33

[a] 100g for the young growing rat
[b] To supply the same amount of cadmium/unit body weight for each species

tion, 1972; Food and Nutrition Board, 1968). For the young growing rat the difference is a little more than tenfold. In general, young growing animals are most sensitive to toxic materials and are used for experimental purposes.

As noted above, the daily cadmium intake for man is about 50 μg, or 83 μg/kg of dry fiber-free diet. A corresponding dietary cadmium concentration for the rat, which would give the same cadmium intake per unit body weight, is 14 μg/kg of diet. This is a level that might produce no detectable adverse effects and could only be studied from the standpoint of cadmium accumulation through the use of radioactive cadmium. These calculations ignore background cadmium contamination in the purified rat diet.

It is obvious, therefore, that in order to study adverse effects of cadmium in experimental animals and produce measurable responses in a reasonable period of time, much higher levels of cadmium must be used. In general 5 mg cadmium/kg diet is the lowest level of cadmium that will produce adverse physiological effects. This is over fifty times more than the typical dietary concentration of cadmium for man. Much higher levels of cadmium are frequently used in toxicity studies.

The turnover of cadmium in experimental animals is very slow (Cotzias et al., 1961). The biological half-life of cadmium in man is still controversial. Estimates generally range between 16–33 years (see review of Friberg et al., 1973, p. 50).

Manifestations of cadmium toxicity

When young experimental animals receive 5–10 mg cadmium/kg of diet or higher levels, the following most notable changes from normal typically occur: slightly depressed growth rate, anemia, poor bone mineralization, hypertension and renal tubular damage. The latter two changes require longer periods of time to develop. In general, large doses of cadmium produce more rapid and more severe responses.

Knowledge of high-level exposure in man comes from occupational health studies of workmen exposed to cadmium and from areas affected by river water contamination from mines in Japan. In the case of the workers, intake was primarily through inhaled dust or fumes. The Japanese were exposed via drinking water and foods, such as rice and soybeans, that acquired cadmium from contaminated irrigation water. The reports of these exposures have been summarized by Friberg et al. (1971; 1973).

Characteristic changes in the workers include depressed appetite, anosmia, anemia and renal tubular damage. In Japan many of the people exposed to cadmium developed renal tubular damage and associated hypercalcuria, glycosuria, proteinuria and amino aciduria. Those exposed to higher levels of cadmium developed a very painful syndrome called "Itai-Itai Byo" or Ouch-Ouch disease. Bone demineralization was severe and death sometimes resulted. The disease occurred in a susceptible population class, multiparous post-menopausal women, who were thought to be deficient in vitamin D and calcium. Schroeder (1964; 1965; 1967) has postulated a causal relationship between cadmium and hypertension in man. An unusually high incidence of hypertension in cadmium-exposed human beings has not been observed.

Table 2—Relationships between cadmium and essential nutrients

	Dietary intake of individual nutrients		
Nutrient	Normal[a]	Deficiency[b]	Excess[c]
Zinc	+	+	+
Iron	+	+	+ (Fe^{2+})
Manganese	+	?	?
Copper	+	+	+
Selenium	+	?	+
Calcium	+	+	?
Ascorbic acid	?	?	+
Vitamin D	?	+	?
Protein	?	+	+

[a] + Cadmium affects metabolism and/or function of the nutrient; ? No relationship has been established.
[b] + A deficiency of the nutrient increases the severity of cadmium toxicity.
[c] + An excess of the nutrient decreases the toxicity of cadmium.

Cadmium and essential nutrient interactions

Table 2 presents a simplified scheme for the relationships that have been observed between cadmium and some essential nutrients, including minerals. A comprehensive treatment in this area is far beyond the scope of this review.

The relationships are considered from three points of view with respect to the required nutrients. The first is whether or not cadmium affects the metabolism and/or function of the required nutrient under "normal" conditions of dietary intake. "Normal" is used in a very imprecise way. Ideally it should refer to experiments in which each required nutrient was present at exactly or only slightly above the requirement level for the particular species. Rather, the studies were carried out with various adequate purified and stock diets which had varying levels of nutrients. A question mark indicates ambiguous findings or lack of studies. Without exception, the data for this table were obtained from studies involving the toxic effects of levels of cadmium in excess of the average man's daily intake.

We have recently studied the effect of dietary cadmium on whole body retention of several mineral elements in young Japanese quail (Jacobs et al., 1972). Whole body analyses for zinc, iron, copper, manganese and calcium were carried out at 7 and 14 days of age. When compared with the control group, the birds fed 75 mg cadmium/kg diet retained significantly less of each of the above mineral elements than they had consumed during the 7-day period. With an intake of 10 mg cadmium/kg, only iron, zinc and calcium were decreased. Based either on tissue analysis or physiological responses under conditions known to be associated with specific mineral functions, numerous workers have reported cadmium antagonisms of zinc, iron and copper. Some of the more detailed investigations were conducted by Hill et al. (1963); Bunn and Matrone (1966); Jacobs et al. (1969); Banis et al. (1969); Fox et al. (1971); Pond and Walker (1972); and Mills and Dalgarno (1972).

Evidence that injections of cadmium can affect the metabolism of injected selenium-75 have been reported by Ganther and Bauman (1962) and McConnell and Carpenter (1971).

Ascorbic acid has not been studied extensively with respect to cadmium and not at all in a species that requires an exogenous source of the vitamin. In young Japanese quail fed 75 mg cadmium/kg of diet there was no effect of cadmium upon the concentration of ascorbic acid in the liver either with or without supplemental dietary ascorbic acid (Fox et al., 1971). Berenshtein et al. (1954) reported that daily subcutaneous injection into rabbits of 1–2 mg cadmium caused, after 1 month, a considerable lowering of ascorbic acid in muscle, liver, spleen, kidney, suprarenals, lungs, cerebrum, cerebellum and eyes. To establish relationships important for man, studies are needed in a species that requires a dietary source of the vitamin.

The second aspect of cadmium-nutrient relationships presented in Table 2 concerns the effects of cadmium in animals receiving a deficient intake of an essential nutrient. This is an area in which less work has been done but one that may be particularly important for man.

Because of similarities in chemical reactivity, zinc and cadmium have been studied extensively in a wide range of model systems, from the molecular to the whole animal level. Supplee (1961) showed that cadmium greatly intensified symptoms of zinc deficiency in turkey poults and that higher levels of zinc in excess of the requirement decreased the adverse effects of cadmium. This mutual antagonism between cadmium and zinc has since been observed repeatedly under

a great variety of conditions, including the recent studies of Petering et al. (1971).

Several studies have been carried out with various combinations of deficient levels of zinc, iron and copper in combination with supplements of the elements alone and in combination with cadmium (Hill et al., 1963; Bunn and Matrone, 1966; Banis et al., 1969). In general, deficiencies of each of these three elements enhanced the adverse response to cadmium, and supplements of the elements had some protective effect against cadmium.

Kobayashi et al. (1971) fed cadmium-polluted rice in low and normal calcium diets. Accumulation of cadmium in the liver and kidneys of mice receiving the low calcium diet was much higher than in mice receiving adequate calcium. A deficiency of calcium has been shown to result in increased concentrations of cadmium in the renal cortex and liver (Larsson and Piscator, 1971). After 2 months, decreased bone mineralization was observed only in rats receiving the low calcium diet and 25 mg cadmium/liter drinking water.

It is thought that the Japanese women who developed Itai-Itai disease were deficient in vitamin D. The effect of vitamin D upon cadmium-115 accumulation in bone was studied in rachitic chicks (Worker and Migicovsky, 1961). Treatment with the vitamin increased cadmium uptake by the tibia. In normal chicks there was no effect of supplemental vitamin D.

The short-term effects of low protein intake were reported by Suzuki et al. (1969). They gave mice a low or a high protein diet for 24 hr before and after an oral dose of $^{115}CdCl_2$. With the low protein diet there was a greater uptake of cadmium-115 by the whole body, the liver and the kidneys.

The effects of excess amounts of nutrients upon cadmium toxicity have been investigated by many workers. It has not always been possible to compare the effect of excess against requirement level of the nutrient.

Some of the most extensive investigations of cadmium-nutrient interactions have been studied in relation to the acute effects of injecting large doses of cadmium. It has been shown that protection against testicular necrosis can be provided by zinc (Parizek, 1957, 1960; Mason et al., 1964), selenium (Kar and Kamboj, 1965; Mason et al., 1964) and cobalt (Kar and Kamboj, 1965). Selenium also affords protection against other acute effects of injected cadmium. These include hemorrhage and necrosis of the ovary in rats in persistent estrus (Parizek et al., 1968c), placental damage and toxemia-like syndrome in pregnant rats (Parizek et al., 1968b), damage to the lactating mammary gland (Parizek et al., 1969) and survival after toxic doses of cadmium (Parizek et al., 1968a, b; Gunn et al., 1968).

The pertinence of the above findings to longer-term toxicity effects of cadmium remains to be established. The effect of several mineral elements upon growth depression and hematocrit was investigated in young Japanese quail fed 75 mg cadmium/kg diet (Fox et al., 1971). Marginal beneficial effects on anemia were reported for zinc and copper. Chromium, cobalt, nickel, selenium and molybdenum had no effects. Beneficial effects of excess levels of zinc, iron and copper may be found in the references cited above in relation to cadmium and diets containing normal and deficient levels of zinc, iron and copper.

An important aspect of the cadmium-iron antagonism related to the greater antagonism of iron (III), supplied by ferric citrate, compared with iron (II), as ferrous sulfate (Fox et al., 1971). In quail fed 75 mg cadmium/kg diet, 30 mg iron (II)/kg diet was beneficial, whereas 100 mg iron (II) almost completely prevented anemia and growth depression due to cadmium. 300 mg of iron (III) had no effect on anemia and a small effect on growth. This difference varies greatly from the requirement of iron (II) and iron (III) for hematopioesis and growth of the quail. Iron (II) of ferrous sulfate is utilized about 1.5–2 times more efficiently than iron (III) of ferric citrate, depending on level of intake (Harland et al., 1973).

Ascorbic acid has been shown to be markedly effective in preventing the anemia, growth depression and pathology caused by a high level of dietary cadmium, 75 mg/kg, in quail (Fox and Fry, 1970; Fox et al., 1971; Richardson and Fox, 1972). The primary effect of ascorbic acid appears to be an increase in the utilization of iron (II) in cadmium-fed birds; however, some aspects of the pathological changes responsive to ascorbic acid appear to be unrelated to either iron or zinc (Richardson and Fox, unpublished data).

The type of dietary protein markedly alters the toxicity of cadmium in young Japanese quail (Fox et al., 1973). When compared with birds fed casein-gelatin or soybean protein, birds fed dried egg white showed markedly less adverse effects on growth, hematocrit and tissue concentrations of zinc and iron when the diet contained 75 mg cadmium/kg.

Mechanisms of cadmium-mineral antagonisms

The biochemical effects of cadmium have recently been reviewed by Vallee and Ulmer (1972). Three general explanations of the adverse effects of dietary cadmium are possible, particularly with regard to antagonism of essential minerals. Cadmium may replace zinc or some metal in an enzyme or at some other site and interfere with a specific metabolic reaction. Cadmium may displace an essential element or the carrier of an essential element in a transport system, thus disrupting such processes as intestinal absorption, transport and storage within the body and excretion. Data from numerous studies suggest that absorption is a critical point in many cadmium-mineral antagonisms. Some of the problems may be related to intestinal enteropathy, which was observed in patients with Itai-Itai disease (Murata et al., 1969) and in cadmium-fed animals (Wilson et al., 1941; Yoshikawa et al., 1960; Richardson and Fox, 1972; Stowe et al., 1972).

Biological effects of cadmium incorporated into food

The majority of studies on cadmium toxicity involve inorganic salts of cadmium, usually administered by injection. A lesser number involve administration of inorganic cadmium in the drinking water or in the diet. Very few studies have been made in which cadmium was fed in a foodstuff of biological origin.

We have compared the effects of cadmium incorporated into oysters with those of cadmium chloride in young Japanese quail (Fox et al., 1972; and unpublished data). For this work oysters were maintained in fiberglass tanks. They were exposed to sea water alone or sea water containing cadmium added to a level of 0.1 μg/ml from cadmium chloride. After 2 wk the oysters receiving cadmium began to show signs of decreased pumping action. They were harvested, and the concentration of cadmium in the tissue was 60 μg/g. Concentrations of iron, zinc, manganese and copper were similar in the high cadmium and control oysters. The oysters were homogenized, freeze-dried and incorporated into purified diets for feeding to Japanese quail.

Because of all the cadmium-nutrient relationships described above, the total concentrations of protein, zinc, manganese, iron, copper, calcium and magnesium were maintained at constant levels. The high-cadmium oysters were incorporated at levels to supply either 5 or 10 mg cadmium/kg diet. The low-cadmium oysters were fed at the same levels, with and without cadmium chloride.

With 10 mg cadmium/kg diet, the oysters supplied 258 mg zinc and 8 mg copper/kg diet. This, plus the zinc and copper present in the purified soybean protein used in the diets, supplied zinc and copper at levels approximately nine and three times the quail's requirement, respectively. By most parameters in a 2-wk period, there was little difference between cadmium supplied by the oysters and that by cadmium chloride, at the same levels of essential mineral intake.

Subsequent studies (unpublished data) have shown that with zinc, copper and manganese at the requirement level, significantly more cadmium accumulated in the kidney than when higher levels of these elements were present in the diet.

This briefly illustrates some of the complications that arise when one attempts to evaluate the biopotency of cadmium in food materials. The toxicity of the cadmium might be affected by the ligand(s) to which it is bound in the food or the presence of other components in the food that can alter the toxicity of cadmium by some means. Clearly, a more sensitive bioassay procedure is needed.

We have made some progress in this direction from observation of cadmium concentration in the quail's duodenal mucosa following very low levels of cadmium intake (0.078 μg/g diet and higher). Cadmium concentrates rapidly in the duodenal mucosa at levels several-fold greater than the dietary concentration. A reproducible log-dose response has been obtained. This may prove to be a useful bioassay; however, the duodenal concentrations need to be correlated with accumulation of cadmium in the liver and kidneys, using cadmium-109 chloride and labeled foods. Also, the comparative biopotencies need to be determined in long-term, low-level cadmium feeding as evaluated by physiological and histological responses.

Standard reference diets

Our present knowledge shows that the toxicity of cadmium may vary greatly depending on the composition of the diet in which it is fed. There is a need to develop one or more purified reference diets for trace element studies in each commonly used animal species. At least one such diet should contain the total amount of each nutrient at the requirement level (or no more than 10% excess) as our current knowledge of requirements permits. Availability of such diets could predictably resolve many of the seeming conflicts that arise between laboratories and in the use of different species of animals.

Needed information about cadmium

Additional information is needed on the following: (1) Acretion rate of cadmium with dose, particularly as related to the age of the animal and to cadmium source; (2) Biopotency of cadmium in food and other sources; (3) Factors that can either decrease or increase the effects of cadmium; (4) Sources and amounts of cadmium contamination; and (5) More precise understanding of cadmium effects.

SUMMARY & CONCLUSIONS

CADMIUM IS a toxic element that is potentially dangerous to man unless exposure is controlled. The toxicity of cadmium may be decreased or increased in response to dietary intake of some essential nutrients, from deficient to levels above requirements. We need better experimental models for evaluating the effects of cadmium in man.

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COLOR OF ANTHOCYANIN SOLUTIONS EXPRESSED IN LIGHTNESS AND CHROMATICITY TERMS.
Effect of pH and Type of Anthocyanin

INTRODUCTION

COLOR is the major quality that anthocyanins impart to food and drink. Commonly used as objective measurements of color in anthocyanin solutions are the specific absorption bands, e.g., the wavelength of maximum absorption (λ max) in the 500–530 nm region, the absorption in the 400–430 nm region and other forms of abridged spectrophotometry (Sudraud, 1958). However, many investigators have recognized that tri-stimulus values are preferable when color must be reported in terms relevant to the human visual response (Ough et al., 1962).

Tri-stimulus colorimetry (Judd and Wyszecki, 1963) can be carried out by appropriate conversion of spectral data, or by use of equipment designed to yield direct tri-stimulus values. One such instrument is the Hunterlab Color and Color Difference Meter (Hunter Associates Laboratory, Fairfax, Va.) (Hunter, 1958). The suitability and advantages of this instrument for the evaluation and specification of wine color have been reported by Robinson et al. (1966). They found that hue and saturation depended upon solution lightness as well as the coloring materials present.

The color of red wines, particularly when young, is due largely to the anthocyanins. Some wines, especially those from hybrid grapes, have a great variety of anthocyanins (Van Buren et al., 1970). The contribution of particular anthocyanins to the overall color is known only to a limited extent. The purpose of the present study has been to extend this knowledge in order to help control and standardize the color of beverages containing anthocyanins.

MATERIALS & METHODS

THE ANTHOCYANINS, except for cyanidin-3-glucoside, were purified by the column chromatography method developed by Hrazdina (1970). Cyanidin-3-glucoside was obtained as the juice from Darrow cv. blackberry (*Rubus fruticosus*) where it constitutes more than 95% of the anthocyanin pigment.

The anthocyanins were dissolved or diluted in a solution containing 120 ml of ethanol, 5 ml of glycerol, 2g citric acid, 3g malic acid and 0.02g 2-chloro-4-phenyl-phenol (a preservative) in 1 liter volume. pH was adjusted with 2N HCl or 2N NaOH. Ethanol was included in this solution in order to make it similar to dry wine. This concentration of ethanol did not affect the color characteristics of anthocyanin solutions. Concentrated solutions of the anthocyanins were prepared the day before the measurements were made, held overnight at 2°C, filtered and allowed to come up to room temperature.

Anthocyanin concentrations were measured by the spectrophotometic method of Niketić-Alekić and Hrazdina (1972) using malvidin-3-glucoside and malvidin-3,5-diglucoside as standards.

Spectral measurements were made on a Beckman DK spectrophotometer using cells with path lengths of 1 and 10 mm.

Tri-stimulus color measurements were made using a cell with a 10 mm light path in the transmission head of a D25 Hunterlab Color and Color Difference Meter. The light source was a tungsten filament. Measurements were obtained as "L," "a" and "b" readings. The "L" values, closely related to the human visual response to lightness or brightness, was an expression of the light transmittance of the sample as viewed through the green filter. The "a" dimension indicates degree of redness on the positive side and blue-green on the negative side. The "b" dimension indicates yellowness on the positive side and purple-blueness on the

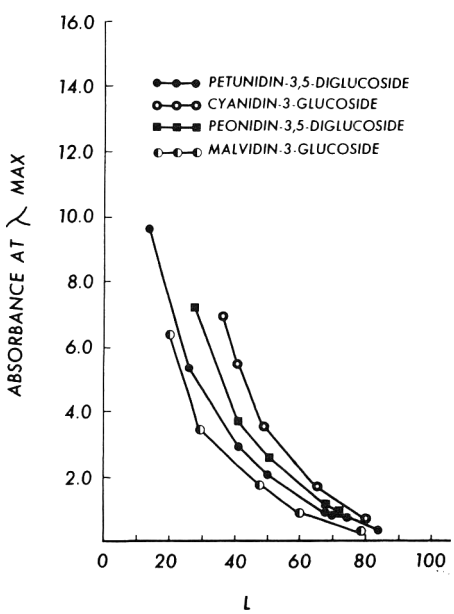

Fig. 1—The relation between absorbance and Hunter "L" values for various anthocyanins. Absorbance determined at λ max in the 500–530 nm range. Solution pH was 3.4.

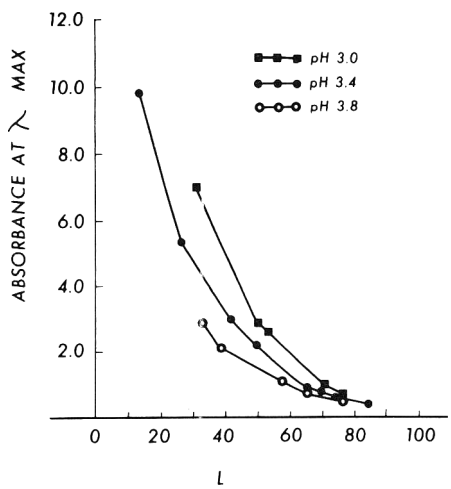

Fig. 2—The effect of solution pH on the relation between absorbance and Hunter "L" values for petunidin-3,5-diglucoside. Absorbance determined at λ max in the 500–530 range.

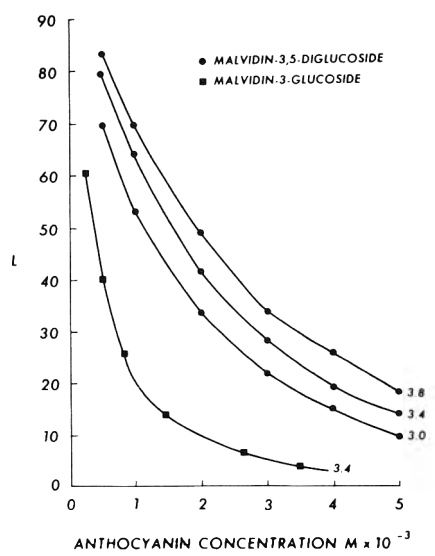

Fig. 3—Relation between anthocyanin concentration and Hunter "L" values for malvidin-3,5-diglucoside and malvidin-3-glucoside. The numbers on the curves refer to pH.

negative side. From "a" and "b" values other chromaticity parameters can be calculated. The angle θ is a parameter that expresses hue and this angle is the arc cotangent a/b. As angle θ increases from 0° to 60° the hue changes from violet-red through red to orange. Saturation (or purity, or vividness) was obtained as $(a^2 + b^2)^{1/2}$.

RESULTS & DISCUSSION

AN IMPORTANT COMPONENT of the color of a solution is its lightness ("L"), a factor related to the intensity of light reaching the observer after passage through a green filter. "L" is therefore related to the spectral absorbance of a nonturbid, nonfluorescing solution. Many previous studies on anthocyanin solutions have included absorbance measurements (Asen et al., 1972; Bayer et al., 1966; Rice, 1964; Sudraud, 1958; Timberlake and Bridle, 1967).

A comparison of spectral absorbance data with the psychophysical "L" values showed that solutions of different anthocyanins have different quantitative relations between "L" and the absorbance measured at the wavelength of maximum absorption (λ max) in the 500–550 nm region (Fig. 1). Furthermore the relation between "L" and absorbance for solutions of a particular anthocyanin was affected by the pH of the solution as illustrated for petunidin-3,5-diglucoside (Fig. 2). It was of interest to determine the relation between "L" and anthocyanin concentration, Figure 3. Equivalent "L" values were obtained with lower concentrations of 3-monoglucoside than with 3,5-diglucosides. Thus 3-monoglucosides had a greater coloring ability in the pH 3.0–3.8 range. This is in agreement with Timberlake and Bridle's report (1967) showing that solutions of 3-monoglycosides had a larger fraction of the anthocyanin in the colored flavylium form at pH 3.0 than was the case for the 3,5-diglucosides. Lowering of the pH of a particular anthocyanin solution decreased L, corresponding to the well known increase in absorbance with decreasing pH due to structural transformations of flavylium compounds (Timberlake and Bridle, 1967).

The λ max was used for obtaining the absorbance because λ max varied for these anthocyanin solutions depending on both concentration and pH. This variation in λ max is shown in Table 1. These results indicate that the variations in λ max with concentration and pH found by Asen et al. (1972) for cyanidin-3,5-diglucoside are general properties of anthocyanins. With a rise in pH in the sensitive 3.0–3.8 range (Rice, 1964) the λ max increases, and with an increase in concentration the λ max increases or decreases depending on the pH of the solution. Concerning this latter point, it appears that there is a critical solution pH for each anthocyanin where a change in concentration does not result in a change in λ max. At a pH below this critical pH the λ max increases as concentration decreases, while when the pH exceeds this critical pH the λ max decreases as the concentration decreases.

Table 1—λ max for anthocyanin solutions of different "L" and pH[a]

		Concentrated solutions			More dilute solutions		
Anthocyanin	pH	"L"	conc[b] × 10^{-3}	λ max	"L"	conc[b] × 10^{-3}	λ max
Malvidin-3,5-diglucoside	3.0	25	2.7	520	71	42	524
	3.4	21	3.0	522	74	.68	526
	3.8	23	4.6	524	79	.62	526
Petunidin-3,5-diglucoside	3.0	31	2.0	520	76	.37	524
	3.4	14	4.2	523	83	.35	525
	3.8	32	2.8	525	77	.66	525
Peonidin-3,5-diglucoside	3.0	25	2.4	515	64	.55	518
	3.4	10	4.7	517	71	.55	519
	3.8	18	3.4	519	85	.33	520
Malvidin-3-glucoside	3.0	19	.95	520	60	.21	522
	3.4	21	.95	523	78	.12	525
	3.8	16	1.2	526	79	.15	526
Cyanidin-3-glucoside	3.0	15	1.7	509	80	.12	514
	3.4	15	1.9	514	80	.15	515
	3.8	15	2.1	518	80	.16	515

[a]Soln were in 12% ethanol (v/v). "L" measured in a 1 cm light path.
[b]Molarity of anthocyanin solution

The variation of λ max with pH was less pronounced at low concentration of anthocyanin. At the higher concentrations shifts as great as 9 nm were seen as the pH was changed from 3.0 to 3.8. These shifts may be related to the increasingly greater proportion of the anthocyanin pigment present in the carbinol and anhydro forms as the pH goes higher. The λ max for absorption by anthocyanin solutions occurs at only slightly shorter wavelengths than the 555 nm λ max for the action spectrum of the "L" color component (Judd and Wyszecki, 1963). Therefore solution λ max shifts to longer wavelengths would be expected to result in a more complete overlap with the "L" function and would decrease the "L" correspondingly to a given absorbance. Thus cyanidin-3-glucoside solutions, with their lower λ max, had a higher "L" at a given absorbance than did solutions of the other anthocyanins. When anthocyanin solutions were examined at different pH's and a constant absorbance, it was found that the longer λ max at high pH was associated with a lower "L" value.

A convenient method of presenting Hunter tri-stimulus color values is by plotting chromaticity factors, such as "a," "b," θ and saturation, against "L" values (Robinson et al., 1966). This not only places the color in a definite location in the color space, but it also provides an indication of the effects of concentration on the perceived color.

The principle pigments of grape wines are malvidin-3-glucoside and malvidin-3,5-diglucoside (Van Buren et al., 1970). The relation between "L" and the "a" and "b" chromaticity dimensions, obtained directly from the color difference meter, are shown for solutions of these pigments, Figure 3a, b. The presence of maxima for both the "a" and the "b" values is an inherent characteristic of the measurement of these dimensions since both opaque and completely transparent solutions have "a" and "b" values of zero. The size of the maximum and the corresponding "L" value can be used to distinguish one anthocyanin from another. Malvidin-3,5-diglucoside had a higher "a" maximum and a lower "b" maximum than malvidin-3-glucoside. A further difference was the minimum found for the diglucoside at high "L" values. These comparisons should be carried out at a particular pH since the "L" vs. "a" or "b" plots were strongly influenced by pH, Figure 3c, d. This figure, as well as the data shown in Table 2, indicates that raising the pH of the solutions lowers the "a" and "b" maximums and lowers the "L" values at which the maximums occur.

Chromaticity values with a close relation to human color perception can be calculated from "a" and "b" values. These are hue, expressed in the Hunter system by the angle θ, and saturation, a description of the purity or vividness of the color. θ is expressed as degrees, and as θ increases from 0° to 60° the corresponding hue changes from violet red to orange. A θ of 15° indicates a red hue.

Plots of θ and saturation against "L" are shown for malvidin-3-glucoside and malvidin-3,5-diglucoside in Figure 4. The higher θ max for malvidin-3-glucoside is due to the higher "b" max and the lower "a" max found for this anthocyanin. This higher θ max indicates that this anthocyanin can show a wider range of hues, towards orange red, than can be obtained

Table 2–Maximum "a," "b," θ and saturation values for anthocyanins in 12% ethanol at pH 3.0–3.8

Anthocyanin	pH	"a" max	"L"	conc[a] × 10^{-3}	"b" max	"L"	conc[a] × 10^{-3}	θ[b] max	"L"	conc[a] × 10^{-3}	Saturation max	"L"	conc[a] × 10^{-3}
Malvidin-3,5-diglucoside	3.0	80	50	1.1	19	35	1.9	15	30	2.2	78	50	1.1
	3.4	75	44	1.9	13	25	2.6	13	20	3.3	75	45	1.8
	3.8	72	40	2.6	8	15	6.1	12	15	6.1	72	40	2.6
Petunidin-3,5-diglucoside	3.0	77	53	1.0	20	35	1.7	16	30	2.1	78	50	1.1
	3.4	74	46	1.5	13	26	2.6	13	20	3.3	74	45	1.5
	3.8	70	41	2.1	9	18	4.8	12	15	5.4	70	45	1.9
Peonidin-3,5-diglucoside	3.0	73	40	1.3	25	48	1.0	22	50	.92	80	45	1.1
	3.4	66	40	1.6	23	40	1.6	19	40	1.6	70	40	1.6
	3.8	71	40	1.7	16	27	2.5	13	25	2.7	73	40	1.7
Malvidin-3-glucoside	3.0	71	45	.33	22	39	.42	18	35	.50	73	42	.38
	3.4	66	43	.45	18	32	.65	16	26	.80	68	40	.50
	3.8	62	37	.62	13	26	.89	14	20	1.1	65	37	.62
Cyanidin-3-glucoside	3.0	72	38	.60	35	52	.35	27	55	.30	78	45	.65
	3.4	69	37	.65	31	47	.45	25	50	.40	75	40	.60
	3.8	67	38	.75	26	41	.65	22	45	.52	72	40	.70
Cranberry juice Howe cv. (Vaccinium macrocarpum)	3.4	72	40	.60	27	45	.45	21	45	.45	77	42	.55

[a] Molarity of anthocyanin solution
[b] Hue angle

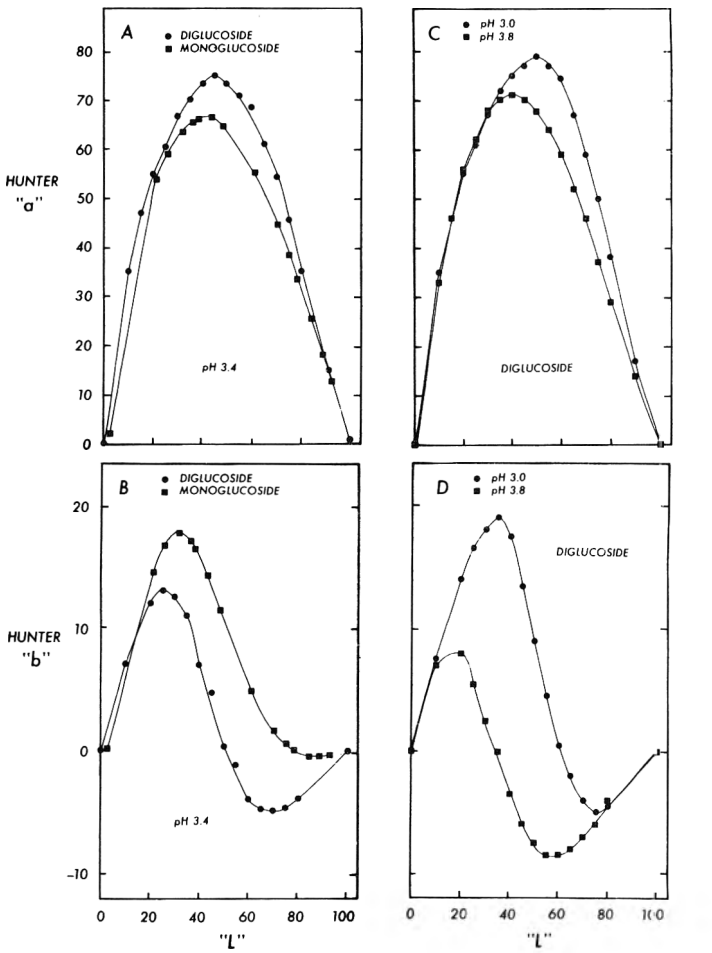

Fig. 4–Hunter "a" and "b" values of malvidin-3-monoglucoside and malvidin-3,5-diglucoside solutions at various dilutions. Dilution increases "L" values. (A) Hunter "a" value of malvidin-3-monoglucoside and malvidin-3,5-diglucoside solutions at pH 3.4; (B) Hunter "b" values of malvidin-3-monoglucoside and malvidin-3,5-diglucoside solutions at pH 3.4; (C) Effect of pH on the Hunter "a" values of malvidin-3,5-diglucoside solutions; (D) Effect of pH on the Hunter "b" values of malvidin-3,5-diglucoside solutions.

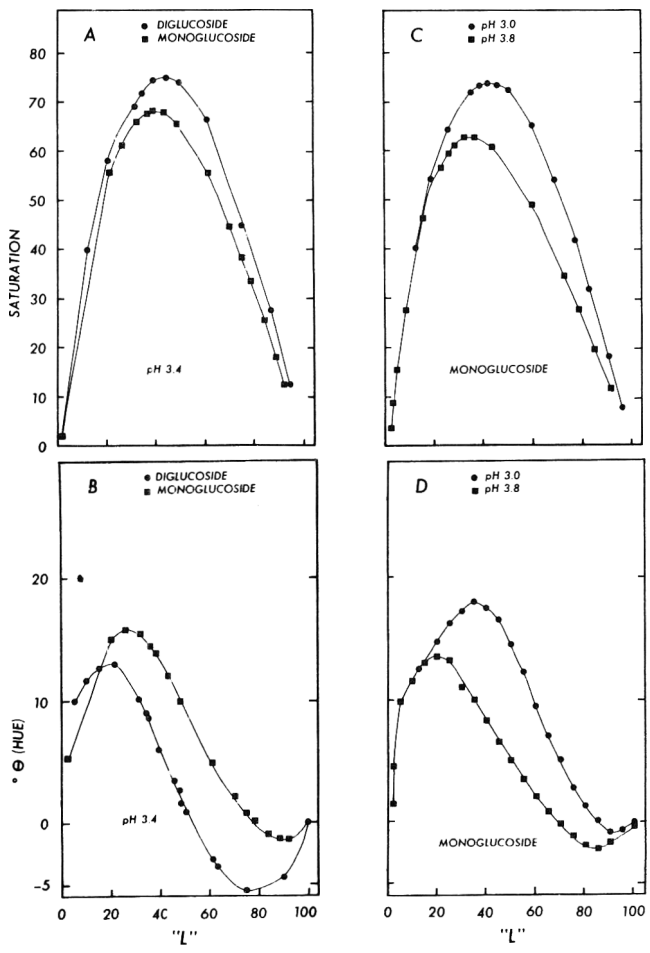

Fig. 5–Color saturation and θ (hue) values of malvidin-3-monoglucoside and malvidin-3,5-diglucoside solutions at various dilutions. Dilution increases "L" values. (A) Saturation values of malvidin-3-monoglucoside and malvidin-3,5-diglucoside solutions at pH 3.4; (B) θ values of malvidin-3-monoglucoside and malvidin-3,5-diglucoside solutions at pH 3.4; (C) Effect of pH on the saturation values of malvidin-3-glucoside solutions; (D) Effect of pH on the θ values of malvidin-3-glucoside solutions.

with malvidin-3,5-diglucoside. The diglucoside has a wider range toward the purple at high "L" values and high pH. Data for other anthocyanins are presented in Table 2. The θ values of very dilute anthocyanin solutions, "L" above 90, leveled off or increased as dilution was continued. The possible hues obtainable in solutions of cyanidin-3-glucoside cover a θ range nearly twice as large as the range obtainable in a solution of malvidin-3,5-diglucoside. Peonidin-3,5-diglucoside solutions had a combination of a large θ range and a large saturation range. They also showed the greatest variation with pH. Mixtures of anthocyanins had chromaticity parameters in between those of the individual pigments.

Variations in "b" between anthocyanins were greater than for "a," therefore difference in θ max values were closely related to differences in "b" values. On the other hand saturation max differences were closely related to "a" values since "a" values were the larger in magnitude and thus played a weightier role in the calculation of saturation. At "L" values less than 10 all the anthocyanins tested had similar "a" and "b" values in the pH range 3.0–3.8.

Color measurements were carried out in cells with a 10 mm light path. If the light path had been larger or smaller we would have obtained different "L" vs. "a" or "b" diagrams. Thus colors of different hue and saturation would be produced. If an anthocyanin solution, such as a red wine, were held in a container shaped so that a wide range of light paths through the solution could be obtained, then the solution would exhibit the variety of hues indicated by its "L" vs. θ diagrams. The fiery hues ascribed to wine in the glass illustrate this effect.

Color parameters of the anthocyanin solutions are related to the chemical structure of the anthocyanins. Values for "a" max were lower and the "b" max were higher (thus θ max was higher) as the number of hydroxyl or methoxyl groups on the B ring decreased. Glycosylation at the 5 position reduced θ max. There seemed to be little difference between B-ring hydroxyls or methoxyls with regard to the chromaticity parameter maximums, thus malvidin and petunidin-3,5-diglucoside had similar values. The types of sugar on the anthocyanins may also have only minor significance. In this regard the pigments from Howe cranberry, consisting largely of the 3-arabinosides and 3-galactosides of cyanidin and peonidin (Fuleki and Francis, 1968) gave chromaticity factor maxima close to what might have been expected if the pigments had been 3-glucosides.

Hue variations between anthocyanin solutions were roughly in the same direction as might be predicted from their spectral absorption characteristics. As the

Table 3—Suggested values[a] for θ maximums of untested anthocyanin solutions, pH 3.4

Anthocyanin	θ max	"L"
Delphinidin-3,5-diglucoside	12	18
Cyanidin-3,5-diglucoside	20	42
Pelargonidin-3,5-diglucoside	32	62
Petunidin-3-glucoside	17	30
Delphinidin-3-glucoside	16	28
Peonidin-3-glucoside	23	48
Pelargonidin-3-glucoside	37	74

[a] Derived by extrapolation considering structural properties of the anthocyanin in relation to an "L" vs. θ max plot of the anthocyanins actually tested.

λ max shifted to longer wavelengths the θ max decreased indicating more purple-red and less orange color. This agrees with earlier visual estimates of the colors of anthocyanin solutions (Harborne, 1967).

These results with anthocyanins might be compared with the "L" vs θ plots for varietal red wines presented by Robinson et al. (1966). Two of the wines (S 128 and S 8355) had θ max values similar to anthocyanin solutions, but a third wine (SV 5-247) exhibited increasing θ as "L" increased. This can be taken as an indication that the third wine contained nonanthocyanin pigments influencing the color in the direction of orange. Furthermore, the θ values for most of the Rosé wines exceeded that expected from anthocyanin alone, indicating that these wines also had a similar nonanthocyanin pigment. The nonanthocyanin pigment may have been brown products resulting from condensation of polyphenols.

The effect of changing pH on the color of anthocyanin solutions can be explained by reference to the "L" vs. chromaticity dimension plots in conjunction with the knowledge that the "L" value of a given anthocyanin solution will decrease as the pH is lowered from pH 3.8 to 3.0. Considering the variation of θ, if a pH 3.8 solution has an "L" value lower than that at its max, and we lower the pH, the "L" will decrease and result in a new "L" corresponding to a lower θ. If a pH 3.8 solution of the same anthocyanin had an "L" value 30 or 40 units higher than "L" at θ max then moderate lowering of the pH would lower "L" values and raise the θ values. Both raising or lowering the pH by 0.4 units of a pH 3.4 solution at its θ max would result in a decrease in θ.

Predictions of tri-stimulus values of solutions of untested anthocyanins might be made by extrapolation from data on anthocyanins already studied, including the assumption that the addition of a sugar to the 5 position of anthocyanin-3-glycosides increased the blueness. Such extrapolations are possible at any "L" value, but the most useful ones might be those where a chromaticity factor reaches a maximum, Table 3. The values suggested fall in line with the general observations made on anthocyanins that increased hydroxylation augments blueness.

CONCLUSION

SOLUTIONS of a pure anthocyanin give a range of colors depending on concentration and the pH of the solution. While there are marked differences between anthocyanins regarding the range of colors obtainable, they impart a similar dark red color to concentrated solutions. The "L" color function increases as concentration decreases, while at a given concentration the lower the pH (in the 3.8–3.0 span) the lower the "L." The relation between "L" and absorbance is different for different anthocyanins. Color chromaticity dimensions, θ and saturation, are fixed by both the "L" and the pH of the solution. Furthermore the θ and saturation show maximum values in the middle "L" region. These maximum values are related to the structure of the anthocyanin.

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ANTHOCYANINS IN BARBERA GRAPES

INTRODUCTION

ANTHOCYANINS are the pigments responsible for the color of red grapes. They are heat labile and undergo oxidative changes readily. Amelioration of the color of quality wines is accomplished by blending with grapes rich in anthocyanin pigments.

Knowledge of the composition and inheritance pattern of the anthocyanin pigments in grapes is extremely valuable in developing new varieties with the desirable color characteristics. Somaatmadja and Powers (1963) isolated six pigments from Cabernet Sauvignon grapes. Chen and Luh (1967) used cation exchange resin and paper chromatography for separation and identification of anthocyanins in Royalty grapes. Koeppen and Basson (1966) identified the anthocyanins in Barlinka grapes as oenin (Malvidin-3-glucoside), mono-p-coumaroyl oenin and 3-glucosides of peonidin, petunidin and delphinidin. Very little is known about the chemistry of anthocyanins in Barbera grapes.

The present work covers the isolation, purification and identification of the anthocyanin pigments in Barbera grapes.

MATERIALS & METHODS

Grapes

80 lb of ripe Barbera grapes (*Vitis vinifera*) were supplied by Professor Harold P. Olmo of the Department of Viticulture and Enology. They were harvested from the University vineyards at Davis, washed with tap water, stemmed and sorted to remove underripe and overripe ones. The grapes were sealed in No. 2½ cans under a vacuum of 16" Hg and then stored at −18°C.

The Barbera grapes had the following characteristics: soluble solids, 21.8° Brix at 20°C; and total acidity, 0.57% as tartaric acid.

400g of grapes were thawed at room temperature and macerated in a Waring Blendor with 200 ml of 0.1% (v/v) conc HCl in absolute methanol for 5 min under a nitrogen atmosphere. The mixture was filtered through Whatman No. 1 filter paper under vacuum. The residue was extracted two more times with the same solvent. To the combined extracts, a sufficient amount of Dowex 50W-X4 cation-exchange resin in the hydrogen form was added (Chen and Luh, 1967). After setting for a short time, the resin was thoroughly washed with distilled water to remove sugars and pure methanol to remove organic compounds other than anthocyanins. The pigments were eluted from the resin with a total of 6 liters of acidified methanol, increasing the concentration gradually from 0.1 to 1.0% (v/v). The eluates were combined and concentrated under vacuum in a flash evaporator at 40°C almost to dryness. The concentrate was redissolved in a small volume of 0.01% HCl in methanol, and stored at −18°C in the dark under a nitrogen atmosphere (Liao and Luh, 1970).

Paper chromatography

The solvent systems for paper chromatography of the components have been described previously (Chen and Luh, 1967; Liao and Luh, 1970).

Whatman No. 3 MM papers (67 × 46.5 cm) were used for purification of pigments. Whatman No. 1 papers (67 × 46.5 cm) were used for identification of anthocyanins, aglycones, sugars and alkaline degradation products. One dimensional paper chromatography was used for preliminary purification of pigments by the bar technique (Smith and Luh, 1965).

Development of the chromatograms was carried out by descending flow of n-Butanol-acetic acid-water (BAW, 4:1:5) system for 18 hr. From the air-dried paper each band was cut for elution in a glass chamber by descending chromatography with methanol containing 0.1% conc HCl. Each fraction was concentrated into a small volume in a flash evaporator. The above procedure was repeated for each concentrate with acetic acid-water-conc HCl (AWH, 15:82:3) as the second developing solvent for 12 hr. The concentrates were kept under nitrogen and used for further analysis.

Two-dimensional paper chromatography

For absorption spectra measurement and for R_f value determination, a small amount of pure pigment was obtained by two dimensional paper chromatography on several Whatman No. 3 MM papers. The paper was irrigated with BAW as the first solvent in the long direction and AWH as the second in the short direction by the descending method. The solvents used for the anthocyanidins were Forestal (water, HOAc, HCl, 10:30:3), in the first direction and BuHCl (n-butanol, 2N HCl, 1:1), upper phase, in the second direction.

From the dried chromatograms, the identical spots were cut, combined, eluted with 0.1% methanolic HCl, concentrated in a flash evaporator to a small volume and stored under nitrogen at −23°C in the dark.

Chromatographic method for detecting mono- and di-glycosides in anthocyanins

The nature of glycosides in the anthocyanins was determined by the ascending chromatographic method described by Ribéreau-Gayon and Peynaud (1958). The paper was placed with the lower edge dipped in an aqueous 0.6% citric acid solution (w/v) for 6 hr. If diglycosides are present they would move faster than the monoglucosides on the paper. Examination under ultraviolet radiation would give a red fluorescence in the presence of diglucosides of malvidin.

Partial acid hydrolysis

Partial acid hydrolysis of the pigments was done according to the method of Abe and Hayashi (1956).

Identification of acyl groups in anthocyanins

After complete acid hydrolysis, the mixture was cooled and extracted several times with anhydrous ether. The ethereal extract was concentrated to a small volume on a low-temperature steam bath, and then spotted on Whatman No. 1 paper.

Authentic samples of caffeic, p-coumeric, ferulic and chlorogenic acids were run as references. The papers were developed in a descending direction with BAW (4:1:5) for 16 hr and with 2% HOAc for 4 hr.

The dried chromatograms were examined under ultraviolet radiation before and after exposure to ammonia vapor (Swain, 1953). They were sprayed with the diazotized p-nitroaniline reagent, left to dry, and exposed again to ammonia or sprayed with 20% Na_2CO_3. The color of the spots was noted for identification of the acylating agent (Chen and Luh, 1967). The procedure was also followed in hydrolysis of the crude extract of the pigments for preliminary information.

Identification of the sugar moiety

The sugars derived from the acid hydrolysis of the glycosides were identified after removal of the aglycone and the acid (Lynn and Luh, 1964; Chen and Luh, 1967). Dowex 50W-X4 cation-exchange resin in the hydrogen form was added to the hydrolysate. The resulting solution was chromatographed on Whatman No. 1 paper (Harborne and Sherrat, 1957). The developing solvents were: BAW (4:1:5) for 36 hr (Rg value), and 17 hr (R_f determination); Bu-Py-W (6:3:1), for 36 hr (Rg value); and Et-HOAc-W (3:1:3), for 15 hr (Rg value).

The sugar spots on the chromatograms were visualized by spraying with aniline-hydrogen phthalate reagent and heating at 105°C for 5 min (Partridge, 1949). The R_f and R_g values of the sugars were compared with those of the authentic sugars glucose, galactose, arabinose and xylose.

Alkaline degradation of the aglycone

The barium hydroxide degradation method described by Luh et al. (1965) was used to study the chemical structures of the anthocyanidins.

Chromatography and identification of aromatic compounds were conducted in the same way as for the acyl group identification.

R_f values of anthocyanins

The purified pigments were chromatographed on Whatman No. 1 papers in a descending direction in three different solvents; BAW (4:1:5) solvent for 18 hr, AWH (15:82:3) solvent for 6 hr, and 1% HCl for 4 hr. The papers were air dried and the R_f values measured. The

fluorescent behavior of the spots was recorded after examination under ultraviolet radiation (3,600 Å unit output, model 1910, Burton Medi-Quip Co., Van Nuys, Calif.).

Color reaction with aluminum chloride

The dried two-dimensional chromatograms were sprayed with 5% aluminum chloride in 95% ethanol. A change in color from red to blue indicates the presence in the B-ring of the anthocyanin molecule of two hydroxyl groups in the ortho position. Anthocyanins such as delphinidin, cyanidin and petunidin give a blue color after spraying with the $AlCl_3$ reagent.

Absorption spectra of anthocyanins

The absorption spectra of pure pigments were recorded with a Beckman DK-2 spectrophotometer. The purified pigment was dissolved in methanol containing 0.01% conc HCl. The same solvent was used as a blank for the visible spectra. The ultraviolet spectra of the pigments were also studied. In this case, a solution obtained by eluting an appropriate blank area of the chromatogram with the same solvent was used as a reference (Mabry et al., 1970).

RESULTS

Paper chromatography

One-dimensional chromatography of the crude extract of the pigments by the bar technique, with BAW as developing solvent, yielded six distinct bands.

It was noticed that the bluish purple color of the two first bands (1 and 2) was less stable. It soon turned into a blue-violet on the dried papers. These two bands were present in small amounts, and caused some difficulties in the separation and purification procedure. Band 3 was the most abundant pigment, with a very intense purple color and some fluorescent behavior under ultraviolet radiation. It almost covered band 4 which showed a characteristic salmon pink color. Bands 5 and 6 appeared as unique widespread bands. The R_f values of the purified bands are listed in Table 1. The anthocyanin pigments in the Barbera grapes were glucosides of anthocyanidins, either acylated or not acylated. They were derived from a very limited number of aglycones. It is helpful to study the number of aglycones liberated by acidic hydrolysis of a mixture of the pigments. The nature, position of attachment and number of sugar molecules in the pigments were then followed.

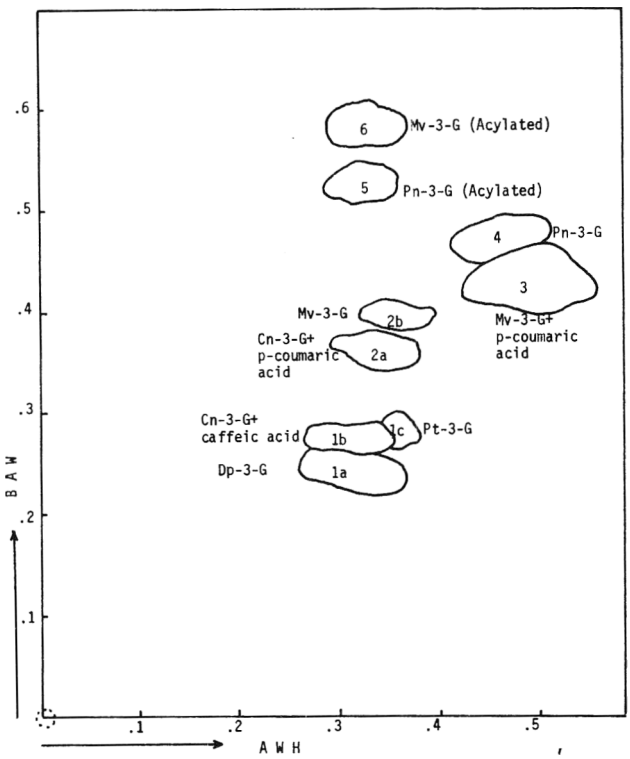

Fig. 1—Two-dimensional chromatogram of the anthocyanins from Barbera grapes.

Two-dimensional paper chromatography indicated the presence of nine pigments. A two-dimensional paper chromatogram of the anthocyanins from Barbera grapes is shown in Figure 1.

A two-dimensional chromatogram was sprayed with $AlCl_3$ to provide information on the presence of ortho dihydroxyl groups in the B-ring. Delphinidin, petunidin and cyanidin would react positively to give a characteristic blue color.

Pigments 1a, 1b, 1c and 2a turned blue after application of the $AlCl_3$ reagent. The test was repeated by adding 1 ml each of 95% ethanol into two separate test tubes. To one tube, several drops of $AlCl_3$ were added. The other tube served as a reference. A few drops of purified anthocyanin pigment solution was added to both tubes, and the color changes were noted. The results were identical to those obtained from the paper chromatogram, but were much more evident and conclusive.

Sugar moieties in anthocyanins

An unidirectional paper chromatographic technique, using a 0.5% (w/v) citric acid as solvent was carried out to test for presence or absence of diglucosides in the anthocyanin pigments. The pigments of the crude extract moved on the chromatogram as unique bands. Examination under ultraviolet radiation failed to show any fluorescent behavior, showing the absence of 3,5-diglucosides (Harborne, 1958; 1967).

The absence of diglucoside in the anthocyanins of Barbera (*V. vinifera*) conforms with the well-established observation that *V. vinifera* varieties contain only monoglycosides.

The R_f and R_g values of the sugar moiety from the pigments were identical with those of an authentic sample of glucose. Glucose was the only sugar found in all the anthocyanins in the Barbera grapes.

Table 1—R_f values and color characteristics of anthocyanins of Barbera grapes

Pigments	R_f value in BAW	Color characteristics		$AlCl_3$ reaction
		Visible	Ultraviolet	
Band 1a	0.23	bluish-purple	dull-purple	+
1b	0.29	bluish-purple	dull-purple	+
1c	0.31	bluish-purple	dull-purple	+
2a	0.35	blue-purple	dull-purple	+
2b	0.41	purple	dull-purple	–
3	0.43	mauve-purple	dull-purple	–
4	0.47	salmon-pink	dull-pink	–
5	0.50	purple-pink	dull-pink	–
6	0.56	purple	dull-purple	–

Table 2–R_f values and color characteristics of the acylated anthocyanins of Barbera grapes

Pigment	R_f values: BAW (4:1:5)	R_f values: 2% HOAc	Color: UV	Color: UV + NH_3	Color: DPNA	Color: DPNA + Na_2CO_3	Identification
1b	0.78	0.34	Blue fluor.	Blue fluor.	Tan	Brownish	Caffeic acid
2a	0.85	0.48	–	Blue fluor.	Yellow orange	Blue gray	p-Coumaric
3	0.86	0.50	–	Blue fluor.	Yellow orange	Blue gray	p-Coumaric
5	–	–	–	–	–	–	(acylated)
6	–	–	–	–	–	–	(acylated)

Table 3–R_f values and color characteristics of the alkaline degradation products of anthocyanins of Barbera grapes

Pigment	R_f value: BAW (4:1:5)	R_f value: 2% HOAc	Color characteristics: UV	UV + NH_3	DPNA	DPNA + Na_2CO_3	Identification
1a	0.61	0.42	–	–	Yellow	Gray brown	Gallic acid
1b	0.78	0.56	–	–	Tan	Gray blue	Protocatechuic acid
2a	0.78	0.52	–	–	Tan	Gray blue	Protocatechuic acid
2b	0.83	0.56	–	–	Orange yellow	Blue	Syringic acid
3	0.82	0.52	–	–	Orange	Blue	Syringic acid
4	0.85	0.55	–	–	Yellow	Violet	Vanillic acid
5	0.87	0.59	–	–	Yellow	Violet	Vanillic acid
6	0.83	0.56	–	–	Orange	Blue	Syringic acid
Spot B	0.69	0.60	–	–	Orange	Deep orange	Phloroglucinol

Standards	BAW	2% HOAc	Color: DPNA	Color: DPNA + Na_2CO_3
Syringic acid	0.82	0.53–0.56	Orange	Blue
Vanillic acid	0.85	0.58	Yellow	Violet
3-Orthomethylgallic	0.76	0.47	Red orange	Red purple
Protocatechuic acid	0.78	0.52	Tan	Brown
Gallic acid	0.58	0.41	Yellow	Gray brown
Phloroglucinol	0.67	0.59	Orange	Deep orange

Anthocyanidins

The R_f values of the anthocyanidins (aglycones), using Forestal solvent were: spot A, 0.40; spot B, 0.60; spot C, 0.67. Since the Forestal solvent fails to separate well cyanidin (0.49) from petunidin (0.46), and peonidin (0.63) from malvidin (0.60), the Bu-HCl solvent was run after the Forestal solvent in the second direction. The pigments were identified from their R_f values and positions on the paper as delphinidin (A1), petunidin (A2), cyanidin (B), malvidin (C) and peonidin (D).

Partial acid hydrolysis

The partial acid hydrolysis method was used to determine the number of sugar moieties in the individual pigments.

An anthocyanin with one sugar per aglycone yields upon chromatography two spots, namely the 3-monoside and the aglycone. A 3-diglycoside would yield three spots, and a 3,5-diglycoside yields four spots. If the pigment contains a sugar in the C5-OH of the A-ring, a characteristic fluorescence under ultraviolet radiation would be seen.

The chromatograms of the hydrolysis products developed with BAW and AWH solvents indicate that pigments 1a, 1b, 2a, 2b, 3 and 4, are monoglucosides. Two spots are present on each chromatogram, the 3-monoglucoside and the aglycone. Examination under ultraviolet radiation did not reveal any fluorescence, indicating the absence of 3,5-diglucosides.

Acyl moieties

Acid hydrolysis can remove the acyl groups from the anthocyanins. Identification of the acyl groups was accomplished by co-chromatography with authentic samples of phenolic acids. The acids commonly involved in acylated anthocyanins in grapes are p-coumaric acid and caffeic acid (Ribéreau-Gayon, 1959; Chen and Luh, 1967), and acetic acid (Anderson et al., 1970a, b).

The characteristic color of the acids after spraying with DPNA (diazotized-p-nitroaniline) and Na_2CO_3 (20% w/v) can also help the identification. Caffeic acid exhibits a very intense blue fluorescence, and p-coumaric also after exposure to NH_3 vapor.

The acyl groups in some of the anthocyanin pigments of Barbera grapes are presented in Table 2. Caffeic acid was found in pigment 1b. The spot became brown after spraying with DPNA and Na_2CO_3. It had the same R_f as that of an authentic sample of caffeic acid. Pigment 2a was acylated with p-coumaric. Pigment 3, the major pigment, was also acylated with p-coumaric acid. There was an indication that another minor pigment, malvidin-3-glucoside acylated with caffeic acid, was present which moved slightly

Table 4—R_f values of the anthocyanins of Barbera grapes

Pigments	R_f values at 20°C 1% HCl (4 hr)	R_f values at 20°C AWH (6 hr)	Identification
1a	0.04	0.17	Delphinidin-3-monoglucoside
1b	0.09	0.34	Cyanidin-3-monoglucoside with caffeic acid
1c	0.05	0.24	Petunidin-3-monoglucoside
2a	0.06	0.26	Cyanidin-3-monoglucoside with p-coumaric acid
2b	0.06	0.33	Malvidin-3-monoglucoside
3	0.08	0.30	Malvidin-3-monoglucoside with p-coumaric acid
4	0.08	0.35	Peonidin-3-monoglucoside
5	0.06	0.31	Peonidin-3-monoglucoside (acylated)
6	0.06	0.31	Malvidin-3-monoglucoside (acylated)

Reported values	1% HCl	AWH
Cyanidin-3-monoglucoside + p-coumaric	0.07[a] (0.17)[d] 0.11[b]	0.26[a]
Delphinidin-3-monoglucoside	0.03[a] 0.05[b]	0.18[a]
Malvidin-3-monoglucoside	0.06[a]	0.29[a]
Peonidin-3-monoglucoside	0.09[a]	0.33[a]
Petunidin-3-monoglucoside	0.04[a] (0.03)[d]	0.22 (0.30)[d] 0.25[c]

[a] Harborne, 1958a
[b] Acylated
[c] Somaatmadja and Powers, 1963
[d] R_f values in parenthesis have been revised by Harborne, 1967.

slower than pigment 3, when BAW was the solvent. This point needs further investigation.

The fluorescent property of the acylated pigments disappeared during partial acid hydrolysis. Albach (1963) found both caffeic and p-coumaric acids present as acylating agents in a group of the malvidin, peonidin, petunidin, and delphinidin pigments in Tinta Pinheira grapes. The pigment acylated with p-coumaric acid was moving ahead of the one with caffeic acid. The high R_f values of the pigments 5 and 6 in BAW solvent and their appearance and position on the chromatogram supports that they were acylated.

Alkaline degradation products of anthocyanidins

Identification of the benzoic acid derivatives from alkali degradation of anthocyanins is an important method for proving the structure of the pigments.

The R_f values and color characteristics of the alkaline degradation products are presented in Table 3. Alkaline degradation was also applied to the unhydrolyzed pigments.

On the chromotograms of the alkaline degradation products, two spots were always present, corresponding to the aromatic acid and phloroglucinol respectively.

Pigment 1a was identified as delphinidin, 1b and 2a as cyanidin, 2b, 3 and 6 as malvidin and 4 and 5 as peonidin. It appears that malvidin is the dominant aglycone of the pigments contributing to the color of Barbera grapes, followed by peonidin.

Chromatographic properties of anthocyanins

Table 4 lists the R_f values of the anthocyanin pigments from Barbera grapes.

Pigment 1a was identified as delphinidin-3-glucoside, 1b and 2a as cyanidin-3-glucoside with acylation. Pigments 2b, is malvidin-3-monoglucoside, and pigment 3 was malvidin-3-monoglucoside acylated with p-coumaric acid. Pigment 4 was peonidin-3-monoglucoside and Pigment 5 was peonidin-3-monoglucoside with acylation. Pigment 6 was malvidin-3-monoglucoside with acylation.

When malvidin-3-glucoside is acylated with caffeic acid or p-coumaric acid, the R_f values may be predicted from the acyl groups present. Acylation causes a decrease in R_f values in HOAc-HCl and 1% HCl solvents and a remarkable increase in BAW. The high R_f values of pigments 5 and 6 indicate that they are acylated pigments.

The R_f values found for pigment 1c correspond to that of petunidin-3-glucoside. Its position on the theoretical diagram corresponds to that for petunidin mono-glucoside.

Spectral properties of anthocyanins

Table 5 lists the spectral properties of the anthocyanins studied. The absorption maxima, the ratio of the optical densities at 440 nm to optical densities at the maxima and the effect of $AlCl_3$ on the spectra are further indications of the identity of the pigments that have been identified by other methods.

Pigment 1a has been tentatively identified as delphinidin-3-monoglucoside. It has an absorption maxima at 533 nm.

Pigment 1b appears to be cyanidin 3-monoglucoside acylated with caffeic acid; pigment 2b resembles cyanidin-3-monoglucoside; and pigment 3 malvidin-3-monoglucoside acylated with p-coumaric acid. Pigment 4 appears to be peonidin-3-monoglucoside, and pigment 5 peonidin-3-monoglucoside with acylation. Pigment 2b was not available for spectral identification. The ratio of OD at 440 nm to that at maximum peak can be used to differentiate the monoglucosides from the diglucosides based on the value of this ratio (Harborne, 1958). The 3,5- and 5-glycosides have only 50% of the ratio of absorption of the corresponding anthocyanin in which the 5-hydroxyl group is free.

DISCUSSION

THE EXTRACTION of anthocyanin pigments from Barbera grapes was easily accomplished with methanol containing 0.1% HCl. Sugars, minerals and other components which would otherwise interfere with the movements of pigments on paper chromatograms were eliminated by the use of a Dowex 50W-X4 cation exchange resin column. The impurities were readily washed away from the column with distilled water followed by methyl alcohol. The anthocyanins can be eluted from the column by gradually increasing the concentration of HCl in the methanol from 0.1 to 1.0%. In order to remove any methanol extractable impurities, the ion-exchange resin should be washed several times, first with 2N HCl, then with distilled water, and finally with methanol. It is important to allow the anthocyanin

Table 5–Spectral properties of anthocyanins of Barbera grapes

Pigment	Max (nm)	$\frac{E_{440}}{E.\ vis\ max}$ %	$AlCl_3$ shift (nm)	Identification
1a	533	23	+8	Delphinidin-3-monoglucoside
1b	525,305,275	28	+22	Cyanidin-3-monoglucoside with caffeic acid
2a	523,278	17	+15	Cyanidin-3-monoglucoside with p-coumaric acid
2b	–	–	–	Malvidin-3-monoglucoside
3	535,300,279	19	0	Malvidin-3-monoglucoside with p-coumaric acid
4	523,295,275	23	0	Peonidin-3-monoglucoside
5	525	24	0	Peonidin-3-monoglucoside (acylated)
6	533,280	28	0	Malvidin-3-monoglucoside (acylated)
Reported by Harborne (1967)				
Cn-3-G[a]	523	24	+18	
Pn-3-G[b]	523	24	0	
Dp-3-G[c]	534	18	+23	
Mv-3-G[d]	534	18	0	
Pt-3-G[e]	534	18	+14	

[a] Cyanidin-3-monoglucoside
[b] Peonidin-3-monoglucoside
[c] Delphinidin-3-monoglucoside
[d] Malvidin-3-monoglucoside
[e] Petunidin-3-monoglucoside

pigments to be absorbed on the resin for a shorter time, preferably less than half an hour. This will greatly facilitate the elution of the pigments from the resin with acidified methanol.

In most cases, arabinose was also observed together with glucose in the acid hydrolysate. It appeared as a brown spot on the chromatograms (Chen and Luh, 1967; Liao and Luh, 1970). It was produced as an artifact by the action of mineral acid in the solvent system on the paper. To determine whether arabinose was present as a sugar moiety or as an artifact, hydrolysis of the total extract, which had not been paper chromatographed, was done. No arabinose was found in the hydrolysate. It appears that glucose was the only sugar moiety present in the anthocyanins of Barbera grapes.

Ribéreau-Gayon (1959) studied the genetic relationship among *Vitis vinifera* varieties with regard to pigmentation. From a study of more than 80 hybrids, he concluded that diglycosides were characteristics of *V. riparia* and *V. rupetris* and that the diglycosides are dominant genetically. The anthocyanins could be divided into two groups: the monoglucosides (a genetic marker for *V. vinifera*) and diglycosides (*V. riparia*).

The present study indicates that the Barbera grapes contain monoglucoside only. Delphinidin, petunidin, cyanidin, malvidin and peonidin were shown to be the anthocyanidins. The presence of p-coumaric and caffeic acyl groups in the pigments is also indicated. The acyl group in pigments 5 and 6 was not positively identified. From the work of Anderson et al. (1970a, b), it is highly possible that both pigments may be acylated with acetic acid. From the above results, the Barbera grape belongs to the *V. vinifera* variety. The variety has been cultivated in California for many years (Amerine and Winkler, 1963). The wines made from it are characterized by a high acidity and deep red color. They are used quite often for blending with other wines.

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COLOR STABILITY OF BETANIN

INTRODUCTION

COLORANTS in food are either naturally occurring pigments or artificial dyes and lakes. Since the number of artificial colors is limited, and the safety of some has been questioned, there is a need to further explore natural pigments as food colorants.

The red beet root (*Beta vulgaris*) is a good source of red and yellow pigments known as betalains. Although similar pigments are found in other plants, the beet is the only food product containing this group of pigments. Because of the high pigment concentration in the beet root, and since beet powder is permitted as a colorant under the 1960 Color Additive Amendment, the pigments of the beet are an obvious choice for further investigation.

It is generally recognized that several factors affect the stability of color in red pigmented foods during preparation, processing and storage. These factors include heat, pH, light, presence or absence of oxygen, metals and other chemicals. Only limited studies are reported in the literature concerning the stability of betalains. However, the chemical nature of betalains has been studied extensively and reviewed by Dreiding (1961) and Mabry (1966, 1970).

Color of canned beets, though subject to change, is generally acceptable because the beet contains a high concentration of pigment. This level generally is great enough to counteract any loss that may occur during the canning process (Mackinney and Little, 1972). Aronoff and Aronoff (1948a, b) studied thermally degraded beets and found that pigments were lost gradually as a brownish material was formed. The effect of pH on color of beets during heat sterilization has been studied by Habib and Brown (1956) and Lusas et al. (1960). These investigators reported that raising or lowering the pH above or below the normal value for beets (approx. 5.5) caused marked changes in color and that minimum changes occurred at approximately pH 5.5. Darkening or browning of beet products by oxidation both before and after the canning process has been reported by Vilece et al. (1955), Habib and Brown (1956) and Lusas (1958). Livingston et al. (1954) studied the role of trace metal contamination in discoloration of beet puree and reported that both iron and copper accelerated darkening. Similar results were reported by Lusas et al. (1960).

This study was designed to investigate how some chemical and physical factors affect the stability of betanin in model systems and in beet juice and puree.

EXPERIMENTAL

Preparation of samples

Betanin and beet juice used in this study were prepared according to the method of von Elbe et al. (1972). The method to obtain pure betanin involves preparing an aqueous extract of beets, separating and purifying the pigment by chromatography, and eventually crystallizing the pigment.

Beet puree was prepared from cooked, diced beets which were blended (Waring Blendor) and packed in 307 × 306 cans. The cans were refrigerated until used.

Preparation of betanin solutions

McIlvaine's citric-phosphate buffer (0.1M) solutions with pH values of 3.0, 4.0, 5.0, 6.0, 7.0 and 8.0 were prepared (Anon. 1968). Buffer solutions with values of 2.0 and 9.0 were prepared by adding 1N HCl and 1N NaOH, respectively, to the McIlvaine's buffer.

pH

Changes in the color of betanin caused by pH were measured using solutions prepared by mixing 1 ml of an aqueous solution of betanin (concentration 0.4 mg/100 ml) and 4 ml of each buffer. The buffered solutions were introduced into 10-ml vials which were flushed repeatedly with nitrogen gas to limit oxygen.

Vials were maintained at 4°C in the dark, and absorbance readings (400–650 nm) were made initially and after 7 days of storage, using a Beckman Acta III spectrophotometer.

Temperature

The rate of degradation of betanin was measured after treatment at 100°C and pH values of 3.0, 4.0, 5.0, 6.0 and 7.0 and at 25° and 50°C at pH values of 5.0 and 7.0. The rate at which betanin degraded in beet juice was measured after treatment at 100°C and pH values of 3.0, 5.0 and 7.0. When the degradation rate of betanin in beet puree was measured, cans of puree were heated at 102, 110, 116, 121 and 129°C for 30, 45 and 60 min.

Model systems were prepared with known amounts of betanin dissolved in each buffer. After mixing, 1 ml of each solution was placed into a 3-ml vial (15–21 vials). Vials were flushed repeatedly with nitrogen to limit oxygen. Similar samples at pH 3.0, 5.0 and 7.0 were prepared using beet juice. Temperatures of 50, 75 and 100°C were maintained in an oil bath, and 25°C was maintained in a constant-temperature water bath equipped with a refrigeration cooling system (Blue M Electric Co., Blue Island, Ill.).

Light and/or air

How light and/or air affected the rate of degradation were determined on solutions of betanin at pH 7.0. The buffered betanin solutions were introduced into 5-ml vials and the vials from which air was to be limited were flushed several times with nitrogen. Samples to be exposed to light were stored using standard daylight with an intensity of 24 ft-c. The samples without exposure to light were stored in vials wrapped in aluminum foil. All vials were stored at 15°C. The betanin content remaining in solution was determined daily for 7 days. All determinations were done in duplicate and each determination consisted of eight paper electrophoretograms.

Analysis of betanin

Betanin contents in model systems, beet juice and beet puree were determined according to the method of von Elbe et al. (1972). In this method, an aqueous betanin solution is subjected to paper electrophoresis. The bands of individual pigments on the electrophoretograms are quantified by comparing peak areas obtained by densitometry to peak areas obtained with known betanin solutions. To measure betanin in beet puree, 100g of puree were washed with water under nitrogen. Washing was continued until no color remained in the eluate. The extract was made to volume and applied directly to electrophoretograms. Kinetic parameters were calculated by the method of Lenz and Lund (1974). This method involves determination of the average retention of betanin in containers heated at different temperatures and selected heating times.

RESULTS & DISCUSSION

Stability of betanin solutions

Effect of pH. Visible spectra (400–650 nm) were obtained for betanin solutions at each pH value, initially and after 7 days of storage at 4°C. The visible spectra for pH 2.0, 5.0 and 9.0 are shown in Figure 1. Absorbance values and absorption maxima (A_{max}) of each sample are in Table 1. No shifts in the absorption maxima of betanin solutions were noted between pH 4.0 and 7.0, and the spectra of these solutions were found to be identical. Below pH 4.0, i.e., pH 2.0, a slight shift (2 nm) toward a shorter wavelength

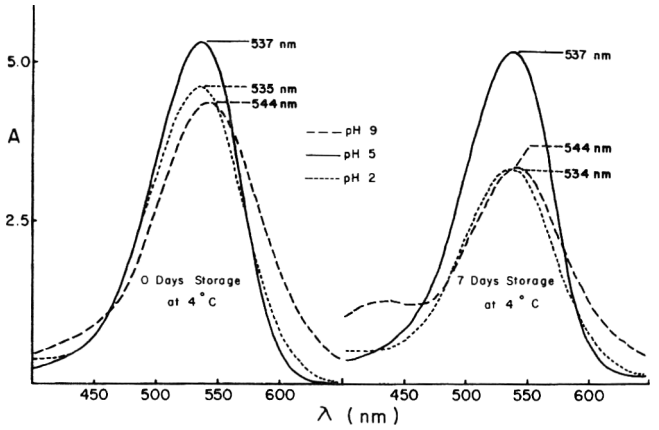

Fig. 1—Visible spectra of betanin at pH 2.0, 5.0 and 9.0.

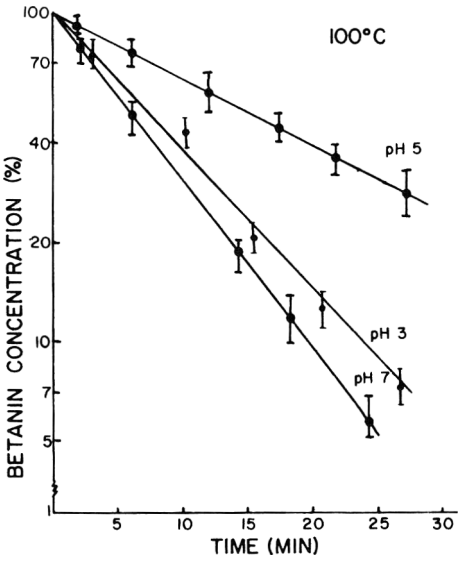

Fig. 2—Degradation rates for betanin in a model system at 100°C at pH 3.0, 5.0 and 7.0.

of 535 nm occurred, and the intesity of the absorbance decreased. In addition, the spectrum increased slightly in absorbance in the 575–650 nm region and the color of the solution changed from red to red-violet.

Above pH 7.0, the absorption maximum shifted toward a longer wavelength (544 nm at pH 9.0), and the intensity of the absorbance decreased. A considerable increase in absorbance occurred in the 575–650 nm and 400–450 nm regions. These alterations were accompanied by a marked change in color from red to violet. The above data agree well with those reported by Nilsson (1970).

Storage of the betanin solutions for 7 days at 4°C had no measurable effect on the absorption maximum of each solution, and the intensity of the absorbance did not change between pH 3.0 and 7.0. However, marked decreases in absorbance occurred when the pH value was below 3.0 or above 7.0 (Fig. 1 and Table 1). The stability of betanin was greatest between pH 4.0 and 5.0.

These results agree reasonably well with those presented by Peterson and Joslyn (1960) who reported that betanin was most stable near pH 4.0. Their data, however, indicated that betanin was more stable at pH values in the range of 1.0–3.0 than at pH 5.0. This observation was not confirmed. Although the temperature of storage and buffers used in the two studies were similar, direct comparison of the data was not possible because the conditions of storage, time of storage and presence or absence of light and/or air, were not reported.

Effect of temperature

When solutions of betanin pigment were heated for various times, the red color gradually diminished and eventually a light brown color appeared. This change of color was studied by determining the decrease in betanin content and calculating the percent of betanin remaining after heating as:

$$\%R = \frac{\text{mg of betanin at X time}}{\text{mg of betanin at 0 time}} \times 100$$

where %R = percent remaining.

The percentages (%R) were plotted on semilogarithmic paper against time. The straight line relationship obtained indicates that the rate of degradation of betanin upon heating followed first order reaction kinetics. The half-life ($T_{½}$) of the pigment can therefore be calculated as:

$$T_{½} = 0.693/k$$

where k is the rate constant, obtained by multiplying the slope of the line by −2.303 (Feliciotti and Esselen, 1957). Figure 2 presents data for pH values of 3.0, 5.0 and 7.0 when the percent of betanin that was remaining in the model system was plotted against time.

Data in Table 2 show the rate constants (k) and half-life ($T_{½}$) values for betanin at pH values of 3.0, 4.0, 5.0, 6.0 and 7.0 and for beet juice at pH values of 3.0, 5.0 and 7.0 when both were heated at 100°C. A graph of half-life versus pH in the model systems and the beet juice is shown in Figure 3. These data clearly indicate that the stability of betanin is greatest between pH 4.0 and 5.0. It is noteworthy that the stability of betanin in beet juice at pH 3.0 and 7.0 is similar to the stability of buffered betanin solutions. However, at pH 5.0, betanin in beet juice is far more stable. This indicates a definite protective effect by the juice constituents.

Table 1—Absorbances at absorption maxima (A_{max}) of betanin solutions[a] at different pH values and stored at 4°C

	Storage time			
	0 days		7 days	
pH value (± 0.05)	Absorbance ± 0.01	A_{max} ± 1 (nm)	Absorbance ± 0.01	A_{max} ± 1 (nm)
2.0	0.47	535	0.34	534
3.0	0.51	535	0.47	534
4.0	0.53	537	0.52	537
5.0	0.54	537	0.53	537
6.0	0.53	537	0.51	537
7.0	0.52	537	0.47	537
8.0	0.49	538	0.37	538
9.0	0.49	544	0.34	544

[a] Betanin concentration: 0.45 mg/100 ml

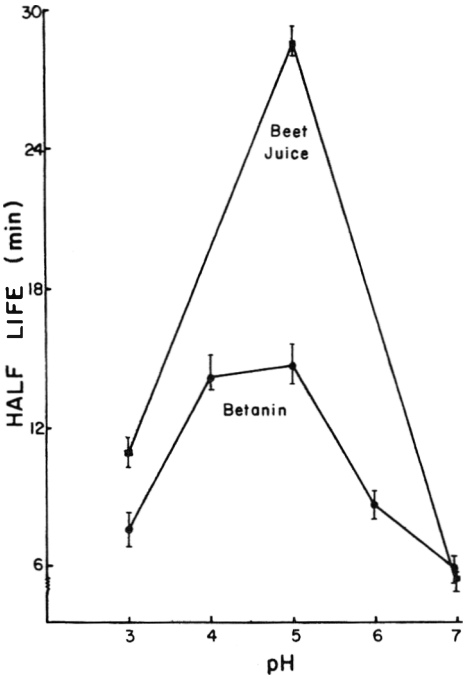

Fig. 3—Half-life for betanin in a model system and betanin in beet juice as a function of pH at 100°C.

The stability of betanin solutions at different temperatures was studied further at pH values of 5.0 and 7.0. These values were chosen because the stability was greatest at pH 5.0 and lowest at pH 7.0. Data on stability of betanin in terms of k, and $T_{½}$ for these pH values at temperatures of 25, 50 and 75°C appear in Table 3. The data clearly show that the heat stability of betanin decreases with an increase in temperature.

The activation energy (E_a) was calculated from a semilogarithmic plot of k vs 1/T and was found to be 12.5 ± 2 kcal/mole. The method of Lenz and Lund (1973) and the data in Table 4 were used to calculate an E_a value for betanin in beet puree and it proved to be 10.0 ± 2 kcal/mole. These two values suggest that the mechanism of degradation in the model system is similar to that in the beet puree. It is of interest to note that the k value (0.02 at 100°C) for betanin in beet puree was identical to the k value (0.024 at 100°C) for betanin in beet juice. This again indicates a protective effect in puree at the natural pH (4.9) when compared to the k value (0.048 at 100°C) obtained for betanin in the model system.

The data on kinetics as calculated for degradation of betanin in model systems would suggest that beet pigments cannot be used as a food colorant, because of their extreme heat sensitivity. Even the protective effect noted in beet juice and beet puree would limit the application. To investigate if a greater protective effect might exist in some foods, beet pigment was added as a colorant to some products (von Elbe and Maing, 1973). Color loss was measured in terms of Hunter reflectance values and expressed as L, a_L, b_L, $\tan^{-1}$ a/b, $(a^2 + b^2)^{½}$ and $\Delta E = [(L_1 - L_2)^2 + (a_1 - a_2)^2 + (b_1 - b_2)^2]^{½}$. Table 5 shows selected data obtained with soy protein, protein gels and two types of sausage (summer sausage and bologna) that were colored with betanin. The sausages were prepared without nitrate or nitrite, and the characteristic cured meat color was produced only by betalain pigments (von Elbe et al., 1974). Data on color indicate that changes during storage were very small and illustrate that the color in products is more stable than the color of the model systems would indicate. Furthermore, they point out that the protective effect in these

Table 2—Degradation rates[a] for betanin solutions and beet juice as a function of pH at 100°C ± 1

pH ± 0.05	k $(min)^{-1} \times 10^{-3}$	$T_{½}$ (min) ± CE[b]
3.0	94	7.4 ± 1
4.0	51	13.6 ± 2
5.0	48	14.5 ± 2
6.0	79	8.1 ± 1
7.0	118	5.9 ± 1
Beet juice		
3.0	79	8.8 ± 1
5.0	24	28.6 ± 3
7.0	135	5.1 ± 1

[a] Average of triplicate determinations
[b] CE = maximum calculated error

Table 3—Degradation rates[a] for betanin solutions as a function of temperature at pH 5.0 and 7.0

Temperature	pH ± 0.05	k $(min)^{-1} \times 10^{-3}$	$T_{½}$ (min) ± CE[b]
25 ± 1	5.0	0.61	1150 ± 100
	7.0	6.2	110 ± 10
50 ± 1	5.0	2.2	310 ± 30
	7.0	13.8	50 ± 5
75 ± 1	5.0	7.8	90 ± 10
	7.0	35	20 ± 2

[a] Average of triplicate observations
[b] CE = maximum calculated error

Table 4—Average percent retention of betanin in beet puree

	Temperature °C ± 1			
Time (min)	102	110	116	129
30	83	68	62	52
45	57	37	35	—
60	46	31	16	—

Table 5—Hunter color reflectance values of food systems colored with beet pigment and stored at 4°C

Hunter color reflectance values	Food product							
	Protein-gel pH 5.0		Soy protein pH 6.4		Summer sausage pH 4.8		Bologna pH 5.8	
	0	16	0	16	0	7	0	7
L	15.4	16.9	58.7	59.6	42.7	45.2	47.9	46.2
a_L	3.9	2.9	21.3	20.0	10.1	11.1	11.7	10.7
b_L	−0.9	−0.3	4.5	5.6	7.2	7.3	10.7	11.1
$\tan^{-1}$ a/b	−77.0	−84.1	78.1	74.4	54.5	56.6	47.5	44.0
$(a^2 + b^2)^{½}$	4.0	2.9	31.8	20.8	12.4	13.3	15.9	15.4
ΔE	—	1.8	—	1.8	—	2.7	—	2.0

protein foods exists and is adequate to make use of betalains as food colorants practical.

Influence of light and/or air

The sensitivity of betanin to degradation by light and/or air was determined by storing betanin solutions at pH 7.0 under air or nitrogen with or without exposure to light for 6 days at 15°C. Presence of air increased the rate of degradation by 14.6 ± 0.5% and light by 15.6 ± 0.5%. The increase in the rate of degradation caused by exposure to air and light was 28.6 ± 0.5% indicating that these effects are cumulative. These results demonstrate the sensitivity of betanin to air and/or light and the need to protect products containing betanin against long exposure to air or light. This agrees well with the observation by Vilece et al. (1955) that a small amount of oxygen (6%) in the headspace of a can of sterilized beet puree was sufficient to cause browning near the surface.

CONCLUSIONS

RESULTS of these experiments indicate that the color of betanin is stable when the pH value is proper and is relatively heat labile in unprotected systems.

The color in model systems is most stable between pH 4.0 and 6.0. The thermostability of betanin in model systems is pH dependent and greatest in the range of pH 4.0 and 5.0. The thermostability of betanin in beet juice and beet puree is greater than that observed with model system, suggesting a protective effect. This protective effect also appeared with some foods that contained added betanin. Based on the data presented, it is concluded that under selected conditions betalain pigments should find application as food colorants.

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INACTIVATION AND REGENERATION OF IMMOBILIZED CATALASE

INTRODUCTION

CATALASE immobilized on different carriers has been reported quite extensively. The carriers used included diethylaminoethyl (DEAE) cellulose (Mitz, 1956), Para-amino-styrene (Brandenberger, 1956), semipermeable collodion microcapsules (Chang and Poznansky, 1968) and cheesecloth (Balcom et al. 1971). The method of immobilization includes entrapping, covalent bond formation and cross-linking through cross-linking agents such as glutaraldehyde. The applications of immobilized catalase in food processing has been discussed by Balcom et al. (1971). A comparison of the performance of different reactors using immobilized catalase was reported by O'Neill (1972). He reported that the selection of reactor types (such as plug flow or stirred tank) depends on the reaction order of catalatic decomposition of hydrogen peroxide. The reaction mechanism of catalatic decomposition of high concentration of H_2O_2 is still undecided in the literature. According to the calculations of Bonnichsen et al. (1947), the data obtained by many investigators do not fit the hyperbolic relationship required by the Michaelis-Menten theory. Moreover, Ogura (1955) reported that through the "quenching" technique, using a rapid flow two-mixer method, the relationship between the rate of reaction and the substrate concentration fitted the Michaelis theory. One of the difficulties in obtaining true kinetic data is the fact that catalase is inactivated rapidly at high hydrogen peroxide concentration.

Here we report catalase immobilized on collagen which has been used to immobilize many enzymes (Vieth et al. 1972a, b; 1973; Wang and DiMarco, 1972; Wang and Vieth, 1973; Ventkatsubramanian et al., 1972). The reactor selected for this study was that of a plug-flow type.

MATERIALS & METHODS

Preparation of the active membrane

Collagen from cow hide was obtained from the USDA Eastern Regional Research Laboratories, Philadelphia, Pa.; beef liver catalase from Worthington Corp., Freehold, N.J.

All other materials were of C.P. grade.

20g of wet collagen was suspended in distilled water to a volume of 200 ml. pH was brought to 4.5 by the slow addition of 1N HCl with constant stirring.

The dispersion was homogenized with a blender until a viscous dispersion was obtained. This step was done three or four times with cooling intervals between them. Beef liver catalase (100 mg) was then added and the dispersion was again homogenized. Degasing was done by vacuum.

The dispersion was cast on a plastic sheet to about 2mm thickness and was air dried at room temperature for 40 hr. After drying, the film was easily detached from the plastic sheet.

In some experiments, the dried membrane was tanned with glutaraldehyde. The film was soaked for 15 sec in 10% glutaraldehyde solution, pH 8.0. After that, the film was washed with running tap water to remove unbound glutaraldehyde.

Preparation of the module

Dried films were layered on a supporting material such as cotton cloth. They were rolled around a glass rod to form a module. The module was then fitted into a glass column to form a reactor.

The substrate solution was pumped to the lower side of the module by a zero-max pump, (type CCW_2 with Sigmamotor model T8). All the experiments were done by continuous flow of substrate through such a reactor. Flow rate in these experiments was 5 ml/min and washing was done by a 2-min flushing at 45 ml/min with 0.01M, pH 6.8 phosphate buffer.

Determination of catalase activity

The method of Herbert (1955) was used. This method is based on an iodometric assay of the H_2O_2 concentration before and after the catalase reaction. To check the free enzyme activity, 1 ml of enzyme solution in 0.01M sodium phosphate buffer pH 6.8 was mixed with 5 ml of 0.01M H_2O_2 in the same buffer. The reaction was stopped 30 sec after mixing by the rapid addition of 2 ml 1N H_2SO_4.

To determine the concentration of H_2O_2 left in the reaction mixture, 0.5 ml of 10% KI was added, followed by one drop of 1% ammonium molybdate. A yellow color developed in about 1 min. A titration was done with 0.02N $Na_2S_2O_3$. When the color had almost disappeared, a drop of starch solution was added and a blue color immediately developed. This color was then titrated with the thiosulfate solution to the end point.

There are two ways in assaying the bound enzyme activity. First, the active membrane in the module was assayed and the specific activity at steady state was determined according to the concentration of the H_2O_2 in the entering and outgoing solutions. The residence time was the measure of the reaction time.

Second, the membrane was ground into powder and dispersed in the phosphate buffer to eliminate diffusional resistance. The dispersion was assayed as the procedure used for the free enzyme.

Recovery of the enzymatic activity of the bound enzyme

The membrane was first inactivated by the H_2O_2 solution at different concentrations. The membrane was then washed with the phosphate buffer and incubated in it for different lengths of time. After incubation in the buffer, the module was washed for 2 min with the substrate solution and the activity again assayed. The washing steps were necessary to remove the buffer or substrate left in the reactor.

RESULTS

Enzyme activity

The following specific activities of different catalase preparations was determined.

Free enzyme. A solution of 0.02 mg/ml catalase in 0.01M phosphate buffer pH 6.8 was made. This preparation had an activity of 1870 international units per mg catalase.

Table 1—Loss of activity during the preparation of the active membrane

Sample	Activity in IU/mg	% Specific activity of the free enzyme
Free catalase	1870	100
Bound catalase before tanning	492	26.3
Bound catalase after tanning	61	3.3

Bound enzyme with no tanning. The catalase was bound to the collagen membrane according to the method described before. The concentration of the catalase in the dried membrane was 3.2% (w/w). A dispersion of 2 mg/ml active film in phosphate buffer was checked for the enzyme activity, and was found to be 492 IU/mg catalase.

Immobilized enzyme after tanning. A dried film was tanned as described and then washed, ground (like the untanned one) in a buffer and the activity was found to be 61 IU/mg catalase.

As shown in Table 1, after the immobilization process, the catalase-collagen membrane complex retained 26.3% of the initial activity. The tanning process reduced the activity retained to 3.3%. At this point, a decision had to be made as to whether tanning is a necessity or not. When an untanned active membrane was continuously washed overnight with 0.01M, pH 6.8 phosphate buffer, the activity retained was 32% of the unwashed membrane. The activity lost (68% of the original activity) was mainly due to the leaching of the loosely bound enzyme. Tanning creates cross-links between protein molecules in the membrane. It is expected that tanning improves the retentability of the enzyme on the membrane. While this process was successfully used in urease, glucose oxidase and whole cell immobilization on collagen (Wang and Vieth, 1973; Vieth et al., 1972a, b; 1973; Saini et al., 1972), it is not suitable for the immobilization of catalase. The tanning process itself inactivates more enzyme than it can save. Consequently, the membranes used in the following experiments were untanned. When such membranes were made and kept at 4°C, they were stable for more than 5 months.

Inactivation of the immobilized catalase during a continuous flow of H_2O_2 through the module

As shown in Figure 1, when 0.01M H_2O_2 was used, there was a gradual decrease in enzyme activity during the first 10 days. No enzyme activity was found in the outgoing solution, and it is assumed, therefore, that the decrease in activity was not due to leaching but to inactivation.

After the first 10 days, the enzyme activity was stable for another 10 days. The stabilized level was about 1/6 of the original activity. In the next 13 days, there was a slow inactivation which stopped at about 1/15 of the original activity. The module was still active when the experiment was terminated after 44 days of continuous operation.

As shown in Figure 2, when 0.1M H_2O_2 was used, inactivation occurred in a few hours. There was no stabilization of activity at two levels, but only a gradual decrease in enzyme activity toward complete inactivation.

Regeneration of catalase activity in a module

Inactivation of the immobilized catalase could be reversed by incubation of the module in 0.01M phosphate buffer, pH 6.8.

The recovery process was studied after inactivation of a module with 0.1M H_2O_2. The modules were washed thoroughly with phosphate buffer after inactivation and then incubated in phosphate buffer for a certain period of regeneration time. The regenerated module was flushed with substrate solution before testing.

As shown in Figure 3, the recovery of activity was very small with a short incubation time. Only after 3 hr of incubation was some measurable recovery detected. The degree of recovery is directly proportional to the length of incubation time. However, the higher the activity regenerated, the steeper is the inactivation curve when the module is exposed to hydrogen peroxide again.

DISCUSSION

Preparation of the active membrane

In using collagen as a carrier for enzyme immobilization (Wang and Vieth, 1973, Vieth et al. 1972a, b; 1973), the first and crucial step is to make a proper dispersion and membrane. Collagenous tissues are not soluble in water, and the dispersion is usually made by using a chemical dispersing agent, such as cyanoacetic acid, lactic acid, hydrochloric acid, citrate buffer (pH 3–4.5), Tris buffer (pH 9–12), etc. When collagenous tissues are treated with these chemicals, only a small portion of the total collagen is solubilized, and what is obtained is an aggregated collagen fibrillar dispersion (Becker, 1956; Hochstadt and Lieberman, 1960). The degree of aggregation seems to depend on the kind of dispersing agent used. This is indicated by the strength of the air-dried membrane when it is contacted with water. The membrane made from cyanoacetic acid, or lactic acid dispersion loses its structure and goes back to a dispersion in water, whereas the membrane made from hydrochloric acid dispersion retains its structure in the same process. (The acidity of the dispersions is pH 4.5). Consequently, when a membrane is made with lactic acid or cyanoacetic acid, it is necessary to go through the process of tanning (Wang and Vieth, 1973), or an annealing process (Vieth et al. 1972a, b; 1973) to create more cross-links between molecules and collagen

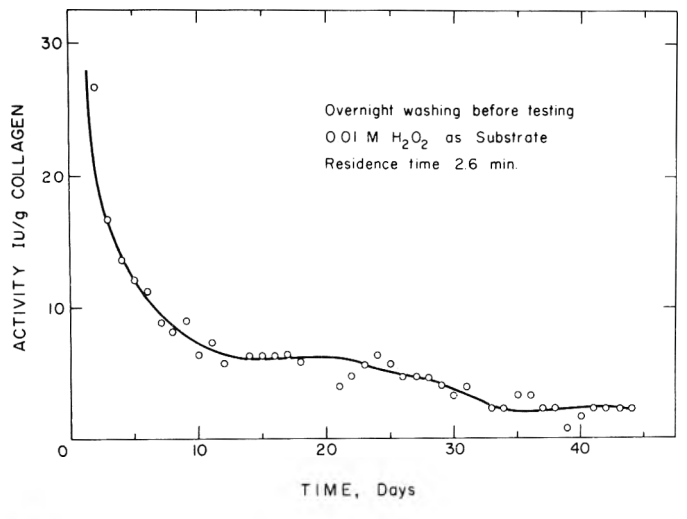

Fig. 1–Inactivation of collagen-catalase modular reactor with 0.01M H_2O_2.

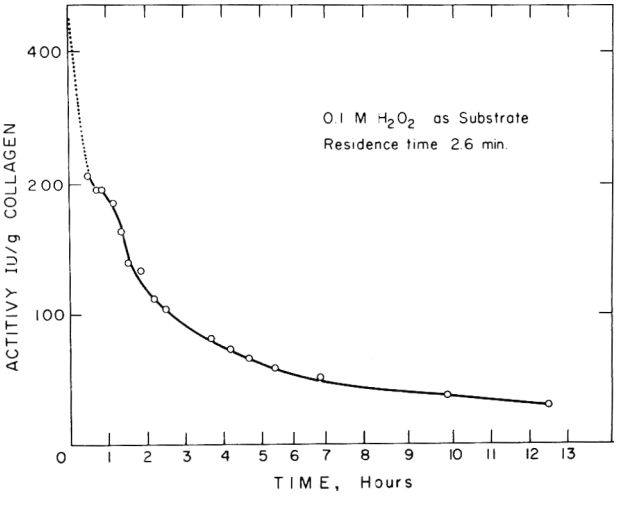

Fig. 2–Inactivation of collagen-catalase modular reactor with 0.1M H_2O_2.

fibrils. But these processes are not necessary for membrane made with hydrochloric acid. It is clearly more favorable to use hydrochloric acid than the other acids from both economical and technical points of view provided that the membranes made possess the same binding capacity for enzymes. An attempt was made to bind more catalase to collagen by tanning the dried catalase-collagen membrane but was not successful because of the inactivation of catalase during the tanning process. Since the membrane made from hydrochloric acid dispersion retains its structure without further cross-linking treatment, the tanning process was eliminated in making the active catalase-collagen membrane.

Inactivation of the active membrane in a module

Inactivation of immobilized catalase on collagen has a higher rate at 0.1M H_2O_2 than at 0.01M H_2O_2. This result is in agreement with that reported by Miller (1958) and George (1947). Balcom and Foulkes (1971) reported the loss of about 70% initial activity after 3500 ml of 0.05% H_2O_2 passed through a column containing 11.5g of catalase-cheesecloth complex made from glutaraldehyde coupling. As shown in Figure 1, the stable limit of catalase immobilized on collagen indicates a loss of 84% of its initial activity after 72,000 ml of 0.01M H_2O_2 passed through the module. This result indicates that catalase immobilized on collagen seems to be more stable than that immobilized on cheesecloth. Nevertheless, both the results reported here and that by Balcom and Foulkes (1971) showed rather rapid inactivation of immobilized catalase as compared to other enzymes immobilized on collagen (Wang et al. 1973). This may well be enough to discourage the use of immobilized catalase in continuous destruction of residual H_2O_2 in milk as part of a continuous cheese making proposition. However, the phenomenon of regeneration of immobilized catalase activity as reported here offers some encouragement.

Regeneration of inactivated immobilized catalase

The regeneration of inactivated immobilized catalase has not been reported before in the literature. Although the regenerations of thermally inactivated free catalase and peroxidase were noted in high-temperature short-time (HTST) processed canned foods (Guyer and Holmquist, 1954; Esselen, 1950; Farkas et al., 1956; Zoueil and Esselen, 1959; Vetter et al., 1959; Yamamoto et al., 1962; Resende et al., 1969) and in model systems (Joffe and Ball, 1962; Wang and DiMarco, 1972). The mechanism of the regeneration of the thermally inactivated catalase is unknown. However, the catalase molecule has been reported to consist of four subunits (Tanford and Lovrien, 1962; Valentine, 1964). The subunits can be formed by lyophilization, acid treatment, alkali treatment and other means. Samejima and Yang (1963) were able to almost completely regenerate the acid denatured enzyme with 90% return of the initial activity provided that the enzyme subunits were not allowed to stand at low pH long enough to allow the formation of precipitates. However, the mechanism of catalase inactivation by hydrogen peroxide at high substrate concentration is also unknown. There is no direct evidence as to the possible dissociation of the catalase molecule into subunits under high H_2O_2 concentration. Nevertheless, the regeneration phenomenon observed here indicates that the inactivation of the catalase molecules which are held to collagen molecules through multiple linkages of hydrogen bondings, electrostatic attractions and hydrophobic bondings, is reversible.

This is an interesting observation, and certainly deserves further investigations to elucidate the mechanisms of inactivation and regeneration.

From the application point of view, the recovery of activity from inactivated catalase might change the idea of giving up the use of immobilized catalase in food processing.

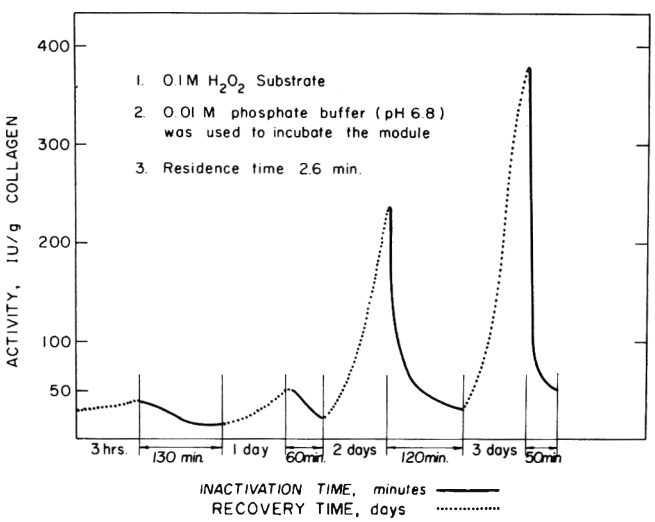

Fig. 3—Serial inactivation and regeneration of collagen-catalase modular reactor.

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SCANNING ELECTRON MICROSCOPE STUDIES ON DRY BEANS. Effects of Cooking on the Cellular Structure of Cotyledons in Rehydrated Large Lima Beans

INTRODUCTION

LEGUME SEEDS have been used as human food for more than 80 centuries and are still one of the most important sources of protein for a large segment of the world population. Commercial, domestic varieties of legumes include the dry seeds of the genus *Phaseolus,* such as large dry Lima beans (*P. lunatus*) and the white and colored common beans (*P. vulgaris*). Cooking of dry beans is necessary not only to tenderize the seed coat and cotyledon and develop acceptable flavor and texture, but also to make the bean protein nutritionally available. Dry beans are usually prepared for food by soaking in water at ambient temperature for 16–24 hr (overnight) and cooking in fresh boiling water with or without table salt or other condiments for 1 hr or longer depending upon the variety, age, storage history and other quality-dependent factors. Recently a simple process has been developed for preparing several types of quick-cooking Lima and other dry beans which develop maximum nutritive value and digestibility after cooking for 15 min or less (Rockland et al., 1971). The process consists of: (1) loosening seed coats by vacuum infiltration in a dilute solution of common inorganic salts including sodium chloride, sodium tripolyphosphate, sodium bicarbonate and sodium carbonate; and (2) soaking and rehydrating the beans in the same salt solution. Empirical variations in the salt formulations, processing time and temperature were used to optimize the process for individual bean varieties. However, the mechanism by which the beans are rendered quick-cooking remains obscure. Therefore it was of interest to utilize the scanning electron microscope (SEM) to characterize changes in Lima bean cotyledon tissue that are associated with the normal cooking process and to determine if they differ from those which occur during cooking of processed, salt-soaked, quick-cooking beans.

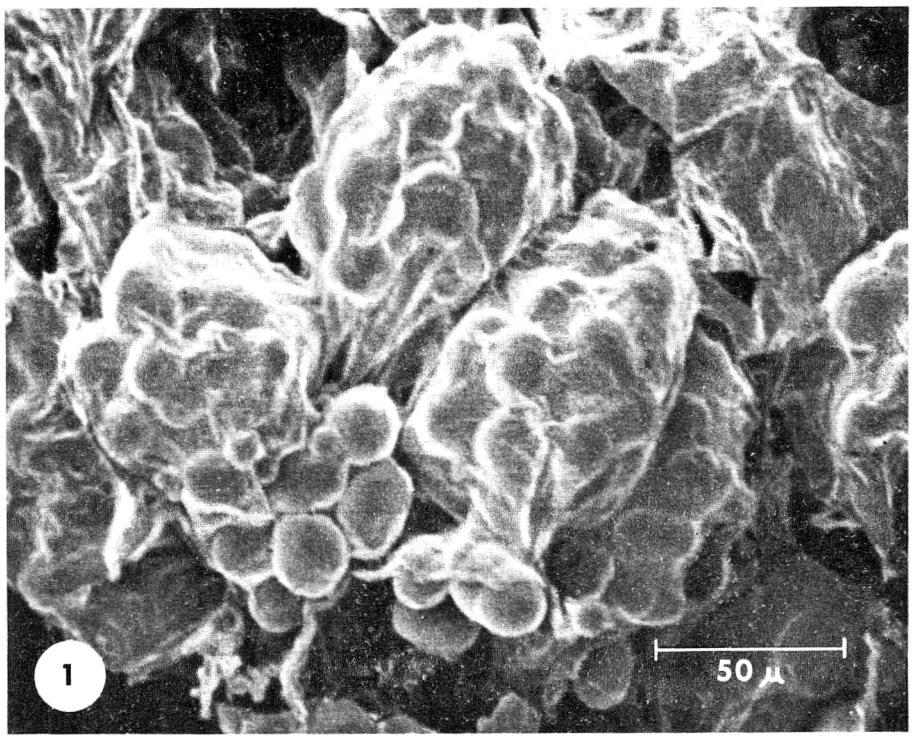

Fig. 1—Whole cells in raw, hydrated Lima bean cotyledon.

EXPERIMENTAL

ISOGENETIC, large dry Lima beans (*P. lunatus*, Var. Ventura) 1970 crop, were obtained from the University of California, Davis. They contained 10.3% moisture and were stored in a sealed polyethylene bag at 10°C.

Rehydration and soaking of dry beans

Standard dry beans were rehydrated by soaking in 5 volumes of distilled water for 24 hr at 20°C. Quick-cooking (salt-soaked) beans were prepared using the Hydravac vacuum infiltration process to accelerate rehydration in a solution containing 1.0% sodium chloride, 0.5% sodium tripolyphosphate, 0.75% sodium bicarbonate and 0.25% sodium carbonate as described previously (Rockland and Metzler, 1967).

Preparation of material for SEM studies

The seed coat was removed from the raw water-soaked or salt-soaked bean and one-half of the cotyledon was fractured near the center of the major axis by a sudden flexure. A 1 mm slice parallel to the ruptured surface was excised, placed on a glass slide and lyophilized. Cooked bean samples were prepared by placing whole, water-soaked beans in boiling water and cooking for specified periods of time or until they became tender and edible. Water-soaked beans required 45 min and salt-soaked beans required 10 min to cook completely in fresh boiling water. The seed coat of each bean was removed by hand and the hot cotyledon was fractured in the same way as the raw beans. A 1 mm slice parallel to the ruptured surface was excised, placed on a glass slide and air-dried. For SEM studies dry bean sections were attached to a glass slide with electrically-conducting cement and were coated with gold in a vacuum chamber to make the sample conducting and to enhance emission of secondary electrons. Isolated cells and cell wall fragments contained in a drop of aqueous suspension were air-dried on a glass slide before being coated with gold.

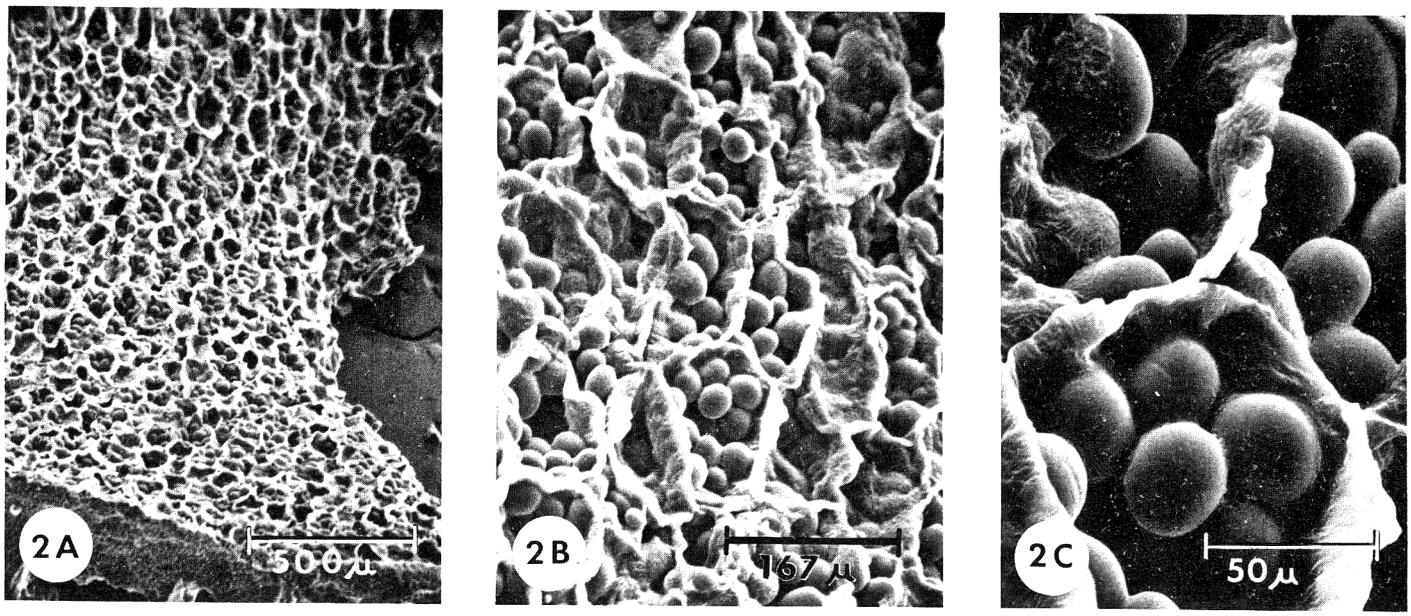

Fig. 2–Section through raw, hydrated Lima bean cotyledon .

RESULTS

Raw beans

Fracturing raw, turgid water-soaked or the more flaccid salt-soaked cotyledons normally ruptured cell walls, exposing the cell contents. However, occasional fields, viewed with the SEM contained nearly whole cells which resembled oblate plastic bags collapsed onto the large, nearly spherical starch granules inside (Fig. 1). No major differences were observed between the cell structures of raw, water-soaked and the corresponding salt-soaked, quick-cooking bean cotyledons. However, under the light microscope, the proteinaceous cytoplasmic matrix of raw cells appeared to be slightly more granular in the salt-soaked sample. Sliced surfaces of raw bean cotyledons were similar to those produced by fracturing. Viewed in the SEM, the sliced surfaces contained nearly spherical starch granules, averaging about 25 μm, closely packed within a honeycomb-like structure (Fig. 2). Irregular matrix particles, presumably proteinaceous, occupied the spaces among the starch granules. Boundaries between the walls of adjacent raw cells could not be distinguished by either optical or scanning electron microscopy. Adjacent cell walls appeared to be continuous with the middle lamella except at the junctions of three or more cells where intercellular spaces occurred (Fig. 2C). Washing the surface of freshly cut sections of raw cotyledons removed starch granules and the cytoplasmic matrix, exposing inner cell wall surfaces. Small pits, arranged in a semi-organized pattern were present in the membranes which lined the interior walls of cells from both water- and salt-soaked beans. These pits were also seen on fragments of isolated cell wall preparations (Fig. 6A). Starch granules, isolated from raw, water- and salt-soaked beans were indistinguishable (Fig. 3).

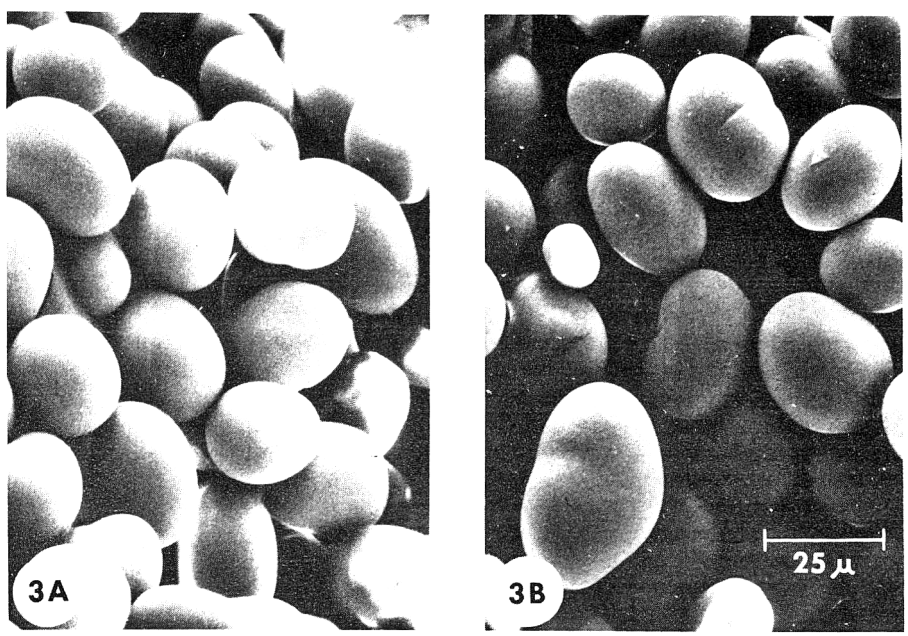

Fig. 3–Starch granules isolated from rehydrated large dry Lima beans: (A) Water-soaked beans; (B) Salt-soaked beans.

Cooked beans

In contrast to raw, soaked beans, in which cell walls ruptured when the cotyledon was fractured (Fig. 4A-1, 4B-1), cooked or partially cooked bean cells separated readily along the surfaces of individual intact cell walls (Fig. 4A-2, 4B-2). Short heat treatment loosened the intercellular matrix of the middle lamella sufficiently to allow separation of individual cells without rupture of cell walls. Dispersions of intact cells were obtained from water-soaked beans cooked 10 min or salt-soaked beans cooked only 2 min by squeezing fragments of the cotyledons between glass slides. There was a marked difference in the appearance of cells in the two partially cooked bean products.

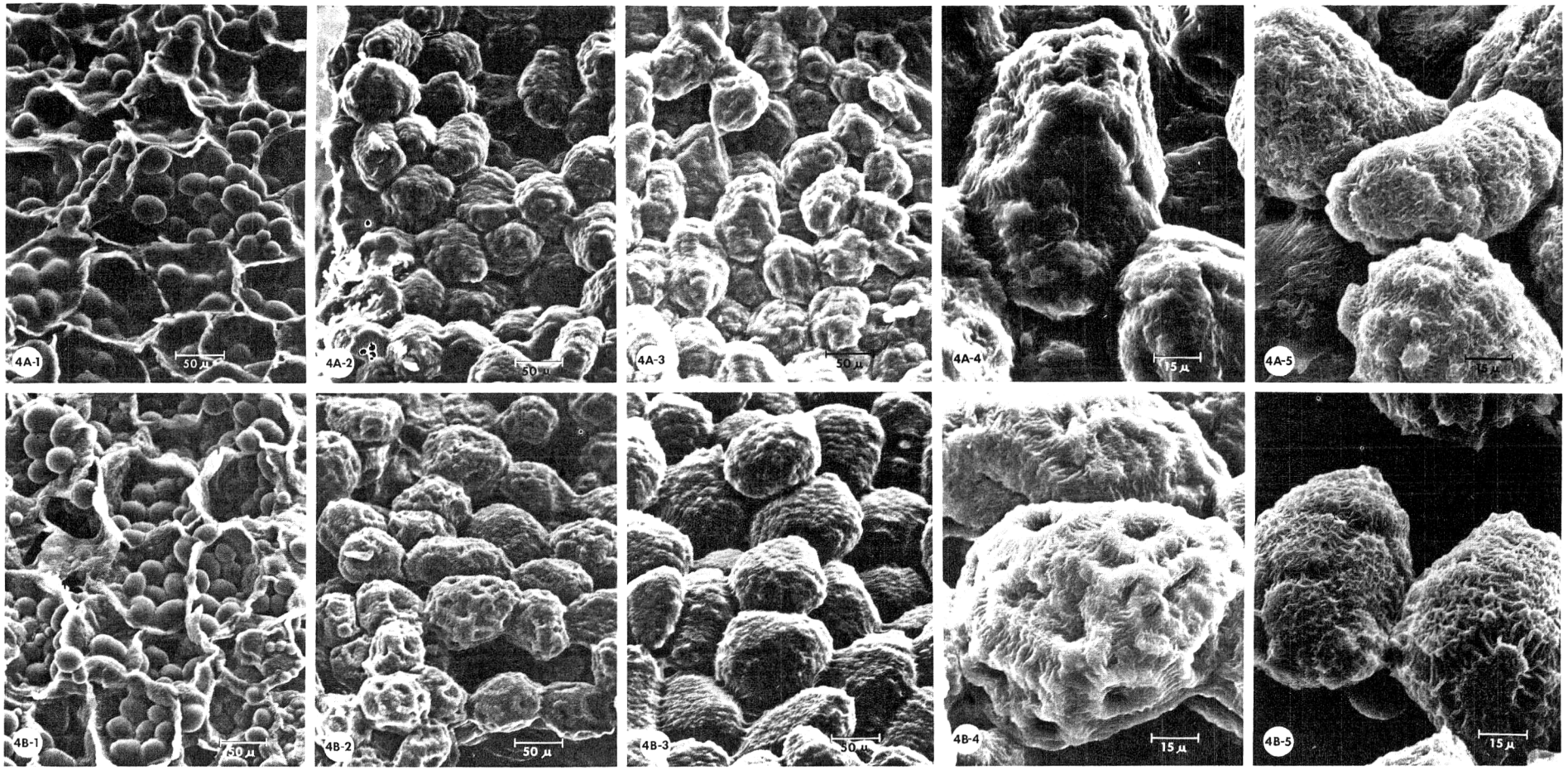

Fig. 4—Effects of cooking on cell structure in hydrated large Lima bean cotyledon: (A) Sections through fractured water-soaked cotyledon; (B) Sections through fractured salt-soaked cotyledon. 4A-1 Raw; 4B-1 Raw; 4A-2 Cooked 10 min; 4B-2 Cooked 2 min; 4A-3 Cooked 20 min; 4B-3 Cooked 3 min; 4A-4 Cooked 20 min; 4B-4 Cooked 2 min; 4A-5 Cooked 45 min; 4B-5 Cooked 10 min.

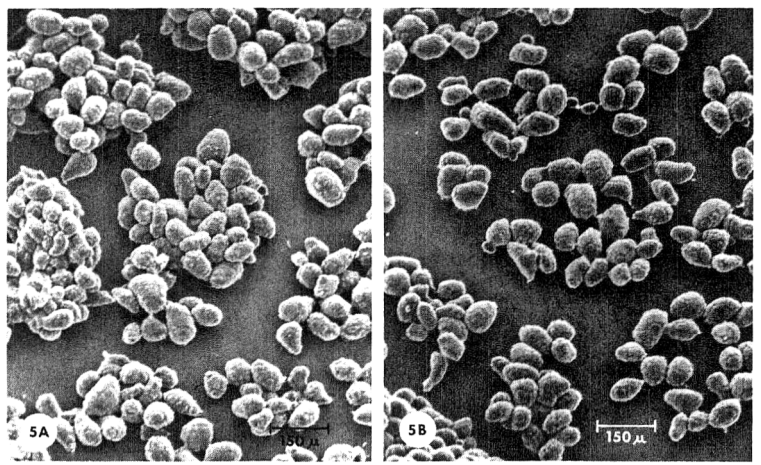

Fig. 5—Dispersion of cells from cooked cotyledons: (A) Water-soaked, cooked 45 min; (B) Salt-soaked, cooked 10 min.

The air-dried cotyledon cells from water-soaked beans had numerous irregular protuberances (Fig. 4A-2) quite different from the "bag of marbles" appearance of the raw, whole cells (Fig. 1). Cells from the salt-soaked beans, cooked only 2 min, were different from both the raw and water-soaked beans. They had an involuted appearance with numerous indentations covering the cell surfaces (Fig. 4B-2, 4B-4). Water-soaked beans required 20 min in boiling water to develop the same involuted appearance (Fig. 4A-3, Fig. 4A-4). The cells in salt-soaked beans lost their involuted appearance, became more spherical and developed numerous semi-regular protuberances after cooking for an additional 1–2 min or a total of 3–4 min (Fig. 4B-3). This configuration was not observed in any of the preparations prepared from water-soaked beans.

After cooking the water- and salt-soaked beans for 45 and 10 min respectively, at which time both preparations were completely cooked, the individual intact cells from both types of beans resembled each other closely (Fig. 4A-5, 4B-5). They had a multi-faced surface covered with wrinkled cell walls and were not distinguishably different in SEM photographs.

Cells of the fully cooked beans were relatively noncohesive. The intercellular bonding material appeared to have dispersed or dissolved, permitting individual cells to separate readily in either hot or cold water. The shape and integrity of the cooked cotyledons would appear to be maintained by the seed coat and by morphologically different membranes covering the convex and concave surfaces of each cotyledon. No differences were apparent in SEM observations of dispersed cells in fully cooked, water- and salt-soaked beans (Fig. 5A, 5B). Visual microscopic examination under polarized light of raw, partially cooked and completely cooked beans indicated that loss of birefringence, characteristic of starch gelatinization, occurred very rapidly at 100°C and was indistinguishable in both types of beans.

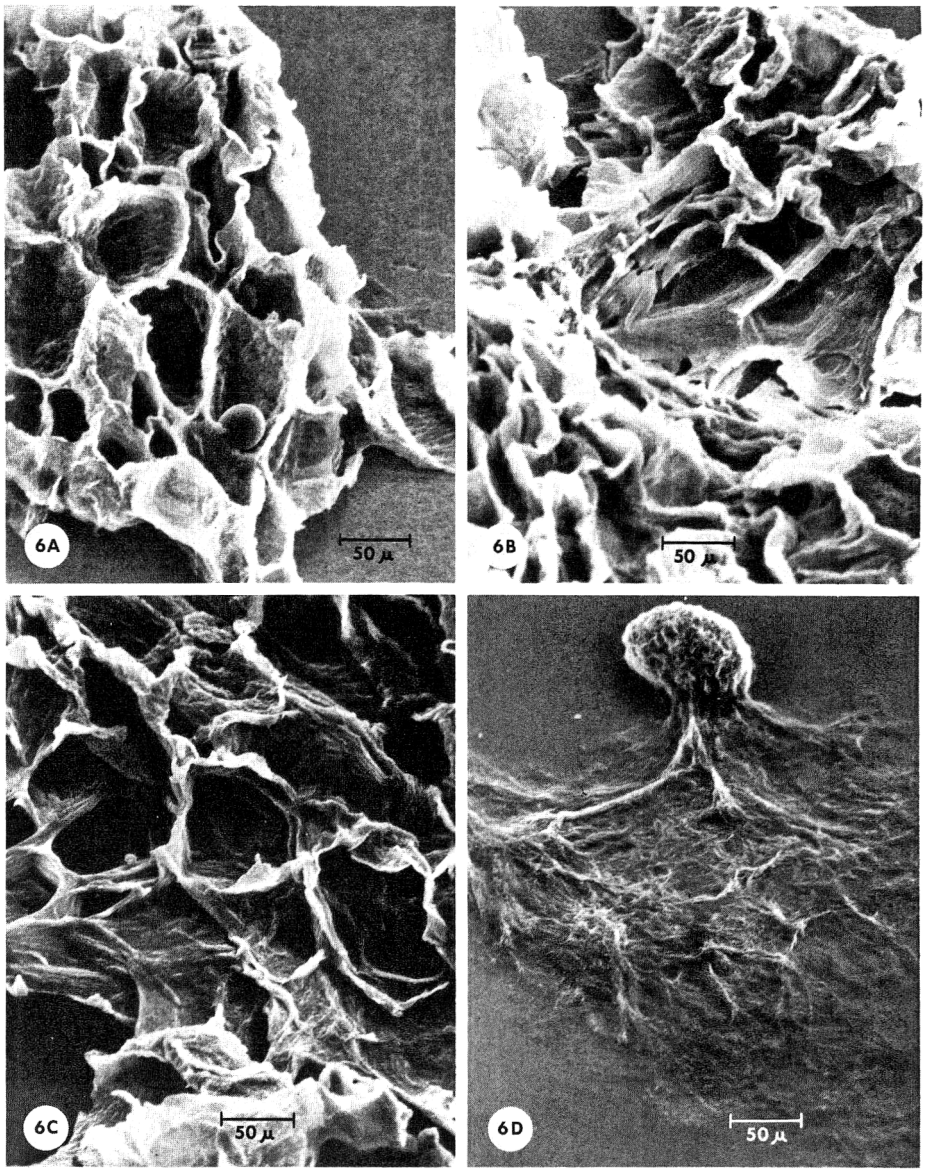

Fig. 6–Lima bean cell wall fragments: (A) Raw, unheated; (B) Heated in boiling water for 45 min; (C) Heated in boiling water for 45 min after preliminary soak in cold salt solution and water wash; (D) Heated in boiling salt solution for 45 min (see text).

Influence of cooking on isolated cell walls

As mentioned previously, cell walls of raw beans ruptured whereas cell walls of cooked beans maintained their integrity when the cotyledons were fractured. This difference in the properties of raw compared to cooked cotyledons can be conceived as being due to the plasticizing effect of boiling water on the middle lamella which relieves stresses imposed on cell walls during mechanical fracture of cooked cotyledons. Therefore it was of interest to study the effects of cooking on fragmented cell walls separated from their cytoplasmic environment. Lima bean cell fragments were prepared by macerating skinned, raw, water-soaked beans in distilled water using a high-shear blender which ruptured cell walls causing intracellular cytoplasm to be discharged into the aqueous slurry. The slurry was filtered through multiple layers of cheesecloth and washed exhaustively until no starch granules settled in filtrates of successive washings. The white fibrous mass was air dried. The preparation was composed of clumps of broken, evacuated cells arranged in an irregular honeycomb-like structure (Fig. 6A). A small proportion of the cells resisted rupture and remained attached to the walls of neighboring evacuated cells.

In contrast to whole cells which separated readily during cooking, no separation of adjacent evacuated cell walls was observed even after boiling the preparation for 45 min. The boiled cell fragments remained attached to adjacent cell walls (Fig. 6B) and appeared similar to the original unheated material (Fig. 6A). The intrinsic factors or properties which influenced the separation of whole cells in boiling water were not present in the isolated cell fragment preparation. When raw cell fragments were added to a boiling solution of the dilute salt mixture used to process quick-cooking beans, the honeycomb structure collapsed almost immediately into a thin, filmy nondescript mass. It did not disperse completely into separate cell fragments even after boiling for 45 min. Random, individual intact cells,

contained within the cellulosic reticulum of the washed, salt-free preparation, retained their integrity and shape, but remained attached to the collapsed cell walls of adjacent cells (Fig. 6D). The localized, transitory effect of the salt solution was demonstrated by cooking a sample of the cell wall preparation that had previously been soaked in cold salt solution for 4 hr and washed exhaustively to remove the salts before cooking. The cooked washed preparation retained the honeycomb structure (Fig. 6C) and resembled the raw (Fig. 6A) and the water-cooked (Fig. 6B) cell wall preparation.

DISCUSSION

DURING NORMAL cooking in boiling water, intercellular material within the middle lamella softens and permits separation of adjacent whole cells. No apparent differences have been found between the cellular structure of cooked, water- and salt-soaked bean cells observed in the scanning electron microscope. Different cooking rates of the two types of beans appear to be related primarily to differential rates at which cell separation occurs.

The middle lamella of plant tissue is generally considered to be composed of pectic substances (Kertesz, 1951) associated with divalent cations such as calcium and magnesium (Letham, 1962), and possibly proteinaceous material (Ginzburg, 1961). Cell abscission (Stösser et al., 1969) and the separation of apple and carrot plant cells (Letham, 1962) and peaches and *Avena* coleoptiles (Shah, 1966) have been related to the loss of calcium and magnesium from the middle lamella. It is suggested that the separation of bean cells during cooking may also be related to the transposition or removal of divalent cations, particularly calcium and magnesium, from bridge positions within the pectinaceous matrix of the middle lamella.

Letham (1962) has also reported that rapid and efficient separation of plant cells may be obtained in a warm, slightly alkaline solution of polyphosphates and other metal chelating agents. Rapid cooking and tenderization of salt-soaked (quick-cooking) dry beans may be due to facilitation of divalent cation transport or removal from the middle lamella of bean cotyledons in the presence of imbibed alkaline tripolyphosphate. Slower cooking of normal, water-soaked beans may be attributed to the more gradual leaching of divalent cations within the middle lamella by phytic acid and other naturally-occurring chelating agents contained within the intracellular cytoplasm. Failure of an isolated bean cell wall preparation to disperse in boiling water may be associated with the unavailability of metal chelating agents in the washed residue of ruptured cells from which the cytoplasm has been removed. Collapse of the honeycomb structure in a raw, cell wall preparation after immersion in boiling alkaline tripolyphosphate solution is consistent with the premise that: divalent cations bridge and support the pectinaceous matrix between bean cells; and that elimination of the cation bridge by a metal chelating agent allows softening of the middle lamella and separation of whole cells.

During cooking of whole beans, mechanical stresses, imparted during starch gelatinization, protein denaturation, swelling and heat convection, may further facilitate cell separation and the development of the uniform, smooth texture in fully cooked beans. Complete dispersion of an isolated cell wall preparation was not effected in boiling salt solution presumably because insufficient mechanical stress was imposed upon the flaccid, ruptured cell residues.

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L-ASPARTYL-L-PHENYLALANINE METHYL ESTER (ASPARTAME) AS A SWEETENER

INTRODUCTION

ASPARTAME (L-aspartyl-L-phenylalanine methyl ester) was found to be about 160 times sweeter than 4% sucrose in aqueous solutions and compared favorably to sucrose in sensory attributes (Cloninger and Baldwin, 1970). Therefore, this study was conducted to establish equivalents to various concentrations of sucrose, to evaluate the effects of selected ingredients on sweetness of the aspartame as compared to sucrose, and to monitor effects on pH. As an example of a typical application, the aspartame was evaluated in a noncarbonated orange-flavored beverage. Since sugar affects texture and body of food products, as well as sweetness (Glicksman and Farkas, 1966), the aspartame was evaluated in combination with gelatin, gum arabic (GA), methocel (MC), and carboxymethylcellulose (CMC).

EXPERIMENTAL

ASPARTAME for this study was supplied by Searle Biochemics, Div. of G.D. Searle & Co., Chicago. All solutions were prepared with water which was redistilled in glass. Except for determinations of sweetness equivalents in water and in noncarbonated beverage, tests were conducted on levels of sweetness comparable to that of soft drinks (12% sucrose) and slightly sweetened tea or coffee (4% sucrose).

Sensory and statistical analyses

All sensory tests were conducted by the ranking method which permitted treatment of the data by chi-square for ranks (Friedman, 1937) as explained in an earlier publication (Cloninger and Baldwin, 1970). Four 20-ml samples at room temperature were served in 6-oz coded glasses randomly arranged on the serving tray. The composition of the four samples depended upon the objective of the test and is included in the description of specific tests. Judges were not required to swallow the samples but were instructed to be sure the sample contacted all parts of the mouth. Charcoal filtered, odor-free water (APHA, 1965) was provided for rinsing the mouth between samples. Judgments for each test were made by at least 20 University of Missouri students or staff members. Tests were conducted in a specially designed taste panel room, and judges were seated in individual booths.

Sweetness equivalents

To determine aspartame concentrations equivalent in sweetness to sucrose concentrations of 2, 4, 8, 12, 20 and 30% (w/v), two sweetness levels were included in each set of four samples. Judges were instructed to rank the samples in order of increasing sweetness. When low and high sweetness for sucrose solutions were confused, judges were considered unreliable and their data were discarded. If a significant ($P < 0.05$) difference was found between aspartame and sucrose within a level of sweetness, aspartame solutions were reformulated and retested.

Aspartame in buffer

Aspartame (0.025% and 0.12%) and sucrose (4% and 12%) solutions were evaluated in a buffer system (pH 3.2) prepared as follows: 84.4 ml 0.1M citric acid + 15.6 ml 0.2M Na_2HPO_4 made to 2000 ml with water redistilled in glass. Sensory evaluation included one set of randomly arranged samples which were ranked in order of increasing sweetness and one set ranked for increasing sourness. To orient the judges to sourness in presence of sweetness, two references were served before the ranking test for sourness. These references were as follows: 4% sucrose in half strength buffer and 4% sucrose in full strength buffer.

Aspartame and gelatin in buffer

Aspartame or sucrose was first dissolved in approximately 50 ml buffer. Gelatin (1.5%) was hydrated in approximately 200 ml buffer, heated to dissolve, and then cooled to 40°C before combining with the sweetener solutions. After further cooling to room temperature, solutions were brought to volume, dispensed into coded glasses and refrigerated (8°C) overnight. Four coded samples representing 0.025% and 0.12% aspartame and 4% and 12% sucrose gels were arranged randomly on trays for judges to rank according to increasing sweetness. Plastic spoons were provided to facilitate tasting.

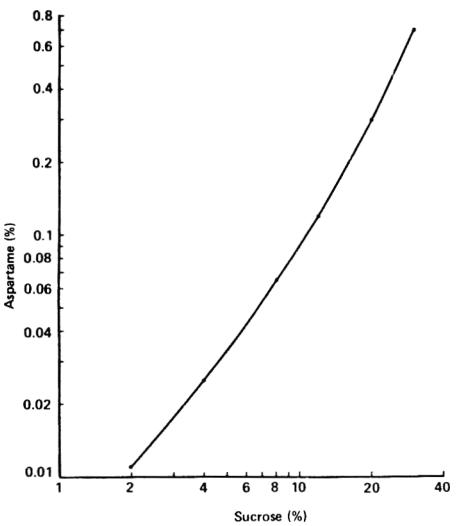

Fig. 1—Relationship of sweetness of aspartame and sucrose (n = 20).

Aspartame and selected gums in buffer solutions

Aspartame and sucrose were dissolved in about 150 ml of buffer prepared as described above. To achieve a 1% dispersion of gum, CMC (Grade P-75-L, DuPont, Wilmington, DE) or GA (USP Type G-150, Meer Corp., North Bergen, NJ) were dispersed in 750 ml buffer, added to the sweetened solutions, and brought to 1000 ml with buffer. The procedure for MC (MC Premium grade, 10 cps, Dow Chemical Co., Midland, MI) was the same except that it was necessary to heat about 200 ml of the 750 ml of buffer to 80–90°C before dispersing the gum in it. Solutions were allowed to stand 24 ± 3 hr at room temperature before use. The procedure described above for combining ingredients was adopted in preference to mixing of gum and sweetener because Batdorf (1964) reported that dry mixing of CMC with sugar resulted in 25% less viscosity than adding sugar to cellulose gum solution.

Four samples representing 0.025% aspartame and 4% sucrose in buffer only and in buffer containing 1% gum, were presented randomly on trays for judges to rank according to increasing sweetness. A second set of four samples was presented for evaluation of increasing thickness. Judges were instructed to consider feel in the mouth, not visual impression. Samples of 0.12% aspartame and 12% sucrose in buffer only and in buffer containing 1% gum were ranked in the same way.

Viscosities (30°C) of these solutions were determined by Ostwald pipet (80–100 sec with water at 20°C). Density was determined with a Westphal balance. Calculations for absolute viscosity were made according to the method described by Sommer (1952).

Aspartame in a noncarbonated orange-flavored beverage

A commercial orange beverage mix and sweetener were mixed prior to adding glass distilled water. The beverage mix was used on a basis of 2.7 g/liter, and aspartame and sucrose were weighed to yield 0.09% and 10% (w/v) solutions, respectively. For this test, only two samples were presented for evaluation at one time. Separate sets of samples (randomly positioned) were presented for ranking of sweetness, sourness and acceptability. This procedure

Table 1—Rank totals[a] for sensory evaluations of sweetened solutions with and without gums, and viscosities of these solutions

Solution[b]	Gum arabic			Methocel			Carboxymethylcellulose		
	Sweetness	Thickness	Viscosity in cps	Sweetness	Thickness	Viscosity in cps	Sweetness	Thickness	Viscosity in cps
	Sweetness equivalent to 4% sucrose								
4% S	41b	42b	1.0	44b	34b	1.0	42a	35b	1.0
0.025% A	60a	40b	0.8	51ab	39b	0.8	57a	31b	0.8
4% S+G	42ab	50ab	1.3	40b	62a	3.1	45a	65a	7.8
0.025% A+G	57ab	68a	1.0	65a	65a	2.5	56a	69a	6.4
	Sweetness equivalent to 12% sucrose								
12% S	49a	54b	1.2	44a	41b	1.2	48a	36c	1.2
0.12% A	55a	41b	0.8	50a	41b	0.8	42a	27c	0.8
12% S+G	50a	71a	1.6	53a	68a	3.8	55a	77a	10.0
0.12% A+G	56a	46b	1.0	53a	50b	2.6	55a	60b	6.5

[a] n = 20 except for gum arabic at the sweetness equivalent to 12% sucrose where n = 21. Comparisons may be made among the four rank totals for sweetness or for thickness listed under each gum within each sweetness level. Totals followed by the same lower case letter are not significantly different ($P < 0.05$, chi-square of ranks, Friedman, 1937). The higher the rank total, the greater the sweetness or thickness.

[b] S = sucrose; A = aspartame; G = gum.

was repeated with 0.075% aspartame and 10% sucrose.

RESULTS & DISCUSSION

Sweetness equivalents

Figure 1 illustrates that the relationship between sweetness of aspartame and sucrose is almost linear when plotted on a log-log scale. The magnitude of the sucrose equivalent of aspartame decreased as level of sweetness increased. Aspartame was 182 times sweeter than 2% sucrose but only 43 times sweeter than 30% sucrose.

Aspartame in buffer

No difference was found between sweetness of 0.025% aspartame and 4% sucrose nor between 0.12% aspartame and 12% sucrose in buffer solutions. Neither were differences in sourness indicated for these solutions, even though there were differences in pH. The aspartame shifted the pH of the solution from 3.2 to 3.3 and 3.5 in the 0.025% and 0.12% solutions, respectively. The pH of both sugar solutions was 3.2. This finding appears to support the statement of Harvey (1920) that both total acid and hydrogen ion concentration influenced the taste of acidic solutions.

Aspartame and gelatin in buffer

In contrast to the above findings, a significant ($P < 0.01$) difference between aspartame and sucrose occurred at both levels of sweetness when buffer solutions were combined with 1.5% gelatin. In these gels, 0.025% aspartame was sweeter than 4% sucrose, and 0.12% aspartame was sweeter than 12% sucrose. However, pH of all four gels was 4.6.

Sweetness of aspartame when combined with selected gums in buffer

There was no significant effect on sweetness of sucrose or aspartame due to combination with GA or CMC at either high or low sweetness levels. However, the solutions containing 0.025% aspartame plus 1% MC were significantly sweeter than the solutions containing 4% sucrose ($P < 0.05$) or 4% sucrose plus MC ($P < 0.01$) (Table 1).

Although there was no significant difference in sweetness between 0.025% aspartame and 4% sucrose in either buffer or in water, 0.025% aspartame was significantly ($P < 0.05$) sweeter than 4% sucrose in buffer when tested along with similar solutions containing GA (Table 1). Trends in this direction, although not statistically significant, were found also with other gums. Stone and Oliver (1966) found a similar trend in research with cornstarch, CMC, MC and tragacanth. In solutions sweetened with sucrose, the more viscous solutions were sweeter than the same solutions without added gum, although differences were not statistically significant. In contrast, Vaisey et al. (1969) reported a masking of the sweetness of sucrose by cornstarch, guar gum and CMC. Also, Arabie and Moskowitz (1971) pointed out that sweetness of both sucrose and saccharin decreased as viscosity increased.

CMC shifted the pH of all test solutions from 3.2 to 5.0. MC had a less marked effect, and pH of these solutions ranged from 3.4–3.6. Solutions containing GA ranged from pH 3.7–3.9.

Thickness and viscosity of solutions containing gums in buffer

Several inconsistencies were observed between viscosities of solutions and sensory ranks for thickness. Sensory rank totals for sucrose and aspartame solutions containing no gums did not differ significantly; but, as expected, sucrose solutions were more viscous than aspartame solutions. Addition of gums was expected to increase both thickness ranks and viscosity. However, solutions containing 1% GA and 4% sucrose were not ranked significantly thicker than 4% sucrose nor 0.025% aspartame solutions, although viscosities varied from 0.8 to 1.3 cps. Also, sensory ranks for solutions containing GA or MC combined with 0.12% aspartame did not differ significantly from those for 12% sucrose or for 0.12% aspartame alone, although viscosities varied from 0.8 to 2.6 cps. In spite of the difference in viscosity between solutions containing 12% sucrose plus CMC (10.0 cps) and 0.12% aspartame plus CMC (6.5 cps), no significant difference in sensory rank totals for thickness was indicated. However, the solution containing 4% sucrose plus CMC was ranked significantly ($P < 0.01$) thicker than the solution containing 0.025% aspartame plus CMC. Viscosities for these solutions were 7.8 cps and 6.4 cps, respectively. Also, 4% sucrose solutions were significantly ($P < 0.01$) less thick according to sensory ranks than solutions containing 0.025% aspartame plus GA although viscosities of both solutions were 1.0 cps (Table 1). Thus, for solutions comparable to those used in this research, viscosity measurements cannot be used to predict sensory response to thickness of solutions.

Szczesniak et al. (1962) classified the mouthfeel of gums according to sliminess rather than thickness and found a correlation between mouthfeel and rheological behavior of gums as measured by Brookfield viscometer (1200 cps, 0.5 rpm). According to these authors, sliminess corresponded to Newtonian behavior of gums. However, gums which behave as Newtonian fluids at one concentration may behave as dilatant or thixotropic

fluids at other concentrations (Glicksman and Farkas, 1966). In addition to differences due to concentration, technological advances in processing have made it possible to produce from one gum several products designed to achieve specific viscosity levels. Thus it is difficult to compare findings of one researcher with another.

Aspartame when combined with a noncarbonated orange-flavored beverage

When 0.09% aspartame was compared with 10% sucrose as a sweetener for a noncarbonated orange-flavored beverage, the beverage was significantly ($P < 0.01$) sweeter and less sour than the beverage containing sucrose. However, there was no significant difference in acceptability between the two beverages. Using 0.075% aspartame as the sweetener resulted in a beverage which did not differ significantly in sweetness, sourness or acceptability from the beverage sweetened with 10% sucrose. Thus, since it required less aspartame to achieve sweetness equivalent to 10% sucrose than expected from the relationship shown in Figure 1, one or more of the ingredients in the beverage mix enhanced the sweetening effect of the aspartame. The beverage mix was an unsweetened product, but the flavoring ingredients probably were plated on sugar in the manufacturing process, and this small amount of sugar may have contributed to the observed enhancement of sweetness.

General discussion

Stability problems were observed when the aspartame was used in soft drinks (Anon., 1973). However, off-flavor, which was encountered in previous work (Cloninger and Baldwin, 1970) when the aspartame was used at levels equivalent to 10% sucrose and above, was not apparent in the material supplied for this study. Also, it was observed that solubility of the material was improved.

In product formulations, variance from the equivalents shown in Figure 1 may result from combination of the aspartame with certain ingredients. For example, enhancement of the aspartame may be achieved by certain ingredients such as the gums, gelatin and a noncarbonated orange-flavored beverage used in this study. The complexity of the latter product makes it difficult to attribute the enhancement to any specific ingredient. Also, use of more than one sweetener in a food permits lowering of the total amount of sweetener ingredients required to achieve a specified level of sweetness (Cloninger and Baldwin, 1970).

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CARBONYL RETENTION IN MODEL SYSTEMS AND BERMUDA ONION JUICE DURING LYOPHILIZATION: EFFECT OF SIMPLE CARBOHYDRATES, BINARY CARBOHYDRATE MIXTURES AND SUCROSE INVERSION

INTRODUCTION

RESEARCH of the past decade has demonstrated increased volatile retention in lyophilized aqueous materials by additions of simple sugars under select processing conditions (Rey and Bastien, 1962; Chandrasekaran and King, 1969; Flink and Karel, 1970a, b; Sauvageot et al., 1969). Flink and Karel (1970a, b) observed variations in retentive capacities of different carbohydrates at equal concentrations. These authors suggested that freezing and freeze-drying cause alignment of carbohydrates into hydrogen-bonded cage-like structures which entrap volatiles but are moisture permeable. The simple adsorption theory of volatile retention during lyophilization, proposed by Rey and Bastien (1962), has been discredited by several researchers (Issenberg et al., 1968; Sauvageot et al., 1969; Flink and Karel, 1970a). Pending the reliability of the "microregion entrapment" theory of Flink and Karel (1970a), the observed retentive capacity differences of simple carbohydrates may be a reflection of trapping efficiency or variations in cage permeability. Hence, the possibility of greater volatile retention by carbohydrate combinations is inherent. Research probing the validity of this assumption has not been reported.

Collapse or melting of the frozen core by high dissolved solids concentrations during freeze drying has been observed by Sauvageot et al. (1969) and Flink and Karel (1970a). Loss of structure resulted in marked volatile losses. Collapse also has been reported in liquid foods containing fructose and invert sugar (King, 1971; Notter et al., 1959). The effect of sucrose inversion on volatile retention during freeze drying has not been determined.

Utilizing model carbohydrate-volatile solutions and Bermuda onion juice, the relative effects of the following variables on volatile retention were compared: (1) carbohydrate type and concentration; (2) binary carbohydrate mixtures; and (3) sucrose hydrolysis.

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Total carbonyls were measured in the onion juice by the method of Schwimmer and Guadagni (1962). Schwimmer and Weston (1961) determined that over 90% of the total carbonyls in mascerated onions is pyruvic acid. Hence, pyruvic acid was selected as the volatile for the model systems.

The total solids content of mature onion bulbs varies from 10–13%, depending on variety (Lee et al., 1970). Schwimmer and Guadagni (1962) have reported soluble solids contents of the juice of several varieties to vary from 6.3–12.9° Brix. Most of the solids content appear to be carbohydrates, as evidenced by high proportions of glucose and fructose, in addition to sucrose, raffinose, stachyose, and two unidentified sugars (Lee et al., 1970). The complexity and high proportions of various simple sugars in onion appear to render this food susceptible to good aroma retention during freeze drying. Studies to determine the validity of this assumption have not been reported.

MATERIALS & METHODS

Samples

Model carbohydrate solutions were prepared utilizing reagent grade glucose, fructose, sucrose and lactose. Water was distilled and deionized. Pyruvic acid was added to the systems to a concentration of 20 μmoles/ml.

Onion juice was prepared from Bermuda onions by a modified method of Schwimmer and Guadagni (1962). The onions were macerated in a Waring Blendor and centrifuged. The supernatant was then filtered with Whatman's No. 1 filter paper. The filtrate was allowed to stand at room temperature for 1 hr to assure maximum development of pyruvic acid. Samples were prepared and subsequently frozen and freeze dried. Solids content was 11.1° Brix.

For all experiments, 5 ml samples in alike 10 ml pyrex Erlenmeyer flasks were utilized, assuring uniform sample thicknesses.

Processing

All samples were frozen by immersion in liquid N_2 (−196°C). Surfaces were not scraped after freezing. Lyophilization was conducted in a Virtis freeze drier at 1.0 torr, ambient platen temperature, and a condensor temperature of −40°C. Chamber pressure was measured by a calibrated Macleod manometer. The prevalent absence of collapse and carbohydrate frothing at the chamber pressure and carbohydrate concentrations utilized was attributed to inert leakage.

Measurement of pyruvic acid and other carbonyl compounds

Pyruvic acid and other carbonyl components were measured before and after processing by the method of Schwimmer and Guadagni (1962). The method involves the determination of total 2,4-dinitrophenylhydrazine-reacting carbonyls. Prior to analysis, the onion filtrate, model systems, and rehydrated freeze-dried samples were diluted 1:40 (vol/vol). 1 ml of the diluted sample and 1 ml H_2O were added to 1 ml of 0.0125% 2,4-dinitrophenylhydrazine (wt/vol) in 2N HCl. After 10 min at 37°C, 5 ml of 0.6N NaOH were added. Absorbance was measured with a Bausch & Lomb Spectronic 20 Colorimeter at 420 nm. The calibration curve was prepared using sodium pyruvate as the standard.

Experimental design

Pyruvic acid retention in model systems and onion juice containing known amounts of carbohydrates were measured before and after freeze drying. Reagent grade carbohydrates were added in the following proportions:

(1) Zero through 20% sucrose, lactose and glucose in 5% increments.
(2) Binary mixtures of sucrose, lactose and glucose whereby the total molarity of the solutions was equal to 0.2775 (~10% sucrose).
(3) Mixtures of glucose and fructose in 10% inversion increments (10 through 100% hydrolysis) and conforming to 10 and 20% sucrose at zero inversion.

Statistical analysis

The degree of treatment differences were ascertained by analysis of variance techniques and illustrated by multiple range tests (Steel and Torrie, 1960).

RESULTS & DISCUSSION

Effect of carbohydrate type and concentration

The effects of carbohydrate type and concentration of pyruvic acid retention in model systems and Bermuda onion juice are illustrated in Table 1. The model system data indicate an increase in percent retention with an increase in carbohydrate concentration and are reflective of the data of several workers (Rey and Bastien, 1962; Chandrasekaran and King, 1969; Flink and Karel, 1970a, b; Sauvageot et al., 1969). Analysis of variance revealed highly significant retention differences between carbohydrate levels,

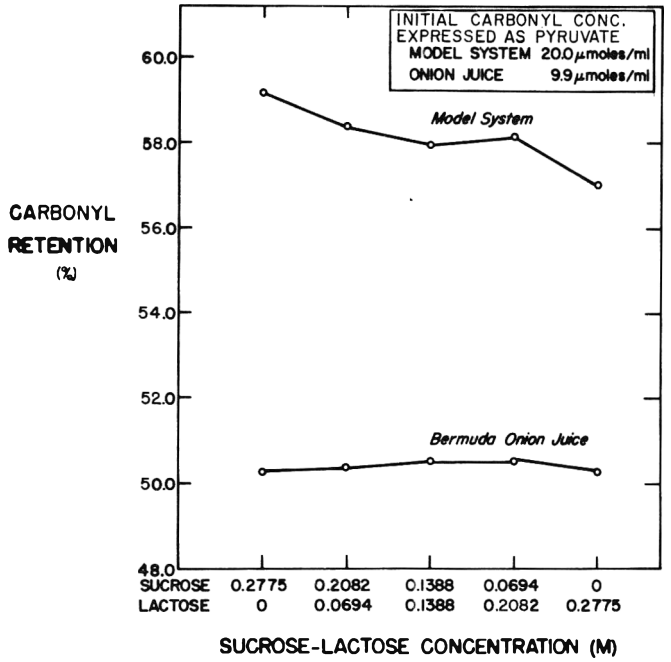

Fig. 1–Effect of 0.2775M sucrose-lactose mixtures on carbonyl retention in freeze-dried model systems and Bermuda onion juice.

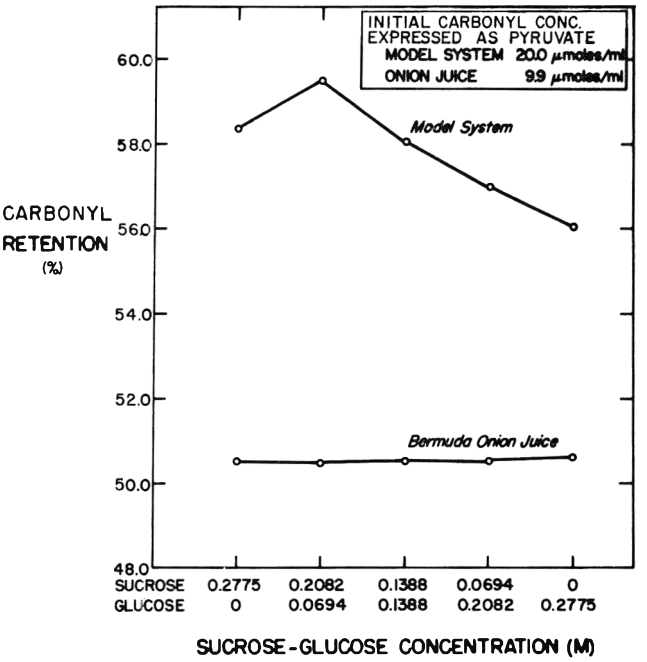

Fig. 2–Effect of 0.2775M sucrose-glucose mixtures on carbonyl retention in freeze-dried model systems and Bermuda onion juice.

excluding the control which contained no carbohydrate. The small differences in retention with increasing carbohydrate content are unlike the reported data of other workers (Flink and Karel, 1970a, b; Rey and Bastien, 1962). However, these workers freeze dried at pressures below 100μ. This experiment was performed at a chamber pressure of 1.0 torr. It is suspected that some incipient melting is inevitable due to freezing point depression by the increase in solids, and an internal pressure buildup adjoining the retreating ice front by escaping water vapor. Data confirming this deduction have been reported by Spiess et al. (1969) and Brockman (1970). A Dunnett value of 2.82% indicated highly significant differences between all treatment means and the control which contained no carbohydrate. The apparent differences in retention by the three carbohydrates at the same concentrations are similar to the results of Flink and Karel (1970a, b).

The effects of carbohydrate type and concentration on retention of carbonyls in Bermuda onion juice are also shown in Table 1. Lactose became insoluble in the juice beyond the 10% level. As with model systems, differences in retention between carbohydrate levels were small. Using Dunnett's procedure (Steel and Torrie, 1960), highly significant differences were found between the control and all samples with added carbohydrate (d^1 = 1.78% for three carbohydrates at two concentrations; d^1 = 1.41% for two carbohydrates at four concentrations).

Table 1–Effect of carbohydrate concentration on retention of carbonyl components in freeze-dried Bermuda onion juice and model system

	Sucrose		Lactose		Glucose	
Carbohydrate conc (%)	Onion juice % Retention	Model system % Retention	Onion juice % Retention	Model system % Retention	Onion juice % Retention	Model system % Retention
0	36.0	0.0	36.0	0.0	36.0	0.0
5	47.6	57.6	47.5	55.5	46.6	54.5
10	51.0	59.0	50.3	57.0	50.1	56.7
15	51.1	58.9		57.6	52.0	56.9
20	51.6	60.0		59.0	53.4	58.5

Initial carbonyl conc expressed as pyruvic acid:			Dunnett value (0.05)	Error mean square
Model system	20.0 μmoles/ml	Model system	2.82%	1.42
Onion juice	9.8 μmoles/ml	Onion juice, all carbohydrates, up to 10% concentration	1.79%	0.59
		Onion juice, sucrose and glucose up to 20% concentration	1.41%	0.37

Effect of carbohydrate mixtures

Sucrose-lactose. The effect of sucrose-lactose mixtures on pyruvic acid retention is depicted in Figure 1. The concentration of added carbohydrate was maintained at 0.2775M, which corresponds to 10% sucrose and lactose. In model systems, retention appeared to decrease with decreasing sucrose and increasing lactose. A least significant difference analysis (LSD) of the treatment means revealed a highly significant difference between the samples containing all sucrose and the samples containing all lactose ($LSD_{0.01}$ = 2.1%, $\Delta\bar{\chi}$ = 2.2%). No significant differences were found between treatment means of the Bermuda onion juice.

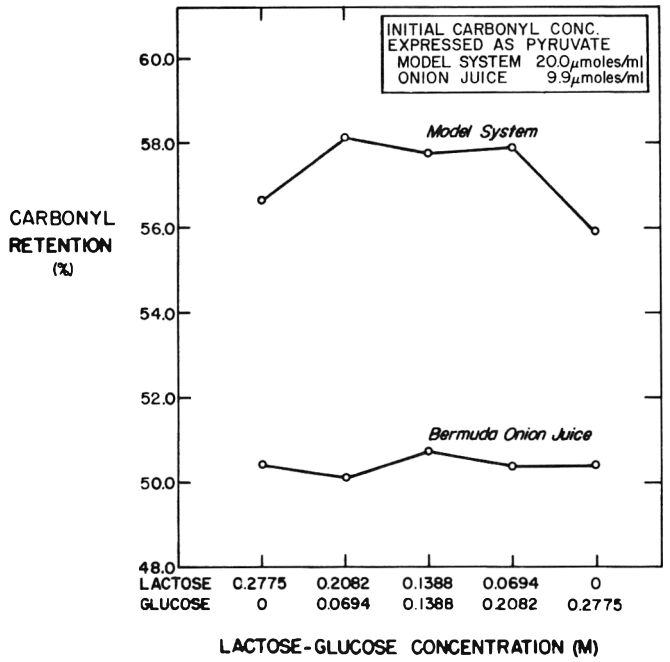

Fig. 3—Effect of 0.2775M lactose-glucose mixtures on carbonyl retention in freeze-dried model systems and Bermuda onion juice.

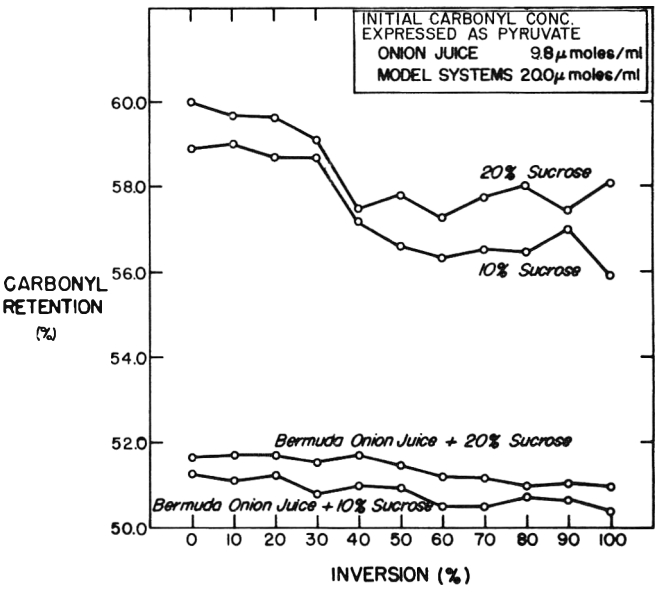

Fig. 4—Effect of sucrose inversion on retention of carbonyl components in freeze-dried Bermuda onion juice and model systems.

Sucrose-glucose. The effect of sucrose-glucose mixtures on pyruvic acid retention is shown in Figure 2. In the model system, the apparent synergistic effect of 0.2082M sucrose and 0.069M glucose was revealed by an LSD analysis. Every treatment containing more glucose than the maximum retention point differed significantly from the maximum retention mean. Although the difference between the maximum retention mean and the retention mean of the samples containing only sucrose was not significant ($\Delta \bar{\chi}$ = 1.1%), it approached the least significant difference at the 5% level ($LSD_{0.05}$ = 1.3%) and far surpassed the all sucrose means at the 10% level ($LSD_{0.10}$ = 0.8%). No statistical confirmation of synergism in the onion juice was found.

Lactose-glucose. The effect of lactose-glucose mixtures on pyruvic acid retention during freeze drying is illustrated in Figure 3. A true synergistic effect in model systems was validated by an LSD analysis. The three retention levels of treatments containing mixtures of lactose and glucose differed highly significantly from the retention mean of the samples containing all glucose ($LSD_{0.01}$ = 1.3%). These three levels also varied significantly ($LSD_{0.05}$ = 0.9%) and highly significantly from the treatment mean of the samples containing all lactose. No synergistic effects were found for the Bermuda onion juice.

Effect of sucrose inversion

Figure 4 demonstrates the effect of increasing sucrose hydrolysis on carbonyl retention in Bermuda onion juice and model systems during lyophilization with initial sucrose concentrations of 10 and 20%. In model systems, two retention levels are evident, with the change occurring between 30 and 40% inversion. This observation was substantiated by LSD analyses for both 10 and 20% sucrose.

Visible differences were observed between freeze-dried specimens at the two spans of inversion. Between 0 and 30% inversion, the freeze-dried residual material possessed structural integrity characterized by a honeycomb-like matrix composed of pores and crystalline walls. Generally, samples freeze dried with 40–100% invert sugar appeared to be in a glassy viscous state and is reflective of the observations of King (1971) and the work of Notter et al. (1959).

Although visible differences in structure of freeze-dried Bermuda onion juice with varying degrees of sucrose inversion were similar to model systems, no LSD confirmation of a two-span retention pattern was evident.

CONCLUSIONS

UNDER the conditions of this experiment, it was concluded that:

1. An increase in sucrose, glucose and lactose concentration in model aqueous systems and Bermuda onion juice results in increased carbonyl retention during freeze drying when carbohydrate concentrations are initially low.
2. Volatile carbonyl components of Bermuda onion juice and model systems are retained in different quantities by different carbohydrates at similar, but low, concentrations.
3. The combination of lactose and glucose synergistically affects the retention of pyruvic acid in model aqueous systems during freeze drying.
4. Pyruvic acid is retained at two distinct levels in lyophilized invert sugar solutions corresponding to single sucrose concentrations. At low hydrolysis levels, pyruvic acid retention is similar. As hydrolysis increases, structural collapse occurs, rendering a lower retention level.
5. In all experiments, differences in carbohydrate concentrations and proportions resulted in slight differences in retention.

The results of these experiments were reproducible. Samples were processed and analyzed for four to six trials per experiment, rendering similar retention and trends.

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RELATIONSHIPS AMONG TITRATABLE ACIDITY, pH AND BUFFER COMPOSITION OF TOMATO FRUITS

INTRODUCTION

ACID CONCENTRATION and pH are important quality and processing characteristics of tomatoes (*Lycopersicon esculentum* Mill.). Several studies have revealed that a proper sugar/acid ratio is paramount to good tomato flavor (Dennison, 1955; Simandle et al., 1966; Stevens, 1972a). Both $[H^+]$ and potential acidity contribute to tartness (Harvey, 1920). The pH is important to processability, as it should be lower than 4.4 to avoid problems with thermophylic organisms (Rice and Pederson, 1954). Higher pH values necessitate longer processing times, increasing the difficulty of obtaining a high quality product.

Total acidity and pH in a tomato should be closely related, but sometimes the relationship between these two factors is not good. Anderson (1957) found that pH and acidity are not always inversely related, and that in some varieties both values are relatively high. Lower and Thompson (1967) also found poor correlation between pH and acidity in certain tomato lines and their progeny. Stevens (1972a) found wide variation in the $[H^+]$/titratable acidity (TA) ratio among 55 divergent accessions and obtained evidence indicating that variation in phosphorus concentration of the fruits is an important factor in the poor relationship between pH and acidity.

It should be possible to explain the relationship between TA and pH using model systems, as the TA is equal to the sum of TAs contributed by the buffers in the fruit. These buffers also establish the pH. The purposes of this paper are to define the relationship between pH and TA in tomato fruits, to establish the contribution of various buffers to this relationship, and to apply this information to predict the effect of various compositions on pH.

MATERIALS & METHODS

Tomato analyses

Fruits from 25 tomato accessions from divergent sources, grown at Davis, Calif. during 1970, were analyzed. A completely randomized design with five replications was used.

A 10-lb sample of prime, mature fruits was harvested from each replication on two picking dates. Each sample was washed, dried, packed in a polyethylene bag, frozen and stored at −10°C until analyzed.

Samples were removed from the freezer the evening prior to analysis. The thawed fruits were blended 1 min at high speed, and seed and skin were removed with a FPECO laboratory pulper. Serum was obtained by centrifuging the puree for 10 min at 1250 × G, and then filtering the supernatant fluid through Whatman 90 paper.

Table 1—Mean phosphate, citric and malic acid concentrations $[H^+]$, and titratable acidity (TA) of tomato fruits from 25 tomato accessions

	mmole/liter[a]			meq/liter[a]	
Accession	Phosphate	Citric acid	Malic acid	$H^+ \times 10^5$	TA
Lutescent mutant of 'Campbell 22'	6.72 a[b]	21.65 k	6.54 def	5.12 efg	63.21 ghi
PI 126436 (Peru)	6.53 ab	53.95 a	4.30 hij	4.43 hij	111.96 b
G2-D1-D5-1-BK-DK	6.50 ab	22.10 k	6.91 cde	5.57 def	61.12 ghij
Walter	6.18 abc	23.85 jk	6.36 ef	3.26 lm	55.87 jk
Pizza	6.03 bcd	30.33 fgh	2.78 l	3.56 klm	56.45 ijk
Crimson	5.95 bcd	30.96 efg	3.26 jkl	3.87 jkl	61.60 ghij
PI 127810 (Peru)	5.92 bcd	23.24 k	4.75 gh	4.27 ij	60.38 ghij
Rutgers Ve	5.85 cde	35.10 d	3.59 ijkl	5.06 fgh	70.72 f
1339-D8-S3 (Florida)	5.67 cdef	28.18 ghi	4.07 hijk	5.73 de	65.79 fg
PI 272691 (El Salvador)	5.53 cdefg	34.36 de	11.52 a	5.09 efg	79.87 e
Red Jacket	5.47 defg	31.55 defg	2.85 l	4.80 fghi	64.29 gh
PI 155369 (Peru)	5.27 efgh	39.06 c	4.94 gh	8.96 a	92.07 d
66D692-3 USDA	5.12 fgh	22.21 k	3.17 jkl	3.86 jkl	47.76 l
Merbein Midseason	5.01 ghi	24.78 ijk	5.65 fg	3.20 m	53.74 k
STEP 375	4.98 ghi	27.25 hij	3.33 ijkl	3.93 jk	58.48 hijk
PI 190188 (Mexico)	4.70 hij	56.97 a	4.41 hi	5.04 fgh	119.87 a
PI 128229 (Bolivia)	4.42 ij	43.15 c	5.65 fg	7.91 b	98.21 c
PI 28223 (Bolivia)	4.33 j	47.53 b	3.65 ijkl	7.99 b	102.11 c
DX 72 (Campbell's)	4.32 j	28.52 gh	3.06 kl	4.14 ijk	63.12 ghi
PI 263713 (Puerto Rico)	4.10 j	32.93 def	2.97 kl	6.64 c	77.04 e
PI 129143 (Peru)	4.10 j	39.88 c	7.41 bcd	6.49 c	91.60 d
Tondo Liscio	3.42 k	23.11 k	8.74 b	5.10 efg	59.52 hijk
Epoch Dwarf	3.23 k	29.58 fgh	4.25 hij	5.79 d	65.82 fg
PI 280597 (Russia)	3.10 k	32.93 def	4.62 ghi	5.69 def	77.24 e
Best of All	3.08 k	22.90 k	8.24 bc	4.75 ghi	57.25 ijk

[a] Mean of 10 samples
[b] Values in vertical columns followed by same letters are not significantly different at P=0.05 by Duncan's multiple range comparisons.

Table 2—Average concentrations and pK_a's of buffers found in tomato fruits.

Buffer	Concentration (mmole/liter)[a]	pK_{a_1}[b]	pK_{a_2}[b]	pK_{a_3}[b]
DL-alanine	0.620	2.348	9.866	
α-amino butyric acid	2.120	2.290	9.830	
L-arginine	2.640	2.010	9.040	12.480
Asparagine	1.940	2.020	8.800	
L-aspartic acid	2.920	2.100	3.860	9.820
L-glutamic acid	7.490	2.100	4.070	9.470
DL-leucine	0.430	2.328	9.744	
DL-serine	1.290	2.210	9.150	
Threonine	0.480	2.150	9.120	
Valine	0.180	2.286	9.719	
Galacturonic acid	0.770	3.200		
Phosphate	5.020	1.959	6.699	12.444
Citric acid	32.240	3.076	4.745	5.398
Malic acid	5.080	3.398	5.046	

[a] From Freeman and Woodbridge (1960) and McClendon et al. (1959) except phosphate, citric acid and malic acid which are from the experiment described in this paper.
[b] From Anonymous (1957) except for α-amino butyric acid and galacturonic acid from Dawson et al. (1969), asparagine and valine from Anonymous (1968) and phosphate from West (1963).

Citrate and malate concentrations were determined by gas-liquid chromatography of trimethylsilyl derivatives as previously described (Stevens, 1972b). Titratable acidity was determined by titrating 10 ml of serum to pH 8.1 with 0.1N NaOH. Soluble phosphorus concentration was determined with the vanadate-molybdate colorimetric method, using serum.

Relationship of titratable acidity to pH

The relationship between TA and pH can be derived from the Henderson-Hasselbalch equation [1].

$$pH = pK_a + \log\frac{[base]}{[acid]} \qquad [1]$$

Equation [1] can be manipulated to obtain the ratio of the [base] to [acid] as shown in equation [2].

$$\frac{[base]}{[acid]} = 10^{pH - pK_a} \qquad [2]$$

For buffers with more than one pK_a, equation [2] can be applied for each pK_a and the resulting ratios combined. The relative proportion of each form of the buffer can thus be computed for any designated pH. The product of the relative proportion times the buffer concentration gives the concentration of each form of the buffer. If this procedure is repeated for both the initial and final (titrated) pH the concentration change for each form of the buffer is obtained. The sum of the changes in titratable H for each form of the buffer is equal to that buffer's contribution to the TA of the fruit. The sum of the contributions from all buffers in the fruit equals the TA of that fruit.

Computer programs were used with the above procedures to predict the contribution of various buffers of tomato fruits to TA and pH. Three computer programs were written. The first program [phosphate, citrate, malate (PCM)] predicted the TA contributed by phosphate, citric acid and malic acid using the pH and concentrations of these buffers in the fruits from the 25 divergent accessions. Individual sample data from the analyses of the 25 accessions were used in model system studies, giving a total of 250 samples. As the predicted TA was less than the observed, a second program [total buffer (TB)] was written to compute the contributions of other buffers known to be present in the fruits. Reported values (Freeman and Woodbridge, 1960; McClendon et al., 1959) were used, as the analytical work had not included these values. A paired t-test was used to compare the predicted TA for a sample of fruits to the observed TA. The TB program was also used to predict the contribution of each buffer at various pH values.

A third computer program [change buffer concentration (CBC)] was written to predict the change in pH caused by changing buffer concentration. This program used the above procedures in an iterative manner, starting at a pH higher than needed and reducing it until the predicted TA equaled the observed. Average buffer and TA concentrations were used in the CBC program. The TA was adjusted for deviations caused by changes in buffer concentration, with the amount of adjustment being equal to the variation caused by the addition or subtraction of buffer at other than the titrated pH (8.1).

RESULTS & DISCUSSION

Application of buffer model to data from tomato analyses

There were significant differences in phosphate, citrate, malate, H^+ and TA concentrations among the 25 accession lines (Table 1).

The average predicted TA using the PCM program was 66.34 meq/liter which is 6.26 meq less than the average observed TA of 72.60 meq. The standard deviation of the difference was 0.436, giving a t-value of 14.37. With 249 degrees of freedom, this is significant at the 0.1% level.

That phosphate, citric acid and malic acid do not account for all of the TA of the fruit is not surprising, as certain amino acids and other organic acids are significant buffers in tomatoes. Mean values from the literature (Table 2) were used in the TB program in an attempt to account for the remaining 6.26 meq of TA/liter. The inclusion of the other buffers resulted in an additional 4.91 meq of TA leaving a differential of 1.35 meq. The standard deviation of the differences was 0.433, giving a t-value of 3.03. This was significant at the 1% level, but not at the 0.1% level. Although there was still significant deviation, the model system accounts for 98% of the TA. It would be unreasonable to expect that a perfect relationship would be found, because the average reported values that were used undoubtedly do not precisely represent the composition of these accessions. A small change in one or two of these buffers could account for the discrepancy in

Table 3—Percent contribution to titratable acidity (TA) by various buffers at pH 4.0, 4.2 and 4.4

Buffer	% Contribution to TA at pH 4.0	4.2	4.4
DL-Alanine	0.030	0.026	0.024
α-amino butyric acid	0.099	0.088	0.081
L-arginine	0.374	0.390	0.417
Asparagine	0.429	0.452	0.488
L-aspartic acid	1.634	1.326	1.053
L-glutamic acid	5.541	4.769	4.000
DL-Leucine	0.024	0.020	0.019
DL-Serine	0.158	0.161	0.168
Threonine	0.061	0.062	0.066
Valine	0.010	0.008	0.009
Galacturonic acid	0.131	0.094	0.068
Phosphate	6.089	6.532	7.109
Citric acid	78.310	79.143	79.731
Malic acid	7.113	6.929	6.769

Table 4—Correlation coefficients for data from 25 tomato accessions

	Citric acid	Phosphate	TA	H^+	pH	Malic + Citric acids
Malic acid	−0.151*	−0.049	0.020	0.069	−0.101	0.004
Citric acid		−0.044	0.904**	0.414**	−0.407**	0.988**
Phosphate			−0.038	−0.255**	0.267**	−0.052
TA				0.574**	−0.579**	0.917**
H^+					−0.981**	0.430**

* Significant at 5% level or ≥ 0.125
** Significant at 1% level or ≥ 0.165

Table 5—Contribution to titratable acidity by the major buffers of tomato fruits at various pH's

	pH					
	4.0	4.1	4.2	4.3	4.4	4.5
	mmoles/liter					
$[H^+] \times 10^5$	10.0	7.94	6.31	5.01	3.98	3.16
Aspartic acid	1.305	1.138	0.983	0.841	0.714	0.603
Glutamic acid	4.426	3.976	3.534	3.110	2.713	2.349
Phosphate	4.864	4.852	4.841	4.831	4.821	4.811
Citric acid	62.556	60.669	58.654	56.468	54.073	51.442
Malic acid	5.682	5.406	5.135	4.864	4.591	4.310
All other buffers	1.050	1.003	0.964	0.933	.907	.886
Total TA	79.883	77.044	74.111	71.047	67.819	64.401

TA. The data from the 25 accessions produced a good test of the buffer model, as there were significant differences among the three major buffers, TA and $[H^+]$.

Use of buffer model to predict those buffers that are important contributors to titratable acidity of tomato fruits

The percent contribution of each buffer to the TA of the tomato fruit at pH 4.0, 4.2 and 4.4 (Table 3) was computed using average concentrations and pK_a's (Table 2). At pH 4.2, phosphate, citric acid and malic acid accounted for 92.6% of the TA. Inclusion of aspartic and glutamic acids added another 6.1% to the theoretical TA, leaving only 1.3% which was divided among the remaining nine buffers. The variation in contribution of a buffer was small in the pH range of 4.0–4.4 when compared to the variation among buffers.

Use of the buffer model to predict effect of various concentrations of phosphate, citric and malic acids on pH

To predict the effect on pH from changing the concentration of one of the buffers, the effect of the change on TA must also be known. The effect on TA can be estimated by considering the characteristics of the buffer and its relationship to $[H^+]$ (Table 4).

When a buffer enters or is synthesized in the tomato fruit, it has some amount of associated H. In the case of a buffer which is translocated into the fruit (e.g., phosphate), its associated H would be determined by the pH of the vascular system solution. For compounds synthesized within the fruit (e.g., citric and malic acids), the net associated H would be the active H of the molecule plus or minus any active H gained or lost during its synthesis.

The correlation between the concentration of a buffer and $[H^+]$ will give an indication of the amount of H associated with that buffer. The effective pH of a buffer will be defined as the pH of entry into, or synthesis within the fruits. The pH of a tomato fruit is defined as the pH of the homogenate of the fruit, and thus is the average pH for all parts of the fruit. The concentration of a buffer whose effective pH is higher than the pH of the fruit would be negatively correlated to $[H^+]$. A buffer whose effective pH is the same as that of the fruit will not change the pH of the fruit, and there would be no correlation between buffer concentration and $[H^+]$. A buffer whose effective pH is lower than that of the fruit would lower the pH of the latter, and its concentration would be positively correlated with $[H^+]$.

Phosphate concentration had a correlation coefficient of −0.255 (significant at the 1% level) with $[H^+]$. This is reasonable, as phosphate is translocated into the fruit at a pH near 7.0 while the pH of the fruit is usually less than 4.5. The net result is that as the phosphate concentration increases, $[H^+]$ decreases, providing quantities of other buffers do not change.

The correlation coefficient for citric acid with $[H^+]$ was 0.414, which is significant at the 1% level. However, that for malic acid was 0.069, which is not significant at the 1% level. The sum of citric and malic acids had a correlation coefficient of 0.430 with $[H^+]$. It can be assumed that the reason malic acid is not significantly correlated is because its effect is masked by the much larger concentration of citric acid. The positive correlation of citric plus malic acid with $[H^+]$ points to an effective pH for these buffers less than the pH of the fruit.

The CBC program used the effective pH of the buffer to adjust the TA of the fruit. All predictions were based on average concentration (Table 2).

As indicated above, an effective pH of 7.0 was used for phosphate. A reduction of phosphate from 7 to 3 mmoles/liter (concentration range among the 25 accessions) resulted in a reduction of pH from 4.29 to 4.21 (Fig. 1). The TA of fruit was reduced from 73.19 to 72.01 meq/liter.

Table 6—$[H^+]/TA^a$ ratios for the major buffers of tomato fruits at various pH's

	pH					
	4.0	4.1	4.2	4.3	4.4	4.5
Aspartic acid	7.65	6.98	6.42	5.96	5.58	5.24
Glutamic acid	2.26	2.00	1.79	1.61	1.47	1.34
Phosphate	2.06	1.64	1.30	1.04	0.95	0.66
Citric acid	0.160	0.130	0.108	0.089	0.074	0.061
Malic acid	1.76	1.47	1.23	1.03	0.87	0.73
Total TA	0.125	0.103	0.085	0.070	0.059	0.049

[a] $[H^+] \times 10^5$ used in computing ratios

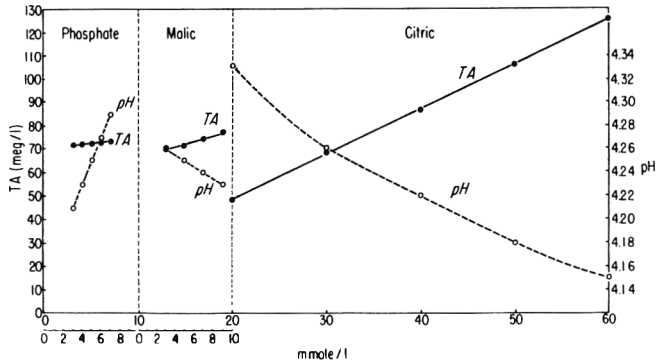

Fig. 1–Change in titratable acidity (TA) and pH with various concentrations of phosphate, malic acid and citric acid in tomatoes.

An effective pH of 4.0 was chosen for citric and malic acids, because of the positive relationship between their concentrations and $[H^+]$. An effective pH of less than 4.0 gave negative TA's for 0 mmoles of citric acid/liter or predicted pH's that were too high or too low. When the citric acid concentration was reduced from 60.0 to 20.0 mmoles/liter (concentration range of 25 accessions) TA was reduced from 126.46 to 48.86 meq/liter, and pH was increased from 4.15 to 4.33 (Fig. 1).

A reduction of malic acid concentration from 10 to 3 mmoles/liter reduced TA from 78.11 to 70.28 meq/liter and increased pH from 4.23 to 4.26 (Fig. 1).

Use of buffer model to predict relationship between $[H^+]$ and titratable acidity

The buffer model can be used to predict the effect of varying pH, on the TA contributed by various buffers. As expected, the TA contributed by each buffer decreased with an increase in pH (Table 5). However, the decrease in contribution by phosphate was at a much slower rate than that by the other buffers. Its contribution decreased only from 4.864 to 4.811 mmoles/liter from pH 4.0 to 4.5. This occurred because the buffering capacity of phosphate is minimal at the pH of tomato fruits.

It is apparent that lack of a constant relationship between $[H^+]$ and TA is due not only to differential buffering capacity among buffers, but also to the change in buffering capacity of each buffer with change in pH. The relationship between $[H^+]$ and TA can only be explained when concentrations of individual buffers and their pK_a's are considered.

A study of the $[H^+]$/TA ratio for the major buffers and for total TA (Table 6) reveals why $[H^+]$/TA is not constant. Even for a single buffer (citric acid) $[H^+]$/TA changes from 0.160 at pH 4.0 to 0.061 at pH 4.5. This decrease occurs with all the major buffers in tomato fruits. For total TA the ratio decreases from 0.125 at pH 4.0 to 0.049 at pH 4.5.

Possible applications to breeding program for lower pH in tomato fruit

The pH in tomato fruits can best be reduced by reducing phosphate or by increasing citric and malic acids. Increasing concentrations of the acids has its drawback, as this also changes the sugar/acid ratio, which may have an adverse effect on flavor, particularly in low sugar varieties. It would be desirable to increase the sugar concentration of these low sugar varieties but this has proven difficult in high-yielding mechanical-harvest types. It seems desirable to maintain a lower acid concentration in these varieties and still have a safe processing pH (< 4.4). This can best be accomplished by decreasing phosphate concentration. The addition of 10 mmoles of citric acid/liter is required to reduce the pH from 4.47 to 4.33. This results in about a 20 meq/liter increase in TA (Fig. 1). A 5 mmoles/liter reduction in phosphate concentration can result in a 0.10 change in pH with only a 1.47 meq/liter reduction in TA. Stevens and Paulson (1973) have demonstrated the variable P concentration of tomato fruits is a heritable trait that can be managed in a breeding program.

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STARCH AND PECTIC SUBSTANCES AS AFFECTED BY A FREEZE-THAW POTATO GRANULE PROCESS

INTRODUCTION

INSTANT DEHYDRATED mashed potatoes, which include potato granules and flakes, comprise the bulk of dehydrated potato products widely accepted in both consumer and institutional markets. Potato granules, the subject of the present study are dehydrated, pre-cooked potatoes in granular form that can be quickly reconstituted to mashed potatoes by mixing with hot or boiling water. In order to retain most of the organoleptic qualities of the freshly cooked mashed potatoes a few critical requirements in the processing of the granules must be observed. Potatoes of high total solids are needed to produce mealy and fluffy mashed potatoes. The cooking of the potatoes also has a profound effect on the process. Both under- and over-cooking have detrimental effects on the potatoes with the results becoming apparent in the subsequent stages of processing (Hendel et al., 1962a). Mashing is one of the most critical steps in processing since it determines the extent to which the cooked potato cells are separated, and consequently, the extent of damage sustained by the separated cells. The ease with which the cooked potato tissue can be mashed depends largely on the variety, specific gravity, starch content and anatomical parts of the potatoes (Reeve and Notter, 1959; Reeve, 1969; Schwimmer and Burr, 1967; Linehan and Hughes, 1969).

Many patents have been issued concerning processing techniques, improvements and equipment used in processing of potato granules. The beneficial effects of freezing and thawing have long been recognized. Rendle (1945) in his patent of the add-back process, introduced freezing and thawing of cooked potato prior to the admixing, as an optional step. He observed that when mashed potato is frozen and immediately allowed to thaw the resulting product is more granular and less gelatinous in texture than when freezing is omitted. Greene et al. (1949) hold a patent on a freeze and squeeze method which was briefly used during the war, but was discontinued due to the organoleptic inferiority and the rather low bulk density of the product. Hendel et al. (1962a, b) patented a method for processing of potato granules without recycling of the dry seed, as in the add-back process, by pre-drying the cooked potatoes to a suitable moisture level prior to conditioning at low temperatures and granulation. Reeve (1954, 1967, 1969) observed that slow frozen cooked potatoes have a slightly greater porosity when dried than the quick frozen ones, and that freezing and thawing of cooked potatoes reduced the swelling capacity of the gelled starch and influenced its textural properties. This had an advantageous effect in the manufacture of granules by rendering them more friable after thawing so that granulation is readily accomplished.

Granulation, i.e., final size reduction before drying to about 6% moisture is also a critical step in the processing of potato granules. The extent of damage, the granule size and the yield of fine granules taken as final product are determined essentially at this stage of processing.

Recently, Ooraikul (1973) introduced the freeze-thaw technique for potato granule production consisting of the following steps: peeling, cooking, mashing, freezing and thawing, pre-drying, granulation, drying, cooling and sifting. This technique consistently gave textural characteristics of the product comparable or better to those available on the market. However, the processing steps applied were not understood well enough in terms of raw material and its response to processing. The present study reports the contents of the two major cell binding constituents responsible for textural quality pectic substances and starch, and their changes induced by processing steps. In addition, the relationship between temperature of mashing and the extent of cell damage on mashing and subsequent stages of processing is presented.

MATERIALS & METHODS

Raw potatoes

The potatoes used were variety Netted Gem grown in Southern Alberta. The average specific gravity was 1.098 which corresponded to 25% total solids and 17.5% starch. Proximate analysis gave the following percentages: protein, 2.1; fat oil, 0.1; total carbohydrates, 19.4; crude fiber, 0.5; and ash, 1.0.

The potato tubers were stored at 4°C and before processing were reconditioned at 18°C for 15 days in order to reduce the amount of accumulated sugars, and to improve the overall processing quality of the tubers.

Processed potato samples

Samples for analysis were taken from a 10-lb batch at various stages of processing in a small laboratory pilot plant. The process used was as follows:

Cooking. Potatoes were peeled, sliced and the strips washed of surface starch, then soaked in a 0.5% sodium bisulphite solution at room temperature for 5 min and steam cooked at atmospheric pressure for 35 min.

Mashing. The cooked potatoes were mashed immediately after cooking in a KitchenAid mixer (Hobart Mfg. Co. Ltd., Troy, Ohio.) equipped with a flat beater at a speed setting of 6 for 2 min.

Freeze-thawing. The mashed potatoes were spread 1/2 in. thick on stainless steel trays, frozen in an air blast freezer (air velocity 3000 cu ft/min, minimum air temperature of −20°C) for several hours, and then thawed at room temperature.

Pre-drying, granulation, drying and cooling steps. These steps were performed in a Manesty Petrie-Fluid Bed dryer (model MP 10E Manesty Machines Ltd. Speke, Liverpool, England) modified by us to have a rotary stirrer immediately above the porous plate. The thawed potatoes were charged into the bowl and pre-dried with an air temperature setting at 93°C and at a stirrer speed of 20 rpm. When the moisture content of the potatoes dropped to a range of 35–42% the granulation step followed by using lower temperature setting, minimum air velocity and a stirrer speed of 500 rpm. After 4–10 min the temperature and the air flow were increased to begin the drying step. After about 5 min of drying the temperature and air flow were again gradually reduced to avoid cell damage due to the abrasion of the granules in the air stream. The heaters were then turned off and the air flow maintained at a low flow rate to reduce the granules' temperature to 20°C.

Analysis of pectic substances

The method of McComb and McCready (1952) was applied with our modification to avoid the interference of free water soluble starch in carbazole reaction for both water and calgon-soluble fractions of pectic substances.

The potato samples were first macerated in ethanol at 5°C using a Waring Blendor at low speed. Subsequent extraction steps with water and a 0.5% calgon solution (sodium-hexametaphosphate, Calgon Consumer Div., Toronto) are presented on Figure 1. From a total of 100-ml potato extract, 2.5 ml-aliquots were taken for determination of free starch, and 2 ml for determination of uronide contents as described

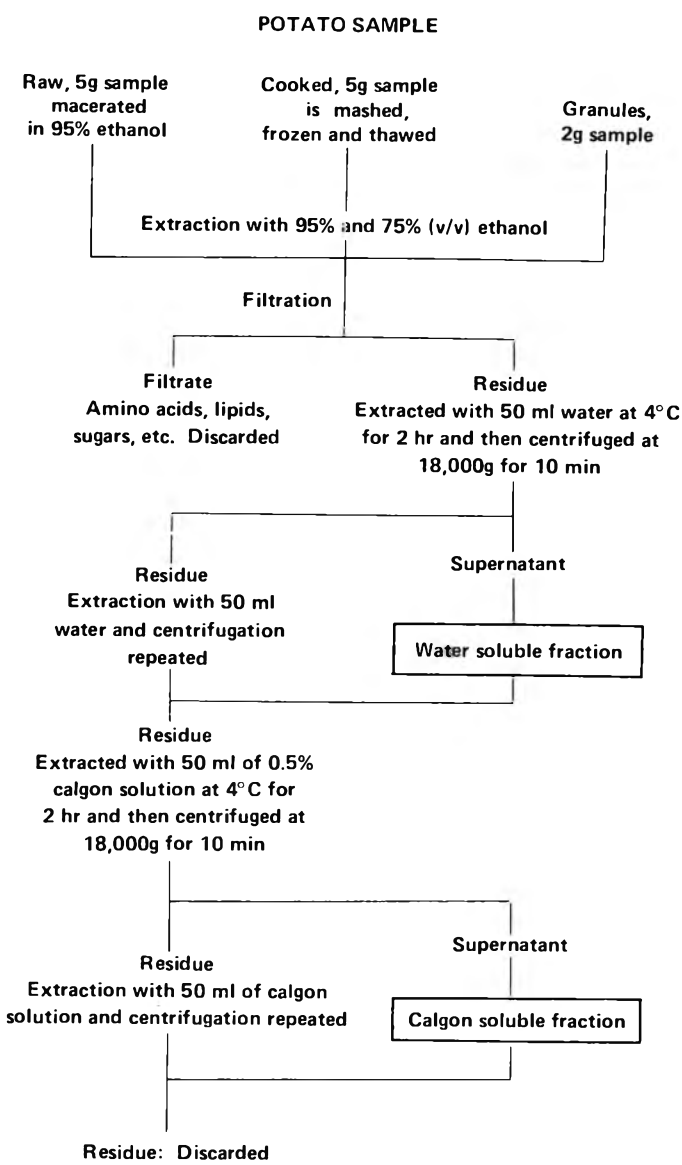

Fig. 1—Flow chart for extraction of pectic substances from potatoes. All extraction steps except the first ethanol step were performed in cold room. All centrifugations were performed on a refrigerated Sorvall superspeed SS-2 centrifuge.

by McComb and McCready (1952). The starch content was determined by mixing the aliquot with 7.5 ml distilled water, heating the solution at 100°C for 5–10 min and cooling to room temperature. Then 0.2 ml of 0.02N KI_3 solution was added and the absorbance of the blue color read at 640 nm.

The uronide values of the extracts were then obtained from the standard curve and corrected for the starch interference using only the linear portion, region A, of the correction curve (Fig. 2). These curves were plotted from data for starch absorbance vs. their carbazole counterparts obtained by carbazole reaction of pure starch solutions, and of D-galacturonic acid, alone and in presence of low and high concentrations of starch, all within the range encountered in potato extracts (see Fig. 3 and 4).

Total starch determination

The amount of starch in raw and processed potatoes was determined polarimetrically after interfering pectin, nonstarch polysaccharides and proteins were removed as outlined by Steiner and Guthrie (1944) and revised by Hadorn and Doevelaar (1960). A 1-g sample was boiled with a 0.3% ammonium carbonate solution, the starch precipitated with iodine, the precipitate decomposed with sodium thiosulphate solution, reprecipitated with iodine, the precipitate again decomposed, then the starch precipitated with 70% ethanol, followed by dispersion in 40% calcium chloride solution, precipitation of traces of protein with uranyl acetate and determination of optical rotation. The starch content was then calculated by using the value $[\alpha]_{25}^{D} = 190.0°$ as obtained below.

Free starch determination

A 2.5-g sample of cooked, mashed or freeze-thawed potatoes, or 0.5g pre-dried or granulated potatoes was agitated for 5 min in 500 ml of water heated to 65.5°C. The slurry was filtered and from clear filtrate an aliquot of 5 ml was transferred to a 125 ml Erlenmeyer flask containing 1 ml of 0.02N KI_3 and 44 ml of distilled water. The absorbance of the blue color developed in the dark at 25°C for 1 hr was read at 640 nm and expressed as Blue Value Index (Mullins et al., 1955).

Examination of intact and damaged potato cells

The potato granule cells were examined under a Leitz Wetzlar light microscope at 100× magnification. The granules were suspended in water heated to 65.5°C and a few drops of such a suspension were mixed with a drop of 0.02N aqueous KI_3 on a slide. The intact tissue cells appeared faintly blue colored with a sharp indication of cell boundary. The damaged cells had a visible broken cell wall portion and a starch gel protrusion from the cell, dark blue in color. A total of at least 1000 cells per slide were counted.

Pure potato starch preparation

The starch was isolated from the same batch of potato tubers used for granule production. About five washed tubers were ground at a low speed in a Waring Blendor with 300 ml ice cold 1% ammonium oxalate containing 100 ppm sodium bisulphite and 1 ml octyl alcohol as anti-foaming agent. The slurry obtained was filtered through a 100 mesh nylon sieve and the residual debris reground and washed with a small volume of water. The combined filtrates were poured through a 200 mesh sieve and further purified by several centrifugations at 2000 rpm. The light brown upper layer of protein was removed while the lower layer was resuspended in distilled water until no impurity was evident under the microscope. Finally the starch was washed with 98% ethanol, ethyl ether and then acetone. It was then air dried. The amylose content of this starch was 21.2% as determined potentiometrically (Schoch, 1964). The specific rotation $[\alpha]_{25}^{D}$ of the starch, which was dissolved by heating at 120°C for 18 min in 40% $CaCl_2$ was 190.0°.

For determination of iodine binding affinity of the starch, 50 mg of starch were boiled for 5 min in 5 ml of 40% $CaCl_2$ solution and cooled to room temperature. Then 2.5 ml of 1N HCl was added and made up to 50 ml with distilled water and filtered. From the filtrate aliquots of 1–8 ml were added dropwise to 100 ml volumetric flasks containing 80 ml water, 1 ml 0.1N HCl and 1.5 ml 0.02N KI_3. The solution was mixed, made up to volume and left in the dark at 25°C for 1 hr. The absorption was then read at 640 nm. The results are given in Figure 5.

RESULTS & DISCUSSION

HOFF AND CASTRO (1969) found that about one-half of the pectic substances isolated from tuber cell wall were polyuronide compounds. The remainder was found to be sugars, predominantly galactose. Further Joslyn (1962) showed that a large quantity of araban and to a lesser extent galactan might be associated with polyuronides in the middle lamella, either as an entrapped admixture in the mesh-

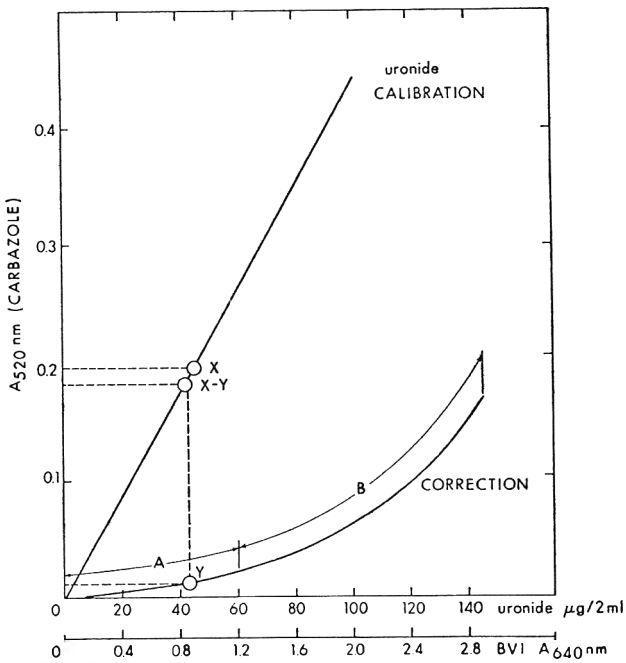

Fig. 2—Calibration and correction curves used in pectic substances determination. The use of curves is illustrated with the following example: when carbazole value (x) = 0.200 and the BVI = 0.800, then the corresponding carbazole value correction (y) = 0.021; hence, the corrected carbazole value (z) = 0.188 and the uronide content in μg 2 ml of extract = 44. The region B of the correction curve does not show linearity for its corresponding starch concentrations; hence the interfering starch content in the extracts of pectic substances, as measured in terms of Blue Value Index must be kept within the linear region A, of the correction curve.

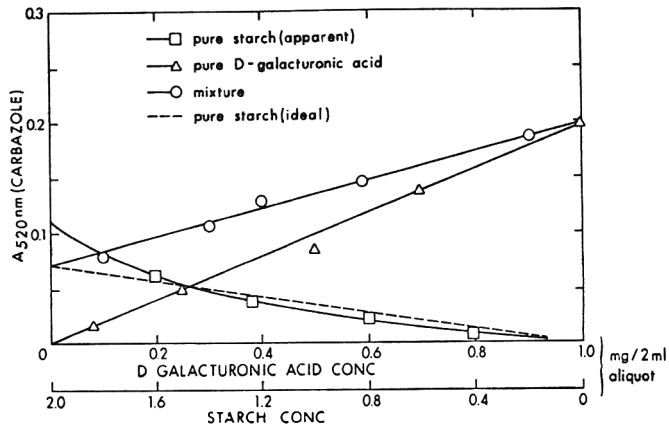

Fig. 4—Effect of high concentrations of starch on carbazole values. Pure starch (apparent) represents the actual A_{520} readings, while the pure starch (ideal) the readings expected. Note that the absorbance of the color developed by the starch present departs from linearity while that by D-galacturonic acid remains linear.

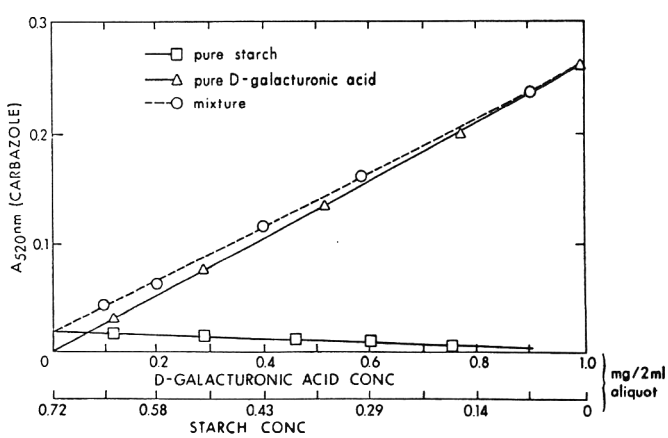

Fig. 3—Effect of low concentrations of starch on carbazole values. Carbazole and galacturonic acid were reagent grade while the starch was prepared from tubers of Netted Gem potato (see text) and applied in concentrations usually encountered in the pectic substances extract from potatoes. Note that the carbazole values increase linearly with the increase in contents of both starch and D-galacturonic acid. The linearity established for starch is in the range of 0—0.036% (w/v) while for D-galacturonic acid 0—0.05% (w/v). In presence of both and within the range given the effect of the starch in carbazole color development is additive.

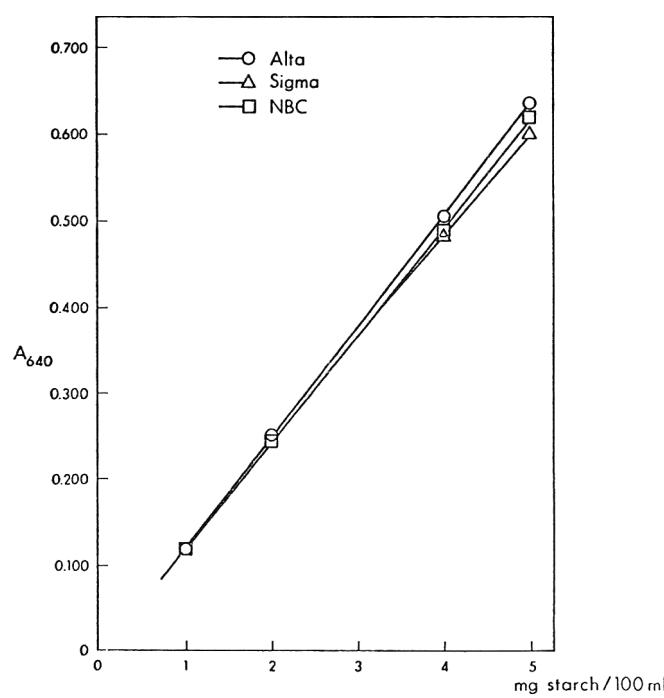

Fig. 5—Blue color intensity of starch-iodine complex as related to starch concentration. [-○- Alta. Netted Gem Potato Starch (Southern Alberta); -△- Commercial potato starch from Sigma Chem. Co., St. Louis, Mo; -□- From Nutritional Biochemical Co., Cleveland, Ohio.]

Table 1—Water and calgon soluble fractions of pectic substances in raw potatoes

Potato batch	Sp.g.	Starch BVI (A_{640} nm)	Correction value	Total	Corrected	Uronide content μg/2 ml Extract	Uronide content mg/100g potato Wet basis	Uronide content mg/100g potato Dry basis
			Water soluble extract					
1	1.096	0.184[a]	0.003	0.017	0.014	3.0	48.0	212
2	1.098	0.388	0.006	0.017	0.011	2.2	35.2	155
3	1.095	0.094	0.002	0.019	0.017	3.4	54.4	240
						Average	45.9	202.4
			Calgon soluble extract					
1	1.096	0.100[a]	0.002	trace	trace	trace	trace	trace
2	1.098	0.167	0.003	0.011	0.008	1.7	27.2	120
3	1.095	0.100	0.002	0.010	0.008	1.7	27.2	120
						Average	18.1	80

[a] This and following data unless otherwise stated are for 5g of potato sample extract made up to 100 ml (see experimental).

Table 2—Water- and calgon-soluble fractions of pectic substances in cooked potatoes

Potato batch	Sp.g.	Starch BVI (A_{640} nm)	Correction value	Carbazole value Total	Carbazole value Corrected	Uronide content μg/2 ml Extract	Uronide content mg/100g potato Wet basis	Uronide content mg/100g potato Dry basis
			Water-soluble extract					
1	1.095	0.488[a]	0.008	0.125	0.117	26.4	295	1296
2	1.098	0.715	0.012	0.078	0.067	15.1	242	1063
3	1.096	1.000	0.016	0.131	0.115	26.0	292	1282
						Average	278.7	1223.8
			Calgon-soluble extract					
1	1.095	0.288[a]	0.005	0.029	0.024	5.2	58.2	256
2	1.098	0.480	0.008	0.035	0.028	6.2	59.5	261
3	1.096	0.400	0.007	0.029	0.023	5.0	48.1	211
						Average	55.3	242.7

[a] This and following data unless otherwise stated are for 5g of cooked potato samples extract made up to 70 ml (see experimental).

work or covalently bound to the polyuronides. In spite of the complex heterogeneous nature of the pectic substances it has been suggested that it is the polyuronide fraction which is mainly responsible for the textural properties of raw and processed potato tissue. Thus Potter and McComb (1957) surmised that the differences in the texture of cooked potato, between soggy and mealy samples are at least in part due to differences in quantity and characteristics of the pectic substances, within the cell wall and between adjoining cells.

In an attempt to relate the extent of cell separation with pectic substances, Bettelheim and Sterling (1955) fractionated these substances into water, calgon and HCl soluble fractions, and determined their characteristics However, these studies showed no clear relationship between texture and pectic substances. In all cases cooking caused an increase in solubility of pectic substances, and a decrease in their methoxyl content and intrinsic viscosity. The greatly lowered intrinsic viscosity, presumably due to depolymerization was observed for all three pectic fractions. The water soluble fraction was least affected. These changes allowed much of the pectic material to diffuse into the cooking medium.

In this study of potato granule processing steam cooking was applied. Under these conditions the bulk of the diffusible pectic substances was retained within the cooked potato strips. After mashing these substances are distributed in the matrix surrounding the separated potato cells. However, a large amount of the water-soluble starch leaching from gelatinized starch granules is also retained in the matrix, and hence it was necessary to develop a method to determine pectic substances in the presence of appreciable quantities of starch.

The starch interference in the carbazole reaction was accounted for by preparing the starch carbazole correction curve (see Fig. 2). A Blue Value Index for the starch was determined for each extract and the corresponding carbazole value due to the starch was taken from the correction curve. This was then subtracted from the carbazole value to give the net value due to the polyuronides alone.

In addition, an improved extraction procedure was used which minimized the soluble starch content in the extracts. A twentyfold excess (w/v) of cold ethanol was used instead of hot, and excessive maceration of tissue was avoided by using the Waring Blendor at the lowest speed. Also the subsequent extractions with water and calgon solution were done at 4°C in order to reduce the swelling and dissolution of the starch granules.

By applying these modifications the enzymatic removal of starch using a mixture of α- and β-amylases, lasting 12 hr, and the subsequent ethanol precipitation of pectic substances as applied by Bettelheim and Sterling (1955) were avoided. The increased speed of analysis makes the modified procedure much more suitable for routine processing studies than the longer enzymatic method.

As seen from Tables 1 and 2 the content of pectic substances in raw potatoes was low in both water soluble and calgon

Table 3—Percentage broken cells in cooked potatoes mashed at various temperatures[a]

Temp at mashing, °C		% Broken cells
After cooking:	80°	2.6
	40°	12.5
	25°	31.9
	10°	45.8
After freezing:	0° (partially thawed)	5.0
	10° (completely thawed)	2.2
	80° (reheated)[b]	2.5
	25° (recooled)[b]	18.5

[a] Mashing was done on steam cooked potato strips with a KitchenAid mixer having a flat beater, at top speed for 45 sec.
[b] Samples were slightly darkened after reheating.

Table 4—Total and free starch contents in potatoes as affected by processing steps

Potato sample	Moisture %	Total starch %		Free starch Blue Value Index ($A_{640\ nm} \times 10^3$)	
		wet basis	Dry basis	Wet basis	Dry basis
Raw	75.5	17.5	68.4	130	516
Cooked Unmashed	79.4	14.0	68.1	75	364
Mashed at 80°C	77.2	15.5	68.0	85	373
Mashed and pre-cooled to 5.5°C	76.1	18.4	77.1	70	293
Freeze-thawed	75.2	19.6	79.0	27	110
Pre-dried	35.0	48.4	83.7	73	112
Granulated	8.1	77.0	83.7	118	128
Final dried unsieved granules	5.0	82.6	83.7	118	123
Sieved granules. Retained on					
18 mesh sieve (1.1%)				90	94
35 mesh sieve (1.8%)				118	122
60 mesh sieve (14.3%)				126	130
below 60 mesh sieve (82.8%)				130	135

[a] Determined according to Mullins et al. (1955) by analyzing 2.5g samples with moisture content above 70%, while for those below 40% the sample weight was 0.5g. The results are means of at least three determinations.

soluble fractions when compared to that in cooked potatoes. This may suggest that pectic substances in the potatoes analyzed are mainly in tightly bound forms. The water soluble fraction appeared to be higher than the calgon soluble fraction. These findings are contrary to those reported by Bettelheim and Sterling (1955) for the same variety of potatoes. However, the apparent total pectic substances, i.e., the sum of the water and calgon-soluble fractions, was quite comparable for the two studies.

The observations that the quantity of calgon-soluble fraction of pectic substances was low and that cooking increased the apparent total considerably, suggest that ionic bonds involving such ions as Ca^{2+} and Mg^{2+} are not important in the structure of protopectin in Netted Gem potatoes. Instead, it appears as suggested by Doesburg (1965) that physical enmeshing of the polyuronides in the cellulosic fibres of the cell walls, and other bonds existing in cell walls and middle lamella are more important. This fraction of enmeshed polyuronides could be solubilized completely by HCl at higher temperatures as found in this work and by Bettelheim and Sterling (1955). However, it was not analyzed in this study because the excessive starch hydrolysis and extraction caused by the HCl resulted in extracts to which the starch carbazole correction curve could not be applied satisfactorily.

The cooking of potatoes brought about a substantial increase in the content of the soluble pectic substances. The water soluble fraction increased sixfold while calgon-soluble fraction increased threefold (Tables 1 and 2). Hence cooking appears to be a most effective method to weaken and dissolve the major portion of the cell wall binding components of the potatoes. The apparent total pectic substances obtained from the cooked Netted Gem potatoes was 1.45% dry basis as compared with 0.7—1.5% dry basis reported by Potter and McComb (1957), 0.084% for Russet Burbank (Idaho) reported by Bettelheim & Sterling (1955) and the same as that of variety Superior reported by Hoff and Castro (1969).

Steam cooking appears to have a much smaller effect on both total and free starch contents than it does on the content of pectic substances. The amount of free starch in the cooked potato was lower than in the raw tubers which indicates that a portion of the free starch may be removed with the drip during steam cooking. Furthermore, it appears that very little leaching of soluble starch from cells in unmashed potato strips takes place during starch granule gelatinization.

Microscopic examination of cooked potato tissue showed that the large swollen starch granules completely filled the cells, and also that almost all of the cell walls remained intact after cooking. Examination of the potatoes mashed at temperatures close to that of cooking indicated that cell separation was easily accomplished with little damage to cell walls. However, as mashing temperature was decreased the percentage of broken cells increased. For example, a reduction of mashing temperature from 80° to 10°C gave an eighteenfold increase in the percentage of broken cells (see Table 3). Simultaneously an increase in free starch Blue Value Index was obtained, which suggests that considerable rupture of gelatinized starch granules occurs as the cell walls are broken.

These observations suggest that the intercellular matrix, which consists of both pectic substances and free starch, has cell binding properties which are dependent on temperature. At lower temperatures the matrix appears to gel and the cell binding forces are so increased that on mashing many of the cells are sheared as the relatively rigid system is deformed, or the cell walls are torn off as the cells are separated.

If it is assumed that the Blue Value Index of free starch in cooked potatoes reflects the presence of amylose and amylopectin in the ratio of 1:3.7 as found in this study for intact starch granules, then the free starch in the cooked potato is 8.1% of total solids. If the free starch is amylose alone, then about one-fifth of this amount of free starch (1.7% of total solids) would be present in the matrix and available for cell binding. This minimum amount of free starch is therefore greater than the amount of pectic substances present in the intercellular matrix even if all of the apparent total of pectic substances, 1.4% of total solids, has a binding role in the matrix. Hence the matrix property should reflect predominantly the properties of the starch constituent, rather than of the pectic substances.

These suggestions are supported by the results of the freeze-thaw experiments (see Table 3). The lowest percentage of broken cells, 2.2%, occurred in potatoes mashed at 10°C after freezing and thawing which would indicate that retrogradation of amylose occurred and brought about a decrease in the strength of the cell binding matrix.

The freezing and thawing has a direct bearing on the success of the straight through process in both the pre-drying and granulation stages (Ooraikul, 1973).

Table 5—Water- and calgon-soluble fractions of pectic substances in potato granules

Potato batch	Sp.g.	Starch BVI (A_{640} nm)	Correction value	Carbazole value		Uronide content		
				Total	Corrected	µg/2 ml Extract	mg/100g potato Wet basis	Dry basis
Water-soluble extract								
1	1.095	0.069[a]	0.001	0.160	0.159	35.5	1136	1190
2	1.098	0.069	0.001	0.126	0.125	28.0	1120	1174
3	1.096	0.066	0.001	0.127	0.126	28.1	1124	1178
						Average	1126.7	1180.8
Calgon-soluble extract								
1	1.095	0.144[a]	0.002	0.035	0.033	7.2	202	211
2	1.098	0.128	0.002	0.018	0.016	33.2	128	134
3	1.096	0.220	0.004	0.024	0.021	5.0	200	210
						Average	173.3	185.0

[a] This and following data unless otherwise stated are for 5g of potato sample extract made up to 100 ml (see experimental).

If the mashed potato was not frozen and thawed it was difficult to stir the potato in the pre-drying step and during granulation it was difficult to separate the cells without incurring so much damage that the broken cell count became meaningless. The introduction of freezing and thawing enabled the pre-drying and granulation to be done at temperatures below the gelling temperature of matrix without noticeable cell damage.

Freezing and thawing had little effect on the level of both water and calgon-soluble pectic substances. The slight decrease was attributed to loss through drips or expressed juice. A small increase in total starch was observed. In contrast freezing and thawing brought about a substantial decrease in the free starch content, equivalent to nearly 200 Blue Value Index units on the dry basis (Table 4).

This also indicates that the important component of the intercellular matrix for successful processing is the free starch rather than the pectic substances.

It is possible to freeze and thaw the potato before mashing, rather than after, as done in this study. Mashing before freezing has the advantage that additives forming clathrates with the free starch can be incorporated, and the freeze thaw step then forces the residual uncomplexed free starch to retrograde. For example, the addition of glycerolmonostearate at 0.1% weight of wet potato reduced the average Blue Value Index from 110 (dry basis) to 60. Antioxidants if required, are also added at this stage.

The dry basis contents of total starch and of water- and calgon-soluble pectic substances were not affected by the pre-drying at 20–30°C, or by the granulation at 15–20°C provided that the moisture content was not less than 35% (wet basis) at the granulation step. Below 35% moisture, granulation gave a two- to fourfold increase in Blue Value Index whereas at moisture contents above 35% the increase in Blue Value Index due to cell damage in the granulation step was only 15%.

Granulation in the range 35–40% moisture had the advantage that it offered the possibility of further retrogradation of free starch. Hellman et al. (1954) reported that the rate of retrogradation of starch gel depends on moisture content and that the greatest rates are in the range of 30–60% moisture. This fact has been applied even in the "add-back" process where the granulation is improved by a decrease of moisture of the moist mix to 35–45% (Olson et al., 1953). Potter (1954) also demonstrated that the granulation step might retrograde the starch. In his study on changes in physical properties of starch in granule production, he observed that as the moisture of the potato decreased, the rate of retrogradation increased until about 30% moisture was reached. Below this, the rate of retrogradation began to increase until about 15% moisture below which there was no measureable change.

Broken cell counts, Blue Value Index and contents of the starch and pectic fractions showed no real change in the final drying stage, which indicates that once the moisture content has dropped below 35%, retrogradation of any remaining free starch or further cell damage by abrasion in air stream does not occur.

The dried potato granules had: total starch 83.7%; apparent total pectic substances 1.4%; bulk density 0.80 g/ml; moisture content 5.0%; and a broken cell count of 2.0% (Tables 4 and 5). The granules with particle size less than 60 mesh comprised 83% of the product. The fine fraction was retained as final product with the remainder being recycled to the pre-drying step of the next batch. The Blue Value Indexes of the coarser fractions were lower than those of the finer fractions (Table 4), which probably reflects the relative amounts of mechanical stress on particles of different sizes during the final processing steps.

In conclusion it appears that the free starch is the more important component in the intercellular matrix during processing and probably also has a more important influence on the textural characteristics of the reconstituted product than does the pectic fraction. The simple straight-through freeze-thaw process presented is such that the free starch content can be readily controlled at all stages of processing to give a product which on reconstitution has a texture closely approaching that of freshly mashed potato of the same batch.

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GLUTAMINE AS A PREDICTIVE MEASUREMENT IN THE QUALITY ASSESSMENT OF PROCESSED CARROT PUREE

INTRODUCTION

PROCESSING TECHNIQUES have produced safe, nutritious foods for the consumer. However it is an accepted fact that some degree of deterioration occurs during the processing procedure. This is believed to be due to certain undesirable changes which take place in the commodity during processing. Such changes as loss of color, destruction of vitamins, and the development of off-flavors are being studied in order to produce better quality products. One compound, 2-pyrrolidone-5-carboxylic acid (PCA) which is formed during processing by the cyclization of glutamine (Archibald, 1945), has been associated with off-flavor formation in various fruits and vegetables.

The conversion of glutamine to PCA is a well-known reaction which occurs in the manufacture of sugar from sugar beets (Goodban et al., 1953). The length of time tomato juice is kept at high temperatures and the amount of heat applied to the product affects the conversion of glutamine to PCA (Rice and Pederson, 1954). PCA present in processed beet puree is formed from glutamine during thermal processing (Shallenberger and Moyer, 1958; Shallenberger et al., 1959). Luh et al. (1969) found that PCA is formed during thermal processing of strained carrots. A drop in pH was also observed. Using radiotracer techniques, Markakis and Amon (1969) found that on pasteurization of grape juice, glutamine is converted to PCA. In work with spinach puree, Lin et al. (1970) found that on processing, PCA is one of the organic acids which increased the most. The formation of PCA has been shown to occur directly from glutamine, and as the temperature of processing is increased, the formation of PCA from glutamine decreased (Clydesdale et al., 1972).

Shallenberger et al. (1959) reported that PCA causes a bitter, medicinal, or phenolic off-flavor in processed vegetables. Studies of PCA on several processed commodities showed that as the amount of PCA present in each commodity increased, it was possible to detect greater differences in the flavor of each commodity.

It is the aim of this investigation to develop a simple quality test whereby knowing the amount of glutamine present in a fresh sample of carrots, the percent conversion of glutamine to PCA upon processing, and the flavor threshold of PCA, the best overall time and temperature for a given process to produce the highest quality product could be predicted.

MATERIALS & METHODS

CARROTS from two separate growing locations, California and Texas, were purchased from a local market. In using carrots from two different geographical areas, it was hoped that any differences in the conversion of glutamine to PCA due to different climates, soils, growing techniques, or fertilizers would be detected. The carrots from each location were analyzed separately. Each batch was washed, peeled and immediately comminuted in a Fitzpatrick Mill (Model M comminuting machine, The W.J. Fitzpatrick Co., Chicago, Ill.) using a fine (No. 40) screen. Due to the nature of the comminuting, a large amount of air was incorporated into the puree. The air was removed prior to filling Thermal Death Time (TDT) tubes by placing the puree in a beaker inside a vacuum desiccator attached to a water aspirator. The carrots were maintained at a vacuum of 23 in. of mercury for 10 min and refrigerated at 35°F. Approximately 4 ml of the puree was placed in each of 80 TDT tubes which had been flushed with nitrogen and the tube sealed with an oxygen flame. The tubes were processed using the methods developed by Gupte and Francis (1964). A temperature controlled glycerol bath was used for processing. The processing temperatures ranged from 240–300°F with 20°F increments and an F_0 value of 4.9. 20 tubes were used for each processing temperature. After processing, the samples were placed in two groups of 10 tubes each. All tubes were frozen at −20°F until analyses were carried out. Each group was analyzed for pH, moisture and PCA content.

A convenient and rapid method of analysis of glutamine developed by Lin et al. (1972) was used. It is based on the analysis of PCA formed from glutamine. The concentration of PCA was determined by using an Automatic Organic Acid Analyzer (AOAA) (Waters Associates, Framingham, Mass.). All data obtained were based on determinations made using this instrument on duplicate samples.

The total glutamine content of the fresh puree was determined. The puree was buffered to pH 6.8 with buffer solution consisting of 4.957g of potassium dihydrogen phosphate and 12.167g of sodium tetraborate in one liter of distilled water. The pH was adjusted to 6.8 by addition of concentrated hydrochloric acid. The sample was hydrolyzed for 2 hr in a boiling water bath. After cooling, the puree was analyzed for PCA using the AOAA. The PCA content in the fresh unhydrolyzed puree was also determined. Since one mole of glutamine is quantitatively converted to one mole of PCA, the total glutamine content was determined by subtracting the amount of PCA present in the fresh sample from the amount of PCA present in the hydrolyzed sample. Hydrolysis studies were also carried out using stock glutamine solutions and mixtures of glutamine solution with carrot puree to check the accuracy of the conversion procedures.

Taste threshold studies were carried out for PCA in both processed carrots and a model system. The model system threshold studies were carried out using stock PCA solutions prepared at various concentrations in distilled water. Carrot puree was prepared by washing, trimming and peeling the fresh carrots. They were then cooked in boiling water until tender, comminuted and cooled. The puree was then divided into seven equal lots. The concentration of PCA was adjusted by the addition of small amounts of PCA stock solution. The actual PCA concentration of each lot was determined by using the AOAA.

In both taste threshold determinations, an untrained in-house panel was used. A multiple paired comparison test was employed. Five samples were presented simultaneously and included one or more hidden controls and a labelled reference sample. The panelists were asked to taste each sample and compare it to a control which had no PCA added. They were asked to indicate when they could perceive a difference between the control and the sample. The occurrence of a taste difference was indicated as a positive response. All threshold tests were carried out in a specially designed sensory evaluation laboratory with isolation booths and facilities for control of environmental conditions such as humidity, light, and temperature.

RESULTS & DISCUSSION

Sensory response to PCA concentration

The percent positive responses obtained from the threshold studies were plotted against PCA concentration. Lines of best fit were determined for the data, 95% confidence limits were established, and the threshold range for each study was determined using the 75% positive response level from ASTM committee

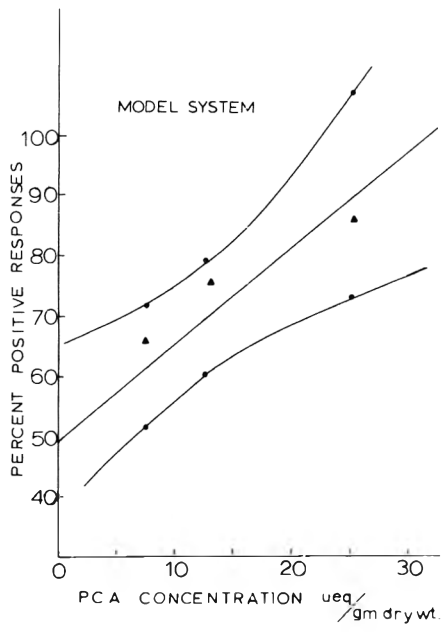

Fig. 1—Percent positive response vs. PCA concentration for the model system taste threshold study. A line of best fit is drawn through the raw data points (▲) and 95% confidence limits (●) are shown.

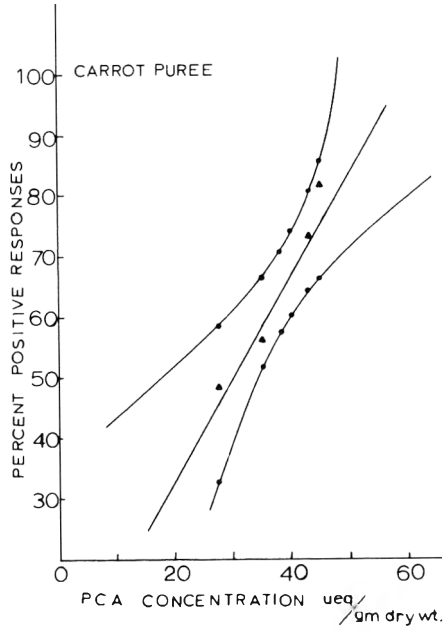

Fig. 2—Percent positive response vs. PCA concentration for the carrot puree taste threshold study. A line of best fit is drawn through the raw data points (▲) and 95% confidence limits (●) are shown.

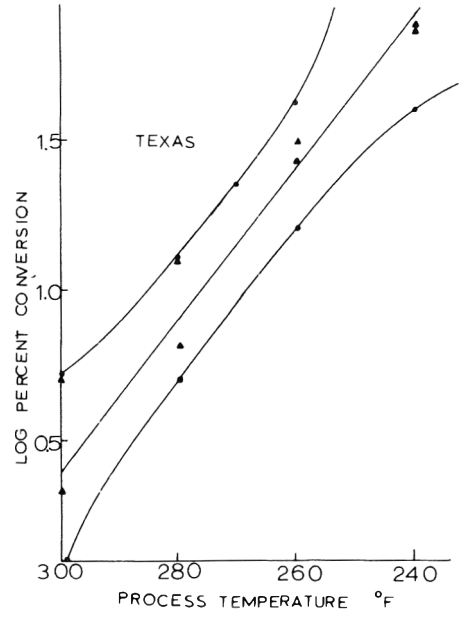

Fig. 3—Log percent conversion of glutamine to PCA vs. process temperature for California carrots. A line of best fit is drawn through the raw data points (▲) and 95% confidence limits (●) are shown.

E-18 publication (1968). The results obtained from the model system study are shown in Figure 1. The coefficient of determination for the line of best fit was 0.8739. The coefficient of determination discussed by Snedecor and Cochran (1967) is defined as the percent variation in the ordinate which may be explained by the variation in the abcissa. The use of this coefficient results in an explicit statistical representation of data. The threshold range of concentration for the model system at this positive response level was between 10 and 24 microequivalents per gram of sample.

In the carrot puree study, according to the ASTM committee definitions for threshold, the 75% positive response level yields a panel taste threshold range of 42–57 microequivalents per gram dry weight of the puree. The results of the carrot puree study are shown in Figure 2 which is a plot of the percent positive responses versus the PCA concentration of the puree. If the 50% positive response level were used, as employed by Patton and Josephson (1957) and others, it would yield a panel taste threshold range of 18–34 microequivalents per gram dry weight of the puree. For this method of constant stimulus (Guilford, 1954), the 50% positive response point is the chance response level and has little meaning in terms of difference threshold. The present authors are in agreement with Powers and Quinlan (1973) that the 70% level (or 75% level as discussed in the ASTM publication (1968)) is more useful.

Conversion of glutamine to PCA

Results of the hydrolysis studies of the model system showed that the percent conversion had a range from 93.7–103.0% with an average percent conversion of 98.6% and a standard deviation of 3.341. The mixture of carrot puree and glutamine solution has a range of 98.24–106.5% with an average percent conversion of 101.96% and a standard deviation of 4.05. From the results, it was concluded that the procedure was reproducible and was not affected by the presence of components of the carrot puree.

The amount of PCA found at each processing temperature was expressed as percent conversion of glutamine to PCA. The 100% level was set as the total amount of PCA formed from glutamine in the hydrolyzed sample. The data from each batch were examined separately. The percent conversion of glutamine to PCA for both California and Texas samples exhibited a logarithmic change with processing temperature. Both sets of data were plotted separately on inverse semilogarithmic plots of the percent conversion versus processing temperatures with the lines of best fit determined. The coefficients of determination were 0.8970 for the Texas data and 0.9710 for the California data. These are shown in Figures 3 and 4, respectively.

The slopes and heights of the two lines were different. Both sets of data were examined together to determine if there were a significant difference between the lines. The data from both batches were combined and using the method of Snedecor and Cochran (1967), it was determined that at the 95% confidence level there was no significant difference between the lines. The combined data were then used to determine a line of best fit and 95% confidence limits. The coefficient of determination for the pooled data was 0.890. The inverse semilogarithmic plot of the percent conversion versus the processing temperature for the combined data is shown in Figure 5.

Predictive mechanism

Using the processing data and the data on the taste threshold range for the carrots, it was possible to formulate a predictive mechanism to determine the best acceptable product in terms of off-flavor due to PCA formation. This may best be explained using a hypothetical situation.

If one assumes that a batch of produce, in this case fresh carrots, has just been received, it would first be necessary to determine the total glutamine content by the method of Lin et al. (1972) which was previously discussed. One would examine the inverse semilogarithmic plot

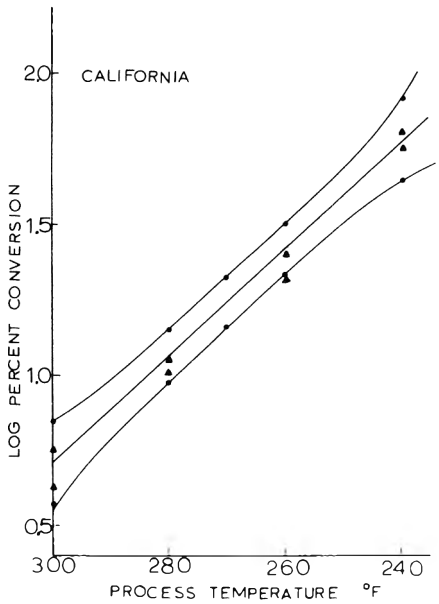

Fig. 4—Log percent conversion of glutamine to PCA vs. process temperature for Texas carrots. A line of best fit is drawn through the raw data points (▲) and 95% confidence limits (●) are shown.

Table 1—Changes in pH of carrot puree due to processing at different temperatures with an F_o = 4.9

Process	Texas	California
	pH	
Fresh	5.91	5.93
240	5.43	5.40
260	5.68	5.71
280	5.74	5.77
300	5.79	5.80

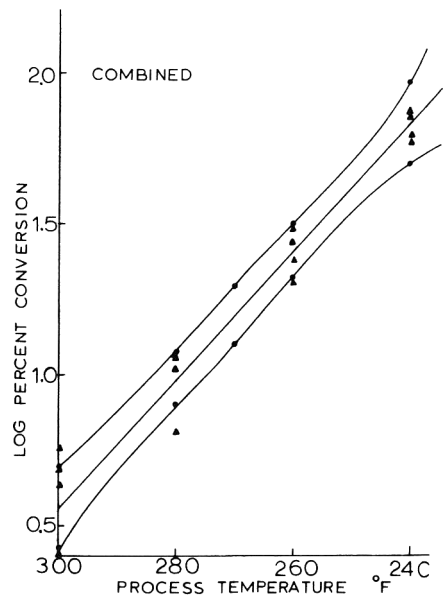

Fig. 5—Log percent conversion of glutamine to PCA vs. process temperature for the combined data. A line of best fit is drawn through the raw data points (▲) and 95% confidence limits (●) are shown.

similar to Figure 5 for the particular processing temperature being employed to determine the percent conversion. This would then be multiplied by the total glutamine content previously determined to yield the amount of PCA which would be formed. A check back to the taste threshold plot, such as the one in Figure 2, and to the taste threshold range would tell us if the amount of PCA which was being formed was above or below the taste threshold concentration. If it was above the threshold, it would be possible, by adjusting the processing temperature, to lower the percent conversion and thus lower the final PCA concentration such that it would be below the taste threshold.

Effect of pH on processing

It has been noted that a change in pH occurs on processing (Luh et al., 1969). Table 1 shows pH changes which were encountered for the various processing temperatures. At the higher processing temperatures, there was a smaller change in pH from the fresh product to the sample than at the lower temperatures. Although this was an overall effect due to all acids formed, PCA, which has been noted as increasing in concentration to the greatest degree during processing (Lin et al., 1970) probably contributed a great deal to this change.

Glutamine is found in many fruits and vegetables (Archibald, 1945). Depending on the degree of thermal processing which the product receives, and the flavor characteristics of the product, the formation of PCA may play a vital role in its quality after processing.

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CERTAIN FUNCTIONAL PROPERTIES OF SUNFLOWER MEAL PRODUCTS

INTRODUCTION

ALTHOUGH SUNFLOWER is a promising new source of food protein (Sosulski and Bakal, 1969), little information has been reported on its functional properties. This may have been due to the presence of chlorogenic acid and hulls in the sunflower meal (Pomenta and Burns, 1971). With a successful method to remove chlorogenic acid from dehulled sunflower kernels (Sosulski et al., 1973), the protein concentrates and isolates obtained may find wider uses in human foods in the future. Although somewhat low in lysine and isoleucine (Smith, 1968; Earle et al., 1968; Sosulski and Sarwar, 1973), sunflower proteins contain high levels of other essential amino acids. However, the functional properties will largely determine its acceptability as an ingredient in prepared foods.

A previous study (Kilara et al., 1972) described such functional properties as nitrogen extractability and moisture adsorption of sunflower meal products. In order to more fully describe the characteristics of these products, the present study presents other basic functional properties including water absorption, fat absorption, emulsification, whippability and foam stability on sunflower flour, protein concentrates and isolates.

EXPERIMENTAL

DEHULLED SUNFLOWER seeds of a single variety, commander, were obtained from Co-op Vegetable Oils Ltd., Altona, Manitoba. One lot of seeds was ground and extracted with Skelly F at 45°C. After desolventizing at room temperature, the defatted meal was reground in a Waring Blendor and sifted through a U.S. 80-mesh screen. The resultant product is called sunflower flour in this paper. Kernels from other portions of the sunflower seeds were cut transversally into two to three pieces with a knife and continuously diffusion-extracted (DE) with tap water adjusted with HCl to pH 5.5 at 60, 80 and 90°C for 4, 1.5 and 1 hr., respectively (Sosulski et al., 1973). After air drying at 50°C for 3 hr, the cut kernels were aspirated to remove the testa and ground as described above. The ground material was extracted with Skelly F, desolventized and reground to pass through an 80-mesh sieve. The defatted products were collected separately as sunflower protein concentrates, DE-60, DE-80 and DE-90. Protein isolate was prepared from the DE-60 protein concentrate by the procedure of Kilara et al. (1972).

The five commercial soy products used in this study as reference proteins included a commercial soy flour (food grade), two concentrates: Isopro (Ralston Purina Co.) and Promosoy (Central Soya Co.), and two isolates: Supro 610 (Ralston Purina Co.) and Promine D (Central Soya Co.). All samples were passed through a U.S. 100-mesh sieve.

The moisture, ash, protein, oil and crude fiber contents of the sunflower samples were determined by the AOAC (1970) method. Total sugars were extracted with 70% ethanol and measured according to the phenol-sulfuric acid procedure of Dubois et al. (1956) using sucrose as the standard. The water-soluble proteins used for the determination of the protein solubility index were extracted following the slow-stirring procedure described in AOCS (1970). Water absorption capacities of soy and sunflower meal products were determined according to the procedure of Sosulski (1962). In the present study, however, the suspensions were centrifuged at 1610 × G (3200 rpm, radius 14.1 cm).

Fat absorption characteristics were measured by adding 0.5-g sample and 3.0 ml of corn oil to a 15-ml conical graduated centrifuge tube. The contents were stirred for 1 min with a thin brass wire to disperse the sample in the oil. After a holding period of 30 min, the tube was centrifuged at 1610 × G for 25 min and the volume of free oil was read. Fat absorption was expressed in percentage as the amount of corn oil bound by a 100-g sample on a 14% moisture basis. Emulsification properties were determined essentially according to the procedure developed by Inklaar and Fortuin (1969). Modifications used in the present study included the addition of 50 ml of corn oil to the dispersed material which was being continuously stirred at 1400 rpm.

Whippability and foam stability were measured by weighing a 6-g sample (14% moisture basis) into a container (14 × 9.5 cm) containing 200 ml of distilled water. The solid material was dispersed in water with a spatula and suspension was whipped for 6 min using a food mixer at a speed set for heavy beating. Volumes were recorded before and after whipping in a 1000-ml graduated cylinder and the percent volume increase due to whipping was calculated according to the method of Lawhon and Cater (1971) and Lawhon et al. (1972). After the total volume of whip was measured, the volume of foam in the standing cylinder also was recorded for foam stability studies at 1, 10, 30, 60 and 120 min after whipping.

All functional properties of soy and sunflower products described in this paper were carried out on an as-is pH basis.

RESULTS & DISCUSSION

THE COMPOSITION of sunflower flour, protein concentrates and isolate is shown in Table 1. These results indicate that the water soluble constituents of the minerals and sugars were largely removed from the sunflower kernels by the DE process in the preparation of the protein concentrates and the protein isolate. Concomitantly, the DE process resulted in slightly

Table 1—Composition of sunflower meal products

Description	Flour	Protein concentrate			Protein isolate
		DE-60	DE-80	DE-90	(DE-60)
Moisture, %	6.5	6.7	7.3	5.3	3.1
Ash, %	8.5	7.8	7.5	7.5	3.5
Protein, %	55.5	67.9	68.3	68.0	90.7
Oil, %	0.5	1.7	1.7	3.6	1.1
Crude fiber, %	3.7	5.2	5.2	4.6	1.4
Total sugar, %	10.0	0.2	0.3	0.3	0.2
Nitrogen-free extract other than sugars, %	15.3	10.5	9.7	10.7	0.0

higher levels of protein and crude fiber, and lower percentage of nitrogen-free extract other than sugars in the protein concentrates. All of the dried sunflower products were bland in flavor and white in appearance except the DE-60 protein isolate which appeared light brown in color. However, color comparisons based on 2% dispersions of sunflower material at pH 9.0 showed that while the protein concentrates were creamy white, the color of the flour and protein isolate were light green and brown, respectively. Apparently, the amount of chlorogenic acid present in the protein concentrates and isolate was almost negligible, while that found in flour was high (Sosulski et al., 1973).

As shown in Table 2, the protein solubility index (PSI) of sunflower flour was 16.1%, as compared to 21.4% for the commercial soy flour. The PSI of sunflower protein concentrates was much lower than the sunflower flour with values ranging from 2.1–3.3%. The protein solubilities of the protein concentrates subjected to higher temperature (i.e., DE-90) were slightly lower than those prepared under more moderate conditions (i.e., DE-60). Similar to soy proteins, sunflower proteins are sensitive to moist heat, the condition widely recognized to denature most natural proteins. Despite these low solubilities, the PSI of sunflower protein concentrates was comparable to the commercial soy protein concentrates tested in this study. The sunflower protein isolate (DE-60) had a similar PSI with one of the commercial soy isolate (Supro 610), but distinctly different from another (Promine D).

The water absorption capacities of sunflower material varied considerably depending on the type of products. The water absorption of sunflower flour was 107.1% of its weight at 14% moisture basis, while that of protein concentrates were 137.8, 166.2 and 203.0% for DE-60, DE-80 and DE-90, respectively, and 155.1% for the protein isolate (Table 2). It should be noted that the water absorption capacity of sunflower protein concentrates increased as the PSI of these products became lower. The results showed that heat denaturation did not lower the water-imbibing capacities of sunflower proteins, but instead improved these properties. A similar effect was reported on the soy proteins (Anon., 1964). The DE-60 sunflower protein isolate had a higher water-absorption capacity than its parent protein concentrate, but still was lower than the high heated concentrates DE-80 and DE-90. In terms of water absorption, all sunflower products other than DE-90 protein concentrate had lower values than their soy counterparts. The higher absorption capacity of water by soy products may suggest that soy proteins are more hydrophilic in nature than the sunflower proteins.

Data on fat absorption clearly showed that soy products had oil absorption values ranging from 84.4–154.5% of their weight at 14% moisture basis, while sunflower products extended from 207.8% for the flour to 256.7% for the isolate. Contrary to the absorption of water, all sunflower products bound more oil than the soy products. In this regard, structurally, the sunflower proteins could be more lipophilic than the soy proteins. Although the mechanism of fat absorption by proteins is unclear, it seems likely that sunflower proteins contain numerous nonpolar side chains that have been believed to bind the paraffin chains (hydrocarbon chains) of fats (Przylecki et al., 1935), thereby contributing to higher absorption of oil.

The results on emulsification properties in Table 2 are based on the percentage of 50 ml of corn oil emulsified in 90 ml water by 5g of soy or sunflower meal products at 14% moisture basis. Sunflower flour which emulsified 95.1% of the added oil was superior to all other products tested. When the oil was added to the sunflower suspension during emulsification, the oil-in-water emulsions appeared in the form of fine foams. These emulsions were very stable even during subsequent heat treatments and the volume tended to increase during heating. Whether nonprotein constituents of sun-

Table 2—Some functional properties of soy and sunflower meal products

Sample	pH 10% dispersion	PSI[a] %	Water absorption[a] %	Fat absorption[a] %	Oil emulsified[b] %
Control					
Wheat flour	5.9	21.7	60.2	84.2	11.7
Soy					
Flour	6.6	21.4	130.0	84.4	18.0
Concentrate (Isopro)	5.0	2.3	227.3	133.0	2.8
Concentrate (Promosoy)	7.0	6.0	196.1	92.0	18.7
Isolate (Supro 610)	7.0	17.4	447.6	154.5	25.2
Isolate (Promine D)	7.1	71.1	416.7	119.2	22.2
Sunflower					
Flour	6.2	16.1	107.1	207.8	95.1
Concentrate (DE-60)	6.3	3.3	137.8	254.9	14.0
Concentrate (DE-80)	6.2	3.1	166.2	239.8	11.3
Concentrate (DE-90)	6.6	2.1	203.0	226.5	10.1
Isolate (DE-60)	7.0	18.2	155.1	256.7	25.6

[a] Mean of duplicate
[b] Mean of triplicate

Table 3—Whippability and foam stability of soy and sunflower meal products[a]

Sample	Vol increase on whipping %	Volume of foam after whipping, ml. 1 min	10 min	30 min	1 hr	2 hr
Control						
Fresh egg white (30 ml)	250.0	620	565	545	520	468
Soy						
Flour	70.0	160	131	108	61	20
Concentrate (Isopro)	170.0	400	28	13	8	5
Concentrate (Promosoy)	135.0	370	265	142	30	24
Isolate (Supro 610)	235.0	670	620	572	545	532
Isolate (Promine D)	230.0	660	603	564	535	515
Sunflower						
Flour	230.0	600	522	487	467	420
Concentrate (DE-60)	220.0	610	522	453	380	170
Concentrate (DE-80)	230.0	600	520	430	321	50
Concentrate (DE-90)	225.0	540	465	375	140	15
Isolate (DE-60)	230.0	630	546	512	493	473

[a] Mean of duplicate.

flower flour aid in the formation of emulsions remains unknown. It seems possible that sunflower flour proteins may collect at the surface of emulsified oil droplets to form a protective barrier that prevents the oil droplets from coalescing thereby causing emulsion breakdown. In terms of emulsification capability, sunflower flour apparently has a unique property that the other products do not have. However, most of the emulsification capacities of sunflower material were lost after the diffusion-extraction process. As seen in the data presented, the sunflower protein concentrates emulsified only 10.1–14.0% of the oil, which was slightly lower than one soy protein concentrate, but higher than the second. The emulsification capacity of DE-60 sunflower protein isolate was 25.6% which was in general comparable to soy isolates.

Data on whipping properties of soy and sunflower meal products were expressed as volume increase due to whipping without addition of sugar (Table 3). Both sunflower flour and protein concentrates increased in volume by about 230%, as compared to 70% for the soy flour and 170% for one of the soy concentrates. However, the sunflower and soy protein isolates had the same increase in volume of 230%. The sunflower flour and its protein isolate had the same whippability, although the protein content of sunflower flour was about 35% lower than that of the isolate. The results indicate that constituents other than proteins may aid in the formation of whipped foam. This is supported by Lawhon et al. (1972) who reported that many water soluble extracts of oilseeds including glandless cotton flour and sunflower flour had a high whipping potential.

Foam stability is important since success of a whipping agent depends on its ability to maintain the whip as long as possible. As seen in Table 3, foams from sunflower flour and protein isolate were much more stable than those obtained from the protein concentrates. Among the sunflower protein concentrates, the DE-90 sample showed less stability than the DE-80 and DE-60 products. Presumably, severe heat treatment which results in heat denaturation of proteins causes unstable foams. The DE-60 protein isolate produced higher whip and more stable foams than its parent protein concentrate DE-60. Evidently, exposure of protein concentrates to extreme alkaline conditions (pH 11–12) during the protein isolation process improved the whippability and foam stability. Soy flour and protein concentrates produced less stable foams than their sunflower counterparts. However, foams from both soy isolates were very stable.

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NUTRITIVE VALUE OF BREAKFAST CEREAL-MILK COMBINATIONS

INTRODUCTION

THE QUESTION of whether breakfast cereals have any nutritive value to warrant their widespread current use or whether they serve merely as a vehicle for nutrients such as vitamins and minerals which are added during processing has exercised nutritionists for years and is still a matter of controversy today. As far as protein quality is concerned, it is well known that some cereal grains either will not support growth, or will support only slow growth when fed as the sole source of protein. It has further been shown by several investigators that certain processed cereals, when fed as the sole source of protein, do not support even the marginal growth that their relatively unmodified counterparts do, as shown, for example, by Sure (1951).

It is also well known, however, that cereals can make a useful contribution to protein nutrition when they are supplemented by their limiting amino acids via milk, meat, legumes, or as the limiting amino acids themselves. As far as breakfast cereals are concerned, Blamberg (1970) investigated five cereals using a chick assay method; half the protein was supplied by the cereal and half by peanut meal. Hackler (1972) studied protein efficiency ratios (PER) by rat assay when 35% of the protein was supplied by the breakfast cereal and 65% by casein. Both investigators reported that some of the cereals may not have contributed much protein for growth. Considering the fact that breakfast cereals are commonly eaten with milk, it would seem appropriate to compare their nutritive value as they are actually eaten, that is, with milk. The proposition would then be whether these cereal proteins have been so badly affected by processing that even with milk they make no useful contribution to the cereal-milk mixture.

Thiessen and Reussner (1958) studied the effect of lysine supplementation on the nutritive value of a wheat breakfast cereal mixed with milk and sugar as normally eaten, that is, 1 oz cereal, 8g sugar (1 teaspoon) and whole dry milk solids equivalent to 4 oz whole milk. This approach seems to be the most logical one to use. We have therefore studied the protein quality of cereal-milk mixtures as compared to that of milk alone, using the slope-ratio assay (Hegsted and Chang, 1965; Hegsted et al., 1968), or, more correctly, by using Allison's nitrogen growth index assay (Allison, 1959) because the slopes of the lines measuring protein quality were calculated without use of the points for zero protein intake (Allison et al., 1959).

EXPERIMENTAL

ELEVEN CEREAL products were studied. An effort was made to select products representing a variety of processing conditions. Cereals used and their protein content (N × 6.25) were as follows: "quick cooking" corn grits (8.87%); corn flakes (7.94%); puffed corn, sugar coated (4.37%); "instant" oatmeal (15.31%); "quick cooking" cream of rice (5.69%); a toasted rice cereal (7.31%); puffed rice (5.94%); "instant" cream of wheat (11.37%); shredded wheat (10.44%); puffed wheat (15.25%); and a rice

Table 1—Weight gains and protein efficiency ratios (PER) of rats fed nonfat dry milk (NFDM) or cereal-NFDM mixtures combined in the proportion of 1 oz cereal with 4 oz fluid milk

Protein source	Dietary protein level (%)	Weight gain[a] (g)	PER
NFDM	5.19	18.6 ± 1.6[b]	1.96 ± 0.13[b]
	7.19	49.7 ± 4.7	2.85 ± 0.17
	9.06	79.3 ± 3.0	2.99 ± 0.10
Corn grits + NFDM	5.31	37.4 ± 4.3	2.90 ± 0.19
	7.19	62.5 ± 1.3	3.18 ± 0.05
	9.31	92.7 ± 7.3	3.07 ± 0.09
Corn flakes + NFDM	4.94	27.5 ± 1.9	2.61 ± 0.13
	6.94	57.4 ± 3.7	3.15 ± 0.09
	8.75	84.7 ± 5.6	3.24 ± 0.10
Puffed corn + NFDM	5.19	38.4 ± 4.3	3.02 ± 0.24
	7.06	56.0 ± 4.0	3.11 ± 0.10
	8.94	81.8 ± 3.8	3.18 ± 0.06
Oatmeal + NFDM	5.06	29.0 ± 2.7	2.52 ± 0.16
	6.64	55.6 ± 2.1	3.18 ± 0.09
	8.69	100.6 ± 3.3	3.50 ± 0.07
Cream of rice + NFDM	5.12	39.6 ± 4.1	3.16 ± 0.19
	7.12	69.3 ± 6.9	3.36 ± 0.17
	9.12	105.7 ± 6.9	3.47 ± 0.14
Toasted rice cereal + NFDM	5.06	21.6 ± 2.0	2.16 ± 0.10
	7.00	46.4 ± 4.3	2.85 ± 0.23
	8.69	72.0 ± 4.1	2.98 ± 0.11
Puffed rice + NFDM	5.31	17.6 ± 2.4	1.86 ± 0.15
	7.62	34.9 ± 2.1	2.35 ± 0.11
	9.37	66.0 ± 3.6	2.68 ± 0.11
Cream of wheat + NFDM	5.19	18.5 ± 1.8	1.84 ± 0.13
	7.19	61.1 ± 5.3	2.98 ± 0.13
	9.12	103.4 ± 5.1	3.34 ± 0.09
Shredded wheat + NFDM	5.19	24.3 ± 2.4	2.38 ± 0.19
	7.12	72.6 ± 4.3	3.45 ± 0.09
	9.31	100.3 ± 4.2	3.29 ± 0.07
Puffed wheat + NFDM	5.06	8.6 ± 2.2	0.99 ± 0.22
	7.12	30.1 ± 3.0	1.94 ± 0.15
	8.56	52.0 ± 3.3	2.42 ± 0.10
High protein flakes + NFDM	5.25	22.1 ± 2.9	2.08 ± 0.14
	7.06	46.0 ± 2.1	2.55 ± 0.03
	9.00	77.7 ± 4.0	2.77 ± 0.08

[a] During a 21 day period. Avg initial weights: 64–65g
[b] Standard error

based wheat gluten-lysine supplemented protein cereal (20.54%). A mixture was made by using each cereal and nonfat dry milk (34.75% protein) in the ratio of 1 oz cereal to 4 oz fluid milk. When necessary, the cereals were ground before use. Each cereal mixture was incorporated into diets to supply approximately 5, 7 or 9% protein. In addition to the cereal-milk mixtures the diets contained, in percent, Jones and Foster (1942) salt mixture 4; nonnutritive fiber 4; vitamin mixture (General Biochemicals, Chagrin Falls, Ohio) 1; corn oil 5; and cornstarch to 100. Protein sources and diets were analyzed for nitrogen by the macro-Kjeldahl method (AOAC, 1965).

The diets were fed for 21 days to young male rats (Sprague Dawley strain from Charles River Breeding Laboratories, Wilmington, Mass.) after a 1–3 day adjustment period during which they were fed a commercial chow diet. Diets of similar protein content containing nonfat dry milk but no cereals served as controls. Rats were weighed weekly, scattered food was carefully recovered and food intakes determined. Protein intakes were calculated using the protein content of the diets as determined by analysis. There were 5–8 rats per level of protein. One group of 7 rats was fed a protein-free diet.

RESULTS & DISCUSSION

DIETARY PROTEIN levels, weight gains and protein efficiency ratios (PER) are shown in Table 1. PER is included for the benefit of those who prefer this measurement of protein quality to that obtained by a slope-ratio assay. Linear regression lines were calculated from the individual data (x = protein intake, y = weight gain) for each protein source. The individual protein intakes varied with the dietary protein levels and the food intakes. A multiple linear regression analysis (including the individual data from all the protein sources) was used to determine the significance of differences between the slopes as is commonly done in the slope-ratio assay (Finney, 1964; Hegsted, et al., 1968). The weight loss of the group fed the protein-free diet (avg 19.7g) was not included in the multiple linear regression analysis because the y intercept is dependent on the particular amino acid deficiency of the diet as pointed out by Hegsted (1972). He further stated that " . . . the slope of the line relating intake and protein deposition when the intake is above maintenance is a true measure of the quality of protein for growth." Allison stated a similar view (1959): "The rate of increase in body weight, with respect to nitrogen intake, was essentially linear at the lower nitrogen intakes so that the slopes of the curves, correlating body weight with nitrogen intakes, are a good measure of the nutritive value of the dietary protein." It should perhaps be stated, however, that significant differences between the slopes were the same whether or not the weight losses of the group fed a protein-free diet was included. Slopes, slope ratios, and standard errors of the slope ratios are given in Table 2 for each protein source. The standard errors of the slope-ratios were computed according to procedures described by Finney (1964).

All of the regression lines were tested and found to be linear except the one for the shredded wheat-milk mixture. In this case the regression line presented here was calculated from data from rats fed the two lower protein levels. The y intercepts from the individual simple linear regression analyses varied from −7.4 to −22.5 whereas the y intercept from the multiple linear regression analysis was −15.96. The slope ratios show that the protein quality of three of the cereal-milk mixtures (shredded wheat-milk, cream of rice-milk, and oatmeal-milk) is significantly higher than that of milk alone, indicating the effectiveness of the amino

Table 2—Slopes of regression lines, slope ratios, standard errors of slope ratios of nonfat dry milk (NFDM) or cereal-NFDM mixtures[a]

Protein source	Number of animals	Slope[b]	Slope ratio[c]	Standard error of slope ratio
NFDM	21	3.79		
Corn grits + NFDM	17	3.90	1.027	0.0242
Corn flakes + NFDM	20	3.96	1.043	0.0243
Puffed corn + NFDM	23	3.95	1.043	0.0236
Oatmeal + NFDM	22	4.06[e]	1.070	0.0240
Cream of rice + NFDM	22	4.14[e]	1.092	0.0237
Toasted rice cereal + NFDM	20	3.72	0.980	0.0240
Puffed rice + NFDM	22	3.38[f]	0.892	0.0239
Cream of wheat + NFDM	24	3.83	1.009	0.0220
Shredded wheat + NFDM[d]	14	4.17[e]	1.100	0.0298
Puffed wheat + NFDM	24	3.12[f]	0.822	0.0233
High protein flakes + NFDM	21	3.41[f]	0.898	0.0218

[a] Y intercept obtained from the multiple linear regression analysis used in the slope-ratio assay procedure was −15.96. Individual Y intercepts obtained from the simple linear regression analyses varied from −7.4 to −22.5.
[b] Slope obtained from the multiple linear regression analysis used in the slope-ratio assay procedure
[c] Ratio of slope of this protein source to the slope of NFDM
[d] Only data from rats fed 5 and 7% protein were used to calculate regression line; see text.
[e] Significantly ($P < 0.05$) higher than for NFDM alone
[f] Significantly ($P < 0.05$) lower than for NFDM alone

Table 3—Contribution of cereal-milk mixtures to RDA (protein) of 8–10-yr-old children[a]

Protein source	Protein			% RDA (protein) for 8–10-yr-old child[d]
	Total[a]	% from milk	Relative potential value[b,c]	
Nonfat dry milk (NFDM)	4.2	100	4.2	10.5
Corn grits + NFDM	6.7	62.9	6.9 (64)	17.2
Corn flakes + NFDM	6.4	65.4	6.7 (60)	16.7
Puffed corn + NFDM	5.4	77.5	5.6 (33)	14.0
Oatmeal + NFDM	8.5	49.5	9.1 (117)	22.7
Cream of rice + NFDM	5.8	72.5	6.3 (50)	15.7
Toasted rice cereal + NFDM	6.2	67.2	6.1 (45)	15.2
Puffed rice + NFDM	5.9	71.7	5.3 (26)	13.2
Cream of wheat + NFDM	7.4	56.9	7.5 (79)	18.7
Shredded wheat + NFDM	7.1	59.0	7.8 (86)	19.5
Puffed wheat + NFDM	8.5	49.6	7.0 (67)	17.5
High protein flakes + NFDM	9.9	42.2	8.9 (112)	22.2

[a] In 4 oz fluid milk, or 4 oz fluid milk + 1 oz cereal
[b] Assuming milk protein has a potency of 100. Figures in parentheses are the percent increase due to cereal.
[c] Total protein X slope ratio (see Table 2). Percent increase over milk alone in parenthese.
[d] RDA for 8–10-yr-old child = 40g protein.

acids of the cereal in balancing the amino acids of milk.

There were no significant differences among the corn cereal-milk mixtures (Table 2). This was surprising because the protein quality of the other puffed cereal-milk mixtures was lower than that of the other mixtures in the same group. There is no good explanation for this finding unless the slightly higher proportion of milk protein to cereal protein, as shown in Table 3, is the answer.

The nutritive quality of the cream of rice-milk mixture was significantly higher than that of milk alone, indicating that rice protein and milk proteins complement each other. The quality of the other two rice cereal-milk mixtures was lower than that of cream of rice-milk, indicating that there were not sufficient amino acids in the milk protein to mask the damage due to processing of the rice.

The high nutritive quality of the shredded wheat-milk mixture, greater than that of cream of wheat-milk, is a reflection of the difference in protein quality of different parts of the wheat kernel (whole wheat vs. endosperm). It is interesting to note that Murlin et al. (1938) in a study of the egg-replacement value of cereals for human subjects found that "wheat endosperm" (cream of wheat) had a biological value of 79.7 and "torn wheat" (shredded wheat) a biological value of 92.8. The milk proteins do not appear to have the necessary amino acids to raise the nutritive value of the wheat endosperm to that of whole wheat.

The applicability to man of the results of studies of protein quality based on experiments with animals has been debated for years. Recently Hegsted (1972) suggested that most proteins would probably be more adequate for man, even for children, than for young growing rats. Using the slope ratios of the cereal-milk mixtures, we have calculated the potential protein value of the usual cereal-milk breakfast and the proportion of the recommended daily allowance of an 8–10-yr-old child (40g) the mixture would supply (Table 3). Also included in the column for potential protein value is the percent increase due to cereal. It can be seen that because of low protein content, and in the case of puffed rice, a comparatively low slope ratio, puffed corn and puffed rice made the smallest contributions to protein nutriture. However, even though the slope ratio of puffed wheat-milk is much lower than those of the other wheat cereal-milk mixtures, the protein content of puffed wheat (15.25%) is such that it is still able to increase potential protein value by 67%.

A normal serving of the other cereal-milk mixtures studied would supply protein in amounts to satisfy 15–22% of the RDA of the 8–10-yr-old child. Although the slope ratio of the high protein flakes-milk mixture is no higher than that of puffed rice, the potential protein value of a serving is similar to that of oatmeal-milk. Information of the type given in the last column of Table 3 would be useful to the consumer in evaluating food combinations as eaten.

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HYDROLYSIS OF LACTOSE IN ACID WHEY BY LACTASE BOUND TO POROUS GLASS PARTICLES IN TUBULAR REACTORS

INTRODUCTION

IMMOBILIZATION of enzymes on particles, fibers, or membranes may allow multiple reuse of the enzyme, control of the selectivity between various reactions in multienzyme reactions, modification of the optimum pH of the enzyme, and may prevent contamination of the product by the enzyme (Katchalski, 1970; Jackson and Edwards, 1973; Wingard, 1972; Zaborsky, 1973).

The low tolerance for lactose of many humans beyond early childhood, the millions of pounds of lactose available annually as an edible by-product in whey, and the processing difficulties associated with its use as a food additive have led to research aimed at the production of new low-lactose foods. Lactose may be hydrolyzed to more digestible glucose and galactose using microbial lactases (beta-galactosidase, EC 3.2.1.23) in soluble (Giacin et al., 1973; Hood, 1971; Kosikowski and Wierzbicki, 1971, 1973; Kosikowski et al., 1972; Olson and Stanley, 1973; Wendorff et al., 1971; Wierzbicki and Kosikowski, 1973) and immobilized forms (Dahlqvist et al., 1973; Eskamani et al., 1973; Giacin et al., 1973; Harper and Okos, 1973; Olson and Stanley, 1973; Stanley and Palter, 1973; Wierzbicki et al., 1973a, b, c; Woychik and Wondolowski, 1972, 1973).

The well-characterized lactases from *Escherichia coli* have also been immobilized and tested with synthetic substrates for scientific purposes (Broun et al., 1973; Bunting and Laidler, 1972; Kay and Crook, 1967; Mattiasson and Mosbach, 1971; Robinson et al., 1971; Sharp et al., 1969) and as a diagnostic tool for intestinal cancer (Stasiw et al., 1972).

This paper summarizes research on the rate of hydrolysis of lactose in acid whey pumped continuously through cylindrical reactors packed with porous glass particles to which lactases from *Aspergillus niger* have been immobilized by diazotization of the glass surface. This work differs from previous work in the combination of the following features. First, the carrier, coupling method and enzyme were selected for optimum stability and activity in acid whey. Second, film diffusion effects on the reaction rate were evaluated. Third, both whole and deproteinized whey of various concentrations were tested as substrates. The results can be used to perform a preliminary design and economic evaluation of a process using immobilized lactases to hydrolyze lactose in acid whey, acid whey concentrates, or deproteinized acid whey.

MATERIALS & METHODS

Preparation of glass-immobilized lactase (LBG)

Methods described earlier (Wierzbicki et al., 1973a, b) were used to prepare a diazonium salt derivative of porous 96% silica glass particles (86.5 nm mean pore diam, 75–125 μm particle diameter; Corning Glass Works, Corning, N.Y.) that had been silanized with 10% γ-aminotriethoxysilane in acetone by a modification of an earlier procedure (Robinson et al., 1971). Lots numbers 2 and 3 of partially purified β-galactosidase (EC3.2.1.23) from *A. niger* (Baxter Laboratories, Chicago) were mixed and coupled to the diazotized glass in phosphate buffer of pH 6.8 containing 2% lactose to produce stable and active glass-bound lactases (LBG) that have been characterized in earlier reports (Wierzbicki et al., 1973a, b). The LBG was stored in acid whey at 4°C until use.

Substrate

In most experiments, lactose in freeze-dried acid whey powder from the Food Science Dept. was used directly or in deproteinized form. The concentration of the reconstituted whey most commonly used in these experiments contained about 4% lactose, 0.8% protein and 1% ash. The whey was deproteinized by boiling for 5 min and then decanting and filtering prior to use (Wierzbicki et al., 1973a, b). Some spray-dried whey was obtained from the Dairy Research and Development Corp., Vernon, N.Y. and reconstituted with distilled water when ready for use. Reported total solids concentrations (TS) of reconstituted whey may be in error by about ±0.5% due to volumetric errors and to water uptake by whey powder before and during weighing as shown by measurements of the refractive index of the reconstituted whey. Toluene was usually added at 5 ml/liter whey to prevent microbial growth. In some cases, whey was pasteurized instead (Wierzbicki et al., 1973a, c).

Assay of enzymic activity

After contact with LBG, 5 ml samples of whole or deproteinized whey were heated to 80°C for 5 min to inactivate lactases, cooled and assayed for the extent of lactose hydrolysis by either one dimensional thin-layer chromatography (TLC) or by a glucose oxidase procedure (GS) which were both described earlier (Wierzbicki et al., 1973a, 1973b).

Continuous flow reactor

Various amounts of LBG were packed between two adjustable piston-like distributors in a jacketed, isothermal chromatographic column with an internal diam of 1.6 cm (Pharmacia Fine Chemicals, Piscataway, N.J.). Whey from a flask in a constant temperature water bath (55°C unless otherwise noted) was pumped peristaltically at various constant rates downward through the column. A second pump circulated water from the constant temperature bath through the column jacket.

During assays of column activity, a third pump supplied fresh whey from a 20-liter reservoir to the flask in the water bath and the reactor effluent was collected for analysis or discarded. Between assays, the reactor effluent was returned to the supply flask in the bath and continuously recirculated through the reactor. When operating in this mode, the spent whey in the recirculating system (about 800 ml) was replaced daily with fresh whey. Periodic backwashing of the column of LBG with 2 liters of distilled water containing 5 ml toluene per liter further served to disinfect the LBG and to remove colloidal matter, including microorganisms, which accumulated during normal operation and led to a high pressure drop across the column after extended periods of operation (several weeks). The hydrolytic activity of LBG showed no apparent effects of toluene treatment.

After continuous flow studies of diffusional effects in columns packed with 1, 5 and 10.5 cm of LBG, most experiments were done with the longest column to minimize the possibility of film diffusion effects. The actual length of the 10.5 cm column decreased from an initial value of 10.8 cm to 9 cm after 3 months of use due mainly to losses of LBG during frequent backwashing. All calculations were based on the actual column length during any given experiment.

RESULTS

Consistency of assays for lactose hydrolysis

Usually both TLC and GS methods were used to determine the extent of reaction, but only GS data are reported in Figures 2, 3 and 4. Figure 1 includes all data in Figures 2, 3 and 4 and shows that TLC indicated consistently higher lactose conversions than the GS assay. Deviations were greatest at intermediate conversions and least at high conversions. Samples at lower total solids (TS) concentrations

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showed smaller systematic differences despite dilution of all samples to similar concentrations before assay. The two methods differed by 10% or less in 20 of the 27 samples of whole whey containing 4–6% TS. Nine data for deproteinized whey showed much better agreement between the TLC and GS methods. The two methods agree very well considering

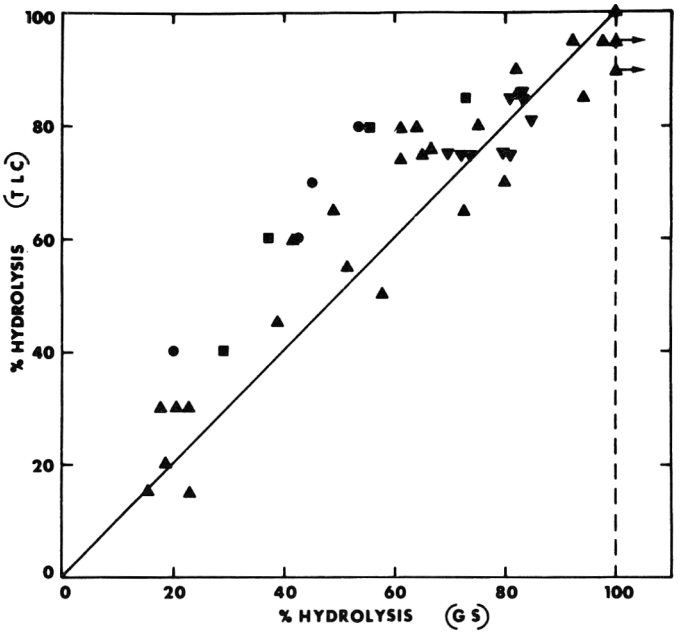

Fig. 1–Comparison of lactose hydrolysis determined on identical samples by both thin-layer chromatography (TLC) and a glucose oxidase method (GS); acid whey: (▲), 4–6% TS; (■), 15% TS; (●), 25% TS; deproteinized acid whey: (▼), 4–6% TS.

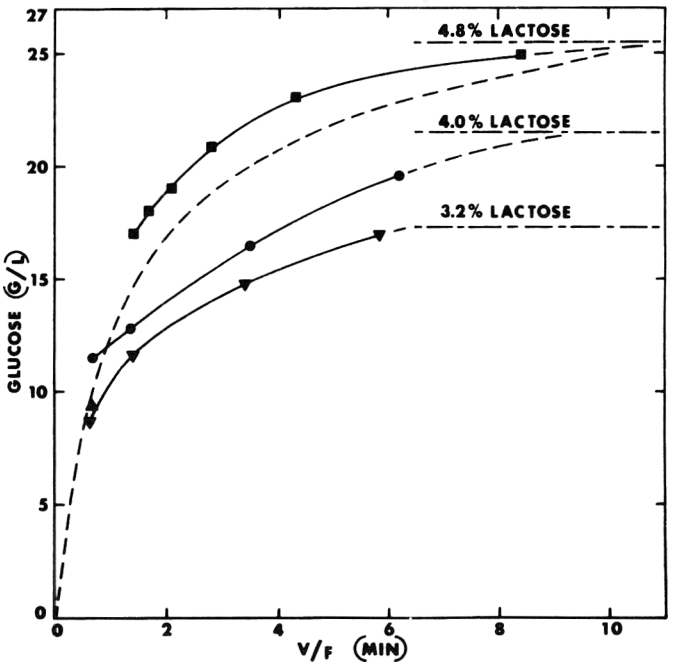

Fig. 2–Glucose production at various fluid residence times in column 4 (1.6 cm diam × 10.5 cm, LBG) from lactose in acid whey, pH = 4.5, T = 55° C. (–▼–), 4% TS; (–●–), 5% TS; (–■–), 6% TS; (· · · ·) composite curve for 5.5% TS.

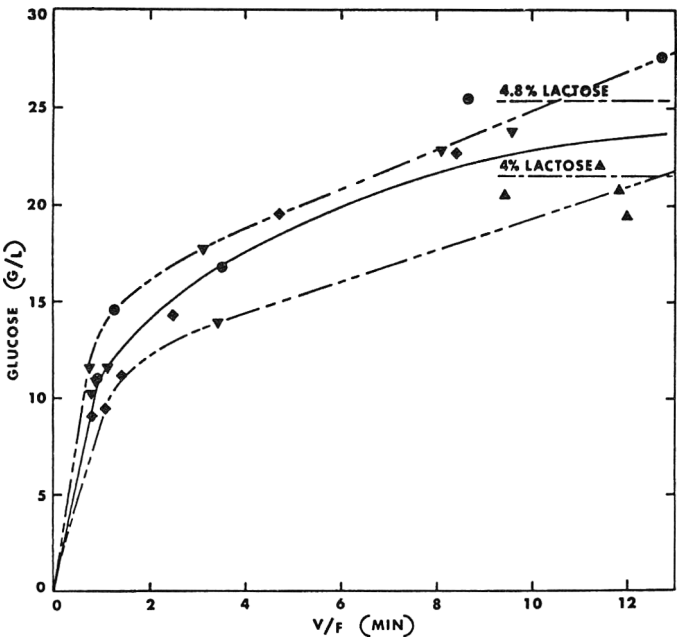

Fig. 3–Glucose production at various residence times in column 4 (1.6 cm diam × 10.5 cm, LBG) from lactose in deproteinized acid whey, pH = 4.5, T = 55° C, 5.5% TS. Experiment number: (●), 1; (▲), 2; (▼), 3, backwashed and repacked before experiment; (♦), 4, approximately 2 wk of semicontinuous operation after repacking of LBG column; (—) composite curve for 5.5% TS.

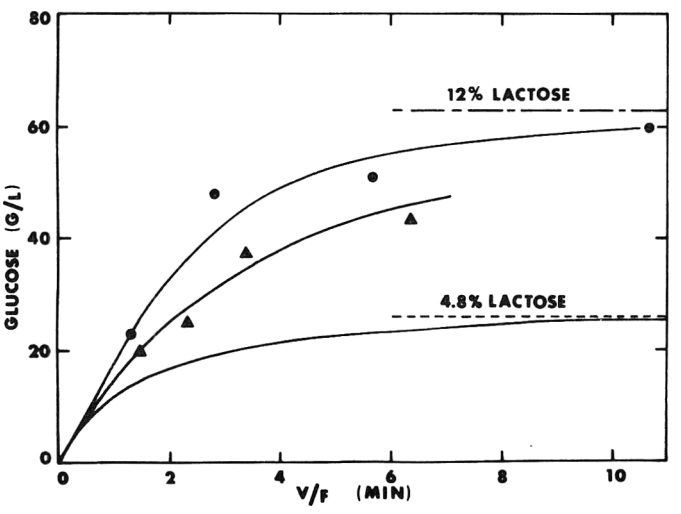

Fig. 4–Glucose production at various residence times in column 4 from lactose in concentrated acid whey; pH = 4.5, T = 55° C. (–●–), 25% TS; (–▲–), 15% TS; (—) composite at 5.5% TS from Fig. 2; (· · · ·) 100% hydrolysis of lactose at 5.5% TS; (–·–·–) 100% hydrolysis of lactose at 15% TS.

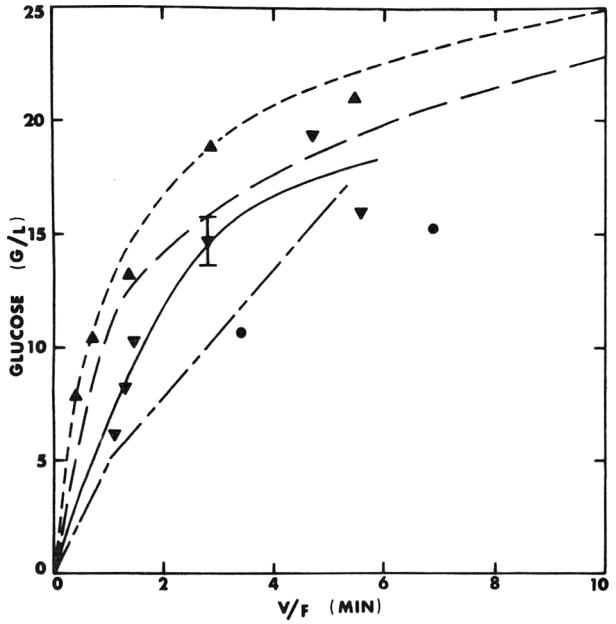

Fig. 5—Summary of glucose production in columns of LBG of 1.6 cm diam and various lengths. (- - - -) composite curve from Fig. 2 for 5.5% TS, whey, column 4 (10.5 cm long); (▲), 5.5% TS, whey, column 3 (5 cm long); (—▼—), 5.5% TS, pasteurized whey, column 2 (1 cm long); (- — — -), 4.5% TS, deproteinized whey, column 1 (1 cm long), correlation of Wierzbicki et al., 1973b; (— — — —), composite curve from Fig. 3 for 5.5% TS, deproteinized whey, column 4, (●) 5% lactose in acid whey, pH = 4.7, T = 37°C, data of Woychik and Wondolowski, 1973.

that the TLC method depended upon visual estimation of percent hydrolysis.

Reproducibility of continuous flow reactions

Variations in conversion at constant flow rate arise from experimental errors, concentration of lactose in the acid whey fed to the column, use of whole or deproteinized whey and channeling due to imperfect packing in the LBG column.

Figure 2 shows three separate experiments with reconstituted freeze-dried whey containing 4, 5 and 5.5% TS. Glucose concentrations were measured by the GS method. The abscissa is the apparent or superficial residence time, which is the ratio of the column volume (V) divided by the flow rate (F). Except for the gradual loss of LBG during backwashing, the column volume was constant and reproducible on a single repacking within the limits of accuracy of our detection method (ruler).

The data show little scatter and trend towards the final glucose concentration expected if all lactose were hydrolyzed in whey solutions containing 4, 5 and 6% TS of which 80% by weight of the original solids was anhydrous lactose. Points for each experiment could be drawn at the origin to symbolize the absence of detectable glucose in untreated controls and the equivalent reactor conditions of infinitesimal contact times between whey and enzyme. The dashed line in Figure 2 is a composite curve of the data of the two experiments at 5 and 6% TS (average = 5.5% TS) and is thus extrapolated to the origin. At a product concentration of 10g glucose/liter, the specific activity from the composite curve for 5.5% TS is 98 μmoles/min/g dry LBG, based on 0.85g dry LBG/ml of packed column.

Figure 3 compares data of four subsequent experiments with the same column using four batches of reconstituted, deproteinized whey which originally contained 5.5% TS. The first two experiments were conducted on successive days. The lower conversion in experiment 2 may be due to a lower initial TS concentration. TS concentrations are accurate to about ±0.5%. Experiments 3 and 4 are in good agreement with the data of the earlier experiments, despite backwashing and repacking of the column, followed by about 2 wk of semicontinuous operation prior to experiment 4 and variability between batches of whey. Because no simple kinetic model fits the data, dashed lines were drawn to enclose most of the data and then a solid line was drawn by eye to maximize representation of all data in Figure 3 and to extrapolate to the origin. At a glucose concentration of 10g/liter, the specific activity from the composite curve in Figure 3 is 78 μmoles/min/g dry LBG, based on a packing density of 0.85g dry LBG per ml of column.

Effects of total solids concentration

At equal fluid residence times in the LBG column, increased lactose concentrations increase rates of lactose hydrolysis (Fig. 2 and 4), showing the hydrolysis reaction is not zero order in whey solids concentration. Glucose concentrations determined by the GS method are reported in Figure 4, although Figure 1 shows that at the higher TS values, the TLC data indicated about 10–20% higher lactose conversions. As lactose concentration is increased, the fractional hydrolysis of lactose to glucose and galactose is less at any given residence time (Fig. 4), showing that the reaction is also not first or higher order in whey solids concentration. The same conclusions apply when conversions measured by TLC are plotted instead of the GS data.

The GS data appear to extrapolate to a common slope at low conversions and holding times, where diffusional effects and product inhibition would be least important and where lactose would also have least effect in the absence of substrate inhibition or activation. However, more experiments at high whey solids concentrations and low conversions would probably show that the reaction is not zero order in whey solids concentration (Fig. 2 and 3).

Subsequent experiments with deproteinized whey also showed higher rates of lactose hydrolysis at higher TS concentrations. The results will not be presented here because they are similar to those in Figure 4.

During these first four groups of experiments, which lasted about 2 months, the LBG column was continuously maintained at 55°C and the length decreased from 10.8 to 10.3 cm. Although a nominal value of 10.5 cm is used throughout the discussion, the actual column length was used to compute the superficial holding times reported in Figures 2–5.

Diffusional effects

Figure 5 compares glucose production in whey at equal fluid residence times for LBG columns of different lengths (Columns 1 and 2, 1 cm; column 3, 5 cm; column 4, 10.5 cm; numbers indicate the time sequence of column preparation and experimental usage). The column and experimental conditions were identical except for the use of differing amounts and batches of LBG and the use of pasteurized whey in column 1. If catalyst and reactant properties are held constant, such a comparison can be used to detect influences on the reaction rate of diffusional resistances in the fluid film surrounding the catalyst particles of LBG

(Levenspiel, 1962). Examination of the curves and data for whole whey shows that conversions were the same for the 5-cm (triangles) and 10.5-cm (dotted line) columns within the limits of experimental error, but that lower conversions were obtained with the 1-cm column (inverted triangles) at all holding times. Confidence limits are shown as bars for one point of the six points shown for the 1-cm column, because that point was based on seven replicate measurements and the student's "t" distribution could be applied to compute the 95% confidence limits on the sample mean (Hoel, 1954). Because conversions were lower at all holding times, it is likely that the intrinsic lactase activity per unit volume was less in the 1-cm column, which was packed with an earlier preparation of LBG that was a 50/50 mix of toluene- and acetone-silanized LBG (Wierzbicki et al., 1973b). Also, TLC instead of GS assays were used to measure percent hydrolysis in column 2, so the differences between the results for columns 2 and 4 are even more significant (Fig. 1).

If film diffusion was entirely responsible for the differences in the reaction rates, the curves for columns 2 and 4 should approach the same limiting slope at sufficiently low conversions and holding times, diverging at higher residence times until very high conversions force convergence again. Because the two assay methods differ most at intermediate concentrations (Fig. 1), film diffusion is thus even less likely to be dominant. However, fluid velocities in columns 1 and 2 are 10 times less than in column 4 at any given apparent residence time (V/F), so diffusion may decrease the reaction rate in the shorter columns for the entire range of data shown in Figure 5. Alternatively, channeling might account for erroneously low conversions in short columns (Li et al., 1972). Intraparticle diffusion effects were not evaluated, but could substantially decrease the apparent activity of LBG, especially in the presence of product inhibition (Jackson and Edwards, 1973). Product inhibition has been demonstrated for *A. niger* lactase in soluble and immobilized form by Woychik and Wondolowski (1972).

Temperature and pH optima of lactase-BG

After more than 100 days of operation of column 4 in a stability experiment (Wierzbicki et al., 1973c), the optimum pH and temperature of the LBG column was determined in continuous flow assays for comparison with batch shake flask results, in which diffusional limitations had been shown to exist (Wierzbicki et al., 1973b). Table 1 summarizes the results of experiments conducted with deproteinized whey containing 5% TS in column 4, which by then was 9 cm long.

Table 1–Glucose production from deproteinized acid whey containing 5% TS by column 4 (after more than 100 days of operation) at V/F = 7.2 min and various temperatures

Temp (°C)	Glucose conc (GS OD at 405 nm)
29	0.12
41	0.25
55	0.32
59	0.35

The glucose concentration in the product stream was only 6.8g/liter or about 17% conversion at the highest temperature of 59°C, due to severe loss in activity during one period of microbial contamination during the stability experiment which followed the experiments already described. Kinetics of lactose hydrolysis were proportional to fluid residence times at lower conversions at all temperatures tested. Although the observed reaction rates do not necessarily obey the Arrhenius equation because no kinetic model could be found that would fit all the data presented here, an activation energy of 6.2 kcal/g mole was calculated from points at 30 and 55°C on a smoothed curve drawn through the data. Operation at 59°C was brief to avoid possible further inactivation of the LBG.

Experiments were next conducted at 55°C and V/F = 7 to 8 min with deproteinized whey containing 5.5% TS and adjusted to pH values between 3.5 (the optimum observed in batch shake flask experiments conducted between pH values of 1.0 and 9.9; Wierzbicki et al., 1973b) and 6.5, a value closer to that most useful in hydrolysis of lactose in sweet whey, skim milk, or whole milk. The highest conversions were at pH values of 3.5, with a 25% rate reduction at pH 4.5, a fourfold reduction at pH 5.5 and a tenfold reduction at pH 6.5, in good agreement with the results of batch assays (Wierzbicki et al., 1973b). Results at lower flow rates led to similar conclusions.

DISCUSSION

FIGURE 5 compares composite curves obtained with column 4 for both whole (Fig. 2, dotted line) and deproteinized (Fig. 3, dashed line) whey with results obtained earlier with the same column packed with smaller amounts of LBG, including a correlation published elsewhere (Wierzbicki et al., 1973b) and data of Woychik and Wondolowski (1973). Their data was obtained with the glutaraldehyde derivative of acetone-silanized porous glass and the same commercial lactase.

Effects of deproteinization

Comparison of the composite curves for whole and deproteinized whey in Figure 5 appears to show a specific activity about 25% lower for the deproteinized whey, which is probably significant (Fig. 1), but cannot be verified statistically by any simple method. A definite conclusion concerning the effect of deproteinization cannot be drawn because the experiments with deproteinized whey were conducted several weeks after the experiments with whole whey and the column may have simply lost some activity during that period. Deproteinization should increase the diffusivities of the substrate and product and lessen the likelihood of fouling of the LBG by precipitation of protein, but may produce inhibitors of lactase (such as delta lactones if heat is used to deproteinize) or remove promoters or stabilizers of lactase.

Comparison with previous work

Figure 5 shows a correlation obtained in the first series of experiments with LBG using toluene-silanized glass and the TLC assay (Wierzbicki et al., 1973b). These results (column 1, pasteurized whey, dashed line) are probably lower because of a lower specific activity of the enzyme preparation.

The data of Woychik and Wondolowski (1973) are also shown in Figure 5, showing about half the specific activity of our preparations. They used the same enzyme and porous glass of the same particle size. Differences that could explain the differences in specific activity include the use of 37°C instead of 55°C and glutaraldehyde coupling to the glass, which reacts primarily with epsilon amino groups (lysine residues) of the protein and secondarily with tyrosine, histidine and perhaps sulfhydryl residues (Tomimatsu et al., 1971). The diazotized glass used here reacts primarily with the tyrosine and other aromatic residues and secondarily with lysine residues (Jackson and Edwards, 1973). The optimum temperature and pH are very similar for both lactase derivatives. Activation energies were also similar; 6.2 kcal/g mole in this work and 4.7 kcal/g mole in theirs. Low activation energies usually imply processes controlled by diffusion or physical adsorption of the reactant and/or products, rather than the reaction itself (Satterfield, 1970). In this case, it is unlikely that the reaction is zero order (Fig. 2–5; Woychik and Wondolowski, 1972), so the low activation energies are probably due to lower reaction rates at higher conversions achieved at higher temperatures.

The mixed order kinetics may be due in part to the product inhibition by D-galactose reported by Woychik and Wondolowski (1972) in 0.1M sodium acetate buffer at lactose concentrations of the same size as used here (up to 100

mM). Calculations by conventional methods (Satterfield, 1970) show that at V/F = 1 min, an average concentration difference of 10 mM lactose between the bulk solution and the LBG particles would be required to sustain a reaction rate of 80 μmoles/(g glass, min), which is the maximum rate observed with column 1 and less than the maximum rates observed with columns 2, 3 and 4 (Fig. 5). Use of smaller particles or less active preparations of LBG would increase the effectiveness of the immobilized enzyme at the expense of higher pressure drops and possibly shorter catalyst lifetimes in the first case and larger reactors in the second.

Olson and Stanley (1973) have immobilized *A. niger* lactase to a phenol-formaldehyde resin by adsorption, followed by cross-linking with glutaraldehyde. They obtained activities of 200 μmoles glucose produced/(min, g drained enzyme resin) at pH 4.0 and 45°C using substantially larger particles (10–40 mesh). They found a pH optimum of 4.0, an increasing lactase activity up to 60°C, but 15% loss in activity of the immobilized lactase at 55°C in 16 hr contact with 0.4M lactose in pH 4.0 sodium acetate buffer. Other results were qualitatively similar to ours, and the results of tests with their immobilized lactase with acid whey will be very interesting.

Other considerations involved in practical uses

Toluene obviously could not be used as a disinfectant in food processing and was not completely effective during the long term stability test with column 4 (Wierzbicki et al., 1973c). The column was contaminated once by a mold and once by a mesophilic yeast during failure of a temperature control system while using pasteurized, toluene-free whey. The second contamination resulted in severe activity loss, but the original LBG still retained significant lactose hydrolyzing activity after 8 months of frequent use. Other solutions to the problems of sanitation are described elsewhere (Wierzbicki et al., 1973c).

The data presented here are consistent with previous and subsequent work and provide a basis for a preliminary design and economic analysis of processes using immobilized lactases to produce low lactose acid whey products. The effects of protein recovery by ultrafiltration are unknown, but prehydrolysis with immobilized lactases would decrease the difficulty of the ultrafiltration process by decreasing the molecular weight of the permeating solutes, although desalting for recovery of the more digestible sugars would be more difficult. Processes using the soluble enzyme are clearly uneconomical at present because of the enzyme cost, but the good stability of LBG suggests that the immobilized enzyme process may succeed economically as well as technically, apart from the advantages of easier quality control and no enzymic contamination of the product. If carrier costs dominate, silica and organic carriers may offer less expensive alternatives.

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EFFECT OF ENZYMATIC HYDROLYSIS ON SOME FUNCTIONAL PROPERTIES OF WHEY PROTEIN

INTRODUCTION

AS A LIQUID by-product of the manufacture of cheese and casein, whey has until recent times been considered to be a process effluent. Heat denatured whey proteins have been commercially available for several years; however, it is only recently that commercial techniques for preparing whey protein concentrates in a less denatured form have evolved (Morr et al., 1973). These developments have been stimulated by the need to reduce environmental pollution (Whey Utilization Conference, 1970) and to better utilize the nutritionally excellent whey proteins (Wingerd, 1971).

Utilization of whey protein isolates has become the subject of intensive investigation. Demott (1972) suggested that whey proteins might serve as a supplement, or partial substitute, for other proteins in food products. Soft drink fortification with whey proteins has recently been demonstrated (Holsinger et al., 1973). Wingerd (1971) discussed the ability of whey proteins to interact and complex with other proteins as well as the potential for whey proteins in the production of textured proteins. The foaming ability of whey proteins has been recognized for many years (Wiechers, 1952). Morr et al. (1973), in preliminary examination of different whey protein concentrates, investigated their foaming, emulsifying and buffering capabilities. Pronounced hydrolysis of the milk proteins to a polypeptide content of 5–40% is described in several British patents as a method for making egg substitutes from milk proteins (Kumetat and Beeby, 1954).

The present study was undertaken to further elucidate the functional properties of whey protein with respect to foaming and emulsifying capacities and to observe the effect of enzymatic hydrolysis on these properties.

MATERIALS & METHODS

Source of proteins

Commercial concentrate of whey protein prepared by gel filtration was obtained from Stauffer Chemical Co., Rochester, Minn. (ENR-PRO 50). Casein and dialysis whey protein concentrate were prepared by precipitation of casein from skim milk with HCl at a pH of 4.6. The precipitated casein was washed three times with water, resolubilized in water by adjusting the pH to 7.5 with NaOH, reprecipitated, washed and freeze dried. Dialysate whey protein concentrate was prepared by dialyzing the whey (pH 4.6) at 2°C for 72 hr against an excess of distilled water. The dialysate was then freeze dried. Grade A, low-heat, nonfat dry milk (NFDM) was obtained from Land-O-Lakes, Minneapolis.

The enzymes used were obtained from the following sources: Pronase, Cal BioChem; pepsin (1:10,000), Cudahy; and Prolase (EB-21), Wallerstein Co.

Gel filtration

Gel filtration studies were performed utilizing a Pharmacia K 15/90 column packed with G-50 Sephadex. A 0.25 ml sample of a 2.5% whey protein sol of enzymatically hydrolyzed whey protein was chromatographed with 0.1M ammonium acetate buffer sol at pH 7.2 at a flow rate of 28 ml/hr and the absorbancy recorded at 280 nm. The samples were adjusted to pH 7.2 before application.

Digestion procedure

Suspensions of whey protein (2.5% w/w) prepared by gel filtration were adjusted with HCl or NaOH to pH 2.0 for pepsin, and 7.5 for Pronase and Prolase EB-21 and were held in a water bath at 50°C for 15 min before the addition of enzyme at a ratio of 1 part enzyme to 75 parts protein. The sol was incubated at 50°C and samples taken at selected time intervals. The digestion vessels were covered with watch glasses. During the course of digestion, pH changed very slightly, i.e., $\approx \pm 0.3$ unit. The sols were not buffered because of the influence buffer salts might have on foaming or emulsifying properties. The extent of proteolysis was monitored by formol titration. A 5.0 ml aliquot of the hydrolyzed protein sol was diluted to 15.0 ml, titrated to pH 8.5 with 0.02N NaOH, 2 ml of neutralized formaldehyde added and again titrated to pH 8.5.

The mg α-amino nitrogen in the sample was calculated from the titration data.

Whipping properties

The whippability characteristics of the protein sols were obtained by diluting an appropriate quantity of protein concentrate in 50 ml H_2O to a final concentration of 4% protein. The pH of the sol was adjusted, heated rapidly and immediately whipped at speed 8 in a Kitchen Aid, Model 3-C mixer equipped with a wire whip. Specific volume was determined by carefully transferring the foam to a tared 160 ml crystallization dish and weighing. After weighing, the stability of the foam was determined by covering the weighed dish with a 1/2 in. mesh screen. The foam was then inverted over a funnel and liquid draining from the foam was collected. The time required for collection of liquid equal to 1/2 of the weight of the original foam was recorded. All measurements were made in triplicate.

Meringues were also prepared by adjusting 100 ml of 10% protein sol to pH 8.5, heating to 80°C and whipping. 40g of sugar were added after the foam had formed and whipping was then continued until it had reached the consistency of a fresh egg-white control. The whey protein concentrate foam was then dipped out, placed on heavy paper, put into a 138°C oven and baked until lightly browned.

The effect of enzymatic hydrolysis was determined by adjusting a 4% sol to the optimum pH for the particular enzyme and held in a water bath at 50°C for 15 min before the addition of enzyme at a ratio of 1:75. After incubation for desired times 50 ml aliquots were heated rapidly to 85°C and immediately whipped.

Emulsion capacity

The emulsifying capacity was determined by a procedure similar to that of Webb et al. (1970). An aliquot of 2.5% protein sol was taken from a standardized whey protein sol which was equivalent to 25 mg of protein. This was added to 100 ml of 1.0M NaCl solution in a 400 ml beaker at 25°C and the mixture tared. Refined corn oil was added from a 500 ml graduated cylinder which had been fitted with a stopcock at the base to facilitate delivery of the oil. The rate of addition was maintained constant at 1 ml/sec during continuous stirring. Electrical resistance, monitored by a voltmeter was used to determine the break point of the emulsion. The emulsifying capacity was calculated as the g of oil required to reach an infinite electrical resistance minus a blank, which consisted of the g of oil required to reach an infinite electrical resistance of 100 ml salt sol (51.2g) divided by the amount of protein (mg).

RESULTS & DISCUSSION

FIGURE 1 reports the relative activities of the proteolytic enzymes on the commercial whey protein concentrate. The data reveal the exceptionally broad specificity of Pronase, a fungal protease of *Streptomyces grisus*. Prolase EB-21, a food grade bacterial protease, exhibited the least activity, whereas pepsin demonstrated a slightly higher activity. It is of interest to compare the rates of hydrolysis in Figure 1 to that of the gel filtration patterns, Figure 2. In the latter fig-

ure, Pronase again demonstrated its broad specificity by the formation of many polypeptides and amino acids. The chromatograph for Prolase EB-21 differed very little from that of the original whey protein. Somewhat surprising was the gel filtration pattern obtained for the whey protein treated with pepsin. This sample did not show an appreciable increase in

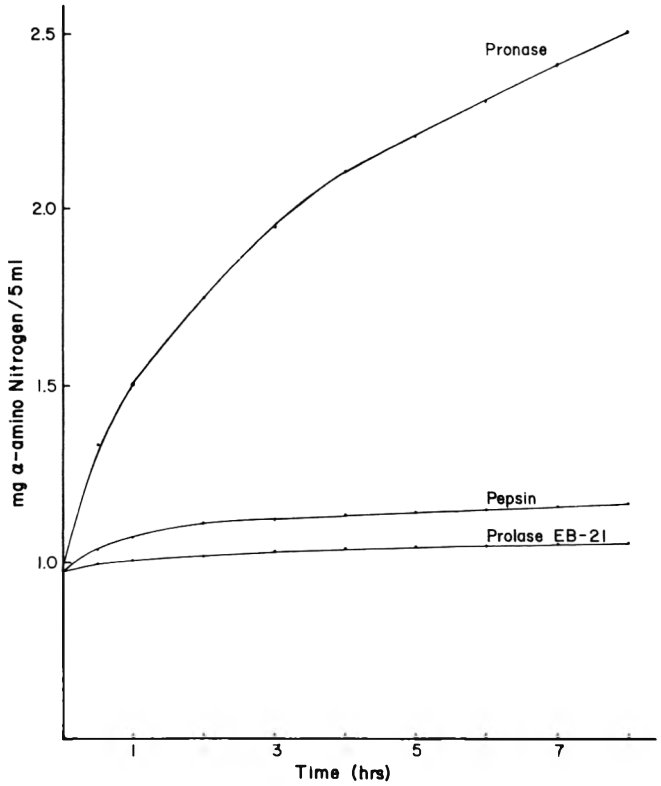

Fig. 1—Hydrolysis of a 2.5% whey protein sol by pronase, pepsin and prolase EB-21.

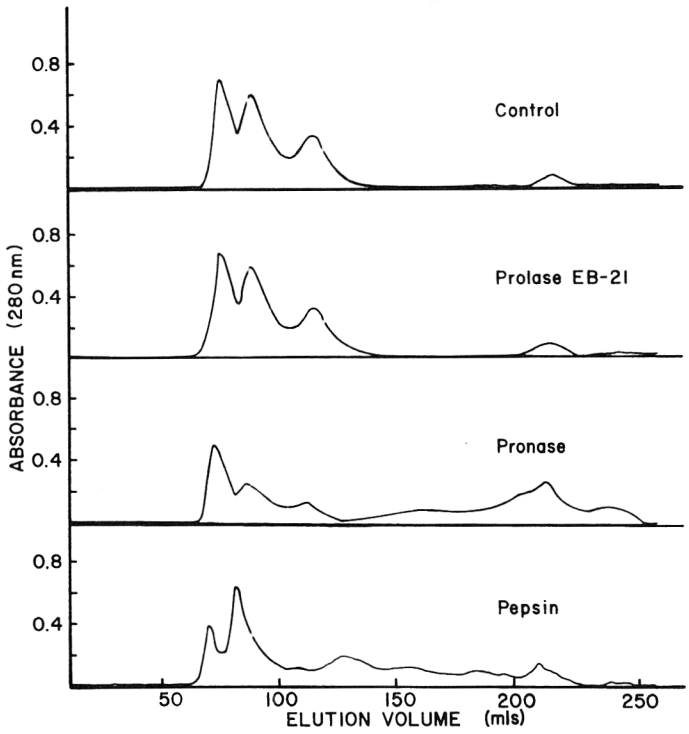

Fig. 2—Comparison of gel-filtration patterns obtained from a 2.5% whey protein sol after reacting with the enzyme for 1 hr.

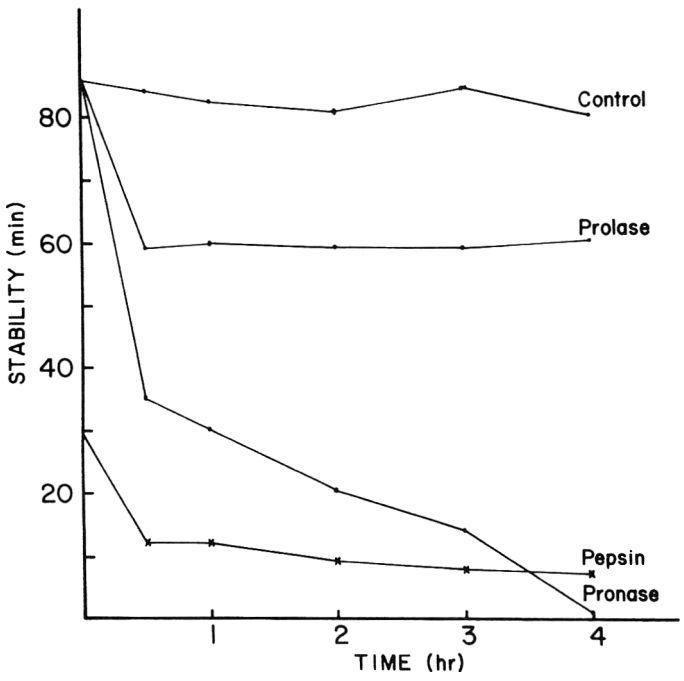

Fig. 3—Effect of enzymatic hydrolysis of whey protein concentrate (Sephadex) on emulsifying capacity.

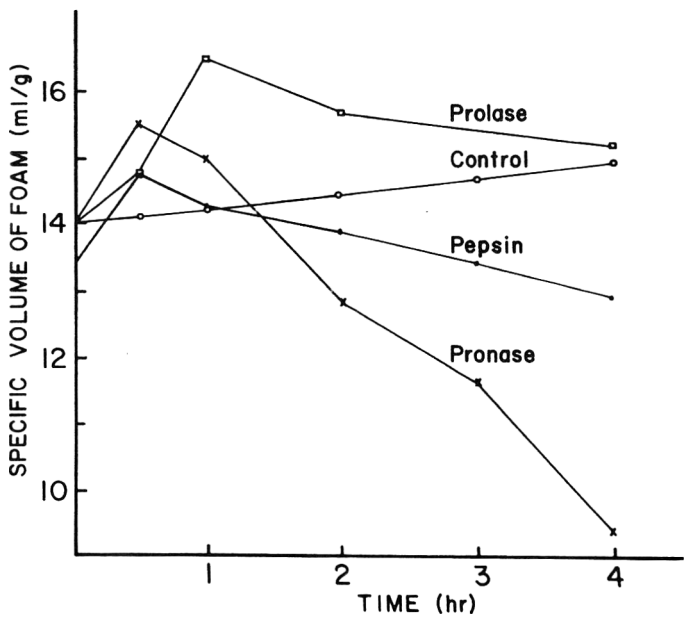

Fig. 4—Effect of enzymatic hydrolysis on specific volume of foam obtained by whipping a heated whey protein sol (4% w/w, 85°C, 6 min whipping).

α-amino nitrogen; however, the chromatogram obtained by gel filtration indicated that extensive proteolysis had occurred. One possible explanation for this was pointed out by Verma and McCalla (1966) who observed that an enzymatic proteolysis under acidic conditions was likely to induce formation of large quantities of polypeptides as intermediates in the reaction. Another peculiarity of this chromatogram was the destruction of the first peak which was composed mainly of β-lactoglobulin. It appears that pepsin preferentially attacked this protein at the pH used to adjust the solution for optimum hydrolysis.

Table 1—Emulsifying capacity of casein, whey protein concentrate from gel filtration, whey protein concentrated by dialysis and nonfat dry milk (NFDM)

Protein source	g oil/mg protein
Casein	1.88
Dialyzed whey protein concentrate	2.99
Sephadex-processed whey protein conc	2.97
NFDM	2.17

Table 1 contains the emulsion capacity data for the different protein sources tested. The dialyzed whey protein concentrate and the gel filtration-processed whey protein concentrate had similar emulsion capacities. These results were in agreement with those of Morr et al. (1973), who reported that different preparations of whey protein concentrate had similar emulsion capacities with the exception of a whey protein complex obtained with carboxymethylcellulose (CMC). Casein showed a lower emulsion capacity than whey protein. The emulsion capacity value obtained for NFDM supported the data obtained for casein and whey protein since the protein of NFDM is approximately 78% casein.

Effect of proteolysis on emulsification is presented in Figure 3. It is apparent from these data that the emulsion capacity decreased as proteolysis continued. This would suggest that there is an optimum mean molecular size of the proteins involved in emulsification which is lower than that of casein.

Table 2—Whippability of whey protein concentrated by gel filtration[a]

	Variable	Specific vol ml/g	Foam stability (min)
I.	Temp of heating (4% sol) °C; no hold; pH 8.5		
	25	8.3	0
	35	10.4	35
	45	11.4	66
	55	11.6	72
	65	11.9	74
	75	12.2	75
	85	14.0	77
	95	14.3	80
	75° (held for 5 min)	14.5	79
II.	Protein content (w/v) 85°C, pH 8.5		
	2%	12.4	65
	3%	13.7	83
	4%	14.0	94
	5%	14.7	102
	6%	15.5	110
III.	Whipping time (min) (4% sol heated to 85°C); pH 8.5		
	2	13.3	60
	4	13.7	73
	6	14.3	74
	8	15.6	98
	10	17.4	126
IV.	pH (4% sol heated to 85°C)		
	2	13.8	29
	3	13.9	28
	4	12.5	80
	5	11.4	77
	6	11.3	62
	7	13.2	85
	8	13.8	101
	9	14.0	107
	8.5, ($Ca(OH)_2$)	12.9	83
V.	Added CMC w/v (4% sol heated to 85°C); pH 6.5		
	0.0	11.4	71
	0.1%	11.0	166
	0.2%	12.4	205
	0.3%	12.9	295
	0.4%	13.2	360
	0.5%	13.3	500
VI.	Miscellaneous		
	Egg whites	9.4–9.5	130 min
	Casein (4% sol heated to 85°C; pH 8.5)	11.6	25 min

[a] Using a 6 min whipping time except Group III, where time was varied

Table 2 summarizes the results obtained for various whipping trials performed on Sephadex-processed whey protein concentrate. Heat treatment of the reconstituted protein concentrate proved to be a requisite for foam stability. The specific volume and foam stability increased directly with temperature of heating. Hansen and Black (1972) reported that heat treatment was detrimental to foam development in a CMC/whey protein complex, and, as they noted, this may have been due to a change in the conformation of hydrocolloid and protein in solution during heat treatments, instead of a denaturation phenomenon. This seems probable since heating whey protein prepared by gel filtration to 75°C for 5 min did not affect development of foam or foam stability.

Using a 6 min whipping time, a concentration of 4% (w/w) protein proved to be the optimal. Concentrations above 4% tended to clog the mixer blades; however, this could be avoided if the whipping time were decreased.

The effect of pH on whippability of whey protein sols was investigated. The data indicate that the greater the net charge the greater the tendency to foam. This was demonstrated by the high specific volume obtained at pH 2 and pH 9 with the lowest volumes obtained in the iso-electric range of whey proteins. Even though the high acid solutions gave good volumes, their stability was poor. Stability increased with increasing alkalinity above pH 6. The addition of 1N $Ca(OH)_2$

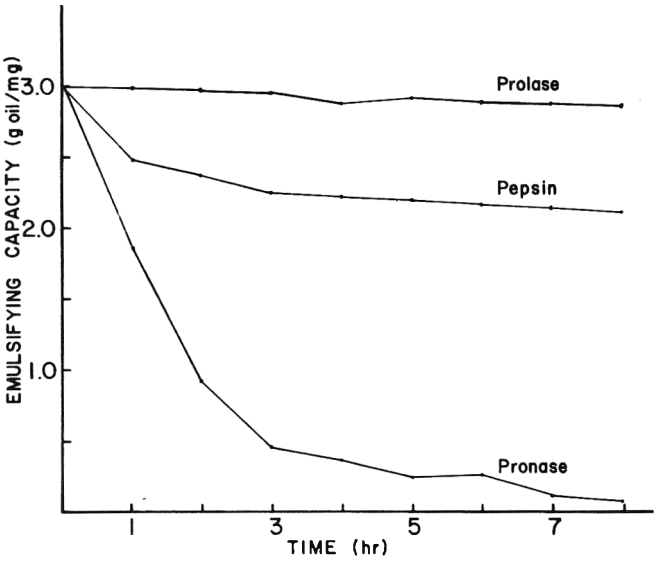

Fig. 5—Effect of enzymatic hydrolysis on stability of foam obtained by whipping a heated whey protein sol (4% w/w, 85°C 6 min whipping).

to pH 8.5 did not increase the specific volume or the stability of the foam. This may be attributed to a relatively high calcium content of the whey protein concentrated by gel filtration. Morr et al. (1973) found this type of whey protein concentrate to have the highest calcium content (0.75%) of any of the concentrates analyzed.

Specific volume and foam stability increased with increasing whipping time through 10 min. However, after 6 min the foam tended to clog the mixer blades, and prolonged whipping gave excessively dry foams.

The effect of added CMC (Hercules, Type 7 HF), was studied. Lighter foams with greater stability resulted from increasing amounts of CMC up to 0.5%, the highest concentration employed.

Four percent casein sols produced foams of poor stability. Fresh egg white foams gave lower specific volumes than whey protein or casein foams but remain more stable foams.

The effect of enzymatic hydrolysis of whey proteins on specific volume and foam stability is shown in Figures 4 and 5. A direct comparison between pepsin and the unhydrolyzed control cannot be made since the sample treated with pepsin was run at pH 2 and the blank at pH 8.5. In all cases, however, the specific volume increased initially and then decreased with time. A limited amount of hydrolysis appears to be desirable to increase the foaming but foam stability is greatly decreased as a result of such hydrolysis. This is probably due to increasing the polypeptide content initially which allows more air to be incorporated. However, the polypeptides do not have the strength required to give a stable foam. The decrease in foam stability manifests itself primarily in the initial 30 min of reaction. Further hydrolysis likely results in peptides which lack the ability to stabilize the air cells of the foam. A limited hydrolysis may be advantageous for utilizing whey proteins in foams since specific volume was increased by 25% by such treatment. The decrease in stability which results from limited hydrolysis can be compensated for by adding stabilizers such as CMC.

Preliminary experiments using 4% solutions of gel filtration-processed whey protein concentrate for preparing meringues were not impressive. In order to make a meringue that would withstand the heat treatment applied during normal baking, the protein content had to be elevated to 8–10%. Even at this level, meringues flattened extensively during baking. Adding 0.5% CMC to a 10% protein solution improved the resulting meringues but these were still not of the same quality as fresh egg white meringues.

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ATLANTIC QUEEN CRAB (Chionoecetes opilio), JONAH CRAB (Cancer borealis), AND RED CRAB (Geryon quinquedens). Proximate Composition of Crabmeat from Edible Tissues and Concentrations of Some Major Mineral Constituents in the Ash

INTRODUCTION

A SURVEY of the literature has revealed that very little analytical data have been reported on the composition of the meats of Atlantic Queen crab (*Chionoecetes opilio*), Jonah crab (*Cancer borealis*) and Red crab (*Geryon quinquedens*). Considerable data are available on the composition of Blue crab (*Callinectes sapidus*) (Farragut, 1965; Kefer and Bauersfeld, 1969; Thompson and Farragut, 1966), Dungeness crab (*Cancer magister*) (Allen, 1971; Farragut and Thompson, 1966; Nelson and Thurston, 1964) and King crab (*Paralithodes camtschatica*) (Kifer and Bauersfeld, 1969; Krzeczkowski et al., 1971). Degens et al. (1967) have reported the concentrations of amino acids and amino sugars in calcified tissues of Portunid crabs (*Callinectes sapidus, Ovalipes ocellatus* and *Carcinides maenas*).

The development of the Queen crab industry has been dealt with in a publication of the Industrial Development Branch, Fisheries Service (1969), and a Code of Practice for the processing of Queen crab has been proposed by Blackwood et al. (1969). As far as the present authors are aware, a report by Addison et al. (1970) comprises the only published results on the composition of Queen crab tissue. The authors are unaware of any data on the composition of Jonah and Red crabs. Information on the life history, distribution and abundance, and size of individual crab species is elucidated in a publication of Wilder (1966).

MATERIALS & METHODS

Collection and preparation of samples

40 live Queen crabs (avg wt 1.6 lb) were obtained from a boat operating in the Bay of Chaleur on Nov. 4, 1971. The crabs were maintained on ice until the boat returned to port (Lamèque, New Brunswick) on Nov. 5, 1971. The crab meat was processed in the plant of a local packer. The crabs were butchered and the sections were pre-cooked for 8 min (100°C) in a fresh water continuous cooker. The sections were then cooled for 10 min (10°C) in a tank of cold, fresh water (continuously flowing). The meat was separated from the sections by hand. The leg and claw meat was kept separate from the body meat. The entire composite samples of leg and claw meat and body meat were then ground in a Hobart Model 84142 Silent Cutter until homogeneous and several separate portions of each type of meat were frozen in polyethylene bags and stored in a cold room (−29°C) until they were analyzed. These samples represented "fresh" crab meat, although, in fact, the meat had been submitted to a precooking operation in order to facilitate shucking.

Two cans (each, 6-3/4 oz net weight, 5-3/4 oz drained weight) of commercially heat-processed Queen crab meat and 1 lb of frozen comercially-processed Queen crab meat were purchased from a local supermarket in Halifax. The canned meat and the frozen meat were each blended in a Waring Blendor and several separate portions of each were frozen in polyethylene bags and stored (−29°C) until they were analyzed.

10 live Red crabs (total wt, 18 lb 10 oz) and 12 live Jonah crabs were obtained from waters off the eastern coast of Nova Scotia. The crabs were pre-cooked in a steam cooker for 10 min after which they were cooled in cold running water. Samples were stored in polyethylene bags as above until analysis.

Analytical methods

All analyses were performed in triplicate. Moisture was performed by oven-drying (100°C) for 18 hr. Protein was determined by the micro-Kjeldahl method (AOAC, 1970). Ash was determined by flame ignition followed by heating in an oven (525°C) for 2 hr. Crude fat was determined by the Bligh and Dyer (1959) chloroform-methanol extraction. Carbohydrate was determined colorimetrically by a method which depends on the formation of a yellow color when sugar is reacted with phenol in sulfuric acid solution (Dubois et al., 1951, 1956; Montgomery, 1961).

Sodium, potassium, calcium and magnesium were estimated by the following method: dried ground crab meat (100g) was placed in an Erlenmeyer flask (25 ml) along with concentrated sulfuric acid (sp gr 1.84; 2.0 ml). The mixture was heated cautiously until the sample was thoroughly charred and it was then cooled to room temperature. Hydrogen peroxide (30%; 10 drops) was added and the mixture was heated for an additional 5 min. The above process of heating, cooling and addition of peroxide was repeated until a clear and colorless solution was obtained (Lindner and Harley, 1942). The reaction mixture was transferred to a volumetric flask and the volume adjusted to 100 ml with distilled water.

The metals were determined on aliquots of these solutions by means of a Unicam Model SP. 90A Atomic Absorption Spectrophotometer. Sodium and potassium were determined by flame emission spectroscopy at 589.0 mμ and 766.5 mμ, respectively. Calcium and magnesium were determined by atomic absorption spectroscopy at 422.7 mμ and 285.2 mμ, respectively. The sample digests and standard solutions which were used for the calcium and magnesium analyses were 1% in lanthanum (added as lanthanum chloride). Appropriate recovery experiments were undertaken at the same time as the actual metal analyses. The operating parameters employed were those described by the manufacturer (Unicam Instruments Limited, 1966).

Phosphate was determined colorimetrically by a method previously reported by Lauer and Baker (1969).

RESULTS & DISCUSSION

THE RESULTS of the gross composition analyses of Queen crab meat are tabulated in Table 1. The results obtained for fresh Queen crab meat were in close agreement with those obtained for Alaska King crab by Krzeczkowski et al. (1971), Dungeness crab by Farragut and Thompson (1966) and Blue crab by Farragut (1965).

A comparison of the results of Krzeczkowski et al. (1971) and the results of the authors of the present work, indicates that the protein content of the leg and claw meat reported by the former authors for King crab is greater than that reported here for Queen crab (20.7% and 15.3%, respectively). The same is indicated for body meat (16.9% and 16.0%, respectively). The authors are aware that the composition of marine species may vary due to physiological, seasonal and environmental influences (Ackman and Eaton, 1970; Krzeczkowski et al., 1971), although Nelson and Thurston (1964) have stated that in the case of Dungeness crab, differences between individual crabs were

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Table 1—Proximate composition of Queen crab (Chionoecetes opilio)

Constituent	Fresh		Canned	Frozen
	Leg & claw meat	Body meat		
Moisture (%)	80.6	82.9	82.5	80.9
Crude fat (%)	1.14	0.86	1.05	0.99
Protein (N X 6.25) (%)	15.3	16.0	14.2	15.6
Ash (%)	2.07	1.71	2.01	2.04
Carbohydrate (%)	0.075	0.054	0.280	0.270

Table 2—Proximate composition of Jonah crab (Cancer borealis) and Red crab (Geryon quinquedens)

Constituent	Jonah crab	Red Crab	
		Legs & claw meat	Body meat
Moisture (%)	78.2	80.8	80.9
Crude fat (%)	1.90	0.99	0.88
Protein (N X 6.25) (%)	16.2	15.0	15.1
Ash (%)	1.47	1.44	1.75
Carbohydrate (%)	3.11	1.59	1.27

Table 3—Mineral constituents in the ash of meats from Queen crab, Jonah crab and Red crab

Sample	Concentration of constituents, g/100g ash				
	Na^+	K^+	Ca^{++}	Mg^{++}	$PO_4^{=}$
Queen crab, fresh (Leg & claw meat)	19.4	13.7	1.31	1.21	12.4
Queen crab, fresh (Body meat)	20.6	12.5	3.90	2.20	11.2
Queen crab, canned	35.0	7.00	2.09	1.22	7.63
Queen crab, frozen	24.8	10.7	1.96	1.21	11.2
Jonah crab	18.8	19.0	6.53	2.22	2.67
Red crab (Leg & claw meat)	23.0	19.3	2.89	2.00	14.6
Red crab (Body meat)	27.4	16.5	2.84	1.84	12.0

greater than differences between crabs from different areas or from different seasons.

The values obtained for protein may be higher than normal due to shell or gill contamination of the meat. Both these tissues, particularly the former, are known to contain high amounts of N-acetylglucosamine, which if present in the meat, would result in relatively high nitrogen values.

The determination of salt as the chloride by the Volhard titration method (AOAC, 1970) yielded the following results for Queen crab meat: 1.09% (fresh leg and claw meat); 1.13% (fresh, body meat); 1.69% (canned meat); and 1.31% (frozen meat). The higher values in the processed meats are probably due to the addition of brine by the packers. Nelson and Thurston (1964) have stated that the loss of protein of commercially packed crab meat could be attributed to contact of the meat with both brine and fresh water during processing. This might account for the lower protein value observed for canned Queen crab meat.

Table 2 comprises the data obtained by the analyses of Jonah and Red crab meats. The results tabulated for Jonah crab are average figures obtained for composite samples of leg-claw and body meats.

The results indicated that the meats of Red crab and Jonah crab had relatively high concentrations of carbohydrate. Since N-acetylglucosamine does not react with the phenol-sulfuric acid reagent, these results cannot be attributed to possible shell contamination of the meat during the shucking procedure.

The results of the ash analyses for sodium, potassium, calcium, magnesium, and phosphate appear in Table 3. Sodium was present in the highest concentration of the cationic constituents analyzed, followed by potassium, calcium and magnesium, in that order. With the exception of canned and frozen processed Queen crab and Jonah crab, the Na^+:K^+ ratios were higher for body meat than for leg and claw meat.

Fox and Cameron (1961) have indicated that the exact amount of sodium (and chlorine) is of little consequence, except in the case of sodium-restricted diets, because the amount of salt added to a food is large when compared with the salt content of the food itself.

All crab ash samples analyzed in the present work had Ca:P ratios of 1.0 or less. Crampton and Lloyd (1959) have stated that the Ca:P ratio in the bone (presumably mammalian) is 1.95:1. These workers have suggested that the optimal Ca:P ratio in the diet lies between 1:1 and 2:1 for normal bone calcification and reproduction. Based on the present results, the ability of crab meat to fulfill these requirements appears doubtful, although it has been suggested (Canada Department of National Health and Welfare, 1964) that the absolute dietary intakes of these two elements is a more significant factor in nutrition than the ratio between them.

Widdowson (1960) has suggested that the following "calorie conversion factors" be used in the determination of the calorific value of a food: protein, 4.1 Cal/g; fat, 9.3 Cal/g; and carbohydrate, 3.75 Cal/g. Thus, the calorific values of the crab meats in the present work are as follows (Cal/100g): Queen crab (leg and claw meats), 73.6; Queen crab (body meat), 72.2; Queen crab (canned), 68.8; Queen crab (frozen), 74.2; Jonah crab, 95.8; Red crab (leg and claw meat), 76.7, and Red crab (body meat), 75.0. These values are appreciably lower than those cited by McCance and Widdowson (1960) for crab (species not specified) (127 Cal/100g) and lobster (119 Cal/100g) meats.

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AMINO ACID, FATTY ACID AND PROXIMATE COMPOSITION OF SNOW CRAB (Chionoecetes bairdi)

INTRODUCTION

SNOW CRABS (*Chionoecetes bairdi, Chionoecetes opilio* and *Chionoecetes Tanner*), also commonly referred to as queen crab, Tanner crab and spider crab, occur broadly from the southeast coast of Alaska westward along the coast (4,000 mi), throughout the Aleutian Islands, in the Bering Sea and the Atlantic Ocean. This species is becoming increasingly commercially important as the supply of other crabs have attained their maximum sustained yield. Although this high protein food has good commercial potential, no compositional data has been reported in the literature on (*Chionoecetes bairdi*). The 1971 domestic catch was 12 million pounds, but it is estimated that the maximum sustained yield could be at least 100 million pounds (Alaska State Dept. of Fish & Game, 1971). The Japanese and Soviet fisheries have harvested 35–70 million pounds annually from the Bering Sea. The fatty acid composition of the raw meat of snow crab (*Chionoecetes opilio*), a related crab, an inhabitant of the Atlantic Ocean along the east coast of the United States and of the Bering Sea, has been reported by Addison et al. (1972); Kizevetter and Gordievskaya (1967) reported the proximate and mineral content of cooked and raw meat.

Public concern is increasing over the nutritional quality including the kinds and amounts of fat present in food. The investigation reported here was undertaken to determine the proximate analysis, amino acid and fatty acid composition, sodium content and potassium content in a commercially canned product, a composite of whole meat and the four types of meat into which snow crab could be processed; namely, body-shoulder, first leg section (Merus), third-second leg sections (*Propodus carpus*) and claws. The various leg muscles are enclosed by a membrane (skin) that may affect the storage and processing properties, thus the merus skin and merus without skin also were analyzed separately for lipid and fatty acid content to determine any significant difference.

MATERIALS & METHOD

60,000 LIVE commercially caught snow crabs (avg wt 2.69 lb) were harvested near Kodiak, Alaska, Nov., 1971, and held in commercial live tanks on boats and at the processor for 5 days before processing. Six crabs were randomly selected, butchered (gills, carapace, entrails and tails discarded), cooked for 26 min in boiling fresh water, then cooled 10 min in chlorinated (0.5 ppm) sea water, according to commercial practice. The meats were extracted by hand to obtain 100% of the meat and weighed separately by type. For comparison the commercial yield was determined by examination of discarded waste at several processors. Weight of meat and percentages of each and estimated commercial yield were as shown in Table 1.

Each type of meat (except tails) was ground separately several times in a meat grinder until homogeneous. A blend in the same proportion (% type) as shown above (except tails) was used to prepare a composite sample to represent whole snow crab flesh.

Ground samples were stored in sealed containers at −51°C until analyzed. Twelve 6-oz cans of commercially canned snow crab from the same catch were provided by a commercial processor that was processing merus, body-shoulder and claws. The commercial product (28% merus and 72% body-shoulder with claws) was removed from the cans, drained, ground until homogeneous and frozen at −51°C until analyzed. Commercially canned snow crab, canned in 1969 and 1970 were also used for the proximate results reported. Commercially cooked merus sections from the above catch were skinned for separate analysis of the skin and skinless meat.

Proximate and fatty acid analysis

Each blended batch was analyzed in triplicate for protein, ash, moisture, lipid and total fatty acids as described by Krzeczkowski et al. (1971). Analyses were made for the presence of glycogen according to AOAC (1945).

Amino acid, sodium and potassium analysis

The drained contents of six randomly chosen commercially packed cans were blended together, subsampled and analyzed by Wisconsin Alumni Research Foundation. Sodium and potassium were determined on a Perkin Elmer Flame Photometer. The amino acids were analyzed according to Moore et al. (1958) on a Perkin Elmer Amino Acid Analyzer. Cystine, methionine and tryptophan were determined microbiologically as described by Henderson and Snell (1948).

RESULTS & DISCUSSION

ANALYTICAL DATA for frozen and canned snow crab may be affected by environmental factors such as season, depth and geographic location of catch. The lipid, in a low-fat animal such as snow crab, is mainly membrane lipid which shows a low degree of seasonal variability. The lipid and its fatty acid composition were determined on several different snow crab meat samples over a 2-yr period and results showed no significant difference in fatty acid composition or % lipid. The proximate analysis of canned snow crab was on samples canned in 1969, 1970 and 1971. These samples showed little variation (±1.5% protein, ±0.3% lipid, ±0.1% ash, ±1.1% water and ±0.2% salt). It is noteworthy, also, that analyses of fatty acids in Alaska pink shrimp canned at monthly intervals for a period of 12 months show little variation (Krzeczkowski, 1970). No attempt was made in this study to evaluate the influence of these factors and the results therefore indicate the composition at the time of sampling only.

The muscle fibers of cooked snow crabs are straight and short. They are approximately half the diameter of king crab muscle fibers, and separate easily from each other during processing to give more shredded meat than observed with king crab. Kizevetter and Gordievskaya (1967) observed a similar fiber difference

Table 1—Snow crab meat type and yield

Type of meat	Pounds[a]	% Type	% Yield	Coml % Yield
Body-shoulder	1.89	34.7	11.7	0–10
Merus	2.10	38.5	13.0	10.0–10.3
Propodus-carpus	0.45	8.3	2.8	Discarded
Claws	0.97	17.8	6.0	2.0–2.4
Tails	0.04	0.7	0.2	Discarded
	5.45	100.0	33.7	12.0–24.7

[a] From six crabs

Table 2 –Proximate analysis of snow crab meat

Type of Meat	% Protein	% Lipid	% Ash	% Moisture	% Salt
Body-shoulder	18.8	1.6	0.4	80.0	0.2
Merus	19.9	1.5	1.2	78.6	0.3
Propodus-carpus	16.6	1.5	1.1	81.4	0.3
Claws	18.0	1.0	1.3	80.0	0.7
Composite (whole meat)	18.8	1.5	1.0	79.4	0.3
Canned[a]	19.5	1.0	1.6	78.2	1.3

[a] Avg of samples obtained in 1969, 1970 and 1971

Table 3 –Amino acid composition of canned[a] Alaskan snow crab

Amino Acid	g/100g Protein
Alanine	5.5
Arginine	9.8
Aspartic acid	10.3
Cystine	1.3
Glutamic acid	15.9
Glycine	5.7
Histidine[b]	2.2
Isoleucine[b]	4.8
Leucine[b]	8.2
Lysine[b]	8.5
Methionine[b]	3.2
Phenylalanine[b]	4.4
Proline	3.9
Serine	4.2
Threonine[b]	4.6
Tryptophan[b]	1.1
Tyrosine	3.9
Valine[b]	4.6

[a] Drained contents
[b] Essential amino acids

in a related species of snow crab (*Chionoecetes opilio*).

Proximate analysis

The proximate analysis shown in Table 2 indicates similarities in the concentrations of lipid and ash among the types of cooked and canned meat with the exception of the body-shoulder meat which was lower in ash and the claws which were slightly lower in lipid. All of the extracted lipids were rich in carotenoids (deep red) except the lipid from the skinless merus which was very light orange in color. The percentages of protein ranged from 16.6 in propodus carpus meat to 19.9 in merus meat. A similar range for protein was reported for king crab (Krzeczkowski et al., 1971). The percentage of moisture varied between a low of 78.2 in canned meat and a high of 81.4 in propodus carpus meat. Kizevetter et al. (1971) reported the proximate analysis of canned Soviet snow crab to be 79.5% moisture, 16.8% protein, 0.1% fat and 2.4% ash. The difference noted between the Soviet and Kodiak canned snow crab is attributed to processing methods and species difference (*Chionoecetes opilio*). The high ash in the Soviet analysis is possibly due to the presence of the tendon in the merus section which was observed in several cans of their canned crab.

The caloric value for the composite snow crab meat was calculated (Atwater and Bryant, 1960; USDA, 1963) as 428 cal/lb as compared to 430 for king crab. Neither snow nor king crab samples contained glycogen, nor did they contain carbohydrate by proximate difference. Such absence may be the result of stress conditions caused by normal commercial live-tanking for several days without food. The thin layer of red pigmented skin that surrounds the muscles contained 3.1% lipid and accounted for about 5.1% of the weight of merus meat. The merus muscle with the skin removed contained 1.0% lipid and is snow white.

Amino acid, sodium and potassium analysis

The amino acid analysis (Table 3) shows that canned snow crabmeat contains a normal distribution of amino acids including the essential amino acids. Glutamic acid, aspartic acid, arginine, lysine, leucine, glycine and alanine were the major amino acids. Allen (1971) reported a similar distribution for dungeness crab (*Cancer magister*) muscle. Thompson and Farragut (1966) reported a similar amino acid content in the body meat of Chesapeake Bay blue crabs (*Callinectes sapidus*) except that alanine and glycine were present in greater quantity. Glutamic acid and aspartic acid are also major amino acids in meal prepared from waste material of king crab (*Paralithodes camptschatica*), Kiefer and Bauersfeld, 1969). The potassium content of the drained canned meat averaged 162 mg/100g or 185 mg/4 oz. The sodium content averaged 516 mg/100g or 585 mg/4 oz. The high sodium value is caused by the addition of salt to the canned crab.

Fatty acid analysis

A wide variety of fatty acids were detected in the extracted snow crab lipids (Table 4). In most respects the relative concentrations of each type of fatty acid were the same in all types of snow crab meat. In the composite whole meat sample three fatty acids (16:0, 18:1 and 22:6) occur in approximately equivalent amounts, i.e., in the 13.5–17.8% range and account for 47.6% of the total fatty acid content. These three acids together with 20:5 (the dominant acid) account for 76.6% of the total fatty acid content. Fatty acids 18:0, 16:1 and 20:4 also occur in approximately equivalent amounts, i.e., in the 3.0–3.9% range and account for 10% of the total fatty acid content. The remaining 22 fatty acids in Table 4 not mentioned above account for only 13.4% of the total.

Addison et al. (1972) reported a similar fatty acid grouping of the raw meat of snow crab (*Chionoecetes opilio*) with the following exceptions: fatty acid 18:1 is reported at 21.7% and fatty acid 18:0 is reported at 2.4%. The difference is small and attributed to specie and environmental factors. In most respects the concentrations of fatty acids in shrimp meat are unaffected by cooking (Krzeczkowski, 1970). This is probably true with crabmeat also. When the skin was removed from the merus meat, higher amounts of fatty acid 20:5 were found. The skin was low in polyunsaturated acids, especially 22:6, and high in monounsaturated acids, particularly 18:1. King crab did not reveal as great a difference between skin and muscle (Krzeczkowski et al., 1971).

The identification of a peak considered to be 23:5 is speculative since the gas-liquid chromatogram for this peak is difficult to interpret due to low peak height and possible interference from traces of nonsaponifiable material. Nevertheless, hydrogenated samples indicated the presence of an acid with this chain length.

In general and for all types of meat, over 50% of the acids were polyunsaturated. A review of the literature on the fatty acid content in over 100 fish and 28 shellfish reveals that only Atlantic cod, gurnard (*Chelidonichths kumu*), king crab, Pacific oyster, scallops and squid have a similar polyunsaturate content (Gruger, 1967; Khalid et al., 1968; Krzeczkowski et al., 1971, 1972; Shimma and Taguchi, 1964).

The deterioration of a marine lipid is greatly influenced by its degree of unsaturation. The relatively large amount of polyunsaturated acids, especially 22:6 and 20:5 (42–46%) must therefore be considered in the maintenance of quality during handling and frozen storage. The skin, because of its high lipid content, is probably more prone to oxidative changes than the muscle which the skin surrounds. Overall the crabmeat is very low in total lipid and therefore does not present a potential lipid oxidation problem in the sense that fatty fish do.

Table 4— Total fatty acid content[a] in canned and various types of cooked frozen snow crab meat

Fatty acids[b]	Whole meat (composite)	Canned	Body-shoulder	Claws	Propodus carpus	Merus	Merus without skin	Skin
Saturated Acids								
10:0	0.3	0.4	0.1	0.1	0.1	0.3	Tr	0.2
12:0	0.4	0.4	0.2	0.2	0.2	0.4	Tr	0.2
14:0	0.4	0.4	0.2	0.6	0.4	0.5	0.5	1.2
15:0	0.7	0.7	0.9	0.9	0.5	0.5	0.5	1.5
16:0	13.5	12.5	13.2	12.5	13.5	13.5	13.7	11.6
17:0	0.9	1.3	1.0	0.9	1.2	1.1	0.9	0.3
18:0	3.1	3.4	3.5	3.8	3.6	2.9	2.8	5.8
19:0	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr
20:0	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr
22:0	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr
24:0	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr
Total	19.3	19.1	19.1	19.0	19.5	19.2	18.4	20.8
Mono-unsaturated acids								
14:1	0.2	0.4	0.4	0.2	0.5	0.4	0.3	0.6
15:1	0.2	0.3	0.1	0.2	0.5	0.3	0.2	0.5
16:1	3.0	3.3	3.4	3.8	3.2	3.3	3.5	4.5
17:1	0.9	0.9	0.9	0.8	0.9	0.7	0.7	1.0
18:1	17.8	16.0	17.5	19.3	17.3	17.0	16.3	21.9
20:1	1.4	1.2	0.5	1.5	1.6	1.0	1.4	3.0
22:1	0.5	0.6	0.5	0.5	0.5	0.5	0.4	0.2
Total	24.0	22.7	23.3	26.3	24.5	23.2	22.8	31.7
Poly-unsaturated acids								
16:2 ω4	0.7	0.4	0.6	0.8	0.6	1.0	0.5	0.8
18:2 ω6	1.1	3.5	2.7	1.9	1.4	1.9	1.1	1.2
18:3 ω3	0.4	0.5	0.2	0.1	0.5	0.4	0.4	0.4
18:4 ω3	0.5	0.6	0.5	0.1	0.5	0.3	0.1	0.2
20:2 ω6	0.4	0.3	0.4	0.2	0.5	0.4	0.4	0.7
20:4 ω6	3.9	4.8	3.7	4.7	6.4	3.7	3.7	8.5
20:4 ω3	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr
20:5 ω3	29.0	28.0	29.6	26.3	26.2	30.0	31.9	22.2
22:3 ω6	1.8	1.4	1.2	1.1	1.1	1.1	1.0	1.1
22:4 ω6	0.5	0.9	0.6	1.0	0.5	0.9	0.8	0.9
22:5 ω3	1.2	1.4	1.5	1.3	1.3	1.1	1.7	1.5
22:6 ω3	16.3	15.0	16.6	15.7	16.5	15.6	15.1	9.4
23:5 ?	0.5	0.5	0.3	0.5	0.5	0.5	1.0	0.5
Total	56.3	57.3	57.9	53.7	56.0	56.9	57.7	47.4

[a] Weight percent of fatty acid methyl esters (C_{10}—C_{23})
[b] Ratio of C Atoms to double bonds

On the basis of these studies it appears that the amino acid content of snow crab meat is similar to that of dungeness and blue crab meat. The fatty acid content and proximate analysis are similar to king crab.

These results show that snow crab contain a considerable amount of meat (33.7%), which is high in protein and nutrition. The four types of meat present in snow crab show no significant difference in fatty acid composition and proximate analysis. Results should be of value to home economists, nutritionists and investigators for improving processing and marketing.

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HEAT STABILITY OF CHICKEN ACTOMYOSIN

INTRODUCTION

MECHANICALLY DEBONED chicken meat holds promise as an important nutritional and functional ingredient in many processed foods. Because of its high protein content, it has important nutritional potential. Functionally, chicken protein has been shown to perform well in many emulsion products such as rolls and frankfurters. Hudspeth and May (1967; 1969) and McCready and Cunningham (1971) found that the salt soluble proteins in poultry muscle tissue (mainly actin, myosin and actomyosin) are of particular functional importance. As with many comminuted food products, the shelf life of mechanically deboned meat is relatively short. Moreover, when mishandled prior to use in processed foods the product is potentially a source of contamination by salmonellae and other harmful organisms (Ostovar et al., 1971; Lillard, 1971). Consequently, it would be desirable to heat process the meat to destroy these organisms and to reduce the total bacterial load. However, when this is done, the proteins which function as emulsion stabilizers are denatured and can no longer function effectively (Young and Lyon, 1973). The objective of this study was to investigate conditions under which one of these proteins, actomyosin, could be made stable to the conditions usually required to heat-pasteurize food ingredients.

EXPERIMENTAL

Preparation of the actomyosin

Fresh hand-deboned chicken breast meat was ground coarsely and extracted overnight with 5 volumes of Weber-Edsall solution (0.6M KCl, 0.04M $NaHCO_3$, 0.01M Na_2CO_3) containing 0.001M EDTA. The extract was diluted with 4 volumes of 0.6M KCl in order to reduce viscosity and centrifuged at 5000 rpm in a Lourdes model A-2 refrigerated centrifuge which was equipped with a number 1605 continuous rotor. The temperature was maintained at 1–3°C. Thereafter the reprecipitation procedure was as described by Briskey and Fukazawa (1971). The protein content of the preparation was estimated by the method of Lowry et al. (1951).

Heat treatment

25 ml of each of the test preparations were placed individually in a 125-ml Erlenmeyer flask, and brought to 65°C (with mixing) in an 85°C water bath. Each flask was held at 65°C in a 65°C bath for 1 min and then chilled to 30°C in an ice bath. The "come up" time was about 40 sec and "come down" time was about 30 sec. This treatment was selected as one which would achieve satisfactory pasteurization of food products (USDA, 1969).

Emulsifying capacity

The index of heat damage used throughout this study was the fat emulsifying capacity (EC) of the protein. The procedure was substantially that of Swift et al. (1961) and was performed in the following manner. 25 ml of the solution whose EC was to be determined (including any precipitate formed during the heat treatment) was adjusted to pH 7.0, except where noted, and then placed in an Omni-mixer chamber which was chilled in an ice bath. The blender was started and after 30 sec corn oil was added at a rate of 15 ml/min until the emulsion "broke" as evidenced by a sudden decrease in viscosity. The total amount of oil added was divided by the milligrams protein in the chamber to yield the emulsifying capacity (EC) per milligram of protein.

Solvent pH

The actomyosin was adjusted to 14 mg per ml in 0.5M KCl. Triplicate 12.5 ml samples were then adjusted to pH 6.5, 7.0, 7.5, 8.0, 8.5, 9.0 and 9.5 with an equal volume of 0.5M tris-HCl buffer. The final volume of each sample was 25 ml. The protein concentration before heating was 7 mg per ml. Each sample was heated as previously described. Since Neelakantan and Froning (1971) found that pH affects EC, the pH was readjusted to 7.0 after heating. The final protein concentration was 6 mg per ml. The EC was determined.

Solvent ionic strength

Triplicate samples of the actomyosin were adjusted to 7 mg actomyosin per ml, ionic strength (μ) 0.2, 0.4 or 0.6. The pH of unbuffered solutions was 6.4. The samples were heat treated as previously described and readjusted to 6 mg protein per ml, μ = 0.6. The EC was determined. Triplicate samples were also tested for heat stability in 0.12M tris-0.2M KCl and 0.12M tris-0.6M KCl, pH 6.5–9.5.

Monovalent cations

Samples of the actomyosin preparation were precipitated by dilution and washed twice with 0.2M KCl, 0.2M LiCl or 0.2M NaCl. They were then adjusted to 0.6M KCl, 0.6M LiCl or 0.6M NaCl. Three 25-ml aliquots of each sample were heated as previously described and EC determined. These data were compared to EC of protein samples with the same salt composition but without the heat treatment.

Divalent cations

Triplicate samples of the actomyosin were adjusted to 0.6M KCl (μ = 0.6), 0.13M $MgCl_2$ – 0.2M KCl (μ = 0.6) or 0.13M $CaCl_2$ – 0.2M KCl (μ = 0.6) and then heated as previously described. Triplicate control samples were prepared in a like manner but unheated. The EC was determined on all heated and unheated samples.

Neutral organic solutes

Hashimoto and Yasui (1966) reported that various sugars and alcohols helped to reduce the heat sensitivity of myosin A adenosine triphosphatase activity. Thus, triplicate 25 ml samples of the actomyosin preparation were adjusted to contain 7 mg protein per ml and 0, 4 or 6% sucrose in 0.6M KCl. The samples were heated as previously described and the EC determined. Control samples were treated similarly but not heated. The EC of the heated and unheated samples were compared.

Inorganic polyphosphates

Polyphosphates have a profound influence on the physical and functional properties of muscle proteins. Hamm (1971) ascribed this influence to (1) change of pH, (2) nonspecific effects of ionic strength and (3) specific effects of the phosphate anion with the myofibrillar proteins. These phosphates might have a beneficial effect on the heat sensitivity of actomyosin. Solutions were prepared in triplicate to contain 8 mg actomyosin per ml, 0.2M KCl and 0.0, 0.2 or 0.6% sodium tripolyphosphate (TP) or sodium pyrophosphate (PP). These concentrations correspond to ionic strengths of 0.20, 0.26 and 0.44 for the TP and 0.20, 0.24 and 0.33 for the PP. The samples were heated as previously described and their EC determined. The EC were compared to the EC of control samples containing the same amount of protein, KCl and polyphosphate but not heated.

Kena

The actomyosin was adjusted to 8 mg protein per ml, 0.0, 0.2 and 0.6% Kena (a commercially available polyphosphate food additive) and 0.2 or 0.6M KCl. Triplicate samples of each solution were heat treated. The EC was determined, and compared to control samples containing the same amount of KCl and Kena but unheated.

RESULTS & DISCUSSION

Solvent pH

It may be seen in Figure 1 that heating the protein below pH 8.5 caused the protein to coagulate and either float on the surface or sink to the bottom of the container. The degree of heat damage was reduced as the pH increased. The damage was never altogether eliminated since even at the highest pH levels tested, the protein in the treated samples formed a floccular precipitate which settled out upon long standing. The protein in the

unheated control did not settle out even after standing over night.

Below pH 8.5, no emulsion formed in the EC test indicating that the protein was denatured to such an extent that it could no longer emulsify the corn oil (Table 1). Above pH 8.5, the EC tests were considerably better. Nevertheless, even at the highest pH levels tested, the emulsifying capacities of the treated samples never exceeded 65% of the unheated controls. Thus, although raising the pH of the solvent above 8.5 improved the heat stability of actomyosin there was still significant damage to the ability of the protein to emulsify fat.

Solvent ionic strength

It may be seen in Table 2 that increasing the ionic strength increased the heat stability of the actomyosin but the EC was lower than that of the unheated protein. pH measurements before and after heating revealed a decrease in pH of from 0.5 to 1.0 units in all samples which indicated that even at the highest ionic strength there was significant denaturation during heating. Since the pH also affects heat sensitivity, the ionic strength experiment was repeated with an added buffer at various pH values to control pH. Measurements taken before and after heating showed that the pH was maintained within 0.1 units. Increasing the pH while maintaining a low ionic strength only marginally improved the heat stability of the actomyosin (Table 3). The end point on the pH 9.0 – 0.2M KCl

Table 3—Effect of pH at high and low ionic strength on the emulsifying capacity of chicken natural actomyosin[a]

pH	Emulsifying capacity[b] (ml/mg protein) 0.12M Tris – 0.2M KCl	0.12M Tris – 0.6M KCl
6.5	–[c]	0.21a
7.0	–	0.19a
7.5	–	0.21a
8.0	–	0.22a
8.5	–	0.20a
9.0	∿0.10	0.23ab
9.5	0.20	0.24b
Control (unheated)	0.26	0.62c

[a] Each value represents the mean of three observations. Heat treatment: 65°C for 1 min; Protein: 7 mg/ml.
[b] Means with the same letters do not differ significantly at $P < 0.05$.
[c] – indicates no emulsion formed in EC test.

Table 1—The effect of solvent pH on the heat stability of natural actomyosin[a]

pH	Emulsifying capacity (ml oil/mg protein)[b]
6.5	–[c]
7.0	–
7.5	–
8.0	–
8.5	0.15a
9.0	0.44b
9.5	0.41b
Control (unheated)	0.68c

[a] Each value represents mean of three observations; Solvent: 0.25M Tris – 0.25M KCl; Heat treatment: 65°C for 1 min; Protein: 7 mg/ml (prior to heating).
[b] Means with the same letter do not differ significantly at $P < 0.01$.
[c] – indicates no emulsion formed in EC test.

Table 2—Effect of variation in solvent ionic strength (μ) on the emulsifying capacity of chicken natural actomyosin[a]

Ionic strength (μ)	Emulsifying capacity[b] (ml oil/mg protein)
0.2	–[c]
0.4	0.23a
0.6	0.33b
Unheated Control (μ = 0.6)	0.39c

[a] Each value represents the mean of three observations. Heat treatment: 65°C for 1 min; Protein: 7 mg/ml.
[b] Means with different letters differ significantly at $P < 0.05$.
[c] – indicates no emulsion formed in EC test.

Table 4—Effect of three monovalent cations on the heat sensitivity of chicken actomyosin[a]

Treatment	Emulsifying capacity (ml/mg protein)[b] 0.6M KCl	0.6M NaCl	0.6M LiCl
Control (unheated)	0.46a	0.45a	0.47a
Heated	–[c]	–	–

[a] Heat treatment: 65°C for 1 min; Protein: 5 mg/ml; pH: 6.4.
[b] Means with the same letter do not differ significantly at $P < 0.05$.
[c] – indicates no emulsion formed in the EC test.

Table 5—Effect of Mg^{++}, Ca^{++} and K^{+} on the heat sensitivity of chicken actomyosin[a]

Treatment	Emulsifying capacity[b] (ml/mg protein) 0.6M KCl[c]	0.13M $MgCl_2$ – 0.2M KCl[c]	0.13M $CaCl_2$ – 0.2M KCl[c]
Control (unheated)	0.36b	0.39c	0.37bc
Heated	–[d]	0.37bc	–[d]

[a] Heat treatment: 65°C for 1 min; pH: 7.0; Protein: 7 mg/ml.
[b] Means with the same letter do not differ significantly at $P < 0.05$.
[c] Ionic strength: 0.6.
[d] – indicates no emulsion formed in EC test.

Table 6—Effect of two levels of sucrose on the heat sensitivity of chicken actomyosin[a]

Treatment	Emulsifying capacity[b] (ml/mg protein) 0% sucrose	4% sucrose	6% sucrose
Control (unheated)	0.36cd	0.37d	0.38d
Heated	–[c]	0.26b	0.34c

[a] Protein: 7 mg/ml; pH: 7.0; Ionic strength: 0.6.
[b] Means with the same letter do not differ significantly at $P < 0.05$.
[c] – indicates no emulsion formed in EC test.

samples was small and quite difficult to see. The stability was partially improved at pH 9.5. The stability of the protein was considerably improved in the buffered, higher ionic strength solvent. This improved stability was evident even at low pH. When the pH was increased, the heat stability also increased slightly. Nevertheless, the EC of heated samples never equaled that of the unheated controls.

Monovalent cations

Replacing the KCl with either NaCl or LiCl had no effect on either the EC or the heat sensitivity of the protein (Table 4). The protein in all heated samples was denatured to the extent that no emulsions formed in the EC test.

Divalent cations

When the heat sensitivity of the actomyosin was tested in the presence of K^+, Mg^{++} and Ca^{++}, the Mg^{++} caused a small though statistically significant improvement in the emulsifying capacity of the control samples (Table 5), but Ca^{++} had no effect. The protein was less heat sensitive in the presence of Mg^{++}. Both Ca^{++} and Mg^{++} are capable of binding with the meat proteins (Inklaar, 1967). Apparently the Mg-actomyosin complex is more stable to heat than the uncomplexed protein while the calcium complex is as heat sensitive as the native protein.

Neutral organic solutes

The presence of sucrose had no effect on the EC of the unheated actomyosin but significantly improved the heat stability of the protein (Table 6). The protein in all heated samples was obviously denatured but the curd appeared softer and more flocculent in those with than in those without the sugar. Even though the sucrose improved the heat stability of the actomyosin, the EC were lower in the heated samples than in the unheated ones. Perhaps higher levels of sucrose or other neutral organic solutes would stabilize the actomyosin further.

Polyphosphates

Adding sodium tripolyphosphate (TP) significantly improved the emulsifying capacity of the control samples (Table 7). However, 0.2% TP was just as effective as 0.6%. The heat stability of the protein was substantially improved by the TP. At 0.6% TP there was no difference in emulsifying capacities between the heated and unheated samples. The effect of sodium pyrophosphate (PP) was similar to that of TP but the response was smaller (Table 8). The PP improved the EC and reduced the heat sensitivity of the actomyosin. No doubt part of these effects was due to the elevated pH and ionic strength caused by the polyphosphates. However, the results in Tables 1, 2 and 3, suggest that the polyphosphate anion has an additional specific effect on the heat sensitivity of the protein. It might be argued that this effect was not the stabilization of actomyosin but rather the conversion of actomyosin to its components actin and myosin by the polyphosphates and the subsequent stabilization of these components. This seems an unlikely course, however, for two reasons: (1) Divalent cations are required for PP to convert actomyosin to actin and myosin (Bendall, 1954). Since all solvents were prepared with deionized water, the Ca^{++} and Mg^{++} content of the actomyosin preparations should have been quite low. (2) TP must be converted to PP + orthophosphate by myosin B triphosphatase in order to dissociate actomyosin and this reaction requires Ca^{++} or Mg^{++}, high ionic strength and an acid medium (Yasui et al., 1964). Since the Ca^{++} and Mg^{++} content should have been low, the ionic strength was less than 0.44 in all samples and the

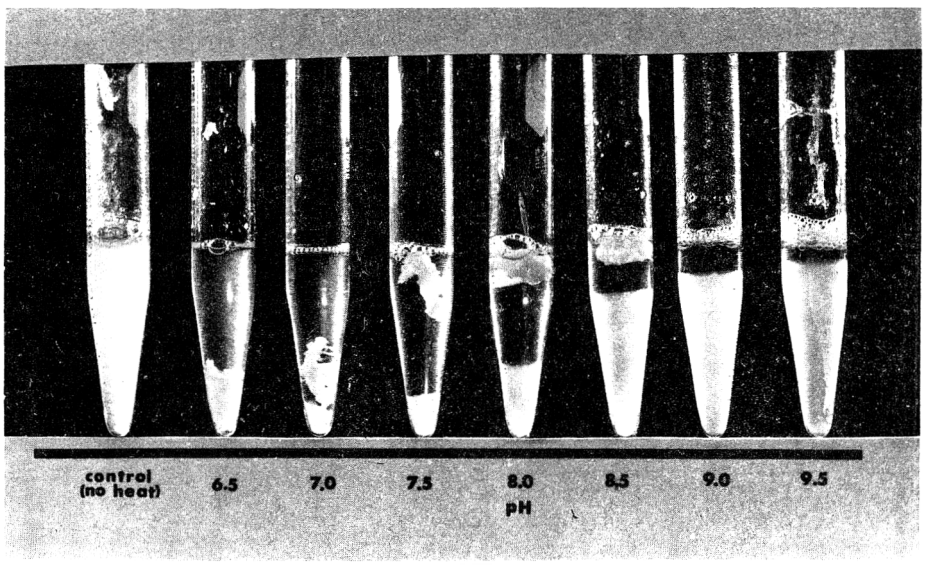

Fig. 1—Effect of pH on the heat sensitivity for chicken natural actomyosin.

Table 7—Effect of sodium tripolyphosphate (TP) on the heat sensitivity of chicken actomyosin in 0.2M KCl[a]

	Emulsifying capacity[b] (ml/mg protein)		
Treatment	**0.0% TP**	**0.2% TP**	**0.6% TP**
Control (unheated)	0.29b	0.51cd	0.52d
Heated	—[c]	0.49c	0.53d

[a] Actomyosin concentration: 8 mg/ml; pH: 0% 7.7, 0.2% 8.6, 0.6% 8.8; Heat treatment: 65°C for 1 min.
[b] Means with the same letter do not differ significantly at $P < 0.05$.
[c] — indicates no emulsion formed in EC test.

Table 8—Effect of sodium pyrophosphate (PP) on the heat sensitivity of chicken actomyosin in 0.2M KCl[a]

	Emulsifying capacity[b] (ml/mg protein)		
Treatment	**0.0% PP**	**0.2% PP**	**0.6% PP**
Control (unheated)	0.25b	0.33c	0.38d
Heated	—[c]	0.35c	0.39d

[a] Actomyosin concentration: 8 mg/ml; pH: 0% 6.6, 0.2% 8.8, 0.6% 9.2; Heat treatment: 65°C for 1 min.
[b] Means with the same letter do not differ significantly at $P < 0.05$.
[c] — indicates no emulsion formed in EC test.

Table 9—Effect of Kena at two KCl concentrations on the heat sensitivity of actomyosin[a]

% Kena	Emulsifying capacity[b] (ml/mg) 0.2M KCl Control (untreated)	Heated	Difference	Emulsifying capacity[b] (ml/mg) 0.6M KCl Control (untreated)	Heated	Difference
0.0	0.31a	–[c]	0.31a*	0.39a	–[c]	0.39a*
0.2	0.51b	0.34b	0.17b*	0.47b	0.41b	0.06b*
0.6	0.49b	0.42c	0.07c*	0.49b	0.41b	0.08b*

[a] Actomyosin concentration: 8 mg/ml; Heat treatment: 65°C for 1 min; pH: 8.2.
[b] Means in the same column with different letters differ significantly at $P < 0.05$.
[c] – indicates no emulsion formed in EC test.
* Indicates significantly ($P < 0.05$) greater than 0.00

medium was strongly alkaline, it does not seem likely that the triphosphate was converted to the diphosphate. A more likely explanation for the heat-stabilizing effect is that the polyphosphates from complexes with the actomyosin that are inherently more stable than the native protein.

Kena

Because of the positive results with the polyphosphates, the effect of Kena on the heat sensitivity of actomyosin in 0.2 and 0.6M KCl was investigated. In heated and unheated samples and at both KCl concentrations, Kena improved the EC of the actomyosin significantly (Table 9). Furthermore, increasing the Kena concentration in the heated samples reduced the heat damage to the emulsifying capacity. At 0.6M KCl, 0.2% Kena was as effective as 0.6% Kena in preventing heat damage, but not at the 0.2M KCl. This difference is likely due to the increased ionic strength and subsequent solubilization of the protein by the Kena in 0.2M KCl. Even though the difference between the EC of the heated and unheated samples containing 0.6% Kena is signficantly greater than zero, this small amount of damage may be tolerable since previous work has shown that a limited proportion of heat-damaged meat may be included in emulsion formulations (Young and Lyon, 1973).

In developing procedures for pasteurizing mechanically deboned chicken meat, it is essential to find methods for reducing the heat sensitivity of the most important functional proteins (actin, myosin and actomyosin). The heat sensitivity of chicken actomyosin is controllable by adjustment of its chemical environment. Efforts to stabilize actin and myosin, might use either of two approaches: (1) develop methods for stabilizing these proteins independently or (2) convert them to actomyosin and stabilize the latter with appropriate additives. Regardless of which approach is taken, any treatment which is applied to make the functional proteins heat stabile might also affect the other quality attributes of the meat. Consideration must be given to the effect of any additives on the color, flavor and storage stability of the product. Polyphosphates influence all of these attributes (Mahon et al., 1971) and the ionic environment influences the color and heat stability of the heme pigments (Snyder and Skrdlant, 1966; Satterlee and Zacharia, 1972). Perhaps after methods are found for stabilizing the entire functional protein complex, the other properties of the meat will be altered to such an extent that process modifications will be necessary to fully utilize the pasteurized product. These considerations warrent further study.

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RELATION BETWEEN ISOMETRIC TENSION, POSTMORTEM pH DECLINE AND TENDERNESS OF POULTRY BREAST MEAT

INTRODUCTION

ALTHOUGH RIGOR contraction has long been considered to influence meat tenderness (Bate-Smith, 1948; Whitaker, 1959), little information is available on the relation between these two factors. Earlier efforts to relate tenderness with the state of contraction of muscle by measuring extensibility or elasticity (Bate-Smith and Bendall, 1956; DeFremery and Pool, 1960; Marsh, 1954), were unsuccessful because length or elastic changes were too small for quantitative measurement (Busch et al. 1972; Jungk et al., 1967).

This paper describes the relation between isometric tension, shear force and pH changes during postmortem aging of chicken pectoralis major muscle. In this study, changes in isometric tension were used as a measure of rigor contraction because these changes correspond to the time-course of rigor and are more sensitive to rigor contraction than are length changes (Goll et al., 1970). In these tests, measurements of rigor tension and shear force were made on the same sample. This procedure minimized the effects of sample-to-sample variability and permitted a direct comparison between these two parameters.

EXPERIMENTAL

TESTS were made on pectoralis major muscles excised from 36 well-rested male chickens (Leghorn, pathogen free, live weight 1.2–2.9 kg) immediately after slaughtering and bleeding. Twenty-four of these birds were administered sodium pentobarbital (35 mg/kg body weight) in the thigh muscles 15 min before slaughtering to obtain muscle tissue having a pH value and high-energy phosphate level comparable to that in live muscle. The remaining 12 birds were not administered sodium pentobarbital and were allowed to undergo various degrees of struggle during slaughtering. This treatment has been shown to cause a greater than normal ante- or early mortem glycolysis and yield tough meat (Khan and Nakamura, 1970).

For tension measurements, two strips of muscle, each 1 cm square in cross section and about 6 cm in length were cut parallel to the fibers from adjacent location from each of the two pectoralis major from each bird. During cutting, care was taken to minimize structural damage. Both ends of the strip were tied with surgical thread leaving approximately 5 cm of tissue between the ties. Isometric tension measurements were made inside a jacketed glass vessel (length 120 mm, diameter 25 mm) having a glass hook on the inside at the bottom to which one end of the sample was tied. The thread at the other end of the sample was attached to a transducer (Model FT 0.3, Grass Medical Instruments, Quincy, Mass.), connected via a strain-gage amplifier to a recorder. Each sample was preloaded initially to a tension of 4g/cm² in order to maintain original conditions (Busch et al., 1972). Liquid was circulated in the jacket to maintain the desired temperature. In these tests an aging temperature of 25°C was used because tension changes at temperatures between 10°C and 25°C have been shown to be minimum and more uniform than that occurring at 37°C or 2°C (Busch et al., 1972). Tension measurements were started as soon as possible after the death of the animal (15–20 min) and continued for up to 20 hr postmortem. All tests were made in duplicate using samples from adjacent location in the muscle.

Since surface dehydration, microbial growth and sample temperature are difficult to control in muscle samples suspended in air, preliminary studies were made to select a liquid medium which would give results comparable to those obtained in air. The following media were tested:

(a) Air at high humidity.
(b) Sodium chloride solution, 0.9% (w/v).
(c) Sodium chloride solution, 0.9% (w/v) + 50 ppm tetracycline.
(d) Sodium chloride solution, 0.9% (w/v) + 1 mM sodium azide.
(e) Sodium chloride solution, 2.5% (w/v) + 50 ppm tetracycline.
(f) Sodium phosphate buffer, 0.2 M, pH 7.0.
(g) Potassium chloride-tris acetate-Mg Cl_2-azide buffer, pH 7.0 (Busch et al., 1972).

The pH of saline solutions were adjusted to 6.9–7.0 with NaOH. Isotonic saline solution containing tetracycline as a bactericidal agent was selected as being the most suitable because this medium did not delay or speed up the onset of tension and gave results comparable to those carried out in air at high humidity (Table 1). This medium was used in all subsequent tests.

Measurements of pH were made with a puncture combination pH electrode on samples similar to those used in isometric tension measurement and held under similar condition. In several cases pH measurements were also made on the same samples used for tension measurements. These samples were removed during different stages of tension development, and minced for pH measurement.

For shear force measurements, samples were removed from the tension measuring apparatus after the desired length of time and cooked to an internal temperature of 82–85°C as described earlier (Khan and Frey, 1971). These samples were clamped in a special mold designed according to DeFremery and Pool (1960) in order to reduce uncontrolled distortion of the muscle during cooking, then vacuum-packaged in plastic bags and cooked in

Table 1—Effect of aging media on time required for development of isometric tension and on the extent of tension produced in poultry breast muscle[a]

Medium	Time postmortem (hr) Start of tension	Full tension	Isometric tension (g/sample) Max	20 hr postmortem
Air, high humidity	3	9	19.8	6.0
Saline, 0.9%	3	9	17.0	5.5
Saline, 0.9% + tetracycline	3	9	17.2	5.0
Saline, 0.9% + sodium azide	2	7	19.5	5.0
Saline, 2.5% + tetracycline	4	13	6.0	0.5
Tris-azide buffer	1	5	22.2	5.8
Phosphate buffer	1	6	25.2	9.0

[a] Values are averages of three birds.

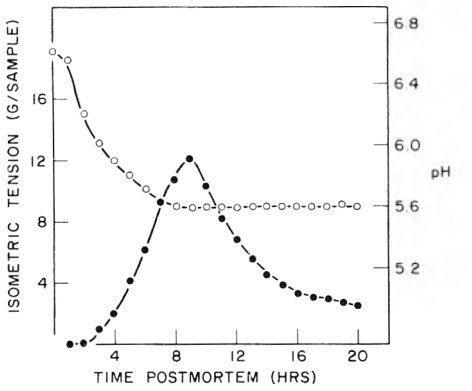

Fig. 1—Typical changes in isometric tension (●) and pH (○) of breast muscle during 20 hr of postmortem aging in 0.9% saline containing tetracycline.

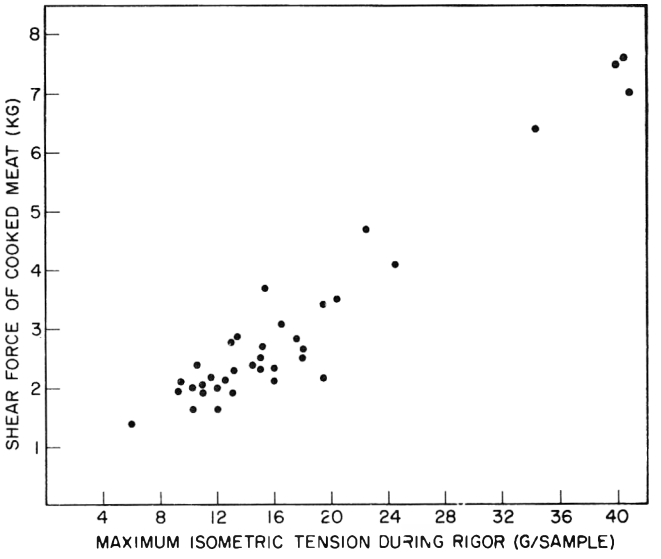

Fig. 2—Relation between maximum isometric tension developed during rigor and shear force of cooked muscle. (Samples were cooked after 20 hr of aging, n = 36, r = 0.97, significant at 1% level.)

a boiling water bath. Strips 50 × 100 mm in cross section were cut and 6–8 measurements were made on each sample using a texture press system (Food Technology Corp., Reston, Va.) equipped with a meat shear cell.

RESULTS & DISCUSSION

TYPICAL CHANGES in pH and tension development in poultry breast muscle samples excised from sodium pentobarbital-treated birds immediately after death are shown in Figure 1. Muscles from both pentobarbital-treated and untreated birds began to develop tension when their pH dropped to values between 6.1 and 6.3, and the tension continued to increase until the pH of the muscle reached its ultimate value (pH 5.5–5.8). Afterwards, the tension started to decline, while the pH of the muscle remained approximately constant. Postmortem time at which muscle samples began to develop tension varied between 30 min and 4 hr, and the time at which they attained maximum tension varied between 2 and 8 hr. Muscle with low postslaughter pH values went into rigor sooner after death than muscle with high postslaughter pH values, and generally developed a higher tension. No attempt was made at this stage to investigate this phenomenon, however. Samples excised from adjacent locations in a single muscle having similar cross section and length varied by ±8% in maximum tension and by ±0.5 hr in time after death at which the tension development commenced or reached a maximum value.

Results of experiments made to determine (a) the shear force of meat during tension rise and decline, and (b) the relation between rigor tension and shear force values are given in Table 2 and Figure 2. Samples cooked at the start of tension development gave the highest shear force values, but as tension increased shear force gradually decreased and reached a minimum value about 20 hr postmortem. Shear force of the meat was lower at the time of maximum tension than at the start of tension development. The effect of cooking meat during various stages of rigor on contractile state of muscle is not understood, but the severe shortening that usually accompanies cooking during rigor (Busch et al., 1967) appears to be maximum at the start of rigor rather than at the height of rigor. The ultimate shear force of meat cooked 20 hr postmortem appear to be directly proportional to the maximum tension developed during rigor (Fig. 2). These results were significant at the 1% level. No such relation could be demonstrated between these factors during the period of development or decline of tension, however.

Isometric tension measurements showed that after the development of full rigor, the ability of muscle to maintain tension is lost. Similar pattern of tension changes has been reported for beef, pork and rabbit muscles (Busch et al., 1967, 1972; Jungk et al., 1967). Since shear force depended on maximum degree of tension developed during rigor and not tension decline, the changes during development of tension appear related to the contractile state of the muscle that influence tenderness. Although the loss of ability of muscle to maintain tension occurred during the period of tenderization, it does not appear to resolve changes which occur during the onset of rigor and influence tenderness. Studies made by measuring extensibility (Bendall, 1960; Marsh, 1954) and sarcomere length (Locker, 1960) have also indicated that muscle does not regain its original state after rigor.

Results indicate that muscle started to develop rigor tension when its pH dropped to a value between 6.1 and 6.3.

Table 2—Shear force of cooked breast meat during development and decline of isometric tension[a]

Postmortem condition	Shear force (kg) (1)	(2)	(3)	(4)
At the start of tension development	4.1	6.5	6.6	4.5
At maximum tension	3.5	3.7	3.6	2.8
1 hr after maximum tension was reached	2.9	3.5	2.8	2.3
20 hr postmortem, tension near minimum	2.3	2.8	2.3	1.9
48 hr postmortem, minimum or no tension	2.3	2.7	2.4	2.0

[a] Results of four separate experiments conducted on four different occasions. Each value shown is the average of two samples.

Earlier studies on beef (Khan and Lentz, 1973) and poultry (Khan, 1971; Khan and Nakamura, 1970) have indicated that postslaughter pH drop to these or lower than these values cause toughness. It appears that muscle having postslaughter pH of about 6.3 or lower starts to develop rigor immediately after slaughter when the carcass temperature is still high. Rapid onset of rigor at near body temperature has been shown to cause toughness (Newbold and Harris, 1972).

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EFFECTS OF PRE-RIGOR TENSION ON TENDERNESS OF INTACT BOVINE AND OVINE MUSCLE

INTRODUCTION

IN THE PAST DECADE a great deal of effort has been put forth in studying the relationship between the contraction state of a muscle and its tenderness, sarcomere length and fiber diameter. Locker (1960) had suggested that tenderness and myofibril striation pattern differences among ox muscles of comparable connective tissue content might be explained by strain imposed on the individual muscles in hanging the carcass before they pass into rigor mortis. This assumption is supported by the work of Herring et al. (1965a) in which he investigated the effect of carcass position at the onset of rigor mortis on sarcomere lengths, fiber diameters and tenderness of selected muscles of the bovine carcass. Results from the 12 muscles studied revealed that for each individual muscle, generally, sarcomeres were longer, fiber diameters smaller and tenderness greater in the orientation producing the greatest tension on that muscle. Since carcasses are normally vertically suspended by a hind shank following slaughter, it would appear that there is considerable potential present for improvement of the tenderness of the longissimus muscle.

Early work carried out by Herring et al. (1965b), Buck and Black (1967), Herring et al. (1967) and Gillis and Henrickson (1969) on the relationship between the postmortem contraction state of muscle and muscle tenderness involved the use of pre-rigor excised muscles or muscle strips. However, Hostetler et al. (1970; 1973) reported significant tenderness improvement in the intact bovine carcass by supporting the carcass by the obturator foramen. Similar findings for lamb carcasses were also reported by Quarrier et al. (1972). Smith et al. (1971) also reported significant tenderness improvement in the intact bovine carcass by altering chilling procedures or carcass suspension, or by disrupting skeletal attachments to the carcass. The effect of skeletal restraint and time delay before freezing on tenderness of various lamb muscles was reported by Marsh et al. (1968) and McCrae et al. (1971). Other methods for improving tenderness of major muscles of intact carcasses were described by Stouffer et al. (1971).

The objective of this study was to investigate methods of applying pre-rigor tension for increasing the tenderness of longissimus muscle of bovine and ovine carcasses after various postmortem aging periods. Methods to tension the longissimus muscle of conventionally suspended carcasses included severing of vertebra and applying weights or mechanical force and mechanical force without severing. The effects of these treatments on shear force values, fiber diameters and sarcomere lengths were observed.

MATERIALS & METHODS

31 LAMBS and seven beef carcasses were utilized in the four experiments of this study. A description of the carcasses and treatments is presented in Table 1. 12 lambs were used in each of experiments 1 and 2, while seven lambs were used in experiment 3 and seven beef animals in experiment 4. The lambs ranged in live weight from 35.8–47.2 kg, and graded average good to high choice. The beef animals ranged in live weight from 347.9–486.3 kg, and graded standard to high choice. Upon completion of the slaughtering process in the Meat Laboratory, Cornell University, both beef and lamb carcasses were split vertically through the backbone and suspended by the hind shank.

In all experiments one side of a carcass was randomly selected to be treated with pre-rigor tension while the opposite side was held as a nontensioned control. The development of tension pre-rigor in the longissimus muscle of the experimental animals was accomplished through weighted or mechanical techniques. The 12 sides to be tensioned in experiment 1 were randomly and equally allotted to one of 3

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Table 1—Description of carcasses and treatments

Experiment	Species	No. of carcasses	Aging periods	Samples: Tenderness	Samples: Histological	Tension: Method	Tension: Level	Vertebra status
1	Ovine	4	48,[a] 120, 240	Rib Loin	Rib Loin	Weighted	4.5[b]	Severed
		4	48, 120, 240	Rib Loin	Rib Loin	Weighted	9.0	Severed
		4	48, 120, 240	Rib Loin	Rib Loin	Weighted	13.5	Severed
2	Ovine	4	48, 120, 240	Rib Loin	Rib Loin	Mechanical	5[c]	Severed
		4	48, 120, 240	Rib Loin	Rib Loin	Mechanical	10	Severed
		4	48, 120, 240	Rib Loin	Rib Loin	Mechanical	15	Severed
3	Ovine	7	48, 168	Rib Loin	–	Mechanical	12.6[c]	Intact
4	Bovine	4	48, 168	Rib Loin	–	Mechanical	11.4[c]	Severed
		3	48, 168	Rib Loin	–	Mechanical	11.4	Intact

[a] Hours postmortem at 2°C before sampling
[b] Weight load in kg
[c] Percent stretch

weighted tensioning treatments. A weight load of either 4.5, 9.0 or 13.5 kg was attached to the carcass by means of either an s-type hook or a bacon hanger which penetrated the longissimus muscle at the level of the sixth thoracic vertebra. Length measurements between last lumbar and sixth thoracic vertebrae along the back-line revealed that the three weighted treatments stretched this section of the vertebral column an average of 10.7%, 14.0% and 17.7%, respectively.

In experiment 2, 12 lamb carcass sides were randomly and equally allotted to one of three mechanical stretching treatments. After an initial length measurement, the tensioned side was stretched either 5%, 10% or 15% of its initial length. The initial and final length was the distance between the ends of the tensioning device. The mechanical device employed to achieve the desired stretching consisted of two flat aluminum strips, 0.6 cm thick by 3.7 cm wide, which slid easily over one another. A set of stainless steel needles was obliquely attached to opposite ends of each strip which served to anchor each end of the tensioning device firmly in muscle tissue. By applying manual force through a removable lever device the metal strips were forced to slide in opposite directions, thereby stretching the muscle. When the desired degree of stretch was attained, the strips were locked securely in place by tightening the two wing nuts present on the bolts of the underlying strip. Tensioning devices were inserted into the lamb longissimus muscle from its dorsal surface with one set of needles implanted anteriorly at the sixth thoracic vertebra while the needles of the opposite end anchored posteriorly near the anterior tip of the ilium.

To facilitate stretching of the longissimus muscle, the tensioned sides of experiments 1 and 2 had the body and spinous process of each vertebra severed beginning with the ninth thoracic and continuing consecutively posterior to the last lumbar vertebra. A small degree of severing of the fascia and tendons overlying the dorsal surface of the longissimus muscle was also carried out.

Experiment 3 consisted of mechanically tensioning the longissimus muscle with no disruption of the vertebral column. No specific tension was obtained, but rather as much stretch as could be comfortably applied without undue force. Measurements revealed that stretching ranged from 8.9–15.6%, with a mean stretch of 12.6%.

Beef carcass sides in experiment 4 were similarly stretched by mechanical means to a comfortable maximum as in experiment 3. The tensioning devices used on the beef carcasses were similar to those described for lamb carcasses, but with longer needles and a greater overall length. In the beef carcasses the anterior area of insertion was the eighth thoracic vertebra and the posterior area of insertion was near the anterior point of the ilium. Stretching of the tensioned sides ranged form 8.7–15.7%, with a mean value of 11.4%. Four of the tensioned sides had their vertebrae severed in a manner identical to the lamb treatments of experiments 1 and 2. This was observed to have little effect on the extent to which the vertebral column could be stretched, and consequently all carcasses were regarded as belonging to the same treatment group.

After tension treatments were applied, all carcasses were placed in a cooler at 2°C within 60–120 min postmortem and stored until samples were removed. Mechanical and weighted

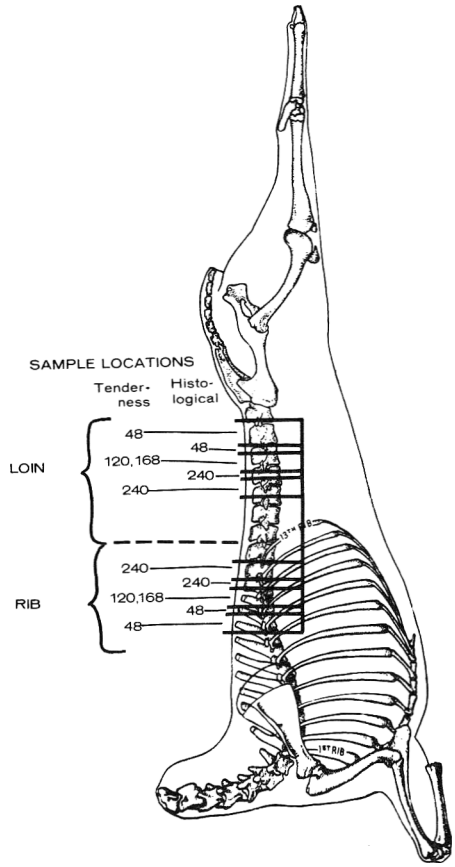

Fig. 1—Location of samples used for tenderness and histological studies. Numbers refer to hours postmortem at sampling.

treatments were normally removed from the tensioned sides approximately 24 hr postmortem. In the interval 24–48 hr postmortem, a section of rib and loin from the ninth thoracic to the next-to-last lumbar vertebra was removed. Samples were taken off of the ends of this section after the appropriate length of postmortem storage for tenderness and histological determinations. Tenderness samples were chops 3.2 cm thick, while histological samples were 0.7 cm slices. Figure 1 illustrates the section removed and location of subsequent samples. Between samplings, the rib and loin section was stored at 2°C in impermeable Cry-O-Vac bags. Each histological sample had a small, equal sized strip of tissue removed from its lateral, medial and dorsal regions. These were fixed in 10% phosphate buffered formalin (pH 6.9–7.1) and stored at 4°C until histological determinations could be carried out.

In experiments 1 and 2, tenderness samples were removed from the muscle segments at 48, 120 and 240 hr postmortem, while histological samples were removed at 48 and 240 hr only. In experiment 3, tenderness samples were obtained at 48 and 168 hr, but no histological samples were taken.

In experiment 4, sections of bovine longissimus with its accompanying vertebrae were excised from the rib (ninth and tenth thoracic) and the loin (third and fourth lumbar) regions of tensioned and nontensioned carcass sides at 48 hr postmortem. Tenderness sample steaks 3.8 cm thick were removed from the excised sections at 48 and 168 hr postmortem. Between sampling periods, the rib and loin sections were stored at 2°C, with the exposed cut surface of the muscle covered with saran.

Chops or steaks were cooked by deep-fat frying in hot beef tallow (135°C), with internal temperatures monitored by means of thermocouples on a Honeywell-Brown Type 602 Recording Thermometer. The ovine tenderness samples were removed from the hot fat when they reached an internal temperature of 60°C, while bovine samples were removed when they had attained an internal temperature of 57°C. Subsequent to removal from the hot fat, the internal temperature increased to 70°C which was the desired end point. Following cooking, all samples were allowed to equilibrate for 4–8 hr in a 4°C cooler before being evaluated for tenderness on a Warner-Bratzler shear device. A 1.27-cm diam core was removed from the dorsal, medial and lateral positions of each ovine sample. Two 1.27-cm diam cores were removed from the dorsal, medial and lateral positions of each bovine sample. Each core was sheared across the grain three times, thus yielding 9 and 18 shear values for ovine and bovine tenderness samples, respectively.

Fiber diameters were determined for all 48 hr samples of experiments 1 and 2. The tissue to be examined was homogenized with 150 ml of cold distilled water at low speed in a Waring Blendor (blades reversed) in a 4°C cooler for 45 sec to dislodge the muscle fibers. The solution of suspended fibers was placed in two petri dishes, which were subsequently examined under an A.O. Spencer binocular microscope. Following a prearranged course in each petri dish, the diameters of 25 fibers were measured with a filar eyepiece micrometer at 100×.

Sarcomere lengths were determined for all 48 and 240 hr postmortem samples of experiments 1 and 2. The tissue was prepared for examination in a similar manner as described above for fiber diameter measurements, except the solution was homogenized for 30 min to dislodge the myofibrils from the muscle fibers. Suspended myofibrils were placed on two glass slides and examined on a Unitron inverted phase contrast microscope equipped with an ocular micrometer. The length of a 10 sarcomere section was measured on each of 20 myofibrils per slide at 1500×. At the same time as sarcomeres were measured, Z lines were also subjectively evaluated in tensioned and nontensioned rib samples at 48 and 240 hr postmortem for nine carcasses in experiments 1 and 2. A Z line was credited as being visible if it could be discerned with moderate ease. If Z lines were not present or very difficult to distinguish, they were recorded as not visible.

The effects of the various factors on fiber diameter, sarcomere length and shear force of muscle were evaluated by an analysis of variance and F-test. Means and standard deviations were computed for each of the variables studied. In certain instances, mean squares and denominator degrees of freedom for tests were synthesized following the procedure of Satterthwaite (1946). Simple linear correlation coefficients were calculated between fiber diameter, sarcomere length and Warner-Bratzler shear force, and tested for significance according to Steel and Torrie (1960).

Table 2–Means, standard deviations and percent decrease in shear force

Tension method	Experiment 1 Ovine Weighted			Experiment 2 Ovine Mechanical			Experiment 3 Ovine Mechanical	Experiment 4 Bovine Mechanical
Tension-level	4.5[a]	9.0	13.5	5[b]	10	15	12.6[b]	11.4[b]
Nontensioned	4.84[c]	4.90	4.62	4.88	4.27	4.66	5.01	4.74
	(1.54)[e]	(1.22)	(1.21)	(1.27)	(1.34)	(1.20)	(1.54)	(1.19)
Tensioned	3.58	3.44	3.20	3.56	3.28	3.61	3.32	3.87
	(0.92)	(0.86)	(0.75)	(0.69)	(0.77)	(0.98)	(0.65)	(0.63)
Decrease in Shear Force (%)[d]	26,0	29.8	30.7	27.0	23.2	22.5	33.7	18.4

[a] Weight load in kg
[b] Percent stretch
[c] Shear force in kg
[d] Percent decrease computed as control minus treated divided by control
[e] Standard deviation in parenthesis

Table 3–Comparison of means, standard deviations and percent decrease in shear force by location and level of tension

Experiment	Species	Method	Tension level	Rib			Loin		
				Nontensioned	Tensioned	Decrease in shear force (%)	Nontensioned	Tensioned	Decrease in shear force (%)
1	Ovine	Weighted	4.5[a]	5.23[c]	3.45	34.0[e]	4.46	3.71	16.8[e]
				(1.55)[d]	(0.97)		(1.41)	(0.85)	
			9.0	4.92	3.48	29.3	4.83	3.40	30.3
				(1.22)	(0.97)		(1.23)	(0.73)	
			13.5	4.76	3.22	32.4	4.43	3.18	29.0
				(1.47)	(1.47)		(0.85)	(0.78)	
2	Ovine	Mechanical	5[b]	5.54	3.76	32.1	4.22	3.36	20.4
				(1.34)	(0.72)		(0.74)	(0.60)	
			10	4.48	3.36	25.0	4.06	3.21	20.9
				(1.57)	(0.81)		(1.01)	(0.73)	
			15	5.27	3.84	27.1	4.06	3.33	18.0
				(1.31)	(1.14)		(0.64)	(0.70)	
3	Ovine	Mechanical	12.6[b]	5.76	3.30	42.7	4.26	3.33	21.8
				(1.64)	(0.64)		(0.95)	(0.66)	
4	Bovine	Mechanical	11.4[b]	4.23	3.56	15.8	5.26	4.18	20.5
				(0.74)	(0.35)		(1.34)	(0.74)	

[a] Weight load in kg
[b] Percent stretch
[c] Shear force in kg
[d] Standard deviation in parenthesis
[e] Percent decrease computed as control minus treated divided by control

RESULTS & DISCUSSION

MEANS, standard deviations and percent decrease in shear force values are presented in Table 2. The application of pre-rigor tension to the intact longissimus muscle in carcasses significantly reduced shear values in experiments 1 and 2 ($P < 0.001$) and in experiments 3 and 4 ($P < 0.01$). These findings are in general agreement with those of Quarrier et al. (1972) and Hostetler et al. (1972) where carcasses were suspended from the obturator foramen. No significant differences in shear force were found among the three tension levels described for experiments 1 and 2. In both cases, the lowest level of tension was as effective in reducing shear values as the intermediate and highest tension levels. This finding agrees with the in vitro studies of Marsh and Leet (1966), Herring et al. (1967) and Gillis and Henrickson (1969) on bovine muscle which demonstrated that a level exists in stretching, beyond which little or no decrease in shear values takes place.

The mechanical application of a non-specific, moderate amount of tension without disruption of the vertebral column in experiment 3, produced shear values comparable to those found in the three tension levels of experiments 1 and 2. Therefore it is concluded in this study that a small amount of tension applied to pre-rigor intact muscle is capable of producing marked improvement in tenderness. There also was no advantage in severing the vertebra or the fascia and tendons overlying the longissimus muscle which agrees with the findings of Smith et al. (1971). The decrease in shear force of beef longissimus in experiment 4 was not as effective as similarly tensioned lamb in experiment 3 (18.4 vs. 33.7%).

The effect of pre-rigor tension on shear values of longissimus muscle was not uniform at all muscle locations as illustrated in Table 3. Tenderness differences between the rib and loin regions of the ovine longissimus muscle were non-significant in experiment 1, very highly significant in experiment 2 ($P < 0.001$), and significant in experiment 3 ($P < 0.05$). Differences between rib and loin regions of the bovine longissimus muscle in experiment 4 were significant ($P < 0.05$). The interaction between location

and treatment was very highly significant ($P < 0.001$) in experiments 1 and 2, significant ($P < 0.05$) in experiment 3 and nonsignificant in experiment 4. This interaction resulted because the tension treatment in ovine muscle was not equally effective in decreasing shear force at both positions. Shear force was generally reduced more in the rib than in the loin. In bovine muscle, shear force was decreased more in the loin than in the rib, but the difference was not as great as in the ovine muscle. Also in experiments 1 and 2 there was a very highly significant ($P < 0.001$) interaction between treatment, level of tension and muscle location. A trend existed in the ovine rib in which the decrease in shear force declined as the level of tension employed increased. This trend was much less pronounced in the ovine loin sample.

Postmortem aging had a very highly significant effect ($P < 0.001$) on shear force values of rib and loin in experiments 1 and 2 and highly significant effects ($P < 0.01$) in experiments 3 and 4. These effects are illustrated in Figures 2 and 3. The improvement in tenderness with aging was parallel between tensioned and nontensioned samples in each experiment. The pattern of postmortem changes is in agreement with Goll et al. (1964) and Smith et al. (1971). The tenderness of the tensioned samples at 48 hr postmortem was equal to that of the control samples after 168–240 hr of aging. The interaction between aging period and muscle location was highly significant ($P < 0.01$) in experiments 1, 2 and 3 and significant ($P < 0.05$) in experiment 4. There was also significant interactions of aging, animal and treatment in experiments 1, 2 and 3. Also in experiments 1 and 2 there were significant interactions between aging, location and treatment.

Pre-rigor tension had the effect of reducing large variable shear values to lower values having much less variability. Least tender samples derived the most improvement from tensioning. However, relatively tender samples also exhibited varying amounts of improvement. Tensioning appears to reduce shear force to a base-line range which is only slightly related to the inherent tenderness of the contralateral nontensioned sample. Below this base-range, factors other than contraction state must exert their influence on shear force.

The application of pre-rigor tension was found to have a very highly significant effect ($P < 0.001$) on the fiber diameter of 48 hr postmortem ovine longissimus muscle. Means and standard deviations of fiber diameters are illustrated in Table 4. These findings are in agreement with those of Herring et al. (1965a). There was no significant difference in mean fiber diameter between the tension levels of the weighted and mechanical treatments although nontensioned values were quite similar and the tensioned values varied markedly. Mechanical tensioning appeared capable of reducing fiber diameter to a greater extent than the weighted treatment. The reason for the dissimilar patterns between responses of the two tensioning methods is not known, but it may be concerned with the differences in the way the two methods exert tension on the muscle.

Fiber diameters in rib and loin regions of ovine longissimus muscle were not found to be significantly different in experiments 1 and 2. Since fiber diameter has been shown to be highly correlated to

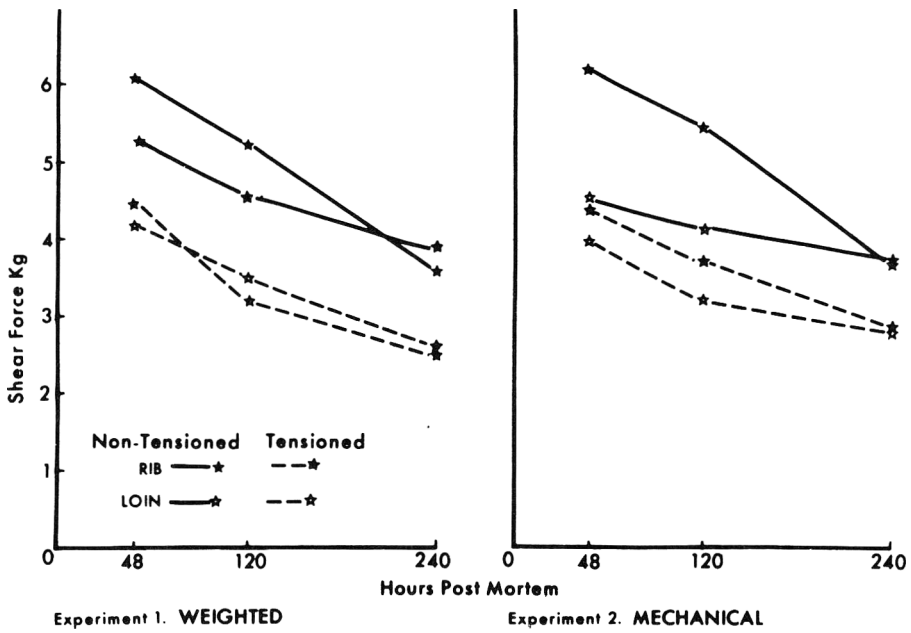

Fig. 2–Effects of tension, muscle location and postmortem aging on shear force values in experiments 1 and 2.

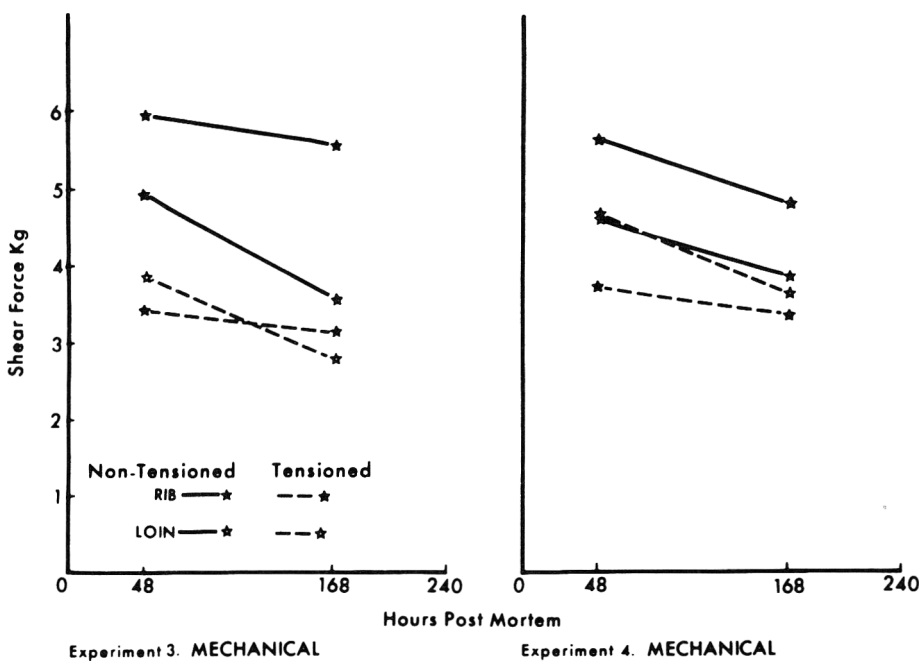

Fig. 3–Effects of tension, muscle location and postmortem aging on shear force values in experiments 3 and 4.

shear force, it is surprising that fiber diameter did not vary between the rib and loin regions of experiment 2 as very highly significant shear force differences were detected between these locations. Evidently factors other than fiber diameter were responsible for the observed differences.

Sarcomere lengths of ovine muscle were observed to be very highly significantly affected ($P < 0.001$) by tensioning the muscle pre-rigor. These results are in agreement with other workers, Eisenhut et al. (1965), Herring et al. (1965a), Hostetler et al. (1970; 1972) and Quarrier et al. (1972). However, no differences could be detected among the tension levels of either the weighted or mechanical treatments. Table 5 demonstrates that sarcomeres were lengthened appreciably when exposed to the lowest tension level of each treatment, but were not significantly affected beyond that point.

Sarcomere lengths were found to differ significantly ($P < 0.05$) between rib and loin region in experiment 2, but not experiment 1. Table 5 reveals that sarcomeres are longer in the loin than in the rib region for both experiments 1 and 2. The lack of significance in experiment 1 appears mainly due to the fact that rib and loin differences of tensioned samples varied in a direction opposite to that observed in the nontensioned samples.

The interactions of weighted treatment with muscle location and mechanical treatment with muscle location were both very highly significant ($P < 0.001$). It can be seen from Table 5 that pre-rigor tension produced larger increases in sarcomere length in the rib than in the loin region. Rib sarcomeres were initially shorter than sarcomeres in the loin region, but following tension treatment no significant difference was noted between them.

Sarcomere lengths were not greatly affected by postmortem storage up to 240 hr. In most cases only minor increases or decreases in sarcomere length were observed during postmortem aging. However, tensioned rib samples of experiment 1 and tensioned and nontensioned loin samples of experiment 2 displayed substantial shortening of sarcomeres with aging. The inconsistent response of the sarcomeres of the rib and loin locations to aging was reflected in significant ($P < 0.05$) aging and location interactions in both experiments 1 and 2.

Simple correlation coefficients between fiber diameter, shear force and sarcomere length at 48 hr postmortem for experiments 1 and 2 are given in Table 6. The correlations were in general agreement but slightly lower than reported by Herring et al. (1965a) and Quarrier et al. (1972).

Means of the percent visible Z lines for the tension and aging combinations are

Table 4—Means and standard deviations of fiber diameters of ovine longissimus muscle

Tension method	Experiment 1 Weighted			Experiment 2 Mechanical		
Tension level	4.5[a]	9.0	13.5	5[b]	10	15
			Nontensioned			
Rib	45.41[c]	43.81	42.64	44.12	42.33	44.15
	(2.47)[d]	(1.94)	(2.21)	(2.89)	(2.92)	(3.90)
Loin	43.11	44.81	42.99	42.91	43.13	43.40
	(2.42)	(1.71)	(1.08)	(2.84)	(1.48)	(1.31)
			Tensioned			
Rib	39.83	36.77	39.74	38.11	36.87	34.26
	(2.97)	(1.22)	(1.02)	(3.76)	(4.38)	(1.39)
Loin	38.41	39.73	39.10	37.08	36.11	34.85
	(3.67)	(2.34)	(3.17)	(4.29)	(2.32)	(1.67)

[a] Weight load in kg
[b] Percent stretch
[c] Microns
[d] Standard deviation in parenthesis

Table 5—Means and standard deviations of sarcomere lengths of ovine longissimus muscle

Tension method	Experiment 1 Weighted			Experiment 2 Mechanical		
Tension level	4.5[a]	9.0	13.5	5[b]	10	15
			Nontensioned			
At 48 hr						
Rib	1.64[c]	1.71	1.78	1.69	1.69	1.69
	(0.06)[d]	(0.06)	(0.18)	(0.05)	(0.03)	(0.06)
Loin	1.99	1.87	1.80	1.97	2.01	1.96
	(0.05)	(0.07)	(0.08)	(0.10)	(0.03)	(0.12)
			Tensioned			
Rib	2.06	2.30	2.31	2.06	2.29	2.20
	(0.14)	(0.16)	(0.03)	(0.19)	(0.15)	(0.34)
Loin	2.00	1.98	2.09	2.15	2.28	2.07
	(0.08	(0.16)	(0.10)	(0.17)	(0.05)	(0.27)
			Nontensioned			
At 240 hr						
Rib	1.67	1.68	1.68	1.67	1.75	1.72
	(0.12)	(0.05)	(0.05)	(0.06)	(0.09)	(0.19)
Loin	1.93	1.88	1.76	1.87	1.91	1.89
	(0.11)	(0.09)	(0.06)	(0.11)	(0.18)	(0.10)
			Tensioned			
Rib	1.96	2.13	2.04	1.97	2.23	2.19
	(0.18)	(0.11)	(0.22)	(0.20)	(0.17)	(0.27)
Loin	2.09	2.06	2.06	2.04	2.17	2.09
	(0.02)	(0.09)	(0.11)	(0.13)	(0.15)	(0.17)

[a] Weight load in kg
[b] Percent stretch
[c] Microns
[d] Standard deviation in parenthesis

Table 6—Correlation coefficients between fiber diameter, sarcomere length and shear value at 48 hr postmortem

Variables	Correlation coefficient Exp 1	Exp 2
Fiber diameter vs. shear force	0.58**	0.36*
Fiber diameter vs. sarcomere length	−0.83**	−0.64**
Sarcomere length vs. shear force	−0.51**	−0.48**

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

presented in Table 7. Tensioning the myofibrils appeared to increase nontensioned and tensioned values as both aging periods were highly significant ($P < 0.01$) and the difference between aging periods was also highly significant ($P < 0.01$). These results agree with reports of others including Davey and Gilbert (1969).

It is concluded that weighted and mechanical methods effectively tension longissimus muscle in intact bovine and ovine carcasses and improve tenderness significantly as measured by the decrease in shear force value. It has been demonstrated that this can be done with only a moderate amount of force and without severing of fasciae, tendon or vertebra.

Tensioned carcasses at 48 hr exhibited tenderness of longissimus muscle equivalent to that of contralateral sides that had been aged for 168–240 hr. Longissimus muscle of tensioned carcasses also improved in tenderness at the same rate as nontensioned carcasses when they were aged for longer periods. Longissimus muscle of tensioned carcasses also exhibited longer sarcomere lengths, smaller fiber diameters and a reduction in the animal-to-animal variability in tenderness. Therefore tensioning by weighted or mechanical method appears to be very practical for commercial application of carcasses conventionally suspended by a hind leg.

Table 7—Percent visibility of Z lines of ovine longissimus muscle[a]

	Postmortem aging	
	48[b]	240
Nontensioned	50	30
Tensioned	69	54

[a] Percent of Z lines visible
[b] Hours postmortem

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TENDERNESS VARIATION IN OVINE LONGISSIMUS MUSCLE

INTRODUCTION

TODAY'S CONSUMER is concerned about the palatability of lamb. Tenderness plays a major role in palatability. Investigations with lamb by Stouffer et al. (1958), Batcher et al. (1962), Carpenter et al. (1964) and Carpenter and King (1965) have reported low relationships of marbling and other traits with tenderness. Improvement of tenderness in lamb through tension has been reported by McCrae et al. (1971) and Quarrier et al. (1972). Carpenter and King (1965) demonstrated that core position was related to tenderness in lamb. Similar variations in core position studies have been reported by Alsmeyer et al. (1965), Carpenter et al. (1968) and Hostetler and Ritchey (1964) for pork and beef.

The present study considered the variation in shear value at three core positions within lamb rib and loin chops and how these relationships were affected by pre-rigor tension and postmortem aging.

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MATERIALS & METHODS

31 LAMBS of approximately 9–12 months of age, and ranging in live weight from 35–47 kg, were utilized in three experiments. After the lambs were slaughtered the carcasses were split longitudinally through the vertebral column and each side was suspended vertically by the hind shank. Tension was applied to one side of the split carcass immediately after slaughter by either weighted or mechanical means, while the contralateral side was held as a nontensioned control. Other aspects of these experiments were described previously by Buege and Stouffer (1974).

Weighted tensioning was accomplished in experiment 1 by attaching weight loads to the muscle and its accompanying vertebral column at the level of the sixth thoracic vertebra by means of either an s-type hook or a bacon hanger. Although tensioned sides were allotted to three different weight treatments, there were no significant differences of shear value, sarcomere length or fiber diameter among the three treatments. Therefore all weighted groups were pooled for this study.

Mechanical tensioning in experiments 2 and 3 was achieved through the use of a device whose ends were anchored in the longissimus muscle in the area of the sixth rib, and near the anterior tip of the ilium immediately after the carcasses were split. Tension was exerted and maintained until 24 hr postmortem. In experiment 2, three levels of tension were utilized but there was no significant difference in shear force, sarcomere length or fiber diameter due to level of tension as reported by Buege and Stouffer (1974). Therefore all mechanical tensioned groups were pooled for this study. In experiment 3, all tensioned sides were subjected to approximately 12% stretch by mechanical tension.

In experiments 1 and 2, the ninth thoracic through the next to last lumbar vertebrae were severed through the body and spinnous processes to facilitate tensioning of the longissimus muscle. Comparison of shear values of tensioned muscle of experiments 1, 2 and 3 revealed no significant difference in response, indicating that severence of the vertebrae contributed little to the objective tenderness scores of tensioned muscle. For this reason, this parameter was not taken into consideration in the presentation of this paper.

Between 60 and 120 min postmortem tensioned and nontensioned sides were placed in a 2°C cooler. In the interval 24–48 hr postmortem, sections of rib and loin from the ninth thoracic to the next-to-last lumbar vertebra were removed from both sides. Samples 3.2 cm thick for tenderness determinations were removed from the extreme ends of the excised muscle segment at 48, 120 and 240 hr postmortem to provide rib and loin samples in experiments 1 and 2. Between each tenderness sample, a 0.7 cm histological sample was removed for use in another study. Tenderness samples were secured in a similar manner in experiment

Table 1—Description of carcass treatments and means and standard deviations for shear force value[a]

Expt	Number of carcasses	Tension method	Vertebra status	Aging period (hr)	Rib		Loin	Loin
					Nontensioned	Tensioned	Nontensioned	Tensioned
1	12	Weighted	Severed	48	6.08	4.49	5.30	4.19
					(1.24)	(0.45)	(1.26)	(0.48)
				120	5.24	3.20	4.59	3.51
					(0.89)	(0.34)	(0.87)	(0.58)
				240	3.57	2.47	3.92	2.60
					(0.77)	(0.24)	(1.02)	(0.40)
2	12	Mechanical	Severed	48	6.20	4.39	4.52	4.00
					(0.74)	(0.89)	(0.78)	(0.38)
				120	5.48	3.72	4.14	3.20
					(1.35)	(0.60)	(0.76)	(0.43)
				240	3.64	2.84	3.66	2.77
					(0.83)	(0.48)	(0.67)	(0.50)
3	7	Mechanical	Intact	48	5.96	3.44	4.95	3.88
					(2.01)	(0.78)	(0.70)	(0.46)
				168	5.58	3.15	3.57	2.79
					(1.13)	(0.41)	(0.61)	(0.24)

[a]Shear force in kg; standard deviation in parenthesis

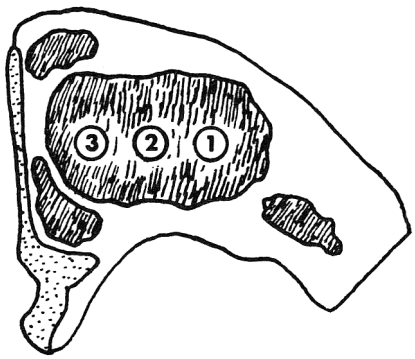

Fig. 1–Positions of cores for determination of shear force.

3 with the exception that samples were removed at 48 and 168 hr postmortem only, and no histological samples were removed. Between samplings, the muscle sections were stored at 1.5°C in impermeable Cry-O-Vac bags.

Within several hours after excision, all tenderness samples were cooked in hot beef tallow (135°C), with their internal temperature monitored by means of thermocouples on a Honeywell-Brown Type 602 Recording Thermometer. Samples were removed from the hot fat when they reached an internal temperature of 60°C. Following removal from the hot fat, internal temperatures were noted to rise 8–12 degrees before starting to decline.

Cooked samples were allowed to equilibrate in a 4°C cooler for 4–8 hr before being evaluated for tenderness. Three cores of tissue 1.27 cm in diameter were removed from the lateral, medial and dorsal areas of each of the rib and loin samples. Figure 1 presents the coring patterns used in the rib and loin samples. Position 1 included samples from the lateral position of the longissimus muscle while position 3 was the position most dorsal to the vertebral column. The middle core sample is referred to as the medial position. Each core was sheared transversely three times in a Warner-Bratzler Shear.

The data were analyzed by an analysis of variance and F-test; means and standard deviations were computed for each of the variables studied. In certain instances, mean squares and denominator degrees of freedom for tests were synthesized following the procedure of Satterthwaite (1946). In appropriate circumstances, means were more closely examined using the least significant difference procedure of Steel and Torrie (1960).

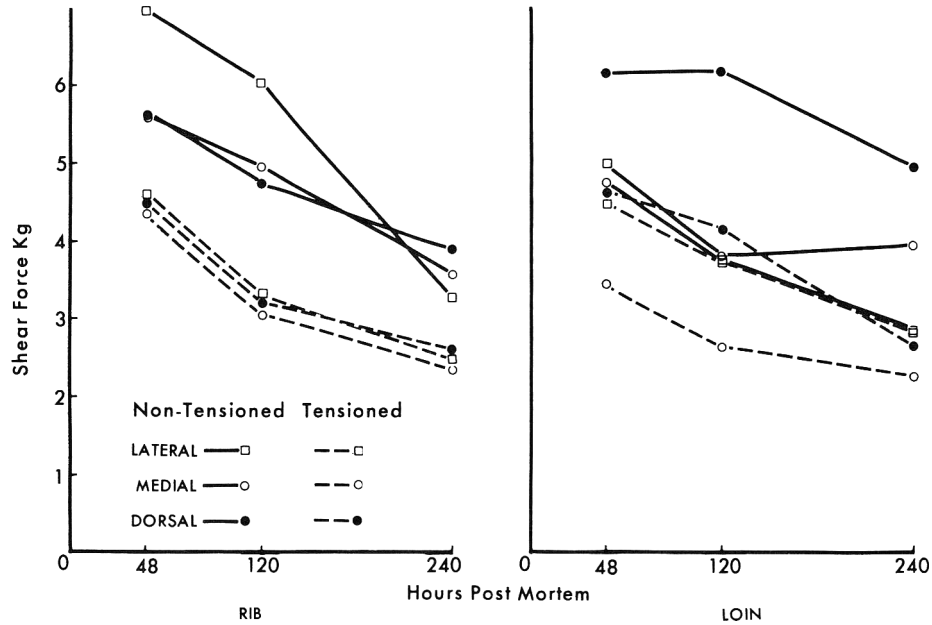

Fig. 2–Effects of aging on shear force value by core position for rib and loin in experiment 1.

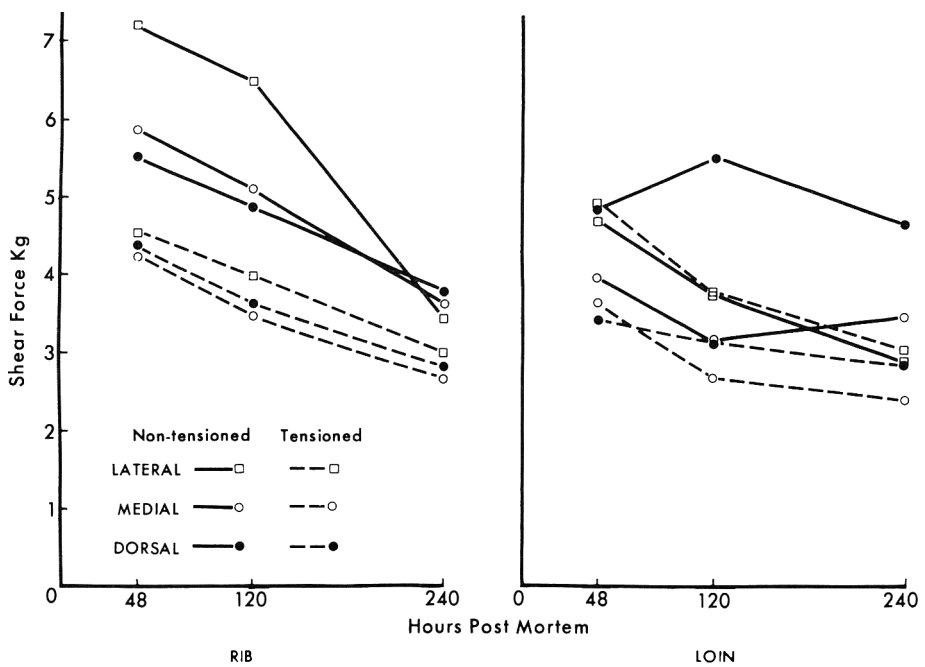

Fig. 3–Effect of aging on shear force value by core position for rib and loin in experiment 2.

RESULTS & DISCUSSION

A DESCRIPTION of carcass treatments as well as means and standard deviations for shear force values of rib and loin chops over the various aging periods covered in these experiments are indicated in Table 1. The average shear force for each sample in each location of all three experiments showed a gradual decrease with aging. The shear force differences due to tension treatment were significant as reported by Buege and Stouffer (1974) in another paper. With few exceptions the standard deviation for each sample decreased with aging. Both methods of tension application, weighted and mechanical, and all levels of tension applied within a method had similar effects on tenderness improvement.

Because shear force data were collected in a very systematic manner in which the identity of each core was maintained, it was possible to study within muscle tenderness variations by evaluating individual core values by position.

The changes in shear value for each core position of rib and loin samples from tensioned and nontensioned chops over aging periods are illustrated in Figures 2, 3 and 4. In all three experiments, core position was found to have a very highly significant effect ($P < 0.001$) upon the shear force of ovine longissimus muscle.

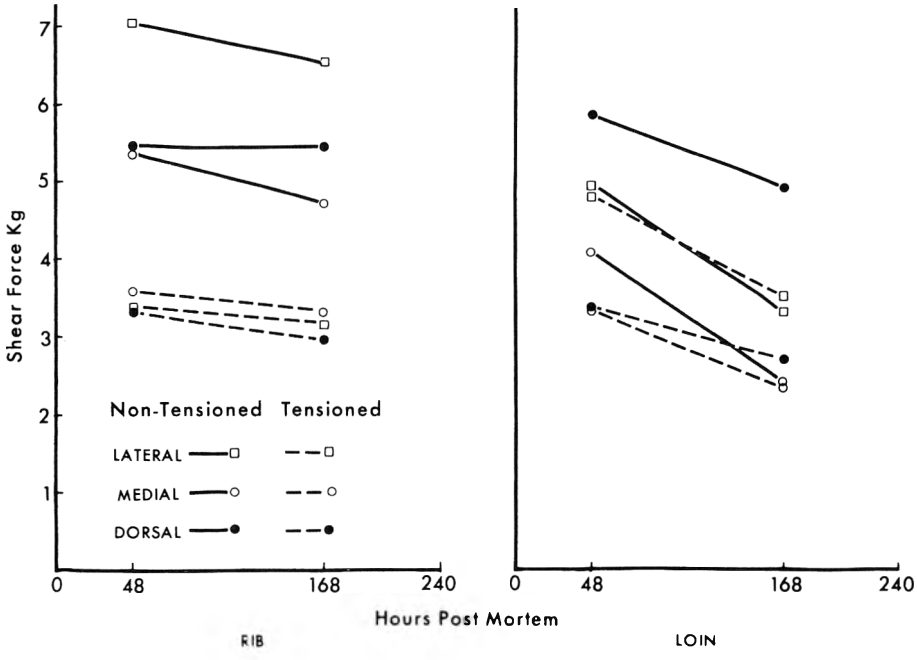

Fig. 4—Effect of aging on shear force value by core position for rib and loin in experiment 3.

The tension treatment also had a very highly significant effect ($P < 0.001$) upon shear force in experiments 1 and 2 and a highly significant effect ($P < 0.01$) in experiment 3. Likewise, the interaction between tension treatment and core, and among location, tension treatment and core were very highly significant ($P < 0.001$) in all three experiments.

The data demonstrate that differences in shear values existed among cores. The patterns of these differences were not the same for rib and loin regions, and were greatly altered by tension treatment. In nontensioned rib chop samples, the lateral core had the greatest shear values, while the medial and dorsal cores were similar and had lower shear values. This finding does not agree with the report of Carpenter and King (1965) who observed in lamb rib chops cooked 48 hr postmortem by deep fat frying that the dorsal core possessed the greatest shear values in the rib. They also found the lateral core to have the smallest shear values, with the medial core being intermediate in shear value between lateral and dorsal cores. Similar studies with beef have produced different opinions concerning the gradient of tenderness within longissimus muscle. Tuma et al. (1962) and Smith et al. (1969) found the lateral position to be the least tender which is in agreement with our findings while Cover et al. (1962) and Sharrah et al. (1965) reported the lateral position to be the most tender which is in disagreement with these results.

In the nontensioned loin region the dorsal core was higher in shear values than the lateral and medial cores, which were comparable. It is possible that the greater shear values of the dorsal core of the loin region were due to excessive rigor shortening in this region. Possibly the vertebral column bears most of the strain in this area, thereby leaving the muscle of this region less tensioned and more susceptible to shortening. Since carcasses were split through the vertebral column in this study, rate of cooling may also have been more rapid at this location, thus enhancing the cold shortening effect.

Pre-rigor tension treatment was observed to affect the cores differently at the two muscle locations. In the rib the tension treatments reduced shear force in each core to a similar level with a slightly greater effect in the lateral area. However in the loin region, tension treatment had no effect on the lateral core while it lowered the shear force of the medial core only slightly, except in experiment 1 where the effect was more pronounced.

Table 2—Comparison of shear force means within a core sample[a]

Expt	Shear position 1[b]	2[c]	3[c]
1	3.95	4.14	4.19
2	3.89	4.11	4.13
3	3.78	4.26	4.25

[a] Shear force in kg
[b] Center of intact core
[c] Center of half cores resulting from initial shear (1)

Tension treatment profoundly decreased the shear force of the dorsal core. These data suggest that in the conventionally hung carcass the longissimus muscle in the lateral position of the loin region is under tension, due to its attachment to the tubera coxae crest of the ilium, while the balance of the longissimus muscle is allowed to shorten and become less tender with rigor. Overall, tension treatment decreases the magnitude of the differences among cores, making the muscle more uniform in tenderness. These data add further evidence to support observations of Eisenhut et al. (1965), McCrae et al. (1971) and Quarrier et al. (1972) that differences in muscles of intact carcasses are due to the degree of stretch or slack imposed on them by their attachments.

Pre-rigor tensioning of longissimus muscle by weighted or mechanical methods reduced the mean and variation of the 48 hr shear values of all rib core samples to a fairly uniform tenderness that was about equal to the control samples at 168–240 hr. The tensioned loin samples were improved but not as markedly. There was essentially no change in the lateral core position, some improvement in the medial position and great improvement in the dorsal position of loin samples.

It was possible in the three experiments in this study to examine whether shear force varied within individual muscle cores. Shear 1 was made approximately through the center of the core's length, while shears 2 and 3 were made on the resulting half-cores, in random order. Table 2 contains the means of the three core shears of experiments 1, 2 and 3. The effect of shear position on shear force was highly significant ($P < 0.01$) for experiment 1, very highly significant ($P < 0.001$) for experiment 2, and significant ($P < 0.05$) for experiment 3. Mean separation analysis revealed that in all 3 experiments shear 1 was significantly different ($P < 0.01$) from shears 2 and 3, but no significant differences existed between shears 2 and 3. Since shears 2 and 3 were randomly chosen half-cores, it was expected that they would not vary greatly. Lower values of shear 1 probably reflect its shorter exposure to heat during cooking, in contrast to areas closer to the surface of the ovine tenderness samples. Ritchey and Hostetler (1965) noted that heating bovine longissimus muscle to higher temperatures resulted in increased shear values.

It is evident that fairly definite and consistent patterns of tenderness occur in rib and loin regions of longissimus muscle of nontensioned ovine carcass sides suspended by the hind shank and placed in a cooler at 2°C 60–120 min after slaughter. Pre-rigor tension improves tenderness in most areas except where optimum tension already exists i.e., lateral position in

loin. Tenderness improvement by this method apparently is independent of the changes that occur in aging up to 240 hr since tenderness improvement of both nontensioned and tensioned samples follow parallel patterns with aging as illustrated in Figures 2, 3 and 4.

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THE AROMA OF CANNED BEEF: PROCESSING AND FORMULATION ASPECTS

INTRODUCTION

THE CHEMICAL and sensory aroma properties in canned beef, heated for different times at 121°C, and the relations between such sets of data have been analyzed and described by us (Persson and von Sydow, 1973; Persson et al. 1973a, b).

It was concluded that the off-flavor in canned beef was due to the formation of certain aldehydes and sulfur compounds.

The purpose of this part of the investigation was to explore the influence of processing parameters and variation in formulation on the aroma, especially to investigate the possibilities to lower the concentrations of the off-flavor producing compounds. In addition, the aroma changes during storage were investigated.

Luh et al. (1964) compared conventionally sterilized meat with aseptically packed HTST-sterilized meat and found a lower amount of hydrogen sulfide and less off-flavor in the latter. Sörman and Kozárová (1969) found that a better sensory quality was obtained in canned pork when applying HTST-sterilization. The sensory changes taking place during storage of canned meat have been described by Leistner and Wirth (1963). They found that the typical meat aroma disappeared and that a stale, sour flavor developed during storage. The appearance became lighter in color and more reddish.

Investigations concerned with improvements of the aroma of canned beef by changing the formulation have not been described in the literature. However, Kazeniac (1961) has found that arginine and carnosine decreased the concentrations of 2,3-butanedione and unsaturated aldehydes in canned chicken meat. Sato and Hegarty (1971) and Sato et al. (1973) have investigated the influence of some additives on the warmed-over flavor in cooked meat. They found that for this flavor problem dihydroxymaleic acid and furmaric acid had no influence, but a mixture of a sugar, e.g., glucose or lactose, and an amino acid, e.g., glycine, lysine or leucine, evidently decreased the warmed-over flavor. Furuhashi and Ayano (1971a, b) found that the stale

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Table 1—Storage experiments performed in this investigation. Storage temperatures: 20°C[a]

Expt. No.	Packaging materials, etc.	Heat treatment: F_c-value	Heat treatment: Retort temp.	Formulation[b]	Storage times (months)	Chemical analyses: Headspace samples: FID[c]	Chemical analyses: Headspace samples: S-FPD[c]	Chemical analyses: VFA[d]	Sensory analyses: Odor quality	Sensory analyses: Appearance
1	Unlacquered can (HD-tinplate) 73 X 55 mm	8	121°C	BFC	0, 6, 12, 18	+	+	+	+	+
2	Lacquered can (with Al-pigment) 71 X 56.5 mm	8	121°C	BFC	0, 6[e], 12, 18	+	+		+	
3	Lacquered can 73 X 55 mm	8	121°C	BFC	0, 6[e], 12, 18	+	+		+	
4	Unlacquered can (Heated without cover) 73 X 28 mm	10.8	121°C	BFC	0, 6, 10	+	+		+	
5	Unlacquered can (Heated without cover) 73 X 28 mm	10.8	131°C	BFC	0, 6, 10	+	+		+	
6	Unlacquered can 73 X 28 mm	10.8	131°C	BFC	0, 6, 10	+	+		+	
7	Flexible pouch of Al-foil laminate (thickness: 16 mm)	10.0	121°C	B	0, 2, 6, 10	+	+	+	+	+
8	Flexible polyamide pouch (thickness: 16 mm)	10.0	131°C	B	0, 2	+	+			

[a] For details about packaging materials and formulations, see text.
[b] B = Beef; BFC = Beef + pork back fat + carbohydrate.
[c] FID = Flame ionization detector; S-FPD = Sulfur specific flame photometric detector.
[d] VFA = Volatile fatty acid
[e] No sensory analyses of the 6 months' samples

flavor caused by aldehydes in cooked rice could be inhibited by adding certain amino acids (e.g., arginine and lysine) which reacted with the aldehydes.

The flavor of sterilized milk and possibly also canned meat can be improved by adding potassium iodate, which lowers the amount of thiol-groups (Samuelsson, 1969). Jocelyn (1972) discusses in general how the SH-group reacts with compounds containing the structure:

$$-\overset{|}{C}=\overset{|}{C}-\underset{|}{C}=O.$$

Fumaric, maleic and sorbic acid are examples of compounds included in this group.

The influence on the aroma of different aseptic sterilization techniques and of HTST-sterilization has been investigated by us. The samples were packed in both rigid cans and in flexible pouches. The influence of some chemical compounds mentioned above has also been tested. Conventionally sterilized beef has been used as reference material. In addition, extensive storage experiments have been performed.

EXPERIMENTAL

Materials

Minced beef from top side round steaks (Biceps femoris and Vastrus labiralis) was used in two formulations:

(B) 79.3% beef + 20% H_2O + 0.7% NaCl

(BFC) 61.3% beef + 13% minced pork back fat + 5% carbohydrate (starch) + 20% H_2O + 0.7% NaCl.

For further details see Persson and von Sydow (1973).

HTST-sterilization

Two packaging materials were used in parallel: deep drawn unlacquered tinplate cans (73 × 28 mm) and sterilizable flexible pouches. The flexible pouches used at 115° and 121°C were laminated with the following layers: polyalkylene (inner), aluminium foil, polyester (outer) (from Continental Can Co.). Size: 185 × 130 mm. At 131°C a heat sealable all nylon film of food quality (0.05 mm) resistant to temperatures up to 180°C was used (from Helinos, Skärhamn, Sweden). Size: 210 × 148 mm. As the latter film has a relatively high water permeability the pouches intended to be used in the storage experiments were packed in aluminium foil pouches after sterilization in order to minimize water loss. Both types of pouches were filled in vertical-slot racks giving a uniform thickness (16 mm). The pouches contained 190g of material. They were sealed under vacuum. Formulation: B (see above).

The cans were processed in small retorts designed for TDT investigations (National Canners Association, 1968) and the pouches in a larger type of retort. The pouches were sterilized in horizontal position in a specially designed rack made of thin wire netting.

In both types of retorts pressure and temperature of the vapor were controlled by a Honeywell Pneumatic controller. To protect the seals and prevent rupturing of the pouches during heat processing and water cooling, superimposed air was used. The temperature and the F_c-value in the cans were measured as described by Persson and von Sydow (1973). The temperature measurement in the pouches was performed by using a similar device designed for this purpose (Ellab Instruments, Copenhagen, Denmark, thermocouple: 1.0 mm thick, 100 mm long: TCK 8; packing gland: TCG 31; teflon guiding rod: TCG 32). The samples were processed to the following F_c values at the following retort temperatures:

F_c-value = 5; temp = 121°C
F_c-value = 10; temp = 115, 121, 131°C
F_c-value = 20; temp = 121°C

Meat sterilized in pouches was stored at +20°C for times up to 10 months and evaluated. The extent of this investigation is given in Table 1 (Expt. No. 7 and 8).

Aseptic canning

In this part of the investigation samples sterilized in sealed cans (normal) and in open cans ("aseptic") were compared.

Direct steam inlet. The beef samples were packed in unlacquered tinplate cans, 73 × 28 mm (see above) and sterilized with or without covers. The small retorts described above were used. The steam used was cleaned by passing it through an Adams TR filter with a Poro-Carbon tube (R.P. Adams Co., Inc., Buffalo 17, N.Y.) and through activated carbon. The steam cleaned in this way gave no off-flavor as measured by tasting the condensed water and by gas chromatography. Cooling to just below 100°C was performed by superimposed (1.5 kp/cm² = 147 k Pa) streaming nitrogen to avoid boiling. The cans without covers were sealed and then chilled in tap-water together with the reference cans. In the cans heated without covers there were no weight losses. The temperatures and the F_c-values were measured as described above. The following F_c-values and retort temperatures were applied:

F_c-value = 10.8; temp = 121°C and 131°C
F_c-value = 21.6; temp = 121°C and 131°C

For each one of the four heating conditions

Table 2—Concentrations, ppb (v/v) of 10 volatile compounds in the headspace gas of specimens of canned beef heated at three different time/temperature relations, corresponding to an F_c-value = 10[a]

		Retort temperature		
		115°C	121°C	131°C
Peak No.	Compound	Conc (ppb)	Conc (ppb)	Conc (ppb)
9	2-Methyl propanal	83	54	39
18	2-Methyl butanal	79	43	30
17	3-Methyl butanal	90	53	40
13a	2,3-Butandione	6.4	6.2	5.8
25	2-Ethyl furan	180	120	89
S2	Hydrogen sulfide	6900	6400	4400
S4	Methyl mercaptan	1400	1200	780
S6	Dimethyl sulfide	810	770	530
S12	2-Methyl thiophene	4.4	3.1	2.2
S13	3,5-Dimethyl-1,2,4-trithiolane	10	11	6

[a] Packaging material: Flexible pouches; Formulation: B = Beef

Table 3—Mean panel intensities and standard deviations (s) for preference value, and six odor qualities for canned beef heated at three different time/temperature relations, corresponding to an F_c-value = 10[a]

	Retort temperature							
	115°C			121°C			131°C	
Odor quality	Intensity	s	Sign. level for dif.	Intensity	s	Sign. level for dif.	Intensity	s
Preference value	5.4	0.3	–	5.4	0.5	–	5.5	0.4
Odor strength	5.9	0.2	0.10	5.4	0.4	0.10	4.9	0.3
"Retort flavor, canned beef off-flavor"	4.8	0.4	–	4.6	0.7	0.05	3.5	0.4
Sulfurous	3.3	0.3	–	3.4	0.6	0.02	2.3	0.2
Burnt	3.7	0.3	0.01	3.0	0.2	0.02	2.3	0.4
Meaty (cooked)	5.0	0.6	0.20	5.5	0.3	–	5.3	0.8
Sharp, pungent	3.7	0.2	0.02	3.1	0.3	0.01	2.4	0.2

[a] Packaging material: Flexible pouches; Formulation: B = Beef

Table 4—Concentrations, ppb (v/v), of 11 volatile compounds in the headspace gas of specimens of canned beef heated at 121°C to an F_c-value = 10.8 or an F_c-value = 21.6. Comparison between specimens heated in cans with and without (aseptic canning) covers (see text)

	Formulation B[a]				Formulation BFC[a]	
	F_c-value = 10.8		F_c-value = 21.6		F_c-value = 10.8	
Compound	With cover Conc (ppb)	Without cover Conc (ppb)	With cover Conc (ppb)	Without cover Conc (ppb)	With cover Conc (ppb)	Without cover Conc (ppb)
Pentanal	5.7	4.3	5.7	3.4	32	14
Hexanal	15	7.5	16	6	90	44
2-Methyl propanal	75	39	84	43	75	38
2-Methyl butanal	53	26	72	41	50	30
3-Methyl butanal	53	27	74	42	84	45
2,3-Butandione	3.8	2.0	3.2	2.2	5.3	2.9
2-Ethyl furan	380	150	420	170	200	190
Hydrogen sulfide	6100	5300	7000	4000	4900	4700
Methyl mercaptan	1400	1100	1700	900	1100	900
Dimethyl sulfide	690	350	790	210	370	270

[a] B = Beef; BFC = Beef + pork back fat + carbohydrate

two formulations B and BFC (see above) were processed in cans with and without covers. The storage changes at 20°C were investigated for some of the combinations: Expt. No. 4, 5 and 6 in Table 1.

Heating under superimposed nitrogen. During sterilization the beef samples were packed in stainless steel cans, 71 × 51 mm (wall thickness = 1 mm) containing 190g, specially constructed for this purpose. The cans were placed at 48 mm depth in an oil bath. When heated with covers the cans were sealed with a stainless steel lid (flange coupled with six bolts and with a combined Teflon/silicon-rubber lip-packing).

The cans heated "without" cover were flange coupled in the same way as above to the open bottom of a gas-proof stainless steel cylinder (200 × 400 mm). To avoid boiling 1.6 kp/cm² (= 157 k Pa) overpressure was kept in the cylinder using streaming nitrogen (1200 ml/min). The nitrogen inlet was at the bottom of the cylinder. The purpose of using streaming nitrogen was to remove the aroma compounds developed during sterilization. The temperature was measured by a specially designed thermocouple which was inserted into the cans through a Swagelok coupling in the lid or in the cylinder. The F-value was determined in the center of the cans. The samples were heated for 75 and 105 min to an F-value = 2.1 and 5.7, respectively. When heating with lid the oil bath temperature was 122°C. Heating "without" lid the oil bath temperature had to be 130°C, the higher temperature was necessary because heat was removed by the nitrogen flow. Two formulations, B and BFC (see above) were used for every combination. After the heat treatment the cans were chilled (still under superimposed nitrogen) in tap water for 20 min. The samples were transferred to unlacquered tinplate cans, 73 × 55 mm, sealed and stored at −90°C until analyzed. The weight loss in the cans heated "without" covers was about 20%. No storage investigations were performed with these samples.

The influence of ingredients

The additives were dissolved in water and added to beef before sterilization (formulation B, see above). The specimens were packed in electrolytical tinplate cans (size 73 × 28 mm) and heated for 45 min at 121°C (F_c = 20). The following amino acids were used in the concentration range 0.5–1.5%: L-lysineHCl, L-arginineHCl and L-histidineHCl (Merck, for biochemistry). Other additives used were: potassium iodate (Merck, pro analysis) in the concentration range 0.005–0.1%; potassium chlorate (Merck, pro analysis) at the concentration 0.05%; disodium fumarate (Fluka, puriss.) in the concentration range 0.1–1.0%; disodium maleate (Fluka, puriss.) in the concentration range 0.01–1.0%; and potassium sorbate (Hoechst) in the concentration range 0.3–1.0%.

Storage changes

The storage experiments performed are given in Table 1. Expt. No. 4–8 have been described above. In Expt. No. 1–3 different types of cans have been compared:

No. 1: Unlacquered hot dip (HD) tinplate (1.25 lb/bb) cans. Size: 73 × 55 mm, holding 195g of material.

No. 2: Electrolytical (El 0.50 lb/bb) tinplate cans. Size: 71 × 56.5 mm, holding 195g of material. Treated with epoxy lacquer mixed with aluminium pigment (0.03g Al/can); stripe lacquered with epoxyphenol lacquer on the inside.

No. 3: The same as No. 2, but without

Table 5—Mean panel intensities for preference value and seven odor qualities for canned beef heated at 121°C to an F_c-value = 10.8 or an F_c-value = 21.6. Comparison between specimens heated in cans with and without (aseptic canning) covers (see text)

	Formulation B[a]						Formulation BFC[a]		
	F_c-value = 10.8			F_c-value = 21.6			F_c-value = 10.8		
Odor quality	With cover Intensity	Sign. level for dif.	Without cover Intensity	With cover Intensity	Sign. level for dif.	Without cover Intensity	With cover Intensity	Sign. level for dif.	Without cover Intensity
Preference value	5.9	–	6.5	5.2	0.20	5.8	3.8	0.20	4.9
Odor strength	5.2	–	5.1	5.5	–	5.2	5.1	0.05	4.0
"Retort flavor, canned beef off-flavor"	3.0	0.10	2.5	3.2	–	2.9	2.5	–	2.1
Sulfurous	3.2	–	3.0	3.5	–	3.0	2.5	–	2.1
Sharp, pungent	2.9	–	2.8	3.4	–	3.0	2.9	0.05	2.4
Sickly	1.9	0.20	1.7	2.4	0.02	1.6	3.0	–	3.1
Fragrant	3.0	–	3.4	3.0	0.20	3.4	1.6	–	1.7
Sour, acid, etc.	2.3	0.05	1.8	2.5	0.05	1.9	2.2	–	2.2

[a] B = Beef; BFC = Beef + pork back fat + carbohydrate

aluminium pigment and with the size: 73 × 55 mm.

The formulation used was BFC (see above). The samples were heat treated at 121°C to an F_c-value = 8 in the retorts described above. The extent of these storage experiments and the analyses performed are given in Table 1 (Expt. No. 1, 2 and 3).

Some of the samples stored for 18 months in Expt. No. 1 were reheated at 121°C for 50 min.

Analysis

All samples described above were stored at −90°C until analyzed.

Gas chromatography. Details about the analytical procedure for analyzing the headspace gas are given by Persson and von Sydow (1973). The quantitative determination of the volatiles in the headspace gas of canned beef was performed using a sampling technique making it possible to analyze large volumes (up to 500 ml) and using an open tubular column gas chromatographic technique. The compounds were detected by a flame ionization detector or a sulfur specific flame photometric detector. To analyze the sulfur compounds a sampling device entirely made from glass and Teflon and a packed glass column were used. In this investigation the glass column was packed with Chromosorb G AW DMCS 80/100 mesh coated with 4% Igepal CA 630 as in the earlier investigation. However, before conditioning about 50 ml of a 10% solution of didecyl phtalate in acetone was forced through the column under a slight nitrogen pressure (Jansen et al., 1971). This procedure gave a column with better resolution and less hydrogen sulfide absorption.

The amounts of the compounds were determined in absolute concentrations (ppb) (Persson and von Sydow, 1973).

The components were identified by analyzing the headspace gas of a low temperature distillate on a combined gas chromatograph-mass spectrometer. Ninety-five compounds were identified.

In some samples the volatile fatty acids (VFA) were determined. The VFA fraction was isolated by steam distillation. The distillation apparatus used consisted of a 250 ml distillation flask with inlet for steam, a 300 mm vigreux column, a condensor and a 500 ml receiver flask (Halvarson, 1972). 25g of the meat sample was homogenized with 25 ml distilled water and then transferred into the distillation flask together with 24g $MgSO_4 \cdot 7\,H_2O$ and 30 ml 0.05N H_2SO_4. Before the distillation was started 6 ml 0.1N NaOH solution was pipetted into the receiver, which was kept at 0°C during the distillation. The steam distillation was stopped when 300 ml condensate had been collected. The distillate was evaporated under atmospheric pressure until 1 ml remained. This rest was added to an ampoule and freeze dried. After the freeze drying 500 µl ethanol (99.5%) and 100 µl conc HCl were transferred into the ampoule which was immediately sealed and then heated for 2 hr at 100°C to obtain the ethyl esters of the VFA (Halvarson, 1972). The ethyl esters were analyzed on a Perkin Elmer 900 gas chromatograph, with injector temperature 200°C, detector temperature 200°C and with the carrier gas (He) flow 30 ml/min. For the analysis of the ethyl esters of the C_1–C_4 acids a 3.2 mm × 3m Porapak Q column at 180°C was used and for analyzing the ethyl esters of the C_5–C_{10} acids a 3.2 mm × 4m 15% FFAP column was used. In the latter case the column temperature was programmed from 80–190°C with 4°C/min after 5 min initial constant temperature. The gas chromatographic system was calibrated using standard solutions of ethyl esters. The total yields of the different acids during steam distillation, esterfication and gas chromatography were determined by adding known amounts of the acids to meat samples. The accuracy was estimated to be about 25%.

Sensory evaluation. For details in procedure, see Persson et al. (1973a). The samples were evaluated using the odor quality technique: a panel was trained to recognize different odor notes in a food item and to estimate the intensities of these. Originally 28 odor notes were used, but in this investigation they have been reduced to 11 by studying the correlation matrix for the odor qualities given by Persson et al. (1973a) and by carrying out in-

Table 7—Influence on the concentrations (ppb) of hydrogen sulfide and methyl mercaptan in the headspace gas of canned beef by adding potassium iodate and the salts of fumaric, maleic and sorbic acid before sterilization[a]

Added agents	Conc (%)	Hydrogen sulfide (ppb)	Methyl mercaptan (ppb)
No additives	0	8300	1700
Potassium iodate	0.005	7400	1500
Potassium iodate	0.02	5500	1300
Potassium iodate	0.05	2100	800
Potassium chlorate	0.05	5100	1700
Disodium fumarate	0.1	5300	1700
Disodium fumarate	0.15	3900	1600
Disodium fumarate	0.30	2500	1200
Disodium fumarate	1.0	760	900
Disodium maleinate	0.06	3800	1400
Disodium maleinate	0.3	740	740
Disodium maleinate	1.0	130	140
Potassium sorbate	0.3	4700	1300
Potassium sorbate	1.0	2200	1100

[a] Formulation: B = Beef

Table 6—Influence on the concentrations (ppb) of aldehydes in the headspace gas of canned beef by adding L-lysineHCl, L-arginineHCl and L-histidineHCl before sterilization[a]

Added amino acids	No additive	L-lysine HCl	L-arginine HCl	L-histidine HCl
		Conc (%)		
Compound	0	1.5	1.5	1.5
Ethanal	890	620	790	890
Butanal	2.2	0.94	1.2	2.1
Pentanal	3.7	1.5	1.9	3.3
Hexanal	6.1	2.1	3.6	4.7
2-Methyl propanal	47	23	30	26
2-Methyl butanal	43	17	24	34
3-Methyl butanal	56	19	33	40

[a] Formulation: B = Beef

Table 8—Influence on flavor properties of canned beef by adding L-arginineHCl, the sodium salts of fumaric acid, maleic acid and sorbic acid, respectively, before sterilization[a]

Added agents	Conc (%)	Flavor properties: Meaty (cooked)	Burnt	"Retort flavor"	Preference value
No additive	0	5.0	5.0	5.0	5.0
L-ArginineHCl	1.5	7.0	3.0	3.0	7.4
Disodium fumarate	0.15	6.3	3.0	3.0	6.4
Disodium maleinate	0.06	6.0	3.0	3.0	5.9
Potassium sorbate	0.3	7.3	2.5	2.5	6.5
L-ArginineHCl + Disodium maleinate	1.5 + 0.15	6.3	3.5	3.0	7.4

[a] Formulation: B = Beef

dividual interviews with the panel members. The intensities of the odor notes were estimated using a 10-point scale. In addition, preference tests were performed. The appearance was estimated for some of the samples in the storage experiments using a scale where 0 = extremely light and 9 = extremely dark.

For the subexperiment investigating the effect of other ingredients, only an elucidatory sensory analysis was performed using five members from the odor quality panel and a reduced list of odor notes. Every sample was assessed twice. The samples were also tasted using the same descriptive words as when smalling them.

RESULTS

HTST-sterilization

In Table 2 the changes in the concentrations for some of the more important aroma compounds are shown. These were obtained when the retort temperature was 115°, 121° and 131°C at the same F_c-value (= 10), i.e., all three samples have obtained the same lethal heat treatment. The results shown were obtained with samples packed in flexible pouches. It can be seen that there was a marked decrease in the concentrations when the temperature was raised, especially in the HTST-sterilized (131°C) samples. The concentrations of the components in the latter sample were even lower than those obtained when heating to the F_c-value = 5 at 121°C. The sample heated to F_c-value = 20 at 121°C had roughly the same concentations of aroma components as the sample with F_c-value = 10 at 115°C. The samples packed in cans were not influenced by the HTST-sterilization to the same degree as when pouches were used although the tendency was the same. The can samples had higher concentrations of the aroma compounds than the corresponding samples sterilized in a pouch. This was due to the larger thickness of the cans and corresponding longer heating time (in mean 25%) which was necessary to reach an adequate center temperature. In Table 3 the changes of some odor qualities used are shown. It is evident that for these odor qualities there is a good correlation with the chemical data in Table 2. The sensory differences for samples in cans were, as expected, much smaller than for those obtained in pouches.

Aseptic canning

Direct steam inlet. Some of the chemical and sensory results obtained with this procedure are given in Tables 4 and 5. The concentrations of the aroma components were lower, especially for the aldehydes, when no cover was used (aseptic canning). These results correlate well with some of the odor qualities in Table 5. The differences in the odor qualities are in general not very pronounced comparing samples processed with or without lids. However, the preference value was higher for all samples without cover. Samples heated for a shorter time at 131°C were also included in this experiment and again, as mentioned above, no special HTST-effect was obtained with the cans of the size used.

Heating under superimposed nitrogen. The same tendency as with direct steam inlet was obtained in this experiment, but the differences were even less marked.

The influence of ingredients

It appears from Table 6 that addition of L-lysineHCl and L-argininеHCl had a pronounced influence on the concentrations of the aldehydes, which decreased to about 50%, while the influence of L-histidineHCl was smaller. In Table 7, it is shown how different compounds react with the two main sulfur compounds, hydrogen sulfide and methyl mercaptan. Potassium iodate had an obvious effect even at very low concentrations, while potassium chlorate had a smaller effect. Potassium iodate decreased the hydrogen sulfide concentration but on the other hand increased the amount of dimethyl sulfide and also the aldehydes. Thus this compound is not a suitable ingredient for canned beef. All the remaining compounds tested caused a marked decrease in the concentrations of the sulfur compounds. The influence on the sensory properties by some of these compounds is shown in Table 8. There is a positive effect on the flavor properties by using these ingredients. L-lysineHCl gave an off-flavor at the concentrations which were necessary to decrease the aldehyde concentrations.

Still better flavor properties were obtained when using combinations of L-argininеHCl and one of disodium maleate and disodium fumarate (Table 8).

Storage changes

In Table 9 the concentration changes are given for 11 aroma compounds obtained when storing canned beef (+ fat + carbohydrate) up to 18 months (Expt. No. 1 in Table 1). Except for dimethylsulfide and 2-ethylfuran there was a very marked decrease in concentrations during storage especially between 0 and 6 months. This is reflected in corresponding sensory data (Table 10). There is a marked parallel decrease in "retort flavor," "sulfurous," "burnt" and "meaty" as well as in the preference value. However, at the same time, the odor qualities "acid, sour" and "like whey butter" increased. The appearance changed towards a lighter, more reddish color. If the 18 months' sample were reheated for 50 min at 121°C much of the storage effect disappeared, but it was still evaluated as somewhat poorer than the 0 month's sample (Table 10). There were no significant differences in chemical or in sensory data when comparing the unlacquered cans (Expt. No. 1) with the lacquered ones (Expt. No. 2 and 3).

The stored samples packed in flexible pouches (Expt. No. 10 and 11 in Table 1) gave a quite analogous pattern in the chemical data (Table 11). However, the changes were more accelerated when pouches were used, especially in those sterilized at 131°C. The sensory changes in the samples packed in pouches and sterilized at 121°C are given in Table 12.

As can be seen from the sensory data (Tables 10 and 12) some odor notes (e.g., "acid, sour," "like whey butter") increased. These can be assumed to be associated with the presence of volatile fatty acids (VFA). The changes in con-

Table 9—Concentrations, ppb (v/v), of 11 volatile compounds in the headspace gas of stored canned beef, heated at 121°C to an F_c-value = 8[a]

	Storage time (months) at 20°C			
	0	6	12	18
Compound	Conc (ppb)	Conc (ppb)	Conc (ppb)	Conc (ppb)
Ethanal	2700	1400	1000	470
Hexanal	76	7.1	3.3	1.8
2-Methyl propanal	110	54	22	16
2-Methyl butanal	71	23	14	8.2
3-Methyl butanal	110	6.3	5.0	2.4
2,3-Butandione	10	6	2	0.6
2-Ethyl furan	250	290	330	160
Hydrogen sulfide	7200	1400	1300	1100
Methyl mercaptan	1300	640	640	540
Dimethyl sulfide	880	930	1100	900
Ethylene sulfide	14	1.6	2.2	0.1

[a] Formulation: BFC = Beef + pork back fat + carbohydrate; Packaging material: Unlacquered cans of HD-tinplate (73 X 55 mm). (Expt. No. 1 in Table 1)

Table 10—Mean panel intensities for preference value and nine odor qualities for stored canned beef, heated at 121°C to an F_c-value = 8[a]

	Storage time (months) at 20°C							
	0		6		12		18	18[b]
Odor quality	Intensity	Sign. level for dif.	Intensity	Sign. level for dif.	Intensity	Sign. level for dif.	Intensity	Intensity
Preference value	3.6	0.01	2.4	0.10	1.9	–	1.9	3.2
Odor strength	5.5	0.01	4.5	0.01	5.3	–	5.1	4.9
"Retort flavor, canned beef off-flavor"	2.5	0.01	1.4	–	1.2	–	1.3	2.4
Sulfurous	2.4	0.01	1.1	–	1.3	–	1.4	2.3
Burnt	2.2	0.05	1.4	–	1.6	–	1.6	2.4
Meaty (cooked)	4.3	0.01	2.2	–	1.8	–	1.8	3.3
Sickly	3.8	–	3.8	–	4.0	–	4.2	3.2
Acid, sour, etc.	3.1	0.05	4.4	–	5.0	–	4.8	3.3
Like whey butter	0.9	0.01	3.5	0.05	4.9	–	4.3	1.8
Appearance (0 = extr. light, 9 = extr. dark)	5.9	0.01	3.8	–	3.9	0.05	3.2	–

[a] Formulation: BFC = Beef + pork back fat + carbohydrate; Packaging material: Unlacquered cans of HD-tinplate (73 X 55 mm) (Expt. No. 1 in Table 1)
[b] Reheated for 50 min at 121°C

centrations of some of the VFA are given in Table 13 (Expt. No. 1 and 7 in Table 1). Most of the acids were present in concentrations close to reported odor thresholds. The amounts increased slowly during storage. The storage experiments corresponding to Expt. No. 2–6 in Table 1 all showed the same pattern both for chemical and sensory data as those described above (Tables 9, 10, 11 and 12).

DISCUSSION

HTST-sterilization

It is evident (Table 2) that a higher temperature and shorter heating time at a constant F_c-value decreased the concentrations of those compounds that could be related to the off-flavors in canned beef (aldehydes and sulfur compounds; see Persson and von Sydow, 1973; Persson et al., 1973b). The differences in concentrations for the sulfur compounds were especially marked between 121°C and 131°C. This was in accordance with the findings by Luh et al. (1964) in their investigations of canned beef, where they found that the H_2S content was three times higher in conventionally sterilized meat than in HTST-sterilized. These observations are of practical interest only where the material is packed in thin containers.

The odor qualities describing off-odors correlated well with the chemical data. There was, for instance, with increasing temperature a marked decrease in negative odor notes: "retort flavor," "sulfurous" and "burnt." "Odor strength" and "sharp, pungent" also decreased in a similar way, while there was no evident improvement in the odor note "meaty" or in the preference value (Table 3). The reason is that the meat samples were packed uncooked and that during the mild HTST-heat treatment there was not time enough for the full meat aroma to develop. This conclusion is in accordance with Luh et al. (1964). This may not, however, imply any practical problems since the meat should be reheated before eating. Furthermore, meat is generally cooked before packing and therefore there will be no problem with raw meat flavor.

In conclusion, a better finished product may be obtained by applying HTST-sterilization provided that the meat is packed in thin layers (in this investigation 16 mm). No significant improvement was obtained with a 28 mm thickness.

A combination of HTST-heating by steam and (a final) dielectric heating (e.g., microwave heating) should improve the result with packages of larger thickness. The central parts of the meat can thus obtain an adequate heating during a shorter time by using microwaves. This is presently under investiation in our laboratories.

Table 11—Concentrations, ppb (v/v), of 11 volatile compounds in the headspace gas of stored canned beef, heated to an F_c-value = 10 at 121°C and 131°C[a]

	Retort temp 121°C Storage time (months) at 20°C				Retort temp 131°C Storage time (months) at 20°C	
	0	2	6	10	0	2
Compound	Conc (ppb)	Conc (ppb)	Conc (ppb)	Conc (ppb)	Conc (ppb)	Conc (ppb)
Ethanal	810	330	480	390	780	200
Hexanal	4.8	4.1	1.9	1.3	16	1.9
2-Methyl propanal	54	24	24	22	39	16
2-Methyl butanal	43	26	18	16	30	4.7
3-Methyl butanal	53	6	9	9	40	2.4
2,3-Butandione	6.2	0.6	0.1	0.1	5.0	0.1
2-Ethyl furan	120	190	74	78	89	85
Hydrogen sulfide	11500	1800	1120	1400	7900	1100
Methyl mercaptan	1200	180	380	410	780	75
Dimethyl sulfide	770	610	600	640	530	440
Ethylene sulfide	13	1.1	1.8	1.7	1.0	0.7

[a] Formulation: B = Beef; Packaging materials: Flexible pouches (Expt. No. 7 and 8 in Table 1)

Aseptic canning

In this part of the investigation the differences between meat samples sterilized with or without covers were investigated. To avoid weight losses it was best to use direct heating with superimposed steam rather than holding the overpressure by nitrogen or air. When using steam, desiccation at the walls was avoided, while when using nitrogen overpressure there was a risk for "burning on" on the walls.

A marked decrease in the concentrations of the off-flavor giving components was obtained for the samples without covers (Table 4). There was an especially large decrease in the aldehydes, while the changes in the sulfur components were less. In all formulations containing fat there are higher concentrations of the aldehydes, e.g., pentanal, hexanal, 3-methyl butanal and therefore the "aseptic" heating has a certain positive influence on those types of samples. This can be seen in Table 5 where the rating of the preference value increased from 3.8 when cover was used to 4.9 when heated aseptically.

It was somewhat surprising that larger differences in concentrations of the volatiles, especially the sulfur compounds, were not obtained between the two types of heat procedures, since sterilizing without cover creates extremely favorable circumstances to distil off the volatiles. This is probably due to the fact that the volatile components are firmly adsorbed on the protein and the fat. The same problem can be found with aseptically heat sterilized milk. This explains why the nasty-smelling volatiles are not released, when heat sterilized food items are cooked, as cooking is an even weaker heat treatment than aseptic canning.

Examples of technical solutions corresponding to these experiments are the Hydropac system (FMC Corp.) in which flexible pouches are sterilized with one side unsealed, thus permitting venting of the volatiles (Brody, 1971).

Other technical solutions to this problem are Swift's Flash 18 system and the Dole system.

Influence of ingredients

The effect of adding amino acids was mainly in accordance with the findings of Furuhashi and Ayano (1971a,b), but L-histidineHCl was almost without any effect on canned beef. This can be due to the fact that L-histidineHCl reacted along different pathways during the severe sterilization than during cooking. The larger amounts of amino acids necessary in canned beef compared with water solutions of pure aldehydes to reach the same effect can probably be explained in the same way.

The effect of the salts of fumaric, maleic and sorbic acid on hydrogen sulfide and methyl mercaptan was very clear and by using one of these three compounds one has a tool to control the concentration of voltile thiol-compounds, especially hydrogen sulfide. If the concentrations of hydrogen sulfide and methyl mercaptan decrease to less than about 10–20% of their original values (given in Table 7), the typical meat aroma disappears to some extent and an off-flavor appears which is similar to the acid, stale flavor in stored canned beef. This shows that adequate concentrations of hydrogen sulfide and methyl mercaptan are important for typical meat aroma.

It is possible that the compounds tested also had some effect on other flavor compounds not analyzable with the sampling technique used in this investigation.

The added compounds have the advantage of being effective even at the severe heating of sterilization. Thus, they can be added before retorting. Addition of the compounds described can certainly be applied to other food items which have off-flavors caused by aldehydes and thiols. Of the additives used, the amino acids, potassium chlorate, disodium fumarate and potassium sorbate are on the GRAS-list.

Storage changes

There were very marked changes in the aroma complex (Tables 9 and 11) during storage. These changes depend on the action of oxygen in the packages. This is supported by Wirth's and Leistner's (1970) finding that the redox potential decreased during the storage of canned beef, which is a symptom of disappearing oxygen. The chemical changes were followed by analogous changes in sensory data. The intensities of most of the sensory properties markedly decreased (Tables 10 and 12). This is valid both for negative odor notes like "retort flavor," "sulfurous" and "burnt" and positive

Table 12—Mean panel intensities for preference value and six odor qualities for stored canned beef, heated to an F_c-value = 10 at 121°C[a]

	Storage time (months) at 20° C						
	0		2		6		10
Odor quality	Intensity	Sign. level for dif.	Intensity	Sign. level for dif.	Intensity	Sign. level for dif.	Intensity
Preference value	5.4	0.01	4.2	–	3.7	–	2.5
Odor strength	5.4	–	5.2	–	5.5	–	5.6
"Retort flavor, canned beef off-flavor"	4.6	0.001	2.0	–	1.4	–	1.3
Burnt	3.0	0.01	1.6	–	1.6	–	1.9
Acid, sour, etc.	2.4	0.01	3.2	–	4.1	–	4.6
Like whey butter	0.2	0.001	1.8	–	3.1	–	3.8
Appearance (0 = extr. light, 9 = extr. dark)	7.7	0.001	6.4	0.01	5.0	–	5.2

[a] Formulation: B = Beef; Packaging materials: Flexible pouches (Expt. No. 7 in Table 1)

Table 13—Concentration of some volatile fatty acids (VFA) in stored canned beef, heated at 121°C to an F_c-value = 8 (BFC) and F_c-value = 10 (B)[a]

	Unlacquered tinplate can (73 X 55 mm) Formulation BFC[a]				Flexible pouch Formulation B[a]			
	Storage time (months) at 20°C				Storage time (months) at 20°C			
Acid (μg/g)	0	6	12	18	0	2	6	10
Acetic	24	25	30	41	21	23	26	26
Propionic	1.5	1.5	1.8	1.9	1.7	1.9	2.1	2.1
Butyric	2.7	2.8	3.0	3.7	4.5	4.4	4.5	4.9
Hexanoic	0.9	1.0	1.1	1.6	0.4	0.8	0.8	0.8
Heptanoic	0.6	0.7	0.7	0.8	0.6	0.6	0.7	0.7
Octanoic	0.2	0.2	0.3	0.5	0.1	0.2	0.3	0.3
Nonanoic	0.1	0.2	0.2	0.2	0.1	0.2	0.2	0.2
Decanoic	0.3	0.3	0.4	0.8	0.1	0.2	0.3	0.3

[a] B = Beef; BFC = Beef + pork back fat + carbohydrate (Expt. No. 1 and 7 in Table 1)

ones like "meaty" and the preference value. The samples lost their typical meat aroma. Simultaneously with these changes there was an increase in some of the negative odor qualities: "acid, sour," "like whey butter" and "sickly." There was also a change in appearance: the color turned lighter and more reddish during storage. The hitherto mentioned changes were common for all experiments (No. 1–8) in Table 1 and in agreement with the findings by Leistner and Wirth (1963). They found that the meat aroma disappeared during the storage and an "old flavor" or "stale flavor" developed. Off-odors as "acid, sour" and "like yeast" were obtained and as in our investigation color changes occurred.

In order to find if the storage changes were reversible the 18 months' samples, from Expt. No. 1 in Table 1, were reheated in the cans at 121°C for 50 min. The sensory data (Table 10) showed that the storage changes evidently were decreased but some of the storage off-odors remained (e.g., "like whey butter"). The color turned darker. Also these results were in agreement with Leistner and Wirth (1963). The reheating temperatures and times used by us have been largely exaggerated to investigate if any changes at all could be obtained. Normally, one does not use such cooking conditions and it is probable that with a milder reheating the storage changes remain more evident than in our experiment.

As mentioned above the off-odors obtained during storage (e.g., "acid, sour," "like whey butter") were suspected to originate from volatile fatty acids. The results of the VFA-analysis (Table 13) showed that there was a slight increase in the concentrations during storage. The acetic, butyric, hexanoic and heptanoic acids seemed to be most important. It is probable, that the the odor from the acids was masked during the initial period of the storage. When the concentrations of the sulfur compounds and the aldehydes decreased the flavor from the acids became of increasing importance. Possibly other components, not analyzed with the chemical techniques used, could have some influence.

During the storage there was no significant decrease in the pH. It varied between 5.9 and 6.0 for all experiments.

From the Tables 9–11 it can be seen that the storage changes seemed to develop more rapidly in flexible pouches than in cans. This is especially evident for those sterilized at 131°C (Table 11). This may be due to oxygen penetration through the pouches.

Other factors than those discussed above might have had an influence on the aroma properties of canned beef. One factor is precooking, which often is applied before filling into the cans. However we found in a separate investigation that precooked beef (final centrum temperature 70°C) did not, to any great extent, differ in chemical concentations from beef packed uncooked. The precooked meat had somewhat higher concentrations of some of the aroma compounds, especially the sulfur ones.

The experiments described have dealt with the possibilities of avoiding off-aroma in canned pure beef added with fat and starch. In industrial formulations, however, other ingredients are often included: spices, onion, etc., and their aroma during sterilization and storage are important to know in order to be able to optimize processes and formualtions. In addition, different cuts and meat from different species and breeds can have an influence on the aroma.

Besides aroma, there are other eating quality properties that are of importance but which have not been investigated in our work. For instance, the changes in water holding capacity and in texture are certainly of significant magnitude during processing and storing of canned beef products.

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RESEARCH NOTES

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A Research Note

EFFECT OF CONCURRENT CALORIC INTAKE ON THE RESPONSE TO ORAL MONOSODIUM L-GLUTAMATE IN SUSCEPTIBLE SUBJECTS

INTRODUCTION

IN A PREVIOUS paper (Kenney and Tidball, 1972) we reported a placebo controlled study of the susceptibility of a group of volunteers to a 5g oral dose of monosodium L-glutamate (MSG) served in tomato juice. Twenty five of the total of 77 subjects tested reported symptoms following the dose of MSG. members of the reacting group revealed a dose-response relationship in the dosage range of 1–5g. In both the initial survey and in the subsequent study, the MSG was administered at least 2 hr after the previous snack (breakfast) and an analysis of the symptom occurrence in relation to the quantity or nature of food taken revealed no effect of previous feeding.

Both culinary and commercial practice in the use of MSG would make the ingestion of 5g unlikely in a single dose of fluid and in fact any ingestion would normally be as part of a meal of significant calorie content. Natural L-glutamic acid moreover, as a constituent of protein, is frequently ingested in quantities in excess of 5g at a single meal.

A study was therefore undertaken to assess the susceptibility of a population of proven reactors to a standard dose (5g) of MSG given as part of a lunch-type snack. In addition the study was designed to test the response to naturally occurring L-glutamic acid in amounts comparable with the test dose of MSG.

MATERIAL & METHODS

THE SUBJECTS for the study were 13 members of the reactor group who had previously been studied in depth. They were acquainted with the objectives of the study to which they had given their informed consent. On each test occasion subjects were provided with a packaged lunch accompanied by a glass of tomato juice. Each subject was given a questionnaire which he or she was required to answer in the 2 hr following the snack.

Four lunch-type snacks were used in the study. The compositions of the snacks were selected to provide 400–470 calories with in one case a high fat, low protein mixture; in another a high carbohydrate, low protein mixture; and in the two other cases high protein content with differing contents of natural L-glutamic acid. The compositions of the snacks were as follows:

Snack A: 50g cream cheese, 2 rye crisp crackers, 10g margarine and 150 ml tomato juice.
Snack B: 75g Wensleydale cheese, 2 rye crisp crackers, 150 ml tomato juice (margarine if desired).
Snack C: 75g Gruyere cheese, 2 rye crisp crackers, 150 ml tomato juice (margarine if desired).
Snack D: 4 oatmeal cookies, 20g jelly or marmalade, 2 saltine crackers, 150 ml tomato juice (margarine if desired).

These snacks provided calories, protein and natural L-glutamic acid in the following amounts:

	Calories (Approx)	Protein (g)	L-glutamic acid (g)
Snack A:	400	4	0.15
Snack B:	420	28	5.5
Snack C:	470	35	7.8
Snack D:	440	6	0.2

Each snack was administered to each subject four times: on two occasions the tomato juice contained 5g of MSG and on the other occasions the juice was lightly salted (0.8g NaCl per 150 ml) to provide an equivalent-taste placebo.

RESULTS & DISCUSSION

RESPONSES RECORDED following the ingestion of either placebo juice or the juice containing MSG differed in no qualitative way from those reported in the earlier study and included sensations of warmth or burning, stiffness or tightness (usually of face or neck), weakness of the limbs, pressure (usually of the head or chest) and tingling. In addition there were reports of more general malaise or headache. The frequency of symptom occurrence is recorded in Table 1. Snack designations including the letter P indicate a snack in which the juice contained no added MSG. Snacks accompanied by the test dose of MSG have the designation M. The latency of symptom onset in this series is consistently longer than when MSG is administered in juice unaccompanied by a snack (Kenney and Tidball, 1972, Table 13). However, the wide variability clouds the possible significance of this observation.

Snack A:	35 min ± 16
Snack B:	32 min ± 12
Snack C:	27 min ± 15
Snack D:	34 min ± 14
Previous series:	22 min ± 7

In the previous study (Kenney and Tidball, 1972) of the dose-reaction relationship of these subjects a placebo response rate of 20% was obtained. It is apparent therefore that the ingestion, along with the placebo juice, of as much as 5g of natural L-glutamic acid in the form of protein produces no additional response in this susceptible population. The slightly higher response seen with snack CP is not significantly different from the other placebo series. However, the response rate to snacks containing 5g of added MSG is significantly lower ($P < 0.01$) in cases B, C and D than in the case of Snack A or the previously determined response to this dose of MSG given without a snack. This protection appears to be afforded equally well by high protein (and high natural L-glutamic acid) snacks (B and C) and high carbohydrate snacks (Snack D). However, the high fat snack A (which is also of low bulk) affords no protection.

Table 1—Frequency of symptom occurrence

Snack[a]	No. of administrations	No. of positive responses	Percentage of positive responses
AP	24	4	16.6%
AM	24	19	79.1
BP	22	3	13.6
BM	23	10	43.4
CP	23	6	26.1
CM	23	10	43.4
DP	24	5	20.8
DM	24	11	45.8

[a] P indicates juice contained no added MSG; M indicates juice contained test dose of MSG.

In practical terms this outcome would suggest that untoward symptoms will be provoked by MSG in a general unselected population even less frequently than was earlier suggested when the condiment is used in the culinary setting of rather small amounts in dishes with a significant calorie content. Of the total population studied earlier 32.5% reacted at the 5g level. In the present study giving a snack with the juice halved the frequency of symptom occurrence at the 5g level (Snacks B, C and D). Furthermore the dose-response characteristics of these susceptible subjects indicates that at a more realistic level of perhaps 1g or 2g, symptom occurrence following the ingestion of MSG in a snack would be no more frequent than in response to an equivalent flavor placebo.

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A Research Note

COMPARATIVE SENSORY EVALUATIONS OF TWO CULTIVATED MUSHROOMS: A. bisporus AND A. bitorquis

INTRODUCTION

DESPITE increasing worldwide consumption of cultivated mushrooms (presently about 1 billion lb), relatively few data on man's preferences for this unique food are available. Although the number and diversity of fleshy fungi suitable for cultivation are considerable (Gray, 1970; Singer, 1961) only three kinds of mushrooms are of worldwide commercial importance. *Agaricus bisporus*, the so-called "cultivated" mushroom or champignon, is the most important cultivated mushroom (600 million lb/yr). The other two mushrooms, the shiitake and the paddy straw mushroom, are produced primarily in Asia.

There is now interest in the cultivation of *A. bitorquis*, commonly called *A. rodmani* in the USA. Except for the 2-layered ring about the stem, *A. bitorquis* is quite similar in appearance to *A. bisporus*. Although *A. bitorquis* probably closely resembles the ancestral form of the cultivated mushrooms (Singer, 1961), it has never been extensively cultivated. Using modern techniques, yields of *A. bitorquis* appear promising (Hasselbach and Mutsers, 1971; Poppe, 1971), and several cultural characteristics could be advantageous for production.

According to Smith (1963) mushroom hunters have long prized the excellent flavor and hard flesh of naturally occurring fruit of *A. bitorquis*; however, its acceptability to the average consumer of cultivated mushrooms is unknown.

Maaker (1971) summarizing the results of a preliminary study of storage and quality indicated that raw cultivated *A. bitorquis* mushrooms had more flavor than those of *A. bisporus;* cooking reduced the flavor difference. Though no details or data were presented, Maaker further states that triangle tests (including a question on preference) did not indicate a clear preference for either mushroom but there was evidence of a slight preference for one *A. bitorquis* cultivar tested. Those tests were done in Holland; the attitudes of American consumers regarding these mushrooms are unknown.

Anticipating general introduction of *A. bitorquis* as a new cultivated species, laboratory scale sensory evaluation of this mushroom was undertaken to learn: (1) Whether nontrained randomly selected persons could distinguish between *A. bitorquis* and the commercial white mushroom, *A. bisporus;* (2) In what ways these species were different in eating qualities, if any; and (3) The preference of those persons who were able to distinguish the species. (Experiments in the cultivation of *A. bitorquis* are in progress at the U.S. Dept. of Agriculture in Beltsville, Md. The strain used in these studies was American Type Collection (ATCC) #24559.)

EXPERIMENTAL

FRESHLY HARVESTED mushrooms of each species were cleaned, sliced longitudinally about 5 mm thick, then cut into approximately 10 mm squares to camouflage size differences. About 38 mushrooms of varied sizes were required for each 255-g sample.

The two samples were simultaneously sauteed for 5 min at 204°C in electric skillets in 2T butter and 1T corn oil (Beck et al., 1961) then very lightly salted. Skillets were alternated for species to minimize possible differences in temperature. Each species was divided into 10 portions. No attempt was made to record or control the temperatures at the time of tasting except that both species were handled simultaneously.

Panel for discrimination and preference

31 panelists (15 men and 16 women) who liked mushrooms were recruited from the USDA staff. About half had experience in evaluating produce on sensory rating scales but none had used triangle tests or evaluated mushrooms. Panels were held in an air-conditioned room in booths with red lighting to mask color differences. Panelists were given three coded samples and told that two of the three samples were identical They were instructed to taste several bites of each mushroom sample. Cool water was provided for rinsing between samples. Panelists were asked to identify the odd sample, to indicate a preference for the odd or matched samples, and to state what factors were used to distinguish the samples. They were instructed to guess if they were not certain of the different sample.

Each species occupied each position as odd sample with equal frequency. In the second replicate, each panelist received the opposite arrangement of his first set. For the third replicate, samples were reassigned so that the odd sample occupied a different position from the previous two replicates. The number of correct selections necessary for significance was determined by χ^2 analysis (or tabular values in ASTM, 1968).

Position effect

During the triangle tests, there appeared to be a tendency to favor the sample to the right side among those panelists who incorrectly identified the odd sample. It was thought that if panelists tasted from left to right (as most did) and were unable to distinguish between the first two samples, they perhaps assumed that the third was different and that they could not taste the difference because of the strong flavor carryover.

After completion of the difference tests and with no change in procedure, 17 randomly-selected panelists were given "blank" triangle sets (all three samples alike). Each received two blank sets of *A. bisporus* and two of *A. bitorquis*, one set per session.

The panelists showing slight positional biases either had all difference sets correct or their errors did not follow the trends demonstrated in the blank sets. Overall, there was no bias favoring any of the positions so the difference-test data were treated according to the standard methods for triangle tests (ASTM, 1968).

Texture profile

Six people trained in the texture profile method (Brandt et al., 1963; Szczesniak et al., 1963) evaluated samples of both mushrooms at one session.

In the profile method, physical characteristics of samples are considered on the initial bite, during chewing, and when the material is ready for swallowing. *Hardness* (the force required to penetrate the sample with the molars) was rated by direct comparison with (1) cream cheese, (2) Velveeta cheese, (3) frankfurter, (4) American cheese, (5) giant green olive and (6) peanut. The differences between anchored points are great enough to be reliably subdivided by the panelists. Chewiness was defined as number of chews to prepare the sample for swallowing. Verbal descriptions and relative intensities were given for factors for which anchored rating scales were not established.

Table 1–Success in discriminating between A. bisporus and A. bitorquis in triangle tests

Replicate	Correct	Incorrect	Total
1	22	9	31
2	20	11	31
3	18	4	22
All	60	24	84

Table 2–Preferences of panelists who correctly distinguished species[a] (numbers of panelists)

Replicate	Preferred A. bisporus	Preferred A. bitorquis	No preference	Total correct
1	11a	10ab	1d	22
2	10ab	7bc	3d	20
3	11a	3d	4cd	18
All	32y	20yz	8z	60

[a] Numbers not followed by the same letters are significantly different at the 0.001 level.

RESULTS & DISCUSSION

Discrimination

Of the 84 judgments (Table 1), 60 correctly identified the odd sample (significant at the 0.001 level), indicating that the species are readily distinguishable. Differences within each replication were also significant at the 0.001 level.) About half of the panelists correctly distinguished the species in all three triangle sets; only three panelists were wrong in all sets. 38% of the correct discriminations indicated use of both flavor and texture; 37%, flavor alone; 23%, texture alone. Of the incorrect responses, 68% indicated that flavor alone was used. These figures suggest that while the two species differ in both flavor and texture, there might be a flavor factor to which some people may be relatively insensitive or perhaps the flavors are simply strong enough to desensitize some panelists before a judgment can be made.

Preference

Of the 60 correct discriminations (Table 2), one-half indicated a preference for the cultivated mushroom *A. bisporus* and one-third, for *A. bitorquis*. Seven panelists (21 discriminations) consistently preferred *A. bisporus* and only two panelists (6 discriminations), *A. bitorquis*. The remainder indicated either no preference or alternated choices, indicating that they had no strong preference. Four panelists expressed a strong dislike for *A. bitorquis* because of an objectionable flavor. Several indicating preference for *A. bisporus* or no preference commented that *A. bitorquis* was somewhat too strongly flavored as a sauteed mushroom but would probably be good as flavoring ingredient with other foods.

The number preferring *A. bitorquis* decreased during the replications. It is not known whether this is a result of changes in the panelists' tastes or changes in the mushrooms with increasing mycelial age.

Texture profiles

Analysis of the first bite indicated that *A. bitorquis* was harder than *A. bisporus*. Although both were between American cheese and olive on the hardness scale, differences within this span can be reliably detected and are of practical significance. *A. bitorquis* was rubbery, nonuniform and slightly tough. *A. bisporus* was slightly rubbery but clean-cutting and even-textured.

During chewing, *A. bitorquis* was appreciably more chewy, fibrous and crisp than *A. bisporus*. *A. bitorquis* broke down into less distinct pieces but took about 20 chews to prepare for swallowing, whereas *A. bisporus* only required 16 chews. Both mushrooms were slippery but *A. bitorquis* seemed less slippery by the time of swallowing.

A. bisporus was buttery and very slightly astringent. *A. bitorquis* was more astringent and had a brothy flavor. They appeared about equal in juiciness.

Observations

When sauteed, *A. bisporus* had a grayish-tan color. *A. bitorquis* had a distinctly pinkish-tan color. The specific differences in flavor were beyond the scope of these panels but from the comments panelists made, there were differences in flavor notes as well as in intensities. Although no tests were made of processed mushrooms, canning might change the flavor and affect the acceptability. Scraps of the mushrooms left in the skillets during panel sessions were sampled by the experimenters after about 10 min, and while *A. bisporus* had continued to soften, *A. bitorquis* was still firm and slightly crisp. This could be a very desirable characteristic for mushrooms to be processed or to be included in foods requiring longer cooking periods, such as soups and casseroles.

Summary

Of the 84 responses, 32 favored *A. bisporus* and 20 favored *A. bitorquis;* the remaining 32 either were incorrect or indicated no preference. These figures could be of importance to food manufacturers considering the use of *A. bitorquis* in their products but it must be reemphasized that these data represent only 31 people.

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A Research Note

DIFFERENTIATION OF PERFECTION, ALSWEET AND ALASKA VARIETIES BY TOTAL STARCH AND AMYLOSE CONTENT

INTRODUCTION

FOOD AND DRUG standards of identity, quality and fill for canned peas as well as the US grades distinguish between two separate pea types: the smooth skinned Alaska varieties (early type) and the sweet wrinkled varieties (sweet type). The alcohol-insoluble solids (AIS) and specific gravity values for a given grade differ in the two types (Anonymous, 1972).

In the dry state (seed) the two types are easily identified. The early type exhibits a smooth skin and simple oval starch granules, while the sweet type has a wrinkled surface and the starch granules contain radical crevices giving them the appearance of composite granules. Crosses between the two types are more difficult to identify since they exhibit characteristics of both types. The popular Alsweet cross exhibits the wrinkled skin of the sweet type yet its starch granule resembles the configuration of the early type. These identifying characteristics enable differentiation between the three varieties in the seed stage. However, in the raw or processed stage, differences in skin characteristics are not apparent and therefore, differentiation between varieties, because of crosses, is impossible.

Thung and Gersons (1966) developed a simple microscopic examination to distinguish between early type (Alaska) and sweet type (Perfection) peas after processing. The method is based on the shape of the swollen starch granules which differ in the two types. The method, however, cannot distinguish between crosses of the two types (e.g. Alsweet). This difficulty in identifying varieties has resulted in some lots being incorrectly graded, since in the grading of peas the packers identification of the type being graded must be accepted.

The amylose content of peas has been the subject of several investigations. Great differences in the amylose content of the starch exist between early and sweet type peas. McCready et al. (1950) reported the amylose content in starch of sweet type peas and early type peas as 69.0 and 37.0%, respectively. Similar results have been reported by Hilbert and MacMasters (1946), Potter et al. (1953), Williams et al. (1970) and Thung (1970). Kooistra (1962) indicated that there is considerable difference in the raw state between all three types mentioned with regard to amylose content. His analysis gives an average (and range) amylose content of 37.9% (35.5–41.1) for early type peas, 65.4% (58.9–68.1) for the sweet type peas, and 24.4% (24.2–24.6) for Cennia and Alsweet varieties. These data would indicate that in the raw state the three types can be distinguished according to the percent amylose in starch.

Thung (1970) worked with processed peas of the different types and sizes, and concluded that the amylose content increased with size and that within a specific size classification the types of peas could be distinguished. The range in percent of amylose content in starch of early, sweet and Cennia peas of the size 7.5–8.2 mm (approximately size 3) was 19.9–28.3, 38.6–45.9 and 12.7–18.5, respectively.

It is the purpose of this paper to determine if the amylose and total starch content could be used to distinguish between Perfection, Alaska and Alsweet varieties with known sizes and tenderometer readings grown in Wisconsin.

EXPERIMENTAL

COMMERCIALLY canned pea samples were obtained of known varieties and tenderometer readings. The varieties included Perfection (sweet type), Alaska (smooth type) and Alsweet (intermediate type). Three lots with three samples for each variety were analyzed.

Moisture as well as AIS content were determined according to the procedures outlined in the *Laboratory Manual for Food Canners and Processors* (NCA, 1968). The method by McCready, 1970 was used to determine the total starch content.

To determine the percent amylose, pure pea amylose was prepared by extracting 150g of AIS powder with 0.2% NaOH. The sample was reextracted until the filtrate showed a negative Biuret reaction. The starch paste was washed with distilled water until free from alkali. The sample was then extracted 5 times with hot 95% ethanol to remove fatty materials. The residue was dried at 70°C in a vacuum oven.

A mixture of 1,400 ml of distilled water, 200 ml of butanol and 50 ml of isoamyl alcohol was heated to boiling on a steam bath and a suspension of 50g of defatted pea starch in 150 ml of distilled water was slowly added with mechanical stirring. The mixture was autoclaved for 2 hr, centrifuged, and the hot supernatant slowly cooled to room temperature by wrapping the container with thick insulating cloth and was centrifuged after 36 hr. The amylose precipitate was purified by repeated (five times) precipitation from a water, butanol and isoamyl alcohol mixture (Schoch, 1942, 1945; Wilson et al., 1943).

A standard titration curve for amylose as well as the amylose content were determined according to the method by Thung, 1970.

RESULTS & DISCUSSION

TABLE 1 presents data of the total starch content and the percent amylose in starch of canned peas of the varieties: Perfection, Alsweet and Alaska of sieve sizes 1, 3 and 5. The high percent of amylose in starch in Perfection peas in all sizes readily distinguishes it from the other two varieties. The lowest amylose content in starch in Perfection peas was 43.3% while maximum amylose content observed in

Table 1—Starch and amylose content of canned peas of sieve size 1,3 and 5

Variety	Sieve size	Starch %[a]		Amylose % in starch	
		Average	Range	Average	Range
Perfection	1	–	–	–	–
	3	44.3	42.8–45.5	48.2	43.3–54.6
	5	43.4	42.8–44.1	67.0	66.2–68.0
Alsweet	1	30.8	–	15.8	–
	3	41.7	39.0–43.7	17.0	14.9–18.9
	5	44.1	43.2–47.7	20.1	18.7–22.0
Alaska	1	51.2	48.2–54.2	20.9	20.6–21.3
	3	57.3	55.8–60.3	21.5	19.1–20.0
	5	64.7	58.7–65.3	24.2	23.5–25.5

[a] Percent calculated on dry basis

Table 2–Starch and amylose content of canned peas of various Tenderometer reading ranges

Variety	Tenderometer reading raw peas	Sieve sizes	Starch %[a]		Amylose % in starch	
			Average	Range	Average	Range
Perfection	100–115	2,3,4	42.1	34.7–47.7	65.4	59.2–69.3
	125–150	3,4,5	45.2	42.5–48.2	63.7	62.1–72.3
Alaska	85–95	1,2	51.2	48.2–54.2	21.0	20.6–21.3
	140–170	4,5	64.6	58.7–70.0	24.1	23.4–25.5
Alsweet	85–95	1,2,3	36.3	35.6–40.5	16.3	15.8–17.4
	100–120	2,3,4,5	41.5	36.7–43.8	19.5	17.6–21.6
	125–150	3,4,5	46.7	45.5–47.7	21.2	19.5–22.6

[a] Percent calculated on dry basis

the Alsweet or Alaska varieties was 25.5. The amylose content in starch in Alsweet and Alaska varieties is very similar, and thus does not serve as an identifying characteristic. However, there exists a difference in the total starch content within a size classification of these two varieties. For example, within size 3 for Alaska and Alsweet the minimum and maximum present total starch values differ by 12 percentage points. A similar relationship exists in the other sizes studied. Thus within a size classification the three varieties can be identified by comparison of the percent starch content and the percent amylose in starch.

Thung (1970) studying smooth, wrinkled and Cennia varieties observed sufficient differences in amylose content in starch within a size classification to permit differentiation between these varieties. Although Alsweet has been considered a type of Cennia (Kooistra, 1962), the results of this study show that the amylose content in starch of Alsweet peas is the lowest among varieties. There is an overlap in the range of amylose content between Alsweet and Alaska varieties. Thus, the amylose content in starch alone within a size may not be conclusive in distinguishing these two varieties.

Since peas are often canned as mixed sizes, size classification as a means of grouping for differentiation between varieties no longer is useful. Therefore, a study was made to determine whether a tenderometer reading classification would provide a workable grouping. The results of this study are shown in Table 2. The high amylose content in starch of Perfection peas again readily distinguishes the sweet type from the other two types. Alaska, and Alsweet varieties could be distinguished within similar tenderometer ranges by percent starch content. Alaska variety had a greater percent starch content compared to the Alsweet variety and the ranges did not overlap. The amylose content in starch again was lowest in the Alsweet variety, however, there was not sufficient difference in content between the two varieties to allow for differentiation.

This study has shown that the three varieties can be distinguished within a size or tenderometer value grouping when percent starch and percent amylose content in starch are known. In both grouping systems, the high amylose content of Perfection variety distinguishes it from Alaska and Alsweet varieties. The Alaska variety may be distinguighed from Alsweet variety by total starch content.

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A Research Note

FLUORESCENT QUANTITATION OF BIOLOGICALLY ACTIVE AMINES IN FOODS WITH 7-CHLORO-4-NITROBENZOFURAZAN (NBD-Cl)

INTRODUCTION

TOXEMIAS resulting from the ingestion of fermented foods containing biologically active amines have been reported (Asatoor et al., 1963). The illnesses have been especially noted in patients treated with monoamine-oxidase inhibitors which block the pathway for catabolism and inactivation of the amines after ingestion (Horwitz et al., 1964). Cheese containing high levels of amines such as tyramine and histamine has been the dietary factor most commonly incriminated for physiological reactions in association with monoamine-oxidase inhibitor treatment.

The separation and quantitation of these amines has usually consisted of thin-layer chromatography for the separation followed by specific chromagenic chemical reactions for quantitation. Reagents forming fluorescent derivatives with the amines have found wide usage for quantitation (Creveling and Daly, 1971). Many chromogenic reagents previously available for forming fluorescent derivatives have possessed a limited flexibility to produce quantitative conjugates under simple experimental conditions with a variety of amines differing in their basic molecular structure.

As a part of a study to identify and quantitate biologically active amines in various foods, several fluorescent chromagenic reagents were examined. The compound 7-chloro-4-nitrobenzofurazan (NBD-Cl), previously described by Ghosh and Whitehouse (1968) and Reisch et al. (1969) as having potential as a fluorometric quantitative reagent for amines and amino acids, was included in the study. Information regarding the use of NBD-Cl for analysis of biologically active amines has been scanty. However, the reagent has previously been shown to be superior to dansylchloride, since it is nonfluorescent itself, has greater stability toward moisture, and fluoresces in the visible region at 464 nm, thus quartz cells are not required for quantitative determinations (Ghosh and Whitehouse, 1968). This note describes the use of NBD-Cl for the quantitation of biologically active amines, and evaluates the reagent in comparison to other commonly used detection reagents.

EXPERIMENTAL

Thin-layer chromatography

Glass plates (20 × 20 cm) were coated with a 250μ layer of silica gel (Kieselgel G, Merck, Darmstadt, Brinkman Instruments, Westbury, N.Y.). The layer was applied as a slurry consisting of 30g of silica gel suspended in 70 ml of distilled water. The plates were dried in an oven at 100°C for 1–2 hr and stored in a desiccator until used. The plates were reactivated at 100°C for 30 min immediately before use. Amines and amino acids used in the study were all obtained from commercial sources. Solutions of the compounds in water:acetone (2:1 v/v) were applied 2 cm from the base of the plates with quantitative capillary pipettes. The solvent system used consisted of CH_3Cl:CH_3OH:NH_4OH (12:7:1). Treatment of the plates after development varied with the different detection methods employed.

Detection reagents

Ortho-phthaldehyde (OPT, Nutritional Biochemical Corp.) derivatives were prepared following the procedure of Aures et al. (1968). The plates were dried carefully following development to insure complete removal of ammonia as the reagent will form a fluorescent derivative with any residual ammonia. After drying, the plates were sprayed with a solution of glacial acetic acid in acetone (1:100 v/v) and air dried for 5 min before being sprayed with acetone containing 0.02% OPT. The plates were viewed either after 30 min under ultraviolet (365 nm) for the presence of histamine or were air dried and exposed to para-formaldehyde fumes at 80°C for 1 hr. The histamine-OPT conjugates required the latter treatment for maximum UV fluorescence.

The NBD-chloride (Aldrich Chemical Co., Atlanta, Ga.) derivatives were simpler to prepare. After oven-drying (1–2 hr at 55°C), the developed plates were sprayed with a freshly prepared 0.02% NBD-chloride-methanol solution and allowed to set for 24 hr at 25°C. The plates were then examined under UV light.

Ethylene diamine (ED, Eastman Organic Chemicals, Rochester, N.Y.) derivatives were formed according to the method of Schneider and Gillis (1965). The spray reagent consisted of ethylene diamine, 5 ml; potassium ferricyanide, 0.1g; absolute ethanol, 45 ml; and distilled H_2O, 50 ml. The plates were viewed under UV after 24 hr at 25°C or after 20 min at 55°C.

Derivatives with 1-floro-2-4-dinitrobenzene (FDNB; Eastman Organic Chem.) were prepared following the same procedure that was used for the ED reagent, except that the spray was 0.04% FDNB in ethanol.

Ninhydrin (J.T. Baker, Phillipsburg, N.J.) derivatives were prepared by spraying the dried, developed plates with a ninhydrin solution (0.3g of ninhydrin, 100 ml of n-butanol and 3 ml of acetic acid) followed by heating for 10 min at 110°C.

Evaluation of detection reagents

The detection reagents were evaluated by a qualitative sensitivity survey. The NBD-Cl reagent was further examined by studying the quantitative behavior of tyramine, tryptamine and histamine derivatives. For the qualitative study, 13 amines at concentrations ranging from 0.1–10.0 μg were applied to TLC plates and chromatographed. After development, the amines were made visual by the procedures mentioned previously.

For the quantitative NBD-Cl study, known concentrations of tyramine, tryptamine and histamine ranging from 0–2.5 mg/g sample were spiked into a Cheddar cheese containing negligible amounts of the three amines. The amines were extracted by the method of Lovenburg and Engelman (1971) and by the procedure of Blackwell and Mabbitt (1965). The extracts obtained from the cheese were spotted directly on TLC plates, chromatographed, and NBD-Cl derivatives formed. The fluorescent amine spots were scraped from the plates and eluted from the silica gel with 5.0 ml of ethyl acetate. The silica gel-ethyl acetate mixture was agitated on a Vortex-Genie for four 30-sec intervals over a 10-min period and centrifuged for 15 min at 5000 × G to remove the suspended silica gel. Fluorescent measurements were determined on a Turner 110 Fluormeter with a primary filter No. 7-60 (365 nm) and a secondary filter No. 4 (465 nm) (G.K. Turner Assoc., Palo Alto, Calif.).

RESULTS & DISCUSSION

THE DATA in Table 1 represent the qualitative evaluation of four of the detection reagents. Ninhydrin and FDNB do not form fluorescent derivatives but were included in the study because of their usefulness as qualitative detection reagents. Lower limits of detectability were not determined for FDNB which formed visi-

Table 1–Comparative evaluation of minimal detectable quantities (μg) of several amines as determined by different chromogenic reagents on TLC

Compound tested	Minimum limits of detectability (μg)			
	NBD	OPT	ED	Ninhydrin
Gamma-amino-butyrate	0.1 F[a]	1.0 F	ND[b]	0.1 NF
Cadaverine	0.4 F	0.6 NF[a]	0.1 F	0.4 NF
Putrescine	0.1 F	0.4 F	0.1 F	0.1 NF
5-methyl-tryptamine	1.0 F	0.6 F	0.6 F	0.6 NF
Serotonin	4.0 NF	0.1 F	0.1 F	4.0 NF
3-hydroxy-tyramine	0.1 F	0.1 NF	0.1 F	0.4 NF
DL-octopamine	1.0 F	0.1 F	1.0 F	1.0 NF
DL-artenol	0.1 F	0.1 NF	0.1 F	1.0 NF
Tyramine	0.4 F	6.0 NF	1.0 F	1.0 NF
Tryptamine	0.4 F	0.1 F	4.0 F	0.4 NF
Histamine	0.1 F	0.6 F	1.0 NF	0.4 NF

[a] Fluorescent (F); nonfluorescent (NF)
[b] No visible derivative formed (ND)

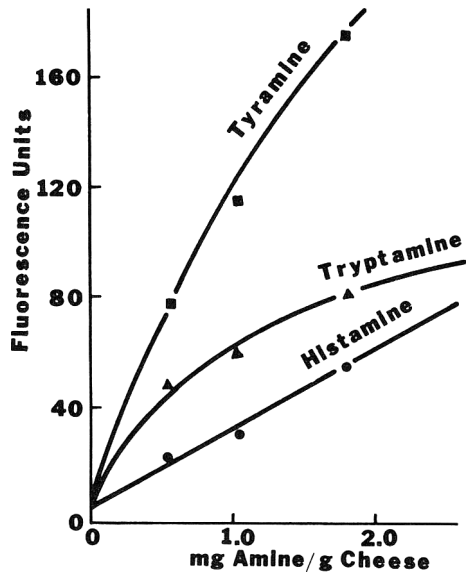

Fig. 1–Relationship between fluorescence and tyramine, tryptamine and histamine concentrations using NBD-Cl as the detection reagent.

ble yellow derivatives with all of the compounds at a level of 10 μg/spot. Ninhydrin was effective at concentrations down to 0.1 μg for many of the amines and was as sensitive in this respect as the fluorescence-inducing reagents.

The lower levels of detectability for NBD-Cl derivatives extended down to 0.1 μg/spot. The only compound which did not form a fluorescent derivative with NBD-Cl was serotonin. The limit of detectability of NBD-Cl with cadaverine, putrescene, tyramine, tryptamine and histamine, which comprise some of the more commonly found amines in fermented products, ranged from 0.1–0.4 μg/spot. Both OPT and ED formed excellent fluorescent derivatives with the majority of the amines. However, OPT does not form a fluorescent derivative with cadaverine and tyramine. Likewise, ED does not form a fluorescent derivative with histamine. In this respect, NBD-Cl seems to be advantageous for use where a single detection reagent is desired for quantitation covering a broad spectrum of amines.

Standard curves prepared for tyramine, tryptamine and histamine following extraction from cheese, TLC separation, and derivatization with NBD-Cl are shown in Figure 1. Histamine fluorescence was linear over a higher concentration range than was noted for tryptamine and tyramine. The fluorescence response for tryptamine decreased markedly from 1 mg to 1.8 mg tryptamine/g cheese. The tyramine derivative gave a sharp increase in measured fluorescence response over the range of amine concentrations used. NBD-Cl derivatives have been used successfully in our laboratory to quantitate biologically active amine content of Cheddar cheese, and this simple to prepare derivative should be considered as a useful tool for amine quantitation.

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A Research Note
TRYPSIN INHIBITORS IN SORGHUM GRAIN

INTRODUCTION

SORGHUM GRAIN is one of the most important crops in the world. This cereal plant is cultivated on all six continents, and widely used as food in Africa and Asia. In Western countries the grain is used as feed and the whole plant used as forage (Wall and Ross, 1970).

Although highly praised as a cereal food in India, the nutritional value of sorghum grain is less than that of maize and barley. The amino acid composition of its proteins shows deficiency in lysine, threonine, methionine and tyrosine, and their digestibility is less than that of other cereal grain proteins. The reason for this low digestibility is not known (Wall and Ross, 1970).

Although the involvement of trypsin inhibitors in the determination of the nutritional quality of a vegetable protein food has not been established (Liener and Kakade, 1969) it was thought of value to know whether sorghum seeds have trypsin inhibiting activity. These substances, known before to be present only in leguminous seeds, have been shown to have a more widespread distribution. They have been found in several botanical families and among cereals, in maize, wheat, barley, rye, oats, buckwheat and rice (Liener and Kakade, 1969; Mikola and Suolinna, 1969).

Evidence for the presence of trypsin inhibitors in sorghum seeds is reported in this paper.

EXPERIMENTAL

Extraction

Sorghum seeds (*Sorghum bicolor* L. Moench) were ground to a fine powder in a Wiley mill. 10g of this powder were extracted for 1 hr with 50 ml of distilled water with occasional stirring. The slurry was filtered through cheese cloth and centrifuged at 8,000 × G for 20 min in the cold. The clear supernatant was saved for further analysis.

Protein and trypsin inhibiting (TI) activity determination

The micro biuret method of Goa (1953) was used throughout the work for the determination of protein concentrations.

The trypsin inhibiting (TI) activity was determined by the Kunitz caseinolytic assay (Kunitz, 1947) using the Folin phenol reagent to detect trichloroacetic acid (TCA) soluble products. One unit of trypsin inhibiting activity (TIU) is defined, in this paper, as the amount of inhibitor that reduces by 50% the activity of a preparation of trypsin that produces an absorbance of 0.500 at 750 nm, in the above assay.

Acid treatment

25 ml of the aqueous extract was acidified with 4N acetic acid to pH 4.0 with continuous stirring, at room temperature. The precipitated proteins were separated by centrifugation at 5,000 × G, for 15 min and discarded. The clear supernatant was brought to pH 5.8 with 0.5N sodium hydroxide.

Heat treatment

Samples (10 ml) of different solutions containing sorghum trypsin inhibiting activity were placed in stoppered glass vials. The vials were then placed in a boiling water bath for the required amount of time.

Sephadex G-100 filtration

A Sephadex G-100 column (2.5 × 39.0 cm) was equilibrated with 0.005μ phosphate buffer, pH 7.0 and the flow rate adjusted to 25 ml/hr. 10 ml of the pH 4.0 supernatant was applied to the top of the column. Eighty 5.2 ml fractions were collected and the absorbance at 280 nm read in a Beckman DU spectrophotometer. The TI activity of pooled fractions was determined.

RESULTS & DISCUSSION

THE TRYPSIN INHIBITORS of sorghum seeds could easily be extracted with distilled water and were soluble in acid pH. During the course of this work an increase of ca. 20% in TI activity was consistently found when the aqueous extract was brought to pH 4.0. This could be due to liberation of inactive or precursor forms in the seed (Table 1).

The trypsin inhibitors were shown to be heat resistant as the activity in the acid pH supernatant was completely unaffected by heating at 100°C during 30 min. Furthermore, dialysis of the acid supernatant indicated that ca. 60% of the TI activity is probably due to low molecular weight compounds (Table 1).

Gel filtration of the acid supernatant showed that the TI activity of sorghum seeds has a broad distribution of molecular weights with the most significant peak of activity being centered around 15,000 daltons. It is interesting to note, in this connection, that a trypsin inhibitor isolated from barley has a molecular weight of 14,400 daltons (Mikola and Suolinna, 1969). It was also shown that ca. 44% of the activity in the gel filtration pattern is due to compounds with molecular weights less than 6,000 daltons. This seems to agree with the dialysis experiment (Fig. 1).

That the sorghum trypsin inhibitors, as separated by Sephadex G-100, are also heat resistant was shown when solutions containing the activity peaks of the gel filtration experiment were heated at 100°C for 30 min, and the original activity remained constant.

Further studies are in progress in this laboratory in order to clarify the type of substance responsible for the TI activity present in sorghum. It is suggested, how-

Table 1—Distribution of protein and trypsin inhibiting activity in extracts of sorghum (Sorghum bicolor L. Moench) grain powder

	Volume (ml)	Protein (mg/ml)	TI activity (TIU/ml)	Specific activity (TIU/mg protein)
Aqueous extract	32.0	14.6	4.6	0.315
pH 4.0 supernatant	27.5	11.4	5.1	0.447
pH 4.0 supernatant, dialyzed	10.2	2.7	1.9	0.705
pH 4.0 supernatant, heat treated	9.2	12.6	5.5	0.441

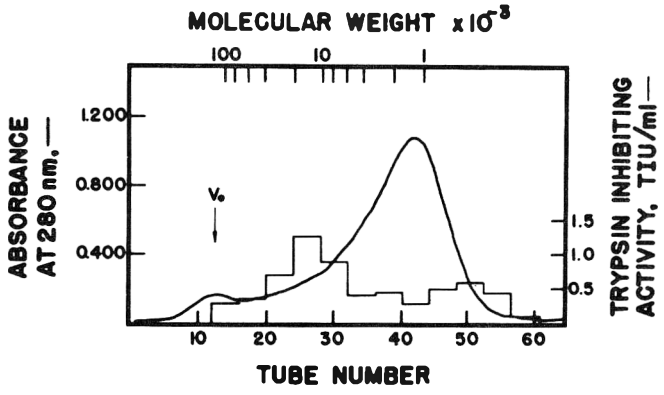

Fig. 1—Gel filtration of the sorghum grain powder acid supernatant in a Sephadex G-100 column (2.5 × 39.0 cm). Eighty 5.2 ml fractions were collected and the absorbance at 280 nm read. The TI activity of pooled fractions was determined by the Kunitz caseinolytic assay.

ever, that at least for the case of the low molecular weight fractions (less than 6,000 daltons), the TI activity is due to tannins present in sorghum seeds that unspecifically bind to proteins (Loomis and Battaile, 1966; Anderson, 1968), and which are known to have a depressing effect on the growth of chicks (Rostagno et al., 1973).

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A Research Note

INFLUENCE OF ODOR SOURCES ON THE ODOR AND FLAVOR OF BEEF

INTRODUCTION

CONSIDERABLE RESEARCH has dealt with the problem of identifying meat flavor and the effects of such things as irradiation, microorganisms, animal diets, pre- and post-slaughter treatment, and packaging (Crocker, 1948; Doty et al., 1961; Thompson and Karmas, 1963; Sjostrom, 1967; Paul and Palmer, 1972) upon this flavor. However, little information is available concerning off-odor and off-flavor caused by various sources during slaughter, storage, shipping, and other handling. The purpose of this project was to determine the influence of odor sources, that might be present in the vicinity of meat, on the odor and flavor of beef.

MATERIALS & METHODS

Phase I

Beef foreshank sections 5.0 cm thick were obtained immediately after slaughter, placed on plastic meat trays and held, by means of a stand, 5.0 cm from the bottom of a 2000 ml capacity plastic container (15 cm diam × 14 cm ht). Each beef section was exposed to a selected odor source and the lid placed on the container and stored 2 days at 3–5°C. The odor sources included the following liquids and solids placed in a petri dish under the beef: ammonia (10 ml as household liquid); the butts and ashes from five cigarettes; corn silage; paint (Dean & Barry Lac-Paint, quick drying enamel, 6A6, liquid on addition); rancid beef grease; kerosene; commercial soap (detergent-germicide Wescodyne; provides 1.6% titratable iodine); hand lotion (Jergens); and beef fecal material. A gaseous odor from the smoke of one cigarette was exhaled into a container prior to sealing. A control was sealed in a similar plastic container and stored in the same cooler with the other samples. The comparisons were replicated five times.

Immediately upon removal from storage the individual samples were cut into two 2-1/2 cm sections. One section was boned and ground for a ground composite sample. The second section was divided into external fat, external muscle surface and internal muscle portions. Each portion was wrapped in aluminum foil and roasted at 177°C for 20 min. After cooking the tissue was divided into 5–10g sample portions, and immediately served warm and uncovered to the panel. Members were asked to evaluate each sample for off-flavor and odor. The panel consisted of eight untrained members who had no knowledge of the treatments (but it was suggested that no samples be swallowed); a maximum of five samples were given per session with one or more being a hidden control. The samples were evaluated on a 10-point intensity scale with 10 representing a very strong off-odor or flavor and 1 no off-odor or flavor (Peryam and Pilgrim, 1957). The panel was likewise asked to identify the off-odor or flavor.

Phase II

In Phase II a section of the inside round weighing approximately 450g was placed in a plastic container in a manner similar to that described in Phase I. However in this part of the project one inside round was removed from the carcass 1-hr postmortem, termed pre-rigor sample, and divided into 12 sections. Each section was placed in a container with two sections designated as controls and the other 10 sections divided so that 2 sections were exposed to each of 5 various odor sources for each trial. After exposure the containers were sealed and stored in a 3–5°C cooler. One sample of each exposure and one control were removed and evaluated after 2 days of storage and the remaining samples were evaluated after 7 days of storage. The second inside round, termed post-rigor sample, was removed from the carcass 24-hr postmortem, and handled in the same manner as the pre-rigor sample. For evaluation each sample was ground twice through a 1/8-in. plate and formed into a small loaf, wrapped in aluminum foil, and baked at 163°C for 1 hr. The panel evaluation was then completed for off-odor and flavor comparable to Phase I. Each odor source was replicated three times at each storage postmortem.

In Phase II the odor sources used were: ammonia (5 ml concentrated NH_4OH); cigarette smoke, ashes and butt from one cigarette;

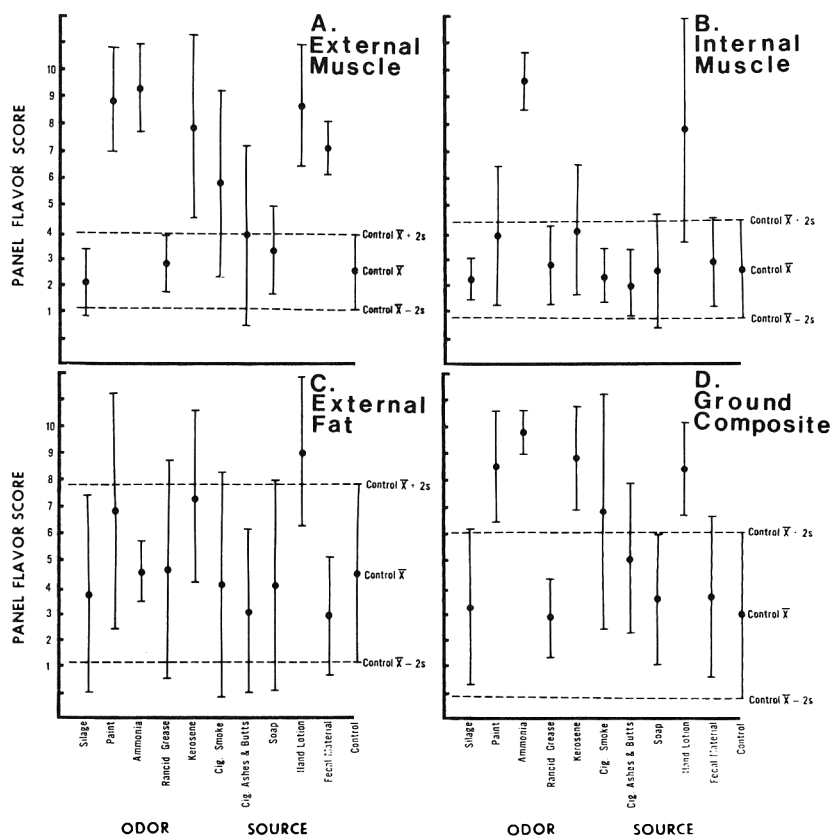

Fig. 1—Mean ± 2 std dev for flavor scores representing: (A) external muscle sample; (B) internal muscle sample; (C) external fat sample; and (D) ground composite sample.

Table 1–Mean odor and flavor scores for each treatment and its respective control

Odor Treatments	Odor		Flavor	
	Treatment	Control	Treatment	Control
Ammonia	8.8**	2.4	8.6**	2.5
Cigarette	6.4**	3.2	7.2**	3.1
Exhaust	5.4**	3.2	6.3**	3.5
Kerosene	6.6**	2.8	8.0**	2.9
Fecal Material	3.4NS	3.1	3.5NS	3.0
Paint	6.5**	3.1	8.1**	3.2
Spoiled Meat	3.6**	2.8	3.6*	2.9
Silage	3.5*	2.9	4.0NS	3.4
Sanitizer	3.4NS	3.2	3.2NS	3.4
Viscera	3.6NS	3.3	3.5NS	3.4

** Significant at 0.01 level
* Significant at 0.05 level
NS Not significant

automobile exhaust fumes; kerosene; beef fecal material; paint (Dean & Barry Lac-Paint, quick drying enamel, 6A6); spoiled meat (beef, 5 days after odor detection); corn silage; a commercial soap (detergent-germicide, Wescodyne; provides 1.6% titratable iodine); and beef viscera. All materials were handled the same as in Phase I except those noted below. The exposure to cigarette smoke, ashes, and butts was accomplished by having the smoke from one cigarette exhaled into the container and the ashes and butt placed in a petri dish under the meat. Exhaust fumes were from an automobile at idle for 1 min into the container of meat and then sealed.

RESULTS & DISCUSSION

Phase I

Figure 1A shows the panel flavor score means (40 panel member evaluation/mean) for the external muscle sample. This figure also illustrates two standard deviation limits for the control and each treatment. Figures 1B, C and D show the same information for internal muscle portion, external fat, and ground composite samples, respectively. From these figures it can be seen that in most cases the outside samples and ground composite samples scored higher in off-flavor intensity than did the inside samples. Some samples such as silage, soap and rancid grease did not show a significant difference from the control indicating there is little evidence that the volatiles from these sources caused off-flavor. The external fat sample was similar to the external muscle portion and the composite sample except the fat exhibited greater variation. The greater variation in scores for the fat samples may be attributed to the panels lack of knowledge concerning the flavor of pure beef fat and possible rancidity development during storage. As might be expected internal muscle off-flavor was less pronounced than that of external samples.

Odor scores followed a similar pattern to those exhibited by flavor scores. Some odor sources were very easy to detect while others were not. Solubility of odor sources in fat and H_2O (muscle) and the intensity of the odor probably play an important part in their transmission to meat.

Phase II

Table 1 shows the mean (96 panel member evaluation/mean) odor and flavor scores for all treatments and their respective controls. Ammonia was found to be the easiest source of odor to detect both by odor and flavor ($P < 0.01$). Cigarette smoke, butts and ashes, automobile exhaust, kerosene, and paint were readily detected by odor and flavor evaluation ($P < 0.01$). Spoiled meat could be detected as imparting an off-odor and off-flavor ($P < 0.05$) but source was not easily identified. Silage imparted an off-odor ($P < 0.05$) which was not easily identifiable nor was it consistently detected in flavor evaluation. The other odor sources of manure, viscera, and Wescodyne did not impart enough odor or flavor to the meat for detection under the conditions of this experiment. If the manure and viscera odors were of bacterial origin then this would agree in part with work by Mast (1971) and Williams (1960) that at least some microorganisms were not detrimental to meat flavor.

The influence of storage time and exposure pre- or post-rigor seemed negligible in most cases. There was one case of an interaction ($P < 0.01$) between odor source and pre- and post-rigor treatments. Silage was the odor source and when exposed to pre-rigor had a flavor mean of 4.81, while the pre-rigor control was 3.13, silage post-rigor 3.22, and control post-rigor 3.65 indicating that meat exposed to silage when warm (pre-rigor) will pick up off-flavor more readily than when cold (post-rigor).

In conclusion it can be said that many sources of odor can impart off-odor and flavor to beef tissue. It would be advisable for the meat industry to keep trucks and cooling systems in repair to avoid exhaust fumes and ammonia contamination. In addition possible sources of odor contamination should be removed from coolers, trucks, slaughter floors and other areas where meat is stored.

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A Reasearch Note

VITAMIN B_6 IN PORK MUSCLE COOKED IN MICROWAVE AND CONVENTIONAL OVENS

INTRODUCTION

MEAT is a good source of vitamin B_6, but little attention has been given to how various methods of preparing meat affect vitamin B_6 retention during heating. How cooking in microwave ovens, used increasingly, affects some of the B-complex vitamins has been studied, but results show no consistent trend. Kylen et al. (1964) reported that beef roasts retained less thiamine when cooked by microwave than by conventional methods, but that pork roasts and beef and ham loaves retained similar amounts by either method. Noble and Gomez (1962) found no significant difference in thiamine and riboflavin retention in lamb roasts cooked conventionally and those cooked by microwaves. Wing and Alexander (1972) reported significantly greater retention of vitamin B_6 in chicken breasts heated for 1.5 min in a microwave oven than for those heated 45 min in a conventional oven.

Because so little information is available, we determined the effect of type of heat and of internal endpoint temperature on vitamin B_6 content of pork muscle.

MATERIALS & METHODS

EIGHT BONELESS, frozen loin sections (cut from the 1st lumbar vertebra to the 7th rib) from eight 200–250 lb barrows were obtained from the Dept. of Animal Science & Industry, Kansas State University. At each of eight periods, a loin section (with back fat) was thawed 4 hr at 4°C and 16 hr at 25°C, then divided into fourths. These treatments were randomly assigned to the four portions: (1) microwave oven, 75°C internal temperature; (2) microwave oven, 85°C internal temperature; (3) conventional electric oven, 75°C internal temperature; and (4) conventional electric oven, 85°C internal temperature.

At each period, the four raw muscle sections (averaging 454g) were placed in Pyrex baking dishes (5 × 9 in.) for roasting: two in an electric oven at 177°C, one to an internal temperature of 75°C and the other to 85°C; and two in a microwave oven (Amana RadaRange, Model RR-2), from which (because of post-oven temperature rise) one was removed when heated to 70°C and the other when heated to 80°C. When their internal temperatures rose to 75°C and 85°C, respectively, the microwave-heated muscle sections were cut (to minimize further temperature rise) and ground for analysis.

Cooking time and cooking losses were calculated. Percentage moisture of ground muscle was determined in duplicate by drying samples 60 min at 121°C (in a Brabender Semi-Automatic Moisture Tester).

Ground samples were freeze dried and total vitamin B_6 was determined by microbiological assay (Toepfer and Polansky, 1970). Each experimental unit (one-fourth loin section) was sampled in duplicate, five dilutions of each sample were prepared, and vitamin B_6 in each dilution was determined in triplicate. A Bausch and Lomb Spectronic 20 was used for transmittancy readings. Total vitamin B_6 was calculated both on the cooked-weight (as ordinarily consumed) and on a moisture-free basis.

The 2 × 2 factorial arrangement of the two types of oven and two endpoint temperatures produced data suitable for analysis of variance:

Source of variation	DF
Oven	1
Temperature	1
Interaction O × T	1
Animal (periods)	7
Error	21
Total	31

That analysis removed animal (cooking period) variations from the desired comparisons and provided ample degrees of freedom for estimating error variance.

RESULTS & DISCUSSION

AVERAGE VALUES for cooking time and losses, percentage moisture, and vitamin B_6 from eight replications are presented in Table 1.

Cooking time (both total min and min/kg) was longer ($P < 0.01$ and $P < 0.05$), as would be expected, and total cooking loss less ($P < 0.01$) for meat cooked in the electric oven than for that cooked in microwave oven. Other workers also have reported greater losses for meat cooked in a microwave oven than for that cooked in conventional type ovens (Kylen et al., 1964; Wing and Alexander, 1972). Muscles heated in the microwave oven had greater drip loss ($P < 0.01$) than did those heated in the electric oven; volatile loss was similar. In comparing endpoint temperatures, the higher one resulted in significantly longer ($P < 0.05$) total cooking time and slight increases ($P < 0.10$) in total cooking loss.

Moisture content was greater ($P < 0.01$) for pork cooked in the electric oven than for that cooked in the microwave oven, probably because that heated in the microwave oven had greater cooking losses. Endpoint temperature did not significantly affect moisture content.

Table 1—Cooking time and losses, moisture and vitamin B_6 in pork muscle

	Microwave		Conventional		Significance[a] of F-value	
Factors	75°C	85°C	75°C	85°C	Oven	Temp
Cooking time, min	13.3	14.3	73.0	80.4	*	NS
Cooking time, min/kg	28.1	28.4	177.0	190.7	**	†
Total cooking loss, %	26.4	29.3	15.6	18.0	**	†
Drip loss, %	7.5	8.4	2.8	3.4	**	NS
Volatile loss, %	18.6	18.6	12.8	17.8	NS	NS
Moisture, %	58.5	57.9	62.9	63.3	**	NS
Vitamin B_6, μg/g of muscle	5.1	5.2	4.8	5.3	NS	NS
Vitamin B_6, μg/g of muscle, moisture free basis	12.3	12.6	13.2	14.4	†	NS

[a] †, Significant at 10% level; *, Significant at 5% level; **, Significant at 1% level; NS, Nonsignificant

Vitamin B_6 content of pork muscle cooked by microwaves has not been reported previously; however, Orr (1969) gives a value of 2.7 μg/g for canned pork, which is lower than the values found in our study. When vitamin B_6 content was calculated on the basis of cooked weight, differences among treatments were not significant; calculated on the basis of dry weight, samples cooked in the electric oven contained more ($P < 0.10$) vitamin B_6. Even though differences were significant, they were small and variation among animals was greater than between oven types.

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A Research Note

APPLICATION OF SDS-ACRYLAMIDE GEL ELECTROPHORESIS FOR DETERMINATION OF THE MAXIMUM TEMPERATURE TO WHICH BOVINE MUSCLES HAVE BEEN COOKED

INTRODUCTION

IN ORDER TO import meat from many countries of the world, the meat must be cooked to 69°C to insure destruction of the foot-and-mouth disease virus, if present. It, therefore, is essential to have a scientific method for quantitating the temperature to which meat has been cooked. Over the last few years a number of methods have been proposed such as, (a) determination of acid phosphatase activity (Cohen, 1969; Czech and Sunseri, 1968); (b) direct spectroscopy of extracted meat pigments (Helmke and Froning, 1971); (c) extractability and coagulation test of meat proteins (Cohen, 1966; Doesburg and Papendorf, 1969); and (d) colorimetric method to measure the degree of denaturation of myoglobin (Roberts, 1972). None of these four methods, however, has proved to be generally acceptable because they are either only applicable within a small temperature range or the measured parameters are susceptible to wide variations with different animals, different muscles or other experimental conditions. In view of these difficulties, the current practice of the USDA is to squeeze meat juice from a piece of meat and to judge it subjectively for the presence of pinkness. Since pinkness is the only criterion used, the processors take steps to fully and excessively cook the meat which is to be imported into the U.S.A. Since this visual method is very subjective, problems arise because there are many situations where red pigments remain even though the meat has been adequately cooked.

This paper describes the development of a reliable and objective method for precisely measuring cooking temperature. This method utilizes SDS (sodium dodecylsulfate)-acrylamide gel electrophoresis.

EXPERIMENTAL

Preparation of samples

Bovine muscles of semitendinosus, semimembranosus, biceps femoris and quadriceps femoris from round cuts of cow beef were trimmed and cut into cylindrical form 8 cm diameter and 8 cm in height. The muscle samples were then aged for different lengths of time (0, 2, 6 and 8 days postmortem) in a cooler of 4°C. After aging, a calibrated thermometer was inserted into each sample so that its bulb was placed in the geometric center of the muscle sample. The samples were then placed in the vinyl tube and cooked to the designated temperature in boiling water. After the samples attained a given temperature, they were held for various lengths of time (0, 10, 20 and 30 min) at that temperature (± 0.5°C). The samples were then transferred to an ice slurry and the temperature change was followed during the cooling. The highest temperature attained was recorded as the final cooking temperature. The temperatures under study ranged from 65 to 90°C with intervals of about 5°C. After complete cooling below 20°C, a 5–10g sample around the thermometer bulb was dissected out, homogenized with 1 volume of distilled water and centrifuged at 18,000G for 15 min. The supernatant was filtered through Whatman #1 filter paper. The resultant supernatant was used for an electrophoretic evaluation.

SDS-acrylamide electrophoresis

To a 3-ml sample aliquot, 0.03 ml of β-mercaptoethanol, 0.03 ml of 1M sodium phosphate buffer (pH 7.0) and 0.15 ml of 10% SDS were added and incubated overnight at room temper-

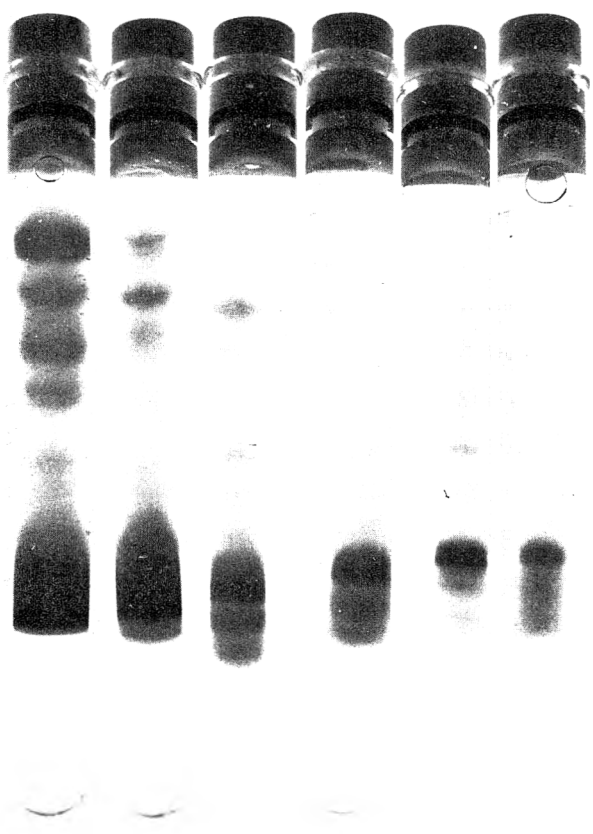

Fig. 1—SDS-acrylamide electrophoretograms of water soluble proteins from beef cooked at 65, 70, 75, 80, 85 and 90° C (from left to right).

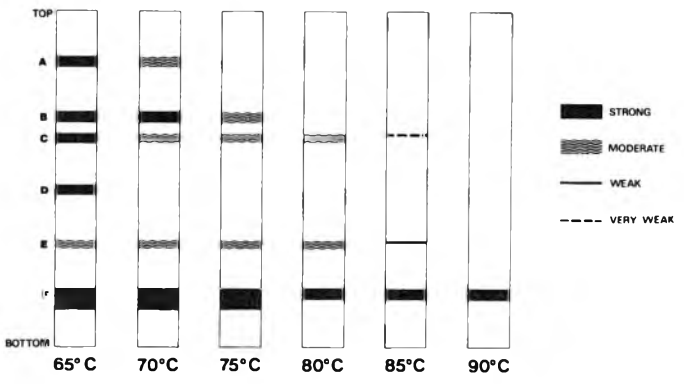

Fig. 2—Schematic diagram of electrophoretic patterns at various cooking temperatures.

ature. The 10% acrylamide gel solution was prepared by mixing 13.5 ml acrylamide solution (22.2g acrylamide + 0.6g methylene bisacrylamide in final volume of 100 ml), 1.5 ml of 1M sodium phosphate buffer (pH 7.0), 0.3 ml of 10% SDS, 1.5 ml of ammonium persulfate (12 mg in deionized water), 13.2 ml of distilled water and 0.03 ml TEMED (N,N,N′,N′-tetramethylethylene-diamine). The gel solution was poured into glass tubes 12 cm in length and 6 mm in inner diameter. A few drops of water were placed on top to produce a flat surface. After complete polymerization of the gels, 50 μl of the prepared samples were applied on top of the gel with 10 μl of bromphenol blue and 10 μl of 8M urea. The gel columns were then immersed in a bath buffer containing 25 ml of 1M sodium phosphate buffer (pH 7.0), 5 ml of 10% SDS and 470 ml of distilled water. Electrophoresis was performed for 6 hr at a constant current of 6 ma per gel with the positive electrode in the lower chamber. After electrophoresis, the gels were removed from the tubes and stained overnight at room temperature with 0.4% coomassie brilliant blue in 50% methanol and 9.2% acetic acid. The gels were then rinsed and destained in a solution containing 50% methanol and 9.2% acetic acid. After complete destaining, the gels were stored in a 7.5% acetic acid solution. The number of bands and their intensities were measured by using a densitometer.

RESULTS & DISCUSSION

SDS TREATMENT was employed in this study as a tool to accomplish electrophoretic separation of proteins solely on the basis of molecular weight, as well documented by Weber and Osborn (1969). An advantage of using SDS is to obtain constant electrophoretic mobilities of proteins independent of isoelectric point and amino acid composition which can be slightly modified during cooking. Thus, the inclusion of SDS gave more consistent and reproducible electrophoretic pattern of proteins in this study.

Figure 1 is the actual picture of the gels showing various numbers of protein bands at various cooking temperatures. For a clear view, a schematic diagram of this electrophoretic pattern is illustrated in Figure 2. At 65°C, at least six distinct bands were observed. Of these, five bands were intense and one band was weak. At 70°C, band D disappeared and only five distinct bands were observed. The intensity of band A decreased to a great extent and band B became more pronounced than adjacent bands A and C. At 75°C, band A disappeared and four distinct bands were observed. The intensity of band F (myoglobin) did not change significantly between 65 and 75°C, indicating no extensive denaturation of meat pigment below 75°C. At 80°C, one of the top bands disappeared and only three distinct bands were observed. At 85°C, the top band was very weak and two distinct bands were detectable at the bottom of the gel. At 90°C, only band F was seen at the bottom of the gel. The intensity of band F was still strong, indicating that even at 90°C there is a fair amount of residual undenatured myoglobin. The electrophoretic pattern obtained at each cooking temperature was highly reproducible regardless of a number of tested variables such as different animals, kind of muscle, length of aging period and length of cooking time up to 20 min at a given temperature.

These results have the following significance: (a) The foot-and-mouth disease virus is inactivated at the moment that an internal temperature of 69°C is achieved (Heidelbaugh and Graves, 1968). The presence, therefore, of four or less distinct protein bands in the present electrophoretic method indicates that bovine muscles have been cooked above 70°C and thus will insure the inactivation of FMD virus; (b) Because of subjectiveness and uncertainty of the current visual method (squeezing meat juice) and the need for safety against regulatory action, there is a current tendency to excessively overcook imported meat, far in excess of 69°C. The employment of the proposed SDS-acrylamide gel electrophoretic method, because of its high degree of accuracy and reproducibility, will make it possible to determine cooking temperature within the range of ± 5°C. This will obviate the need to overcook beef to merely insure the absolute disappearance of pinkness. Cooking operations can be constantly monitored more precisely. The significance of pink juice, if it occurs, can be checked and clarified.

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