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ABSTRACTS:
IN THIS ISSUE

CATHEPTIC ACTIVITY OF FISH MUSCLE. P.K. REDDI, S.M. CONSTANTINIDES & H.A. DYMSZA. *J. Food Sci.* 37, 643–648 (1972)–Catheptic activity was found to be localized in the lysosomal fraction of skeletal muscle of winter flounder (*Pseudopleuronectes americanus*). The enzyme hydrolyzed hemoglobin, globin, bovine serum albumin and endogenous proteins. The hemoglobin splitting activity of the enzyme under different conditions was determined by gel electrophoresis. An optimum pH of 4.0 was found for both the hemoglobin breakdown and autolytic activity on the endogenous proteins. The enzyme was highly thermostable, dialysis had no effect on its activity, and the enzyme was inhibited by sodium chloride, cysteine, ATP, NAD and phenyl methyl sulfonyl fluoride. Its molecular weight was found to be about 32,000 using sucrose gradient centrifugation.

DETECTION OF FROZEN FISH DETERIORATION BY AN ULTRAVIOLET SPECTROPHOTOMETRIC METHOD. A.A. DANOPOULOS & V.L. NINNI. *J. Food Sci.* 37, 649–651 (1972)–The existence of an induction period in the autoxidation of the oil in frozen fish of the species *Pagelus erythrinus* held at −20°C has been shown by the study of its ultraviolet absorption at 232 or 268 nm.

RIGOR-STRETCHED TURKEY MUSCLES: EFFECT OF HEAT ON FIBER DIMENSIONS AND SHEAR VALUES. P.V.J. HEGARTY & C.E. ALLEN. *J. Food Sci.* 37, 652–658 (1972)–Fiber dimensions and shear force values were measured in folded, prerigor and rigor stretched semitendinosus and sartorius muscles from unaged adult turkey carcasses. The unheated rigor muscles can be extended in situ but the extent is significantly less than prerigor stretching. Extensibility was detected by increases in sarcomere length. Prerigor and rigor stretching caused significantly smaller fiber diameters. Heat (70°C and 82°C) decreased sarcomere length of stretched muscles, but produced no significant change in folded controls. The stretched muscles had either significantly higher shear force values (semitendinosus) or were not different (sartorius) from the folded controls. All treatments gave reduced fiber diameters on heating. The relevance of this observation to meat tenderness is discussed. Light microscope evidence showed little damage in the rigor-stretched or heated fibers.

METABOLISM AND HISTOCHEMISTRY OF SKELETAL MUSCLE FROM STRESS-SUSCEPTIBLE PIGS. R.A. SAIR, L.L. KASTENSCHMIDT, R.G. CASSENS & E.J. BRISKEY. *J. Food Sci.* 37, 659–663 (1972)–The metabolism and histochemical characteristics of muscle from stress-susceptible and stress-resistant animals were studied. ATP values were higher and lactic acid values lower in muscle of stress-resistant animals than stress-susceptible animals at the time of exsanguination. Results from oxygen uptake studies revealed that muscle from stress-susceptible animals had a lower ability for aerobic metabolism or alternatively was more labile to damage during the isolation procedure. Our oxygen uptake values for pig muscle were lower than those reported for muscle from other species. The muscle from stress-susceptible animals, when incubated under anoxic conditions, produced more lactic acid than did muscle from stress-resistant pigs under conditions where the muscle from both types of animals had similar CP and ATP levels. Samples for histochemical analysis were examined from restrained and unrestrained muscle strips. There was a trend for the restrained samples to have somewhat smaller fibers. The muscle fiber size from Poland China animals was greater than that from Chester White animals. A large number of intermediate-type fibers, on the basis of DPNH-TR and phosphorylase staining, were found in the muscle of stress-susceptible animals.

PRODUCTION AND BINDING OF MALONALDEHYDE DURING STORAGE OF COOKED PORK. W.R. BIDLACK, T.-W. KWON & H.E. SNYDER. *J. Food Sci.* 37, 664–667 (1972)–Cooked pork samples were stored at 3°C and analyzed for malonaldehyde (MA). MA increased to a maximum and remained there, typical behavior for a food but contrasting with pure lipid oxidation in which MA increases and then decreases as volatile MA is lost. The failure to lose MA in food products may be attributed to MA binding. Water extracts of cooked pork samples (representing approximately 1/3 of the total MA) were fractionated on G-10 Sephadex and analyzed for MA. With increasing storage time, a definite change in elution pattern from free MA to bound MA took place; the binding was not to protein or amino acids.

EFFECT OF SODIUM NITRITE CONCENTRATION ON N-NITROSODIMETHYLAMINE FORMATION IN FRANKFURTERS. W. FIDDLER, E.G. PIOTROWSKI, J.W. PENSABENE, R.C. DOERR & A.E. WASSERMAN. *J. Food Sci.* 37, 668–670 (1972)–The effect of nitrite concentration on N-nitrosodimethylamine (DMNA) formation using frankfurter emulsion was investigated. Sodium nitrite was usually added to the emulsion to give levels of 150, 750, 1050, 1500 and 2500 mg/kg of meat. The frankfurters were cooked with light smoke for either 2 or 4 hr. DMNA was determined in all samples by GLC with an alkali flame ionization detector and confirmed by GLC-MS when sufficient amounts were present. Residual nitrite analysis indicated that about one-half of the sodium nitrite added was still present after processing. 19 μg DMNA per kg meat was found in frankfurters that contained 2500 mg sodium nitrite per kg of meat and processed for 2 hr. Indications of DMNA in levels of 3–14 μg/kg meat were obtained with frankfurters containing 750–1500 mg sodium nitrite per kg meat and processed for either 2 or 4 hr depending on the nitrite level. There appeared to be some tendency for an increase in DMNA formation on increasing processing time.

FLAVOR SENSITIVITY OF SELECTED ANIMAL PROTEIN FOODS TO GAMMA RADIATION. S. SUDARMADJI & W.M. URBAIN. *J. Food Sci.* 37, 671–672 (1972)–Raw protein foods derived from 20 different species of animals of different biological classifications were irradiated with gamma irradiation over a range of doses. The intensity of irradiated flavor developed in each case was determined by an expert panel. Relative flavor sensitivities and threshold doses were estimated.

DEVELOPMENT OF A MATHEMATICAL MODEL FOR OXIDATION OF POTATO CHIPS AS A FUNCTION OF OXYGEN PRESSURE, EXTENT OF OXIDATION AND EQUILIBRIUM RELATIVE HUMIDITY. D.G. QUAST, M. KAREL & W.M. RAND. *J. Food Sci.* 37, 673–678 (1972)–The rate of oxidation of potato chips as a function of oxygen partial pressure, extent of oxidation and equilibrium relative humidity was determined. We used the results to build mathematical models with the rate of oxidation as the dependent variable and the other three factors as independent variables. A mixed approach was used in model building which included incorporation of the knowledge of kinetics of lipid autoxidation mechanisms in food products as well as empirical relations. We developed a relatively simple model with only four constants. Statistical analysis indicates a good fit of this equation to the experimental results and suggests an experimental design allowing evaluation of the constants with less experimentation. The equation developed is useful for package simulation and optimization and storage life prediction, and we expect that the model can be applied to other food products.

COMPUTER SIMULATION OF STORAGE LIFE OF FOODS UNDERGOING SPOILAGE BY TWO INTERACTING MECHANISMS. D.G. QUAST & M. KAREL. *J. Food Sci.* 37, 679–683 (1972)–A mathematical model was developed to describe the deterioration of potato chips, a model of dry food products in flexible packages. In this product, deterioration occurred by two mechanisms acting simultaneously with interaction between them. The mechanisms were: oxidation by atmospheric oxygen and textural changes due to water absorption. We found that the extent of oxidation and the equilibrium relative humidity increase due to water absorption could be predicted as a function of time. The technique can be applied to investigate the storage life of products for different package configurations, initial conditions and environmental conditions.

GAS CHROMATOGRAPHIC DETERMINATION OF VOLATILE NITROSAMINES IN FOODS. J.M. ESSIGMANN & P. ISSENBERG. *J. Food Sci.* 37, 684–688 (1972)–Methods are presented for determination of certain volatile nitrosamines which have been reported to occur in foods. Nitrosamines were removed from foods using a Likens-Nickerson extractor; 60% of added dimethylnitrosamine and 70% of added diethylnitrosamine were recovered from 10 ng/g aqueous solutions. Potential gas chromatographic (GC) interferences were removed from the extract by a simple acid extraction step and, when needed, by liquid column chromatography. Dilute solutions containing nitrosamines were analyzed directly with a GC solvent stripping technique. Nitrosamines were detected with a Coulson electrolytic conductivity detector operated in the pyrolytic mode and with a flame ionization detector. Sensitivities of these detectors are compared for selected volatile alkyl and heterocyclic nitrosamines of potential significance in foods. The specificity of the Coulson detector is demonstrated for analysis of extracts of smoked fish and a number of processed meats. Additional clean-up of concentrates from foods is required to insure unambiguous identification of nitrosamines by combined GC-mass spectrometry.

CHEMISTRY OF THIAMINE DEGRADATION: 4-Methyl-5-(β-Hydroxyethyl) Thiazole from Thermally Degraded Thiamine. B.K. DWIVEDI, R.G. ARNOLD & L.M. LIBBEY. *J. Food Sci.* 37, 689–692 (1972)–A compound isolated from thiamine solutions brought to pH 5.0, 6.0 and 7.0 with 1N NaOH or with 0.1M phosphate buffer and heated in a boiling water bath open to air or in sealed tubes, or autoclaved at 121°C for 30 min, has been identified as 4-methyl-5-(β-hydroxyethyl) thiazole. Infrared, NMR and mass spectral data of this compound are presented and discussed.

CRYSTALLIZATION IN SOLUTIONS SUPERSATURATED WITH SUCROSE AND LACTOSE. T.A. NICKERSON & K.N. PATEL. *J. Food Sci.* 37, 693–697 (1972)–Changes in properties of sucrose solutions as a result of substituting lactose for a portion of the sucrose were studied at room temperature (27°C). The influence of a seeding regimen on the properties also was investigated. Seeding with sucrose or lactose favored crystallization of the sugar of higher concentration, whereas seeding a mixture of sucrose and lactose to less supersaturated solutions resulted in rapid crystallization of both sugars. Crystal habit of each sugar was influenced by increasing the concentration of the other. Solution viscosity following complete crystallization increased with the percentage of lactose. As the percentage of lactose in the supersaturated solution was increased, the proportion of lactose in the crystalline mass also increased, the proportion of sucrose decreased, and the hardness of the crystalline mass decreased. Type of seeding influenced the type and number of crystals and therefore influenced the properties of the system, but this was minor compared to the influence of composition. Application of this work to produce softer, smoother sugar products is suggested.

DIRECT ENZYMATIC CONVERSION OF LACTOSE TO ACID: GLUCOSE OXIDASE AND HEXOSE OXIDASE. A.G. RAND JR. *J. Food Sci.* 37, 698–701 (1972)–The conversion of lactose directly to an acid by glucose oxidase and hexose oxidase as a method of milk acidification was studied. Under the appropriate conditions, both enzymes demonstrated the capability to catalyze the conversion of sugar in milk to an acid, producing coagulation. A uniform distribution of oxygen under quiescent conditions was essential for the reaction and could be accomplished with the addition of hydrogen peroxide in the presence of catalase. Commercial samples of glucose oxidase caused a gradual decrease in pH of skim milk containing added glucose or lactase, (beta-galactosidase). Hexose oxidase, which can react with galactose and lactose as well as glucose, produced acid at a very slow rate in skim milk alone. When lactase was incorporated into the milk, the rate of reaction increased significantly.

INHIBITION OF o-DIPHENOL OXIDASE BY DICHLORODIFLUOROMETHANE. H.C. WARMBIER, O. FENNEMA & E.H. MARTH. *J. Food Sci.* 37, 702–705 (1972)–Dichlorodifluoromethane (f-12) can effectively and irreversibly inhibit the catalytic activity of o-diphenol oxidase (tyrosinase, polyphenol oxidase) in a simple buffered system. The degree of inhibition was influenced by concentration of and duration of exposure to f-12 and by time and vigor of agitation. Under the conditions tested, maximum inhibition of o-diphenol oxidase was obtained using 2.9 mole % f-12 and agitating samples at room temperature and 180 cpm for 20–40 min.

LOW-TEMPERATURE DESTRUCTION OF Trichinella spiralis USING LIQUID NITROGEN AND LIQUID CARBON DIOXIDE. R.E. RUST & W.J. ZIMMERMANN. *J. Food Sci.* 37, 706–707 (1972)–The destruction of *Trichinella spiralis* by low temperature treatment has been demonstrated to be an effective tool in combatting this parasite in fresh pork products. Current procedures, however, require a holding period under varying times and temperatures to accomplish this destruction. Freezing with cryogenic materials offers the opportunity to attain ultra low temperatures and, thus, eliminate post-freezing holding periods. Four trials were conducted using trichina-infected fresh pork patties approximately 9 mm thick and 88 mm square. In trials 1, 2 and 3 the patties were frozen with LN_2 using a modified Heath freezing tunnel to final equilibrated temperatures of −12°C, −14°C, −20°C, −23°C, −25°C, −28°C, −29°C, −39°C and −47°C. In trial 4 the patties were frozen in a Certified Multideck tunnel using liquid CO_2 as a refrigerant to −10°C, −17°C, −23°C, −29°C and −39°C. Patties were thawed immediately and checked for viable *T. spiralis*. No positive samples were found at temperatures of −29°C or below.

PHYSIOLOGICAL AND CHEMICAL STUDIES OF CHILLING INJURY IN PEPPER FRUITS. N. KOZUKUE & K.OGATA. *J. Food Sci.* 37, 708–711 (1972)–Physiological effects of low temperature (1–6°C) on pepper fruits were studied during and after exposure for various periods. The CO_2 production of the fruits stored at low temperature increased abnormally after transfer to 18°C. There was an accumulation of α-keto acids in chilled fruits: fumaric, succinic, citric and malic acids were detected and malic increased remarkably during low temperature storage. Using paper chromatography, chlorogenic acid was found to be a main phenolic substance in the pepper seeds: its content increased immediately after exposure of the peppers to low temperature and decreased rapidly during subsequent cold storage. The content of shikimic acid in chilled seeds showed a similar tendency to that of chlorogenic acid; phenylalanine ammonia-lyase (PAL) activity increased rapidly after 2 days' cold storage, then decreased sharply; tyrosine ammonia-lyase (TAL) activity was fairly low compared with PAL activity.

ACID-SOLUBLE NUCLEOTIDES OF JUICE VESICLES OF CITRUS FRUIT. C.R. BARMORE & R.H. BIGGS. *J. Food Sci.* 37, 712–714 (1972)–Nucleotides from fruit of "Orlando" tangelo (*Citrus reticulata* × *C. paradisi*) and "Hamlin" and "Pineapple" sweet oranges (*C. sinensis*) extracted with perchloric acid, were separated by anion-exchange chromatography using formic acid:aqueous ammonium formate as the eluting solvent. Tentative identification of the various fractions was made by comparing the retention time of unknowns with standards fractionated by the same procedure and by adsorption spectral analyses. With the use of these methods, acid-soluble nucleotides were found to be present in appreciable quantities in mature fruit. Those in concentration that could be tentatively identified were CMP, AMP, CDP, UMP, ADP, CTP, GDP, UDP and ATP. The latter was further assayed by the luciferin-luciferase method. The possible significance of the nucleotides to fruit quality was discussed.

ABSTRACTS:
IN THIS ISSUE

EFFECT OF GAMMA RADIATION ON WHEAT PROTEINS. H. SRINIVAS, H.N. ANANTHASWAMY, U.K. VAKIL & A. SREENIVASAN. *J. Food Sci.* 37, 715–718 (1972)–An observed higher degree of autolysis in irradiated (20–200 Krads) wheat is apparently due to increased susceptibility of proteins to protease action. Total amino acid profiles of wheat and of isolated gluten reveal no appreciable changes on irradiation up to 1 Mrad. However, there is an overall increase in free amino acid levels in wheat irradiated at 1 Mrad. Lysine availability in wheat is not affected by radiation treatment. Studies on radiosensitivities of wheat proteins show a shift in molecular weight distribution to lower values.

STABILIZATION OF CALCIUM SENSITIVE PLANT PROTEINS BY κ-CARRAGEENAN. B.K. CHAKRABORTY & H.E. RANDOLPH. *J. Food Sci.* 37, 719–721 (1972)–The stabilizing effect of κ-carrageenan on 0.15% solutions of coconut, glandless cottonseed, peanut and soy protein isolates was investigated at neutral pH ranges. Addition of 0.01M calcium decreased protein stability by 75–88% in carrageenan-free solutions and only by 30% in solutions containing 0.2g carrageenan per g protein. The order of mixing carrageenan, Ca^{++}, and protein solutions adversely affected stability only when carrageenan was added last. Electron microscopy of stable fractions revealed the presence of many 100–500 Å protein globules, both complexed with κ-carrageenan and free in solution. Protein stabilization appeared to be related to the formation of double helix junction zones by κ-carrageenan.

FREE ARGININE CONTENT OF PEANUTS (Arachis hypogaea L.) AS A MEASURE OF SEED MATURITY. C.T. YOUNG & M.E. MASON. *J. Food Sci.* 37, 722–725 (1972)–The level of arginine, determined by a modified Sakaguchi method, was used to establish the degree of immaturity in freshly harvested and cured peanuts. Precision and accuracy of the method is reported. Calibration curves were plotted to estimate the degree of immaturity in peanuts. The method was tested under field conditions and found to be an accurate measure of immaturity.

THE GRADING OF LOW-ESTER PECTIN FOR USE IN DESSERT GELS. S.A. BLACK & C.J.B. SMIT. *J. Food Sci.* 37, 726–729 (1972)–Dessert gels made from a commercially available low-ester pectin were examined for firmness with a modified Sunkist Exchange Ridgelimeter and for breaking pressure using a Marine Colloids Gel Tester. Data are presented to show the effect of temperature, pH, pectin concentration, cooking time and calcium level on these two quality characteristics. Based on the data obtained a suggested grading procedure in terms of low-ester firmness (F) and low-ester strength (S) was developed.

THE EFFECT OF DEMETHYLATION PROCEDURES ON THE QUALITY OF LOW-ESTER PECTINS USED IN DESSERT GELS. S.A. BLACK & C.J.B. SMIT. *J. Food Sci.* 37, 730–732 (1972)–Low-methoxyl pectins were prepared from a high molecular weight pectin by acid demethylation in an HCl-isopropanol-water suspension and by alkaline demethylation using NH_4OH or NaOH in solution. The demethylated samples were analyzed, graded and used to make dessert type gels. The properties of the resulting gels were compared.

INTERRELATIONSHIP BETWEEN CERTAIN PHYSICOCHEMICAL PROPERTIES OF RICE. K.R. BHATTACHARYA, C.M. SOWBHAGYA & Y.M. INDUDHARA SWAMY. *J. Food Sci.* 37, 733–735 (1972)–Water uptake at 80°C and starch-iodine blue value at 65°C, both expressed as percentage of the respective parameter at 96°C, gave good inverse estimates of the gelatinization temperature of rice. The equilibrium moisture content on soaking in water was 28–29% in *indica*, and 30–31% in dwarf *indica* and *japonica* rice. Similarly the fraction of total amylose soluble in water (96°C) was around 50% in *indica* and *japonica*, but 35% in dwarf *indica*. Dwarf *indica* rice viscogram showed a rather high setback value. The blue value of the excess cooking water of rice gave no useful information. Varieties with less than 25% amylose gave C-type alkali reaction.

THE INFLUENCE OF AGING BEEF IN VACUUM. D. MINKS & W.C. STRINGER. *J. Food Sci.* 37, 736–738 (1972)–U.S. Choice grade beef loins and ribs were aged to determine the effect of vacuum packaging for 7 and 15 days at 32° and 40°F on sensory characteristics, microbial counts and weight loss. Vacuum packaging had no significant influence on tenderness, flavor and juiciness but produced a highly significant reduction in weight loss during aging. Surface bacterial counts were significantly less on the vacuum packaged portions than the portions not packaged.

PROTECTIVE PACKAGING MATERIALS FOR FRESH BEEF SHIPMENTS. R.H. REA, G.C. SMITH, Z.L. CARPENTER & K.E. HOKE. *J. Food Sci.* 37, 739–742 (1972)–Five test shipments were conducted involving 98,000 lb of beef quarters and primal cuts transported 270–1350 mi and requiring from 19–120 hr intransit. Trailer temperatures were affected by loading patterns, product densities, positions of palletized product or paper partitions, thermostat settings and air conveyance systems and correspondingly, the temperature conditions observed were generally inadequate for maintenance of beef quality. The use of polyethylene bags or polyvinyl chloride film significantly ($P < .05$) reduced intransit shrinkage as compared to unprotected cuts in 7 of 16 comparisons. Considerations of costs for materials, package application and removal and the monetary value of reduced weight loss suggest that the concept is economically feasible from the standpoint of savings in weight loss alone. Beef protected by polyethylene or polyvinyl chloride exhibited more desirable lean color and subcutaneous fat cover scores at shipment destination in comparison to beef which was unprotected during transit. Although bacterial counts did not generally differ among treatments, the surfaces of cuts protected by plastic materials were more moist than corresponding surfaces on unprotected cuts. Since increased moisture is conducive to enhanced microbial growth, prolonged storage in the plastic packaging materials should be avoided. No compensatory weight loss was observed during subsequent cooler storage after protective packaging materials were removed.

DEVELOPMENT OF A PROCESS FOR PREPARING A FISH PROTEIN CONCENTRATE WITH REHYDRATION AND EMULSIFYING CAPACITIES. B.F. COBB III & K. HYDER. *J. Food Sci.* 37, 743–750 (1972)–A hot-solvent extraction process for preparing fish protein concentrate (FPC) with rehydration and emulsifying capacities is described. The process employed extraction at pH 2.5 in order to prevent protein-protein interaction. Equal volumes of ethanol and hexane at reflux temperatures were employed as the extracting medium. Gel formation was controlled by the addition of NaCl. The FPC produced by this method had an amino acid spectrum similar to that of the fish, formed gels when added to water and emulsions when the rehydrated material was blended with vegetable oils. Both emulsification and rehydration capacities of the FPC were pH dependent with minimum uptake of water and oil in the range of pH 5–8.

TEXTURE MEASUREMENT OF INDIVIDUAL COOKED DRY BEANS BY THE PUNCTURE TEST. M.C. BOURNE. *J. Food Sci.* 37, 751–753 (1972)–The Instron Universal Testing Machine was set up to puncture test a large number of cooked dry beans, one bean at a time, by using the distance cycling controls on the machine. The punch is caused to cycle between two pre-set distance limits at a predetermined speed, permitting the operator to devote full attention to placing and removing beans in the puncture cup. 10 beans per minute can be conveniently tested by this procedure. The number of peak force heights in 20 selected force ranges are counted off from the chart, making for rapid retrieval of data. Tests on cooked dry beans show that the number of beans in each texture range follows approximately a normal distribution pattern, except for the presence of a few exceptionally hard beans which cause the distribution curve to tail off into the high puncture-force range.

RATE LIMITING MECHANISMS IN CAUSTIC POTATO PEELING. D.M. McFARLAND & W.J. THOMSON. *J. Food Sci.* 37, 754–759 (1972)–The caustic peeling rates of potatoes is considered from an analytical and experimental point of view. The analysis considers the process to be governed by the competing rates of mass transport within the potato (diffusion) and chemical reaction. The chemical reaction between caustic and chlorogenic acid is singled out for analysis since the product of this reaction is a yellow salt which is easily measured by colorimetry. Simplified equations for the concentration of the yellow salt as a function of time is obtained for the two limiting extremes of diffusion controlled rates and reaction controlled rates. Experiments on Idaho Russetts indicate that, over the first 2–3 min of immersion in caustic, the process is diffusion controlled. It is also shown that it is the outer skin which presents the large diffusion resistance. For reasonable times after this initial period the process is reaction controlled and very sensitive to temperature. The implications of these results with respect to processing techniques (e.g., the dry peel process) and sequences is considered and discussed.

SURVEY OF FOOD INGREDIENT–DDT REACTIONS UNDER THERMAL PROCESSING CONDITIONS. J.W. RALLS & A. CORTES. *J. Food Sci.* 37, 760–763 (1972)–Aqueous solutions or suspensions of single or multiple food ingredients were heated at 100°C for 2 hr with carbon-14 labeled DDT. Thin-layer chromatography on alumina plates resolved the resulting mixtures into regions corresponding to R_f values for five known transformation products or analogs of DDT. The radioactivity of each plate region was related to the quantity of each product formed. DDT is relatively stable in heated water or buffer solutions of pH 3 to 8 as shown by recoveries of DDT (98–100%); at pH 8.1, approximately 4% of DDE was formed. The compositional changes in radioactive DDT due to heating with water-soluble vitamins, disphosphopyridine nucleotide, yeast nucleic acid, amino acids, peptides and casein were determined. The maximum reduction in DDT content of a suspension heated with a single component (cysteine hydrochloride) was 10%. A mixture of DDT with nine components heated for 2 hr produced a 20% reduction in DDT content.

EVALUATION OF TRITICALE FOR THE MANUFACTURE OF NOODLES. K. LORENZ, W. DILSAVER & J. LOUGH. *J. Food Sci.* 37, 764–767 (1972)–According to the literature the protein nutritive value of triticale is higher than that of wheat. For this reason, and others, the possibility of using triticale in noodles was investigated. Regular noodles and egg noodles were prepared from all-purpose flour, durum flour, triticale flour, as well as from semolina and were evaluated by panels for flavor and firmness after cooking times of 10, 15, 25 and 30 min. Regular noodles made from triticale flour had the shortest cooking time and the best cooking tolerance. There were no statistically significant differences in the flavor of the noodles among the flours used. The regular triticale noodles had a higher cooking loss compared to the other noodle samples. However, the addition of eggs to the noodle recipe eliminated any statistically significant differences in cooking loss between noodles made from the different flours. It was concluded that triticale, which at present is used in animal feeds, can be used for the manufacture of noodles.

COLORIMETRIC FURFURAL MEASUREMENT AS AN INDEX OF DETERIORATION IN STORED CITRUS JUICES. H.L. DINSMORE & S. NAGY. *J. Food Sci.* 37, 768–770 (1972)–Furfural arising in citrus juice as a result of deterioration during storage may be recovered and concentrated by rapid distillation and then colorimetrically determined by reaction with 10% aniline in acetic acid-ethanol. As little as 25 μg per liter of furfural can be measured. The development of noticeable furfural parallels the development of off-flavor and provides a useful numerical index for the extent of degradation. Results of a brief storage study on orange juice are reported.

NITROGEN EXTRACTABILITY AND MOISTURE ADSORPTION CHARACTERISTICS OF SUNFLOWER SEED PRODUCTS. A. KILARA, E.S. HUMBERT & F.W. SOSULSKI. *J. Food Sci.* 37, 771–773 (1972)–Diffusion-extracted (DE) sunflower meal and protein isolate were compared with untreated samples for their nitrogen extractability and moisture adsorption. Nitrogen extractability values of 90% were found at pH 7 or above in the untreated meal, whereas a maximum solubility of only 70% was achieved at pH 9.0 in the DE meal prepared at 60°C. DE meal prepared at 80°C had low solubility over a pH range of 1–11 which indicated substantial denaturation of the sunflower proteins. The untreated isolate showed a sharp minimum solubility point in contrast to the low solubility of the DE isolate over a pH range of 3–7. Moisture adsorption values for the samples held at 5, 20 and 30°C indicated little difference in moisture contents at relative humidities of 11–55%. At higher levels, however, the untreated meal adsorbed more moisture than the DE meal. A reverse trend was noticed for the isolates as the moisture content of the DE isolate was higher than the untreated sample. The rate of moisture uptake by sunflower kernels was slower than that observed with rapeseed or soybean meats and lower total moisture contents were observed after a 4 hr soaking period.

PALATABILITY OF PANCAKES AND COOKED CORN MEAL FORTIFIED WITH LEGUME FLOURS. D.G. GUADAGNI & D. VENSTROM. *J. Food Sci.* 37, 774–777 (1972)–Various types of laboratory preparations of navy and pinto beans and commercial soy flours were used as fortifying agents to improve the nutritional quality of some relatively low protein foods–corn meal products and pancake mixes. The detectable level of legume products in these foods by a trained laboratory panel varied from about 10–25%. Sensory evaluation of the fortified and unfortified foods by paired comparison and hedonic scale methods indicated that up to 50% of some legume products could be added without significant loss in palatability.

EVALUATION OF A PROTEIN CONCENTRATE PRODUCED FROM GLANDLESS COTTONSEED FLOUR BY A WET-EXTRACTION PROCESS. J.T. LAWHON, L.W. ROONEY, C.M. CATER & K.F. MATTIL. *J. Food Sci.* 37, 778–782 (1972)–Wet-extracted concentrate spray dried at two pH levels was evaluated for use in protein-fortified bread and as a component in meat loaf to reduce juice and fat cook-out during baking and to reduce meat requirement. Bread loaves containing air-classified glandless conc, 100% wheat flour and LCP glanded flour were significantly larger in volume than loaves from other blends, but there was no real difference among loaf volume of breads containing these three treatments. pH of spray drying had a marked effect on baking properties of the wet-process conc; pH 4.5 yielded a poor quality loaf while pH 6.8 loaf volume was equal to bread containing glandless cottonseed flours. Taste panel evaluations showed meat loaves containing 25% wet-extracted protein concentrate to be quite acceptable: milder or more bland than all-meat loaves and somewhat softer or smoother in texture.

SPORE COUNTS OF THERMOPHILIC AEROBIC BACTERIA IN SOIL. M.L. FIELDS. *J. Food Sci.* 37, 783–784 (1972)–Soil from four temperature zones (temperate, subtropics, tropics and desert) were analyzed for spores of thermophilic aerobic sporeforming bacteria. Spore counts (incubated at 65°C for 24 hr after boiling for 30 min) were positively correlated with soil pH and organic matter (in one set of samples). When all data were considered, soil pH was positively correlated ($P < 0.01$) with spore counts.

ABSTRACTS:
IN THIS ISSUE

NITROSAMINES AND THE INHIBITION OF Clostridia IN MEDIUM HEATED WITH SODIUM NITRITE. A.E. WASSERMAN & C.N. HUHTANEN. *J. Food Sci.* 37, 785–786 (1972) During sterilization of a culture medium in the presence of $NaNO_2$ an inhibitor for the growth of *Clostridium* was observed. The possibility this might be certain volatile N-nitroso compounds was investigated using GC-MS. The minimum inhibitory concentration (MIC) of NO_2^- in the medium autoclaved with NO_2^- was 40 ppm; in medium to which NO_2^- was added aseptically, the MIC was 640 ppm. Chromatograms showed peaks with retention times similar to N-nitroso compounds; however, structure confirmation with MS showed none contained a nitrosoamine and additional experiments confirmed nitrosamines had no inhibitory effects to growth of *Clostridia.*

SOME REACTION PRODUCTS FROM NONENZYMATIC BROWNING OF GLUCOSE AND METHIONINE. R.C. LINDSAY & V.K. LAU. *J. Food Sci.* 37, 787–788 (1972)–Column and thin-layer chromatographic analysis revealed that acetone, butanal, ethanal, methanal, methional, furfural and 2-propenal were in the monocarbonyl fraction from heat induced glucose and methionine nonenzymatic browning reaction mixtures. Methional was found to be responsible for the characteristic boiled potato-like aroma of the reaction mixture. 2-Propenal was identified as the 3-carbon aldehyde degradation product of methionine.

STABILITY OF INOSINIC ACID, INOSINE AND HYPOXANTHINE IN AQUEOUS SOLUTIONS. J. DAVÍDEK, J. VELÍŠEK & G. JANÍČEK. *J. Food Sci.* 37, 789–790 (1972)–The stability of inosinic acid, inosine and hypoxanthine in aqueous solutions at 90°C was studied. Inosinic acid, inosine and hypoxanthine were separated by ion exchange chromatography and spectrometrically estimated. Reaction rates in the region pH 2–12 were calculated. The activation energy at pH 3, 4 and 5 were 34.0, 30.4 and 28.1 kcal/mol, respectively. Results show that inosinic acid contents of heat-preserved meat products and enzyme-free systems would stay practically unchanged during normal storage conditions.

DETERMINATION OF BROMINATED VEGETABLE OIL CONCENTRATIONS IN SOFT DRINKS USING A SPECIFIC ION ELECTRODE. D.L. TURNER. *J. Food Sci.* 37, 791–792 (1972)–A method for quantitative determination of brominated vegetable oils at the 15 ppm level in soft drinks was developed. Extracts of beverages were subjected to a debromination reaction using sodium borohydride and a palladium-on-charcoal catalyst. The resulting bromide was measured with a specific ion electrode. At the 15 ppm level of brominated vegetable oil, the accuracy and precision of this method was $\pm < 1$ ppm and ± 1 ppm, respectively.

EFFECT OF SONIC ENERGY ON THE AIR DRYING OF APPLE AND SWEET POTATO CUBES. R.A. CARLSON, D.F. FARKAS & R.M. CURTIS. *J. Food Sci.* 37, 793–794 –Cubes (2 cc) of apple and sweet potato were dried with and without sound energy in air at velocities of 5–20 fps and temperatures of 125–175°F. A stem jet or electronic whistle provided sound energy at 9,800 and 13,000 Hz with an intensity up to 135 dB. Results indicated that sonic energy had no effect on drying rate under all experimental conditions.

FILM OBSERVATIONS AT AN OIL-WATER INTERFACE. J.C. ACTON & R.L. SAFFLE. *J. Food Sci.* 37, 795–796 (1972)–An experimental technique was utilized which allows the observation and photographic recording of film formation at an oil-water interface. Salt soluble protein of cow meat and beef hearts, sodium caseinate and gum acacia showed extensive interfacial film development. No films were observed from solutions of soy sodium proteinate or propylene glycol alginate.

BIOCHEMISTRY OF TEA FERMENTATION: FORMATION OF t-2-HEXENAL FROM LINOLENIC ACID. J.G. GONZALEZ, P. COGGON & G.W. SANDERSON. *J. Food Sci.* 37, 797–798 (1972)–U-^{14}C-Linolenic was shown to be transformed in part to *t*-2-hexenal during the conversion of fresh tea leaf to black tea. In fact, *t*-2-hexenal was the only volatile compound which was formed during the conversion process. Further, the formation of *t*-2-hexenal was shown to be dependent on the enzymic conversion process since no *t*-2-hexenal was formed when the leaf enzymes were inactivated prior to adding the ^{14}C-linolenic acid. Since linolenic acid is the major fatty acid present in fresh tea leaf and *t*-2-hexenal is a prominent component of black tea aroma, it must be concluded that this reaction helps to determine the character of black tea aroma.

AN APPARATUS FOR MEASUREMENT OF CONTRACTILE PROPERTIES OF PORCINE SKELETAL MUSCLE. D.R. CAMPION, R.G. CASSENS & F.J. NAGLE. *J. Food Sci.* 37, 799–800 (1972)–Describes the design of apparatus and development of a test procedure to measure the contraction time, one-half relaxation time, net twitch tension/g wet weight muscle, net tetanus tension/g wet weight muscle and twitch-tetanus ratio in muscle of porcine animals weighing up to 100 kg.

SHELF LIFE STABILITY AND ACCEPTANCE OF FROZEN PACIFIC HAKE (Merluccius productus) FILLET PORTIONS. D.L. CRAWFORD, D.K. LAW & L.S. McGILL. *J. Food Sci.* 37, 801–802 (1972)–Fish portions prepared from frozen blocks of Pacific hake fillets stored vacuum sealed in moisture-vapor proof film at –15°F for 12 months were shown to have a high degree of acceptance by flavor panels with very little change occurring during storage. Antioxidants applied in an aqueous solution achieved through the use of propylene glycol and a surface active agent and sodium tripolyphosphate added either alone or in combination with antioxidant did not improve flavor panel scores. Development of oxidative rancidity during storage as indicated by TBA-analysis was minimal. Sodium tripolyphosphate appeared to exert a slight prooxidative influence, which was somewhat reduced by the application of antioxidants. The degree of acceptance and shelf-life stability observed seems to indicate a good potential for using this under-utilized species for human food.

IFS BASIC SCIENCE

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CATHEPTIC ACTIVITY OF FISH MUSCLE

INTRODUCTION

PROTEOLYSIS of hemoglobin and other protein substrates by tissue extracts has been attributed, by convention, to the action of a group of hydrolytic enzymes, the cathepsins found in the lysosomal fraction (DeDuve, 1963; Shibko and Tappel, 1965; Coffey and DeDuve, 1968). A study by Caldwell (1970) suggested the joint action of several muscle cathepsins on endogenous proteins. Huang and Tappel (1971) showed that cathepsin D is the most important of the cathepsins since it initiates protein hydrolysis and produces peptides that are further broken down by other cathepsins such as cathepsin C.

Skeletal muscles as a source of lysosomal hydrolases have received little attention (Fruton, 1960), probably because of their low lysosomal content. Bate-Smith (1948), in a review article, suggested that proteolysis by cathepsin was the most probable cause of tenderness developed during the post-mortem aging of meat. Zender et al. (1958) and Sharp (1963) also postulated a relationship between autolysis and muscle tenderization.

Fish muscle cathepsins have not been studied in great detail. Siebert (1958) found that the cathepsin activity of fish muscle was ten times greater than that of mammalian tissue; and the role of cathepsin in spoilage of fish muscle was stressed (Siebert, 1962). Purification studies on fish muscle cathepsins were carried out by different workers. Groninger (1964) purified a proteinase from albacore muscle and Ting et al. (1968) partially purified two cathepsins from salmon muscle. Makinodan and Ikeda (1969) reported the existence of a proteolytic enzyme in the muscle of a few fish species which acts at a slightly alkaline pH range, in addition to the other proteinases, which act at an acid range. Cathepsin D has also been purified from porcine muscle (Parrish and Bailey, 1966); bovine muscle (Bodwell and Pearson, 1963; Parrish and Bailey, 1967); chicken muscle (Martins and Whitaker, 1968) and from bovine uterus (Woessner and Shamberger, 1967). Bird et al. (1969) studied the distribution of cathepsin D and other lysosomal enzymes in the white muscle of the goldfish.

In the present study, experiments were undertaken to study the intracellular localization of catheptic activity in the skeletal muscle of winter flounder, (*Pseudopleuronectes americanus*) and to investigate the physical and chemical properties of this enzyme as related to autolytic spoilage.

EXPERIMENTAL

Preparation of cell fractions and cathepsin

Freshly caught winter flounders (*Pseudopleuronectes americanus*) were killed by decapitation and eviscerated immediately. The muscle from the back part, free of bones and skin, was weighed, cut up into small pieces in 0.25M cold sucrose solution containing 1 mM ethylenediamine tetraacetate (EDTA), pH 7.0. The pieces were introduced into a Potter-type homogenizer (smooth-glass-walled tube and teflon pestle) and homogenized at 4°C, using a homogenizing drill (Craftsman, Sears) with four up and down strokes, in sufficient sucrose solution to make a 1:8 (w/v) suspension. Extraction of the enzyme was completed within 4 hr after

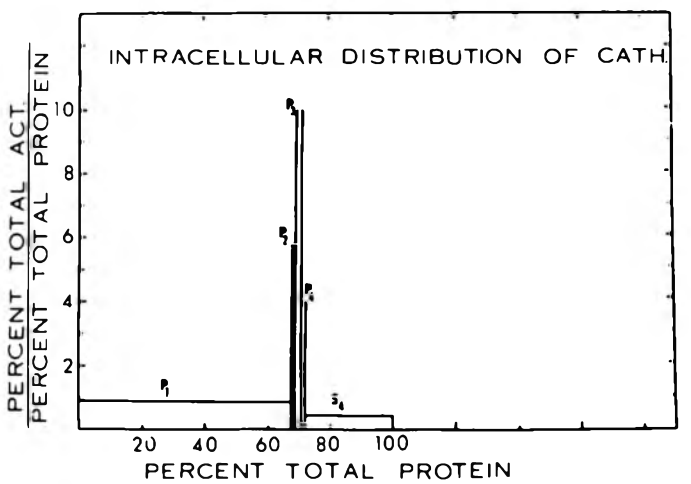

Fig. 1—Intracellular distribution pattern of cathepsin D in fish muscle. The fractions along the abscissa from left to right are: unbroken cells and nuclei (P_1), mitochondria (P_2), lysosomes (P_3), microsomes (P_4) and final supernatant (S_4). These are represented by their percentage of the total protein in the homogenate. Along ordinate, their relative specific activities are plotted as Percent of total activity/Percent of total protein.

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the death of the fish. All the processes were conducted at 4°C.

The homogenate was centrifuged in a Sorvall RC 2-B refrigerated centrifuge using an SS-34 rotor at 755g for 10 min to separate the nuclei and cell debris (P_1). The supernatant was centrifuged at 9,750g for 15 min to sediment mitochondria (P_2). The supernatant obtained was centrifuged at 27,000g for 30 min to separate lysosomes (P_3). The remaining supernatant was centrifuged at 78,480g for 90 min in an L3-50 Spinco preparative ultracentrifuge with a type 30 rotor to separate the microsomes (P_4). The final supernatant (S_4) contains all kinds of soluble proteins. In this study these proteins were termed as "endogenous" when used as substrates to catheptic activity. These endogenous proteins were not characterized.

The lysosomal fraction, as well as other pellets, were resuspended and hand homogenized in 0.1% Triton X-100 in 0.01M imidazole buffer pH 7.2 to release the enzyme. The protein content of all fractions was estimated by the Lowry method (Lowry et al., 1951). In some cases activity was measured in the whole homogenate.

Preparation of denatured hemoglobin

Denatured hemoglobin was used as a substrate for catheptic activity. The preparation was a modification of the method used by Ting et al. (1968). A 10% hemoglobin (Bovine II, Sigma) solution was prepared in 8M urea. Denaturation was allowed to take place at room temperature for 4 hr. The denatured hemoglobin was then diluted with water to make a 2.5% solution. It was filtered through Whatman No. 1 filter paper and dialyzed against several changes of distilled water for 48 hr to remove the urea. After dialysis, 1 mg of trimersol (Sigma) was added to 40 ml of solution and the preparation was stored at refrigeration temperature until used.

Assay of catheptic activity

The method used was a modification of the method of Anson (1938) as modified by Tallen et al. (1952). Assays were carried out in a water bath at 37°C. The assay mixture contained 4 ml of 2.5% hemoglobin solution, 1 ml of 0.2M acetate buffer, pH 4.0, 1 ml of muscle homogenate or 0.5 ml of P_3 (lysosomal fraction) and sufficient 0.1N HCl or 0.1N NaOH to maintain the pH. Water was added to make the total volume to 7 ml. After 2 hr of incubation, 5ml of 0.3M TCA was added, the flasks were swirled by hand for 20 sec and then replaced in the bath for 20 min. The precipitate was removed by filtration through Whatman No. 1 filter paper. The absorbancy of the TCA filtrate was measured at 275 mμ. Blanks were run in the same manner, except that TCA was added immediately before the homogenate or the lysosomal fraction.

Recently a fluorimetric (DeLumen and Tappel, 1970) and a radioactive method (Roth et al., 1971) were reported for assay of cathepsin D activity. We made a comparative study of the sensitivity of all the available assay methods for cathepsin D activity, the results of which will be published elsewhere. Some of the experiments reported here were repeated with the new methods above and the same results were obtained.

Assay of lactate dehydrogenase

Beef heart lactate dehydrogenase was assayed by using a direct spectrophotometric measurement of the disappearance of NADH. The assay mixture contained 0.42 ml of 0.1M imidazole buffer, pH 7.5, 0.004 ml of 0.02M sodium pyruvate and 0.036 ml of 4 mM NADH in 0.01M NaCl. Enzyme solution and water were added to make a final volume of 0.5 ml. The final assay mix contained 0.084M imidazole buffer, pH 7.5, 1.6×10^{-4}M sodium pyruvate and 2.88×10^{-4}M NADH. The production of NAD was measured at 23°C in a Gilford 240 recording spectrophotometer using microcuvettes at 340 mμ.

Sucrose density gradient centrifugation

Sucrose density gradient centrifugation was carried out as described by Martin and Ames (1961), except for slight modification. 5% and 20% sucrose solutions in 0.01M imidazole buffer, pH 7.2, were used to prepare the 30 ml gradients, using the Buchler gradient mixing device. A solution of 1.2 ml of enzyme preparation with 25 μl of beef heart lactate dehydrogenase (10 mg/ml) was layered on the gradients. The gradients were centrifuged in the SW 25.1 rotor in a Spinco ultracentrifuge at 25,000 RPM for 50 hr at 4°C. Immediately after centrifugation, twelve 2.5 ml fractions were collected by puncturing the bottom of the tubes with a 16 gauge needle (Buchler puncturing device). All the fractions were assayed for catheptic activity and for the marker enzyme lactate dehydrogenase.

Gel electrophoresis

A 7% polyacrylamide gel electrophoresis prepared by a modification of the method of Ornstein and Davis (1962) was used to demonstrate the activity on hemoglobin under different conditions. About 0.4 ml of the sample

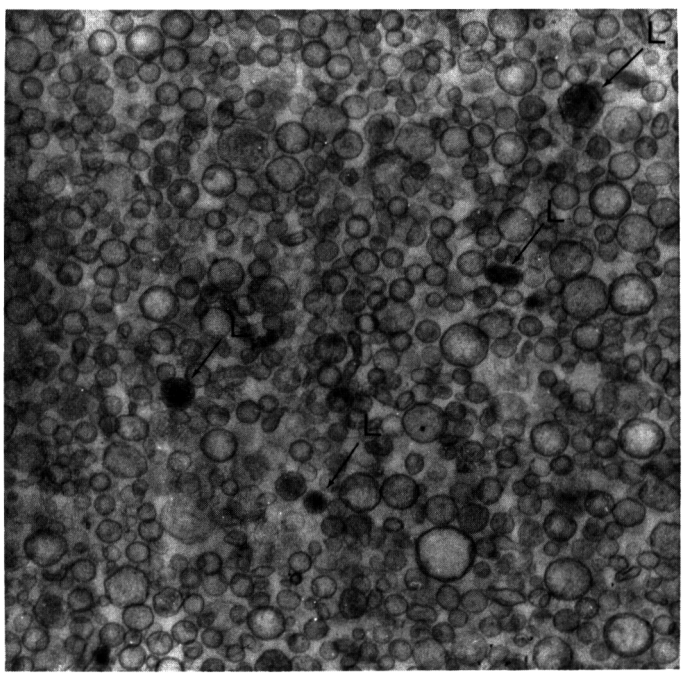

Fig. 2—Electron micrograph of lysosomal fraction (P_3). Magnification 16,856×. L indicates intact lysosomes. The dark staining is characteristic to lysosomes. Immediately after decapitation of living fish, skeletal muscle was taken and the crude lysosomes were separated at 4°C. This fraction also contains some mitochondria and microsomes due to contamination with P_2 and P_4 fractions.

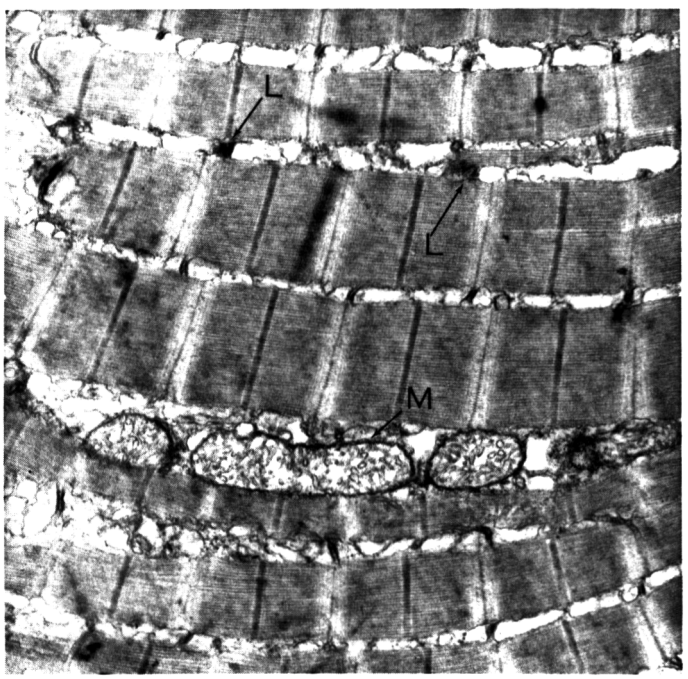

Fig. 3—Electron micrograph of flounder muscle. Magnification 13,063×. L indicates lysosomes, M represents mitochondria. The tissue was fixed immediately after decapitation of the living fish. There was hardly any time for post-mortem disintegration of lysosomes.

Table 1–Activity of cathepsin (P_3) on different substrates[a]

Substrate	Specific activity A/2 hr/mg
1. Hemoglobin, denatured	0.130
2. Hemoglobin, native	0.125
3. Globin, native	0.071
4. Endogenous proteins (S_4)	0.055
5. Bovine serum albumin	0.025

[a]The proteolytic activity of P_3 fraction was carried out on different substrates at pH 4.0 and at 37°C for 2 hr. The assay mixtures contained 1 ml of 0.2M acetate buffer, pH 4.0, 4 ml of 2.5% substrate solutions (S_4 = 4.8 mg per ml protein), 0.5 ml of P_3 and 1.5 ml of water. Assays were carried out as described in the text. The protein concentration is the same for all substrates used, except for the S_4 fraction. The term "endogenous proteins" refers to the soluble proteins present in the final supernatant (S_4) fraction obtained during fractionation of fish muscle homogenate.

with 2% sucrose was layered directly on the top of the polymerized upper gel. The current used for protein separation was 2 ma per tube in the beginning and 3 ma per tube after the front migrated into the upper gel. Total run was about 80 min. The gels were stained for protein in Amidoschwartz solution for 30 min. The stained gels were destained by repeated washings in 7% acetic acid.

Preparation of tissue and lysosomal fraction for electron microscopic examination

Intact muscle tissue was fixed in 2% formaldehyde and 2.5% glutaraldehyde in 0.1M cacodylate buffer, pH 7.2, containing 0.05% $CaCl_2$ followed by post-fixation in 1% OsO_4 in 0.1M phosphate buffer, pH 7.2. The lysosomal fraction was fixed in 5% glutaraldehyde in 0.1M phosphate buffer, pH 7.2, followed by post-fixation in 1% OsO_4 in 0.1M collidine buffer, pH 4.7. From this point, both samples were treated similarly. The samples were dehydrated in a graded ethanol series, followed by propylene oxide, and embedded in Epon 812. Blocks were sectioned using glass knives on a Sorvall MT-2 Porter Blum ultramicrotome. Sections were stained with 6% uranyl acetate and Reynold's lead citrate. Electron micrographs were taken on an RCA EMN-3G electron microscope.

Table 2–Effect of pH on cathepsin D activity of fish muscle lysosomal fraction (P_3) on endogenous muscle proteins (S_4)[a]

pH	Specific activity A/2 hr/mg × 10^2
3	2.5
4	5.2
5	4.4
6	4.0
7	2.3
8	0.5

[a]The term "endogenous proteins" refers to the soluble proteins present in the final supernatant (S_4) fraction obtained during fractionation of fish muscle homogenate. Increase in absorbancy was determined at 275 mμ.

RESULTS & DISCUSSION

THE INTRACELLULAR proteolytic activity is present mainly in the lysosomes of cells ranging from protozoa to the tissues of higher animals. It is normally detected in the tissue extracts by the degradation of hemoglobin at acid pH values. It was first examined in detail by Anson (1938) under the name 'cathepsin.' Later Tallan et al. (1952) by making use of synthetic substrates of low molecular weight, found the existence of cathepsins A, B and C, corresponding in specificity to pepsin, trypsin and chymotrypsin respectively.

In due course other workers showed the existence of a powerful acid proteinase, known as cathepsin D, which is quite distinct from cathepsins A, B and C. It has no action on synthetic substrates of cathepsins A, B and C and can act on proteins like hemoglobin, albumin and casein. It is clearly the enzyme originally described as 'cathepsin' by Anson (1938). Cathepsin E differs from cathepsin D in having a higher molecular weight and being more active at pH 2.5.

In the present study primarily cathepsin D activity is measured on hemoglobin at pH 4.0; but other cathepsins may also be present in the enzyme preparation.

Subcellular distribution of catheptic activity

The intracellular distribution of catheptic activity is shown in Figure 1. Cathepsin had its highest relative specific activity in the lysosomal fraction. However, the mitochondrial and microsomal fractions were also contaminated with some catheptic activity. Similar observations were made by Bird et al. (1969) in the muscle of the goldfish. This could be due to the fact that the lysosomes are heterogenous in size ranging from the size of mitochondria to that of microsomes. Figure 2 shows the intact lysosomes in

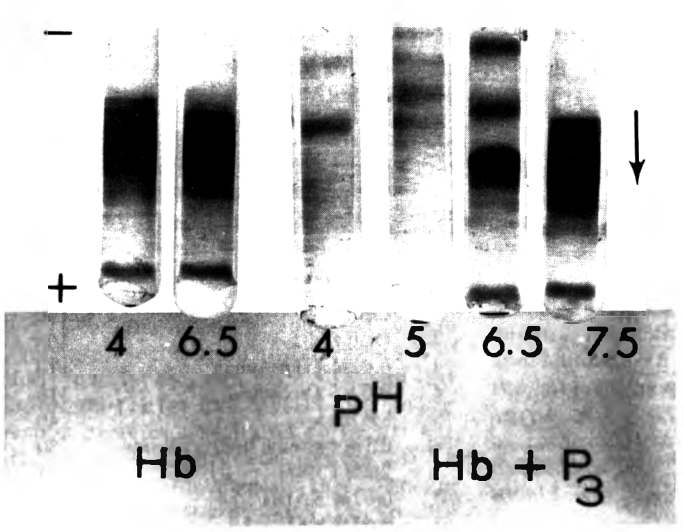

Fig. 4–Polyacrylamide gel electrophoresis showing proteolytic activity of cathepsin on hemoglobin. Effect of pH on the activity of cathepsin. Experiments were conducted as described under "Experimental." The control and samples were incubated under standard conditions for 40 hr and then subjected to electrophoresis. The enzyme fraction (P_3) did not contribute to any protein band formation because the protein concentration was too low.

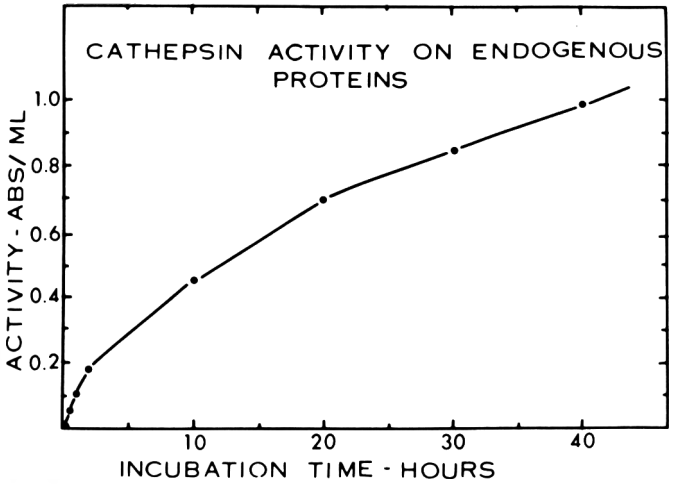

Fig. 5–Effect of time on autolysis in fish muscle homogenate. 5 ml of fish muscle homogenate (1:2) in distilled water was incubated with 1 ml of 0.2M acetate buffer pH 4.0, after adjusting the pH to 4.0 with 0.1M HCl and adding sufficient water to make 7 ml. 5 ml of 0.3M TCA was added to precipitate out the proteins at different time intervals. Absorbancy at 0 times was the control. Difference in the absorbancy of the TCA filtrates of the control and samples at 275 mμ represents activity. Here the term "autolysis" refers to the breakdown of all types of fish muscle proteins present in the homogenate.

the lysosomal fraction (P_3) separated from the fish muscle. The lysosomal fraction also contains some small mitochondria and some microsomes due to contamination. In the electron micrograph only a few particles are exhibiting the characteristic dark staining of intact lysosomes. Others might have lost their proteins due to rupture or leakage during their separation, fixation and preparation for the electron microscopic examination. Figure 3 shows an electron micrograph of fish muscle where the muscle fibers, mitochondria and lysosomes are distinct.

The lysosomes were separated as described under 'Experimental' and the enzyme extract was prepared by disrupting the lysosomal fraction in 0.1% triton X-100 in 0.01M imidazole buffer.

Effect of pH on catheptic activity

The optimum pH for enzyme activity was found to be 4.0 using the assay procedure. Furthermore, gel electrophoresis was used to study the breakdown products of hemoglobin at different pH. Lysosomal enzyme was incubated with hemoglobin at different hydrogen ion concentrations and the gel electrophoretic pattern of products was observed (Fig. 4). At pH 4.0 most of the substrate was digested into peptides, oligopeptides and into smaller fragments; at pH 6.5 the breakdown was less and at pH 7.5 there was no apparent breakdown of hemoglobin. Similar results were obtained using paper and thin layer chromatography. At the optimum pH amino acids and small peptides were released and stained on the chromatograms. The gel electrophoretic procedure used was able to pick up only the fragments of peptide or oligopeptide dimensions, whereas paper and thin layer chromatography separated the smaller breakdown compounds such as amino acids. The formation of small peptides and amino acids indicate that the activity measured is due to the combined effects of all the cathepsins.

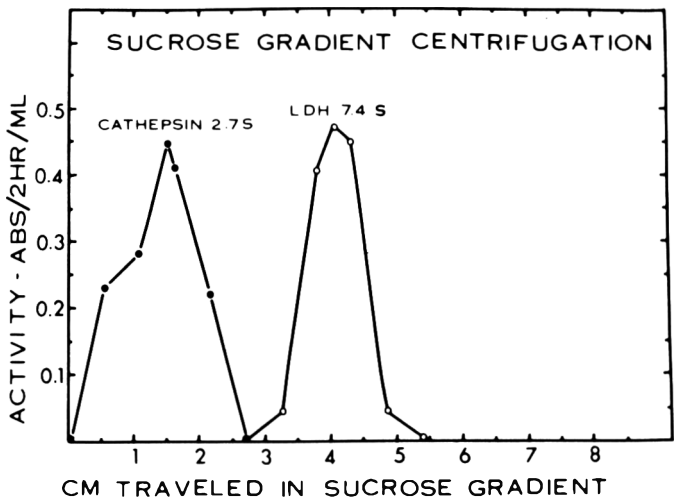

Fig. 6–Sucrose gradient centrifugation of fish muscle cathepsin. Centrifugation was carried out using SW 25.1 rotor for 50 hr at 4°C at 24.5 × 10^3 rpm. Fractions were assayed at 23°C. Beef heart LDH was used as the marker enzyme.

Action of cathepsin on different substrates

Table 1 shows the fish muscle catheptic activity on different substrates. The enzyme was found to act on urea-denatured hemoglobin, native hemoglobin, globin, bovine serum albumin and also on cytoplasmic proteins (endogenous) from the S_4 fraction. Here the term "endogenous proteins" refers merely to the soluble proteins present in the 78,480g supernatant fraction obtained during the fractionation of fish muscle homogenate. These proteins were not characterized such as actin or myosin. Sharp (1963), Bodwell and Pearson (1964) found that mammalian cathepsins were most active on sarcoplasmic proteins. Denatured hemoglobin gave the highest specific activity, probably due to the fact that urea-denatured hemoglobin is unfolded and enzymatic attack is facilitated along the open polypeptide. The activity was almost the same on both denatured and native hemoglobin at pH 3.5. This is probably due to the acid denaturation of native hemoglobin at pH 3.5.

Catheptic activity on endogenous proteins

The enzyme hydrolyzed endogenous proteins in addition to other proteins (Table 1). Fish muscle homogenate of known concentration was incubated with buffer (pH 4.0) at 37°C for the desired duration of time, and the reaction was stopped with 0.3M TCA. The absorbancy

Table 3–Thermal stability of cathepsin D from fish liver and fish muscle[a]

Temp °C	Duration of heat treatment (min)	% Control activity: Incubation time in the assay mix (min) 3	5	8	10
5	15	50	68	84	100
37	15	50	68	84	100
68	15	40	57	68	83

[a]The lysosomal fraction (P_3) was incubated at the temperatures given above. At different intervals aliquots were drawn and assayed for catheptic activity at 37°C. Protein content of the sample was 2.4 mg per ml. Activity of the refrigerated enzyme (5°C) after 10-min incubation in the assay mix was considered as control and all other activities were represented as percent control activities. The assays were conducted by a modified Anson's method as described in the text and also by fluorescent hemoglobin method. Both methods gave similar results.

Table 4–Effect of various agents on catheptic activity[a]

	Treatment	% Control activity
1.	Control	100
2.	Dialyzed	100
3.	0.02M Tryptophan	110
4.	0.02M Tyrosine	110
5.	8M urea	40
6.	0.1M cysteine hydrochloride	30
7.	2mM PMSF	25
8.	0.01M NADH	75
9.	0.01M NAD	25
10.	0.01M ATP	20
11.	0.01M AMP	18
12.	5% NaCl	5

[a]To 1 ml of P_3 sample the various compounds were added at the indicated concentration and the samples were incubated at room temperature for 20 hr. Assays were made as described in the text.

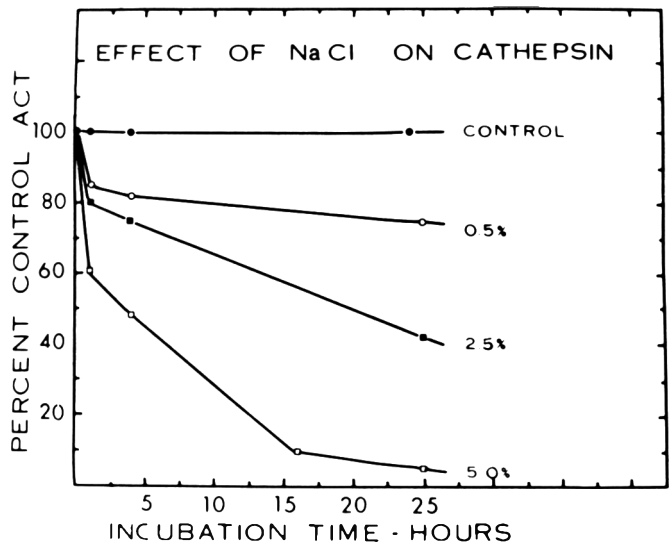

Fig. 7—Effect of concentration of NaCl on fish muscle catheptic activity. P_3 sample was incubated at room temperature with different concentrations of NaCl and aliquots were taken at different time intervals. Catheptic activity in 1 ml aliquots was measured on denatured hemoglobin as described in the text.

of the TCA filtrates was read at 275 mμ. Blanks were run in the same way except that TCA was added at the beginning. The difference in absorbancy between the sample and the blank was proportional to the breakdown of endogenous proteins. Here the term "endogenous proteins" refers to all types of proteins present in the homogenate. The rate of hydrolysis of endogenous proteins was linear with time during the first 2 hr of incubation (Fig. 5).

The activity of lysosomal (P_3) fraction on the supernatant (S_4) fraction was studied as a function of pH. The P_3 fraction had a low protein content and high catheptic activity, whereas S_4 was rich in protein and poor in catheptic activity, so S_4 could be used as a source of protein substrate for catheptic activity. The results in Table 2 show that cathepsin D can hydrolyze the endogenous proteins of fish muscle within a broad range of hydrogen ion concentration. The breakdown products obtained may be further hydrolyzed by cathepsins A, B and C as suggested by Huang and Tappel (1971). This autolytic process would facilitate the subsequent deterioration of fish muscle by bacteria. Although the optimum pH for hydrolysis of the endogenous proteins was found to be pH 4.0, the range of activity was between pH 3.0 and pH 7.0. This suggests that autolysis of the endogenous proteins can proceed at and below pH 6.5 which is the average pH of fish muscle after capture (Amlacher, 1961). After death the pH drops further.

Effect of temperature on enzyme stability

The enzyme is more stable at 5°C than at higher temperatures; however, it was interesting to find that 68°C for 15 min decreased the activity to only 83% (Table 3). Gel electrophoresis further verified the fact that the heated enzyme could still break down hemoglobin into peptide fragments. Makinodan and Ikeda (1969) reported a protease of carp muscle active in slightly alkaline pH range, which retained about 70% of its activity after heating for 60 min at 60°C. This relative stability to high temperatures is important in the heat processing of fish muscle since inadequate heat treatment will cause enzyme activity to be retained. On the other hand this property could be effectively utilized in the purification process of the enzyme as most of the other proteins may be separated out by a slight heat treatment leaving catheptic activity relatively unaffected.

Sedimentation velocity experiments

The sedimentation coefficient of fish muscle cathepsin was determined to be 2.7S, using beef heart lactic dehydrogenase (7.4S) as the marker enzyme (Fig. 6). Using the equation $M_1^{2/3}/M_2^{2/3} = S_1/S_2$ derived from the equation of Scheraga and Mandelkern (1953), and considering the molecular weight of lactic dehydrogenase as 145,000, the molecular weight of cathepsin from flounder muscle was found to be 32,000. The molecular weight was the same for both the liver and the muscle enzyme. This is close to the molecular weight of other proteolytic enzymes such as pepsin, trypsin and rennin. So far there is no conclusive data regarding the molecular weight of cathepsin.

Effect of activators and inactivators

As shown in Table 4 the amino acids tryptophan and tyrosine had very little activation effect on the enzyme. The effect of these two and other amino acids on catheptic activity was tested because the amino acids are the ultimate end products of protein degradation in the body. NADH, NAD, AMP, cysteine, phenylmethyl sulfonyl fluoride (PMSF) and sodium chloride inactivated the enzyme. Siebert (1962) also observed a similar inactivation with cysteine on cod muscle proteases. The stability of this enzyme in 8M urea and toward thermal inactivation as well as its molecular weight of 32,000 suggest that the enzyme under study is probably a single polypeptide, not undergoing significant structural alterations in the presence of urea or heat. Dialysis did not bring about any change, indicating that there are no dialyzable inhibitors, or activators which could affect the enzyme. The inactivation of catheptic activity by ATP is particularly important. The post-mortem degradation of fish follows a similar pattern as that observed for mammals, in which ATP is converted rapidly to ADP then to AMP and AMP is further degraded to IMP and finally inosine is formed (Jones and Murray, 1961; Tomlinson and Geiger, 1962; Tarr, 1965; Briskey et al., 1966). After capture the ATP level declines and thus the inhibitory effect of ATP (Table 4) upon cathepsin may be removed. In this case ATP together with other metabolites may function as a controlling mechanism of enzyme activity. Work is in progress to study this effect and the various metabolites on cathepsin release and activity.

Sodium chloride as an inactivator of cathepsin

From Figure 7 it is seen that sodium chloride inactivated the enzyme. The inactivation was evident, using hemoglobin and endogenous proteins as substrates. Further experiments showed that the main effect of salt was on the enzyme and not on the substrate used. Sodium chloride is known to preserve fish muscle by controlling microbial spoilage. As an inactivator of catheptic activity, it may also be controlling autolytic spoilage.

REFERENCES

Anson, M.L. 1938. The estimation of pepsin, trypsin, papain and cathepsin with hemoglobin. J. Gen. Physiol. 22: 79.

Amlacher, E. 1961. In "Fish as Food," ed. Borgstrom, G., Vol 1, p. 403. Academic Press, N.Y.

Bate-Smith, E.C. 1948. The physiology and chemistry of rigor mortis, with special reference to aging of beef. Adv. Food Res. 1: 1.

Bird, J.W.C., Berg, T., Milanesi, A. and Stauber, W.T. 1969. Lysosomal enzymes in aquatic species. 1. Distribution and particle properties of muscle lysosomes of the goldfish. Comp. Biochem. Physiol. 30: 457.

Bodwell, C.E. and Pearson, A.M. 1964. The activity of partially purified catheptic enzymes on various natural and synthetic substrates. J. Food Sci. 29: 602.

Briskey, E.J., Kastenschmidt, L.L., Forrest,

J.C., Beecher, G.R., Judge, M.D., Cassens, R.G. and Hoekstra, W.G. 1966. Biochemical aspects of post-mortem changes in porcine muscle. J. Agr. Food Chem. 14: 201.

Caldwell, K.A. 1970. Autolytic activity in aqueous extracts of chicken skeletal muscle. J. Agr. Food Chem. 18: 276.

Coffey, J.W. and DeDuve, C. 1968. Digestive activity of lysosomes. 1. The digestion of proteins by rat liver lysosomes. J. Biol. Chem. 243: 3255.

DeDuve, C. 1963. In "Lysosomes," ed. DeReuck, A.V.S. and Cameron, M.P., p. 1. Little Brown, Boston.

DeLumen, B.D. and Tappel, A.L. 1970. Fluorescein-hemoglobin as a substrate for cathepsin D and other proteases. Anal. Biochem. 36: 22.

Fruton, J.S. 1960. Cathepsins. In "The Enzymes," ed. Boyer, P.D., Lardy, H. and Myrback, K., 2nd ed., Vol 4, p. 233. Academic Press, New York.

Groninger, H.S. 1964. Partial purification and some properties of a proteinase from albacore (Germ alalunga) muscle. Arch. Biochem. Biophys. 108: 175.

Huang, F.L. and Tappel, A.L. 1971. Action of cathepsin C and D in protein hydrolysis. Biochem. Biophys. Acta. 236: 739.

Jones, N.R. and Murray, J. 1961. Nucleotide concentration in codling (Gadus callarias) muscle passing through rigor mortis at 0°. Z. Vergl. Physiol. 44: 174.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. 1951. Protein measurement with Folin phenol reagent. J. Biol. Chem. 193: 265.

Makinodan, Y. and Ikeda, S. 1969. Studies on fish muscle protease. 2. Purification and properties of a proteinase active in slightly alkaline pH range. Japanese Soc. Sci. Fisheries 35: 749.

Martin, R.G. and Ames, B.N. 1961. A method for determining the sedimentation behavior of enzymes: Application of protein mixtures. J. Biol. Chem. 236: 1372.

Martins, C.B. and Whitaker, J.R. 1968. Catheptic enzymes and meat tenderization. 1. Purification of cathepsin D and its action on actomyosin. J. Food Sci. 33: 59.

Novikoff, A.B. 1963. Lysosomes in physiology and pathology of cells: Contribution by staining methods. In "CIBA Foundation Symposium of Lysosomes," ed. DeReuck, A.V. and Cameron, M.P., p. 36. Little Brown and Co., Boston.

Ornstein, L. and Davis, B. 1962. Disk Electrophoresis. Parts 1 and 2. Distillation Products Industries, Rochester, N.Y.

Parrish, F.C. Jr. and Bailey, M.E. 1966. Physiochemical properties and partial purification of porcine muscle cathepsin. Agr. Food Chem. 14: 232.

Parrish, F.C. Jr. and Bailey, M.E. 1967. Physiochemical properties of bovine muscle particulate cathepsin. Agr. Food Chem. 15: 88.

Roth, J.S., Losty, T. and Wierbicki, E. 1971. Assay of proteolytic enzyme activity using a ^{14}C labelled hemoglobin. Anal. Biochem. 42: 214.

Scheraga, H.A. and Madelkern, L. 1953. Consideration of the hydrodynamic properties of proteins. J. Amer. Chem. Soc. 75: 179.

Sharp, J.G. 1963. Aseptic autolysis in rabbit and bovine muscle. J. Sci. Food Agr. 14: 468.

Shibko, S. and Tappel, A.L. 1965. Rat kidney lysosomes: Isolation and properties. Biochem. J. 95: 731.

Siebert, G. 1958. Protein-splitting enzyme activity of fish flesh. Experientia 14: 65.

Siebert, G. 1962. Enzymes of marine fish muscle and their role in fish spoilage. In "Fish in Nutrition," ed. Heen, E. and Kruezer, R., p. 50. Fishing News (Books) Ltd., London.

Tallan, H.H., Jones, M.E. and Fruton, J.S. 1952. On proteolytic enzymes of animal tissues. 10. Beef spleen cathepsin C. J. Biol. Chem. 194: 793.

Tarr, H.L.A. 1965. Pathways of glycogen breakdown. In "The Technology of Fish Utilization," ed. Kruezer, R., p. 34. Fishing News (Books) Ltd., London.

Ting, C.T., Montgomery, M.W. and Anglemier, A.F. 1968. Partial purification of salmon muscle cathepsins. J. Food Sci. 33: 617.

Tomlinson, N. and Geiger, S.E. 1962. Glycogen concentration and post mortem loss of adenosine triphosphate in fish and mammalian muscle: A review. J. Fish Res. Bd. Can. 19: 997.

Woessner, J.F. Jr. and Shamberger, R.J. Jr. 1971. Purification and properties of cathepsin D from bovine uterus. J. Biol. Chem. 246: 1951.

Zener, R., Lataste-Dorolle, C., Collet, R.A., Rowinski, P. and Mouton, R.F. 1958. Aseptic autolysis of muscle: Biochemical and microscopic modifications occurring in rabbit and lamb muscle during aseptic and anerobic storage. Food Res. 23: 305.

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DETECTION OF FROZEN FISH DETERIORATION BY AN ULTRAVIOLET SPECTROPHOTOMETRIC METHOD

INTRODUCTION

FREEZING is considered the best method for preservation of fish for a very long time. Nevertheless, frozen fish is perishable and its deterioration at temperatures usually encountered in commercial chain storage and distribution occurs after a time period which depends upon fish quality, freezing and frozen storage conditions. Usually frozen fish can be held several months at temperatures of −20 to −30°C with little undesirable changes (Dyer, 1968).

With respect to fish lipids, the most serious deterioration is rancidity which is due to autoxidation of the lipids (Banks, 1938). This reaction is especially favored in fish lipids because they contain a considerable amount of polyunsaturated fatty acids (Lovern, 1964).

This deterioration also injures fish proteins because at the early stages of autoxidation free radicals and relatively stable hydroperoxides are formed; these subsequently react with proteins causing polymerization of proteins and destruction of amino acids (Roubal, 1970). Another consequence of the aforesaid lipid-protein interaction is a decrease of the quantity of the oil which can be extracted from the fish tissue by diethyl-ether and nonpolar solvents (Almquist, 1956). At the advanced stages of autoxidation, hydroperoxides are decomposed forming low molecular weight carbonyl compounds responsible for the "off-flavors" of rancidity and high molecular weight derivatives responsible for the decrease of iodine value and for the increase of viscosity (Evans, 1961).

Since autoxidation has an adverse effect on the quality of frozen fish, a number of objective and subjective methods have been proposed and used in order to follow the progress of autoxidation in fish oils. Chemical reactions, as the Kreis test, TBA test, etc., designed for the detection of rancidity in usual oils and fats, have also been used for fish oils with less reliability than in usual oils and fats (Banks, 1967).

Peroxide value is more useful than the other methods, because it gives information about the extent of oxidation in a fish oil. A disadvantage of the method is the fact that rancid oils do not always show high peroxide values, because the highly unsaturated hydroperoxides of fish oils are extremely unstable and break down into flavorous components at very low concentrations (Banks, 1967).

In this case further information concerning the extent of autoxidation can be obtained from ultraviolet spectrum of the oil. Oxidized fish oils contain conjugated acids which show high absorption in the region 230–268 nm (Tsuchiya, 1961). On the contrary, fresh fish contains no conjugated acids; therefore, they do not exhibit appreciable absorption in this region (Klenk and Brockerhoff, 1958).

In the present work the oxidative changes occuring in the oil of frozen fish of the species *Pagelus erythrinus*, kept at −20°C, have been determined by ultraviolet spectrophotometry and from the experimental results of this study the induction period of the oil has been estimated.

EXPERIMENTAL

Materials

Fresh fish of the species *Pagelus erythrinus* were frozen at −20°C (without any further process) in the freezer of an ordinary refrigerator and stored there for 6 months. At regular 30-day intervals, samples of the frozen fish were defrosted by exposure to a temperature of 2–5°C for 24 hr. Three samples of fish were combined in one sampling stage in order to reduce experimental error. Each sample was divided into two parts: the first consisting of skin and scales and the second of the flesh without head, stomach and intestines.

Extraction of oil

Since it was known that drying of wet fish tissues and evaporation of solvents at high tem-

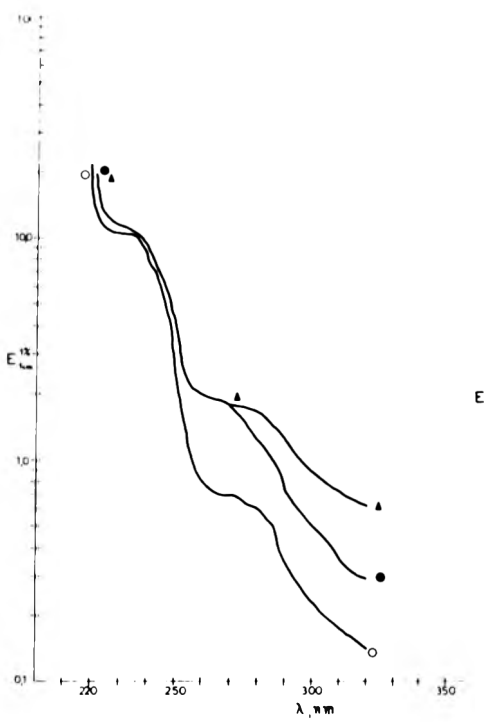

Fig. 1–Effect of extraction on absorption spectrum of fish lipids: ▲ lipids extracted with diethyl-ether by Soxhlet method in diffuse day light at 35°C; ○ lipids extracted with cold mixture of $CHCl_3$:CH_3OH at ca. 20°C; ● the preceding lipids aged 15 days in petroleum ether solution.

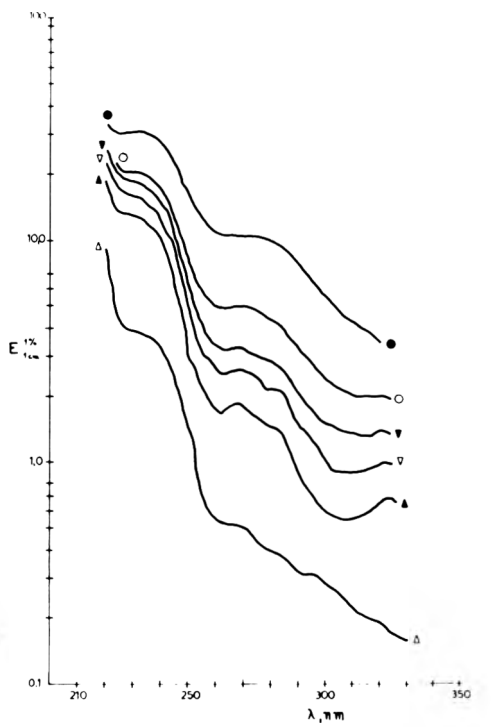

Fig. 2–Effect of oxidation on absorption spectrum of fish lipids at ca. 20°C: △ fresh fish lipids, extracted with $CHCl_3$:CH_3OH; ▲ aged 2 days in open air; ▽ aged 4 days; ▼ aged 7 days; ○ aged 16 days; ● aged 21 days.

peratures could oxidize fish oil (Banks, 1967), some precautions, such as drying the wet tissues by mixing with sodium sulfate and evaporation of solvents in an atmosphere of nitrogen, were taken to avoid oxidation of the oil during extraction.

Accordingly, fish oil was extracted from the samples by using the following two methods:

Soxhlet method. A sample of fish flesh was ground to pulp in a Sorvall Omni-mixer. About 10g of ground flesh was dried by addition of 100g of anhydrous sodium sulfate, well mixed, transferred quantitatively into a paper thimble and extracted by peroxide-free diethyl-ether for about 8 hr in a Soxhlet apparatus. The extract was put in a distilling flask immersed in a water bath, held at 20°C, and the solvent was removed under vacuum in a stream of nitrogen.

The solvent-free oil was immediately used for determining ultraviolet absorption.

Extraction of oil by cold chloroform-methanol mixture 2:1 (v/v). Extraction was carried out by shaking about 10g ground fish flesh with 100 ml of extractive mixture for 30 min. This procedure was repeated four times. The combined extracts were filtered and reduced to a small volume under vacuum in a stream of nitrogen. The concentrated extract was transferred quantitatively to a separatory funnel containing 1g of sodium chloride dissolved in 100 ml of distilled water. The oil was extracted by shaking three times with 50 ml of peroxide-free diethyl-ether. The combined extracts were washed with 20 ml distilled water, dried by addition of 5g anhydrous sodium sulfate and filtered. The filtrate was put in a distilling flask immersed in a water bath, held at 20°C, and the solvent was removed under vacuum in a stream of nitrogen. The solvent-free oil was used immediately for determining ultraviolet absorption.

Determination of ultraviolet spectrum

About 0.1g of freshly extracted oil was accurately weighted, dissolved in petroleum ether (bp 40–60°C), transferred quantitatively to a 10.0 ml glass-stoppered volumetric flask and the solution diluted to volume with the same solvent. Absorbance readings were taken by diluting the original solution so that observed absorbance was always between 0.10 and 0.50.

Table 1–Specific extinction coefficients of oil extracted from fresh fish

Sample no.	$E^{1\%}_{1\ cm}$ 232 nm		$E^{1\%}_{1\ cm}$ 268 nm		Conjugated trienoic fatty acids[c]	
	F[a]	S[b]	F	S	F	S
1	9.88	16.80	0.505	0.655	1.93	8.29
2	5.79	8.62	0.490	0.765	0.69	3.03
3	8.52	12.09	0.721	1.316	1.05	8.29
4	8.84	10.20	0.741	1.366	4.92	12.89
5	10.12	–	0.720	–	–	–
6	10.70	–	0.708	–	–	–
$\bar{x}$[d]	7.98	11.92	0.648	1.026	2.147	8.125
s^2[e]	6.92	12.56	0.014	0.136	3.687	16.239
s[f]	2.63	3.54	0.118	0.368	1.92	4.03

[a]Flesh oil
[b]Skin oil
[c]Expressed as mg per cent
[d]Mean
[e]Variance
[f]Standard deviation

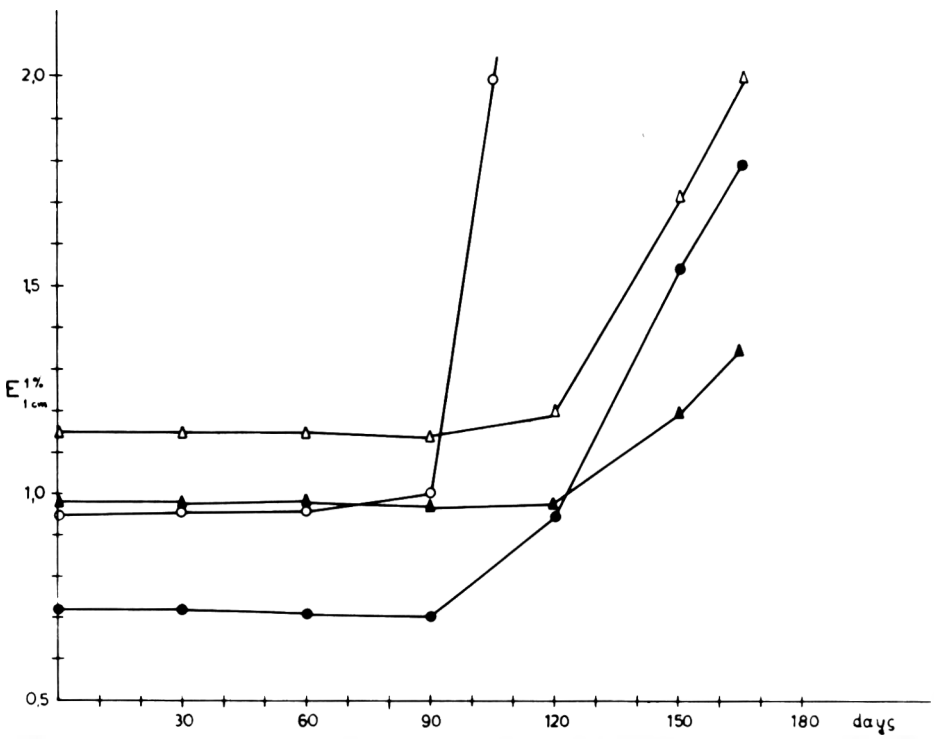

Fig. 3–Effect of freezing storage at −20°C on ultraviolet absorption of fish lipids: • flesh lipids $E^{1\%}_{1\ cm}$ 268 nm; ○ skin lipids $E^{1\%}_{1\ cm}$ 268 nm; ▲ flesh lipids $E^{1\%}_{1\ cm} \cdot 10^{-1}$ 232 nm; △ skin lipids $E^{1\%}_{1\ cm} \cdot 10^{-1}$ 232nm.

RESULTS & DISCUSSION

Comparison between Soxhlet and chloroform-methanol mixture extraction methods

The ultraviolet absorption study of oils extracted by both methods from identical samples of fish flesh, was applied in the research on autoxidative alterations occuring during the extraction process. Figure 1 shows that there was a general rise in the absorption spectrum near 270 nm for the oil extracted by the Soxhlet method as well as for the aged oil extracted by chloroform-methanol mixture.

It is therefore concluded that oil extracted by chloroform-methanol mixture is more suitable for spectroscopic studies than oil extracted by Soxhlet method and that the absorbance readings must be taken as soon as possible.

Effect of autoxidation on ultraviolet spectrum

The influence of autoxidation on ultraviolet absorption has been investigated by exposing 0.5g fish oil extracted by chloroform-methanol mixture to the air at a temperature of about 20°C. As is shown in Figure 2, a rapid general increase is observed of the ultraviolet absorption with a band near 232 nm and another near 268 nm.

Therefore, ultraviolet absorption provides an objective measure of fish oil autoxidation suitable for following the progress of autoxidation in fish oil.

Ultraviolet absorption of oil extracted by chloroform-methanol 2:1 (v/v) from fresh fish

The normal distribution of the specific extinction coefficient of the oil at 232 and 268 nm has been determined on samples of fresh fish of the species *Pagelus erythrinus*, at regular monthly intervals during a year. The approximate percentage of conjugated trienoic acids has been calculated from absorbance readings at 262, 268 and 274 nm according to AOCS (1970).

From the statistical study of the experimental data the following conclusions

can be drawn (Table 1): (a) The specific extinction coefficient of the fresh fish oil cannot exceed the following upper limits: skin oil $E^{1\%}_{1\,cm}$ 232 nm < 19.0 and $E^{1\%}_{1\,cm}$ 268 nm < 1.76; flesh oil $E^{1\%}_{1\,cm}$ 232 nm < 13.2 and $E^{1\%}_{1\,cm}$ 268 nm < 0.88. Consequently, deterioration of the fish oil can be estimated from its specific extinction coefficient at 232 or 268 nm; (b) The skin oil always has higher ultraviolet absorption than flesh oil; (c) There is a weak absorption band near 268 nm expressed as conjugated trienoic acids which is more intense in skin than in flesh oil.

Effect of aging of fish stored at low temperature on the ultraviolet spectrum of its oil

The effect of fish aging on the ultraviolet spectrum of its oil, Figure 3, shows the time of storage plotted against specific extinction coefficient at 232 and 268 nm. From this graph it is apparent that no considerable change of ultraviolet absorption takes place during the early time of storage, but after a certain time, which may be characterized as the induction period of tissue oil, ultraviolet absorption increases rapidly both in flesh oil and skin oil. After the exhaustion of this period, fish oil acquires a rancid taste and odor.

Another effect on oil of fish aging, occuring during storage at low temperatures, is the fact that the observed difference in ultraviolet absorption between skin and flesh oil (Table 1) increases rapidly after the end of the induction period (Fig. 3). This probably happens because the oxidation rate of its oil is faster than that of flesh oil since skin is more liable to atmospheric oxygen than flesh.

In conclusion, ultraviolet absorption constitutes a reasonably satisfactory measure of the extent of oxidation for tissue oil in frozen fish. This method is applicable not only at the early stages of oil oxidation but also at the advanced stages, when peroxide value determinations cannot be used because of rapid decomposition of hydroperoxides.

REFERENCES

Almquist, H.T. 1956. Changes in fat extractability and protein digestibility in a fish meal during storage. J. Agr. Food Chem. 4: 638.

AOCS. 1970. "Official and Tentative Methods," Cd 7-58. Am. Oil Chem. Society, Chicago, Ill.

Banks, A. 1938. The storage of fish with special reference to the onset of rancidity. J. Soc. Chem. Ind. 57: 124.

Banks, A. 1967. Deteriorative changes in fish oils. In "Fish Oils," ed. Stansby, M.E., p. 151. The Avi Publishing Co., Westport, Conn.

Dyer, W.J. 1968. The effect of freezing and frozen storage on the proteins of fish muscle. In "Low Temperature Biology of Foodstuffs," ed. Hawthorn, J. and Rolfe, E.J., p. 333. Pergamon Press, London.

Evans, C.D. 1961. Chemical changes accompanying flavor deterioration of vegetable oils. Proc. Flavor Chemistry, Campbell Soup Co., Camden, N.J.

Klenk, E. and Brockerhoff, H. 1958. Die C_{18} und C_{22} Polyensäuren von Heringoel. Z. Physiol. Chem. 310: 153.

Lovern, J.A. 1964. The lipids of marine organisms. Oceanogr. Macr. Biol. Ann. Rev. 2: 169.

Roubal, W.T. 1970. Trapped radicals in dry lipid-protein systems undergoing oxidation. J. Am. Oil Chem. Soc. 47: 141.

Tsuchiya, T. 1961. Biochemistry of fish oils. In "Fish as Food," ed. Borgstrom, G., p. 238. Academic Press, New York.

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RIGOR-STRETCHED TURKEY MUSCLES: EFFECT OF HEAT ON FIBER DIMENSIONS AND SHEAR VALUES

INTRODUCTION

RIGOR SKELETAL muscle is generally considered to be inextensible. The basis for this conclusion, reviewed in detail elsewhere (Hegarty, 1972), include the apparent inextensibility of carcasses and limbs in rigor; demonstrations of changes in extensibility, hardness, elasticity, contractility and excitability as muscle goes into rigor mortis; x-ray diffraction diagrams showing a reorganization of the helical arrangement of the myosin cross bridges in binding with actin in rigor muscle compared with prerigor muscle undergoing a normal contraction; and no sarcomere length changes when weights are applied to rigor muscle strips. Goll (1968) has categorized two aspects of rigor muscle inextensibility–macroscopic and molecular. Macroscopic inextensibility is the loss of ability of a muscle fiber or a bundle of muscle fibers to stretch under the influence of a given weight or force. Molecular inextensibility is the inability of actin and myosin filaments in a single sarcomere to slide past one another. Goll (1968) further states that the molecular phase, but not the macroscopic phase, may be partially reversible with the onset of the resolution of rigor.

Initial investigations on mouse skeletal muscle indicated that an increase in sarcomere length could be obtained by stretching rigor limbs from a maximally folded to a maximally stretched position (Hegarty, 1972). Examination of the rigor stretched fibers under the light microscope showed no structural damage. This observation has been confirmed by electron micrographs which also show that the rigor stretch effect has produced a sliding of the actin and myosin filaments (Herlihy et al., 1972). Further unpublished light and electron microscope investigations in this laboratory indicate that certain limb muscles of the turkey and pig can be stretched in rigor mortis. This conclusion was also based on an increase in sarcomere length in the rigor-stretched muscle compared with the folded control muscle.

Many workers have reported a high correlation between long sarcomeres (determined on uncooked muscle samples) and tenderness in meat animals. The observations were made on muscles that had undergone excision, tension or prerigor stretching, restraint, or cold-shortening (Herring et al., 1967; Smith et al., 1971; and others). The purpose of the present investigation was to determine if the increase in sarcomere length due to rigor stretching produced an increase in tenderness in commercially important thigh muscles in the turkey. The turkey is ideal for this type of study because the thigh muscles are a convenient size for stretching and the entire muscles can be easily dissected.

EXPERIMENTAL

Experimental animals

Adult tom turkeys, obtained from the University of Minnesota poultry farm, were exsanguinated by cutting the carotid artery. The birds were placed in an inverted metal cone which prevented excessive movement of the wings. This procedure also facilitated the control of leg movement during the death struggle. It was considered important to standardize the degree of struggling at death. Ma et al. (1971) reported significant differences between the time course of rigor mortis in the breast muscle of turkeys which struggled compared to restrained birds. The carcasses were not scalded, de-feathered, or eviscerated.

Muscle sampling procedure

Two thigh muscles, semitendinosus and sartorius, were chosen because of their ability to change from a folded to a stretched state when certain stretch tensions are applied to prerigor limbs. Therefore, it was reasoned that the same method of stretch would also extend these muscles when in rigor mortis. Different methods of stretching the limbs had to be used because of the different anatomical location of these muscles. Six turkeys were used for each of the two muscles in the following experiments.

Experiment I (Rigor stretched muscles). Both limbs were folded inwards maximally, and tied in that position, immediately after exsanguination. The carcasses were allowed to go into rigor at 20–25°C. Completion of the rigor process was determined on muscle strips by isotonic myotron measurements (Forrest et al., 1969) on peroneus longus. Monitoring the development of rigor mortis in this muscle was considered to be a good indication of the rigor state of the semitendinosus and sartorius since all are red thigh muscles. Rigor generally occurred 5–6 hr postmortem. The skin on the folded thigh was then removed and the entire semitendinosus and sartorius muscles dissected according to the nomenclature of George and Berger (1966). Care was observed not to cause any unnecessary movement of the limb. The folded semitendinosus on the other limb was stretched caudad maximally and then cephalad. The limb was then moved laterally maximally away from the body of the bird. The method of rigor stretch used for the folded semitendinosus produces no statistically significant stretch in the sartorius. Therefore, in another six turkeys the sartorius was extended in rigor by hanging the turkey vertically by the limb which is to be stretched and then exerting a downward pull on the carcass. The folded sartorius was obtained by the procedure as outlined above for the semitendinosus.

Experiment II (Pre-rigor stretched muscles). The semitendinosus muscle was stretched immediately after exsanguination by moving the limb cephaled maximally and attaching the foot to a wooden board. The other limb was folded inwards maximally and tied in that position. When the muscles were in rigor, (as determined in Experiment I), the folded and stretched muscles were dissected. The sartorius was stretched prerigor by hanging an additional six turkeys vertically by one limb immediately after exsanguination. The other limb was folded inwards maximally and tied in that position.

Experiment III (Rigor stretched muscles). The six birds used in this group were treated as described in Experiment I except that the semitendinosus muscle was bisected longitudinally from tendon to tendon for the subsequent heat treatment described below.

No tearing of muscles or joint dislocations were produced in either the prerigor or rigor stretched limbs. All muscles were stored in polyethylene bags and frozen to −20°C. After 1–3 wk the muscles were thawed at 20–25°C for approximately 6 hours.

Tenderness evaluation

All muscles were held in the type of adjustable clamp shown in Figure 1. The clamps were then placed in "Scotchpak" polyester bags and the muscles in Experiment I and II were heated to an internal temperature of 82°C (measured by a thermocouple temperature recorder) at an oven temperature of 163°C. Average heating time was approximately 8 min for the stretched muscles and 10 min for the folded muscles. One portion of the longitudinally bisected semitendinosus muscles in Experiment III was heated to an internal temperature of 82°C. The object of heating the other portion to 70°C was to determine the effect of this temperature on sarcomere length and fiber diameter. The cooked muscles were held at 2°C for 24 hr and then shear values were determined on the samples heated to 82°C. Strips (1.2 cm × 1.0 cm) were cut from the center of the muscle. Mean Warner-Bratzler shear values were obtained from five shears of each muscle.

Sarcomere length determinations

Samples from raw and cooked muscles were taken from the external mid-portion of the muscle. This sample location was chosen because recent evidence indicates that sarcomere

length is relatively constant in the midportion of mammalian skeletal muscle during normal development (Williams and Goldspink, 1971). Variation in sarcomere length occurs near the tendons due to the formation of new sarcomeres. Therefore, the mid-portion of a muscle should be the area of least variation in sarcomere length. Individual unfixed muscle fiber fragments were separated by a Polytron homogenizer (Brinkmann Instruments, Inc.) using the procedure outlined by Hegarty and Naude (1970). The suspending medium was a Ringer solution containing 100 mM NaCl, 2 mM KCl, 2.5 mM $CaCl_2$, 3 mM $MgCl_2$ and 8 mM Tris buffer pH 7.0. It should be noted that this solution contains no calcium chelating agent. No contraction could be detected in the unheated fibers isolated in this solution (Fig. 2a, c). This is regarded as further proof that the muscles were in rigor when the stretching was performed. Undamaged fibers cannot be separated by this technique from muscle samples greater than approximately 25 mg. Therefore, because of the small size of the sample and the large size of the muscles, sarcomere length measurements were made on a pool of three samples from each raw and cooked muscle. Twenty randomly selected fibers from each muscle were projected onto white paper (× 1,000) by a mirror attached to the eyepiece of a Zeiss Large Universal microscope and a mercury gas-discharge light source. Sarcomere length was determined by counting the number of A-bands along 100μ of each fiber. Counts were made to the nearest half sarcomere.

Fiber diameter determinations

Fiber diameter measurements were made with Vernier calipers on the same fibers used for sarcomere length measurements (× 1,000).

Statistical analysis

The data were statistically analyzed by a paired comparison test as outlined by Steel and Torrie (1960).

RESULTS & DISCUSSION

THE WIDTH OF the folded and corresponding rigor stretched semitendinosus and sartorius muscles is shown in Figure 1. The observable decrease in the width of the entire muscle indicates that rigor muscle is capable of macroscopic extensibility. Therefore, paraphrasing the definition of macroscopic extensibility given by Goll (1968), it is proposed that the molecular extensibility of the rigor stretched muscle is probably due to the ability of a muscle fiber or a bundle of muscle fibers to stretch under the influence of a given weight or force. Because of the decrease in the width of the muscle due to stretch, an increase in length would be expected if the volume of the muscle remains the same. Abbott and Baskin (1962) showed that the volume of frog muscle remained constant during contraction. Since rigor is a mild contraction, and since the folded and rigor stretched muscles went into rigor in the same post-mortem environment, any positive stretching should therefore produce an increase in muscle length. Though it was difficult to accurately dissect both the semitendinosus and sartorius from tendon to tendon, especially in the folded limb, nevertheless an observable increase in the overall length of the rigor stretched muscle can be noted in Figure 1.

Effect of pre-rigor and rigor stretching on unheated muscles

The effect of rigor stretching on the semitendinosus and sartorius muscles is discussed separately because they were stretched differently due to the anatomical location of these muscles (see Experimental). Furthermore, the amount of force required to stretch the rigor limb was not quantified. Such values would be of little use for comparative purposes. Because the muscles were stretched in the intact limb, the restraining or facilitating influence of the surrounding muscles to a given force may be different for the two muscles studied. Also, the considerable difference in the mean sarcomere length of both these muscles in the folded rigor state (2.43μ in the semitendinosus and 2.28μ in the sartorius, unpublished observations) may produce a different stretch effect when stretched with the same amount of force. The degree of stretch in a muscle was controlled by the natural restraint of the limbs to over-stretching. It is conceivable that the effect of the stretch tension may be different in the two muscles.

Semitendinosus

A significant increase ($P < 0.01$) in sarcomere length was produced in the rigor stretched unheated muscle in Experiment I compared to the folded control (Table 1). This resulted in a significant decrease ($P < 0.001$) in fiber diameter. A similar finding ($P < 0.01$) was obtained for both sarcomere length and fiber diameter when the muscle was stretched prerigor (Experiment II). The decrease in fiber diameter in the muscles stretched either prerigor or in rigor was expected on the basis that muscle maintains a constant volume during contraction (Abbott and Baskin, 1962) and from the appearance of the muscles in Figure 1. Similar results were reported by Herring et al., (1967) and others for pre-rigor stretched muscles from farm animals, and by Hegarty (1972) for rigor stretched mouse muscle. On comparing the sarcomere length values for the folded muscle in Experiments I and II, no statistically significant difference was obtained. In both these experiments the procedure for folding the limbs immediately after slaughter and the subsequent post-mortem environment was the same. The lack of a statistically significant difference in sarcomere length indicates the degree of uniformity in the prerigor folding procedure between different groups of turkeys. Comparison of the sarcomere length in the rigor stretched (2.72μ) and prerigor stretched (3.23μ) semitendinosus muscle indicates that even though this muscle is rigor extensible ($P < 0.01$), it does not attain the same degree of extensibility as prerigor muscle ($P < 0.01$). This is in agreement with the original findings of Hegarty (1972) on mouse muscle. However, since the sarcomere length of the folded mouse and turkey muscles are approximately the same (2.1 and 2.5μ) the question still remains as to the degree of extensibility that can be obtained from in situ muscles with sarcomere lengths greater or less than this range. There was no statistically significant difference in the fiber diameter between the folded muscles or the stretched muscles in Experiments I and II. Fiber diameter is correlated with the developmental stage of the bird and may be related to certain post-mortem envi-

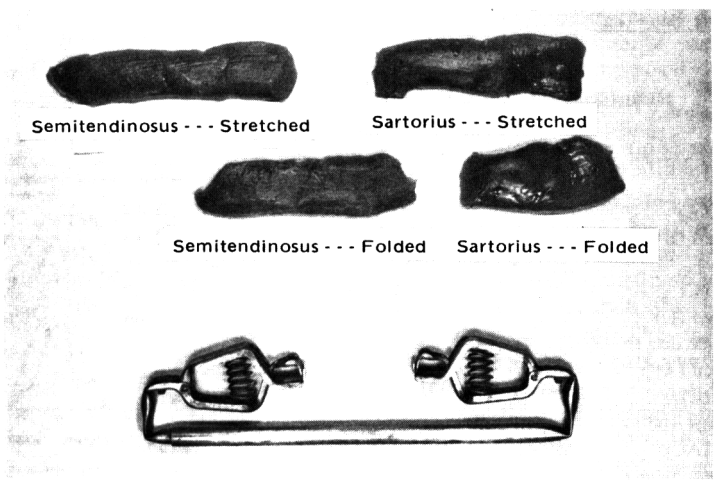

Fig. 1–Unheated folded and rigor stretched turkey semitendinosus and sartorius muscles. The muscles were held isometrically by the clamp during heat treatment.

ronmental factors (review by Hegarty and Hooper, 1971). The lack of a significant difference between the fiber diameter of the two groups of birds in Experiment I and II indicates the uniformity between groups of turkeys.

Sartorius

Prerigor and rigor stretching had the same effect on the sartorius as reported above for the semitendinosus (Table 1). However, a statistically significant difference ($P < 0.01$) was obtained in fiber diameter values between the folded muscles and between the stretched muscles in Experiments I and II. This indicates that the two groups of birds were less uniform in muscle mass than those used for the semitendinosus studies.

Effect of pre-rigor and rigor stretching on heated muscles

Semitendinosus. No statistically significant difference was obtained between the sarcomere length of the folded (2.43μ) and stretched (2.13μ) heated muscles in Experiment I (Table 1). It is interesting to note that the sarcomere length of the rigor stretched muscle decreased from 2.72μ in the unheated state to 2.13μ when heated to 82°C ($P < 0.01$). However, the sarcomere length of the folded muscle remained unchanged by heating (2.45μ unheated and 2.43μ heated). There was no statistically significant difference between the fiber diameter of the folded and stretched heated muscle. When heat was applied to both the folded and to the rigor stretched muscle a significant decrease ($P < 0.001$) occurred in fiber diameter. This is due to the coagulation of the myofibrillar proteins which results in a loss of water-holding capacity in the muscle (reviewed by Hamm, 1966). This occurs primarily between 40–50°C.

When prerigor stretched muscle was heated (Table 1, Experiment II), a significantly higher ($P < 0.05$) sarcomere length was obtained relative to the folded and heated control. The folded muscles showed no significant difference in sarcomere length due to heat. The values for the heated stretched muscles decreased significantly ($P < 0.001$) compared with the unheated prerigor stretched control muscles. Heating produced a significant difference between the fiber diameters of the folded and the stretched muscle ($P < 0.05$), but this difference is less than in the equivalent unheated state ($P < 0.01$). Heating produced a significant decrease in the fiber diameter of both the folded ($P < 0.001$) and the stretched ($P < 0.01$) muscles.

In comparing the rigor stretched and heated muscles (Experiment I) and the prerigor stretched and heated muscles (Experiment II) in Table 1, no significant difference was found between the sarco-

Table 1—Mean sarcomere length, shear force and muscle fiber diameter of prerigor and rigor stretched turkey muscles

	Muscle and treatment					
	Semitendinosus			Sartorius		
	Exp. I Rigor stretched	Exp. II Prerigor stretched	Significance between experiments	Exp. I Rigor stretched	Exp. II Prerigor stretched	Significance between experiments
Sarcomere length (μ)						
Unheated						
Folded (A)	2.45 ± 0.05	2.51 ± 0.08	N.S.	2.18 ± 0.12	2.16 ± 0.10	N.S.
Stretched (B)	2.72 ± 0.08	3.23 ± 0.08	**	2.52 ± 0.07	3.28 ± 0.10	**
Heated						
Folded (C)	2.43 ± 0.06	2.48 ± 0.06	N.S.	2.00 ± 0.08	2.11 ± 0.10	N.S.
Stretched (D)	2.13 ± 0.08	2.86 ± 0.10	**	2.23 ± 0.10	2.88 ± 0.07	**
Significance due to stretching						
Unheated (AB)	**	**		**	**	
Heated (CD)	N.S.	*		*	**	
Significance due to heating						
Folded (AC)	N.S.	N.S.		*	N.S.	
Stretched (BD)	**	***		**	**	
Shear force value (kg)						
Folded	10.56 ± 0.98	9.53 ± 1.42		8.59 ± 0.87	11.57 ± 1.73	
Stretched	14.11 ± 1.55	13.64 ± 1.98		10.20 ± 0.87	9.37 ± 0.90	
Significance	**b	**c		**	N.S.	
Fiber diameter (μ)						
Unheated						
Folded (A)	73.46 ± 0.81	77.38 ± 1.49	N.S.	76.38 ± 1.59	63.67 ± 1.06	**
Stretched (B)	64.18 ± 1.05	66.61 ± 1.53	N.S.	66.40 ± 1.98	55.17 ± 1.33	**
Heated						
Folded (C)	54.92 ± 1.57	61.41 ± 2.09	N.S.	65.46 ± 2.70	61.64 ± 2.15	N.S.
Stretched (D)	55.12 ± 1.37	53.70 ± 1.64	N.S.	61.41 ± 1.02	55.03 ± 2.28	N.S.
Significance due to stretching						
Unheated (AB)	***	**		***	**	
Heated (CD)	N.S.	*		*	**	
Significance due to heating						
Folded (AC)	***	***		*	*	
Stretched (BD)	***	**		*	N.S.	

[a]All Mean values are presented with a standard error of the mean.
[b]Mean of 13 turkeys
[c]Mean of 9 turkeys
*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$

mere length of the folded muscles. The values for the stretched muscles were significantly higher ($P < 0.01$) in the prerigor stretched muscles compared with the rigor stretched muscle. This is the same order of difference as that found in the unheated controls. There was no statistically significant difference in fiber diameter between the folded or between the stretched heated semitendinosus in Experiment I and Experiment II.

It is of interest to note that heat produced the same significant decrease in fiber diameter in both the folded and the stretched muscles which were either rigor stretched or prerigor stretched ($P < 0.001$) except for the prerigor stretched muscle ($P < 0.01$). However, the extent of the decrease is greater in the folded muscles in both treatments.

Significantly lower ($P < 0.01$) shear force values were obtained for the folded muscles compared with the rigor stretched muscles in the thirteen turkeys investigated in Experiment I (Rigor Stretched) (Table 1). This result was unexpected because of the generally accepted positive association between long sarcomeres and tender meat. However, the majority of these comparisons are made between sarcomere lengths in uncooked muscle and the shear force of cooked muscle. Bendall and Voyle (1967), Goll (1970) and others have suggested that caution must be exercised in such comparisons. Sarcomere length of the cooked muscle would be a more meaningful comparison with shear value. In Experiment I (Table 1) no statistically significant difference was obtained between the sarcomere length of the folded and the rigor stretched heated muscle. The values for the prerigor stretched semitendinosus are more relevant (Experiment II, Table 1) because all previous research has been conducted on the relationship of prerigor treatments to muscle tenderness. The lower sarcomere length values in both the unheated and heated prerigor folded muscle would suggest a lower shear value in the prerigor stretched muscle. In fact a significantly higher value was obtained on the nine turkeys studied ($P < 0.01$). More turkeys were used for shear force determinations on both the pre-rigor and rigor stretched muscles than for the fiber dimension measurements because large variations in tenderness within experimental groups has been reported (Varadarajulu and Cunningham, 1971). Considerable variation in shear force values was also obtained within groups in the present investigation (Table 1). Differences were rather uniform in the same direction but the magnitude differed.

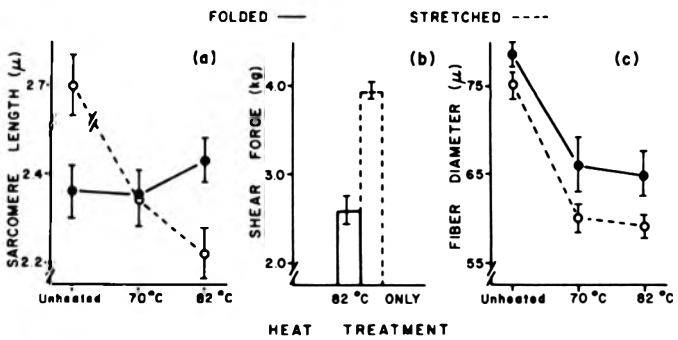

Fig. 2–Heat effect of sarcomere length (a), shear force (b) and fiber diameter (c) of rigor folded and stretched turkey semitendinosus muscle.

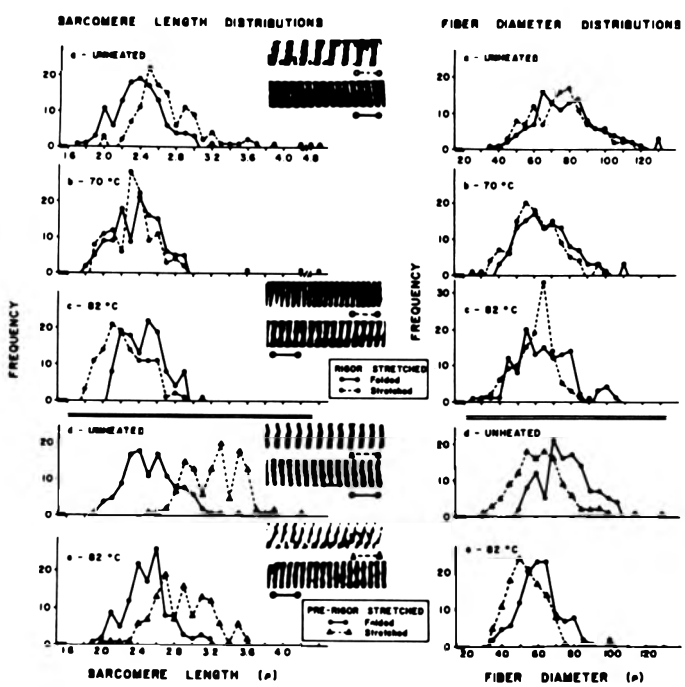

Fig. 3–Sarcomere length and fiber diameter frequency distributions for unheated and heated, folded and stretched (prerigor and rigor) turkey semitendinosus muscle. Portions of the isolated fibers from the different treatments illustrate the changes in the microscopic appearance (X 360).

Sartorius. In general the relationships are similar to those obtained in the heated semitendinosus. The following are the exceptions: the heat effect on sarcomere length was not as great in the rigor stretched sartorius relative to the folded control as it was in the similarly treated semitendinosus (Experiment I, Table 1); shear force values were not significantly different in the prerigor treated sartorius (Experiment II, Table 1) whereas the equivalent semitendinosus gave higher values for the stretched muscles ($P < 0.01$); the significance due to heating on the fiber diameter of both the prerigor or rigor treatments was less in the sartorius when compared with the semitendinosus (Experiments I and II, Table 1).

Effect of different heat treatments

Because of the unexpected results obtained in Table 1, further investigation seemed necessary. The semitendinosus muscle was prepared as outlined in Experiment III (Rigor Stretched Muscle) in the 'Experimental' section. The results are presented in Figure 2. Sarcomere length decreased significantly ($P < 0.01$) on heating the rigor stretched muscle to 70°C. The change from 70°C to 82°C was not significant. In contrast, there was no statistically significant change in the sarcomere length of the folded muscles due to the two heat treatments. However, the data in Figure 2a, b show that the rigor

stretched muscle which had a significantly higher ($P < 0.05$) shear force also had a significantly shorter ($P < 0.01$) cooked (82°C) sarcomere length than the corresponding folded control muscles. Shear values were not determined on the samples heated to an internal temperature of 70°C because of the unequal heat effect between the periphery and the center of the sample. Fiber diameter decreased significantly on heating the folded and rigor stretched muscles to 70°C ($P < 0.05$; $P < 0.01$, respectively). There was no statistically significant change between the values obtained from muscles heated to 70°C and to 82°C.

Heat effect on sarcomere length and fiber diameter distributions

Mean values for sarcomere length and fiber diameter give no indication of the distribution of these parameters within a group of animals. In the present investigation it was considered necessary to present the results in frequency histograms to determine if the stretching effect and the application of heat had a uniform or differential effect on all the sampled fibers from the rigor stretched and prerigor stretched experimental groups. The distribution curves in Figure 3a–c were obtained from the semitendinosus muscles used in Experiment III (Rigor Stretched) and Figure 3d, e were obtained from the semitendinosus muscle in Experiment II (Prerigor Stretched). The sarcomere length decreased significantly ($P < 0.01$) in the rigor stretched muscle on the application of heat (Fig. 3a–c). The distributions are essentially normal in the unheated and heated muscle. However, the spread of the distribution suggests that the fibers with the longest sarcomeres were most responsive to heat. This observation is confirmed by examining the sarcomere length distribution for the corresponding folded muscles (Fig. 3a–c). The folded muscles also had a normal sarcomere length distribution but the application of heat caused no statistically significant change in either the mean sarcomere length (Fig. 2) or the distribution. Similar distributions were obtained for all treatments on the sartorius. The effect of stretch on the unheated rigor muscle gave a few fibers with a sarcomere length greater than 3.6μ. Wilkie (1969), in reviewing the work on the ultrastructural dimensions of vertebrate skeletal muscle, states that the length of the thick filaments, and hence the A band, is 1.5–1.6μ long. The length of the thin filaments vary more from one species to another, ranging from 2.05–2.6μ if measured from tip to tip and thus including the Z line. As far as the authors are aware, no quantitative evidence on the length of the thin filaments has been presented for turkey muscle. Therefore, the maximum and minimum values for the length of the thin filaments and the length of the A band, give sarcomere length values of 3.55μ and 4.20μ, respectively. Examination of the unheated rigor stretched muscle in Figure 3a shows that seven fibers had a sarcomere length longer than 3.55μ, while two fibers had a value greater than 4.20μ. Heating the muscle to 70°C greatly reduced the number of fibers with long sarcomeres (Fig. 3b). After raising the temperature to 82°C, no fibers had sarcomere lengths longer than 3.1μ (Fig. 3c). Since all samples (unheated, 70°C and 82°C) were obtained from the same muscles, there was a high degree of uniformity maintained between the different heat treatments.

Examination of the sarcomere length distribution curves for the prerigor stretched semitendinosus muscles (Fig. 3d, e) indicate that the distribution pattern and heat effect on the folded muscles was the same as that found in the similarly treated folded muscles in Figure 3a–c. Comparison of the unheated prerigor stretched muscle (Fig. 3d) with the unheated rigor stretched muscle (Fig. 2a) indicates how a longer sarcomere length is achieved when the muscle is stretched in the prerigor state. Applying the same dimensions used above to express minimum overlap between the thick and thin filaments, it can be seen in Figure 3d that no fibers had a sarcomere length longer than 4.2μ and 19 had values larger than 3.55μ. When these muscles were heated to an internal temperature of 82°C only two fibers had a sarcomere length greater than 3.55μ (Fig. 3e). It seems therefore, that the heat effect was again greatest in the fibers having the longest sarcomere lengths.

Previous studies have reported a decrease in sarcomere length in heated bovine longissimus dorsi muscle (Schmidt and Parrish, 1971). A study of different temperatures and different time periods at given temperatures on the length and width of muscle fragments from bovine longissimus dorsi was reported by Hostetler and Landmann (1968). These authors did not measure sarcomere length. When isolated fibers from the muscles used in the present investigation were heated in a similar manner, no cross striations could be observed at 82°C and many of the fibers were twisted or wavy. All the fibers viewed from the intact muscles which were heated between the clamps did not exhibit this phenomenon. However, Hostetler and Landmann (1968) found that the greatest effect on fiber width and length occurred below 70°C. Similar observations were made in the present investigation.

The differential heat effect on sarcomere length in the folded and the stretched muscles (Table 1, Fig. 3a–e) is of interest since no similar comparative study has been reported in muscles from meat animals. These prerigor and rigor stretched turkey muscles had a sarcomere length within the 2.4–3.7μ range which Locker (1959) described as Type I (relaxed) in ox muscle. The values decreased significantly when heated to 70°C or 82°C. The sarcomere lengths in the folded fibers were within the Type I range in the semitendinosus and the Type II range (1.9–2.4μ) in the sartorius. The sarcomeres in both these muscles when folded did not increase significantly when heated. Presumably the effects of heat on the ultrastructure of skeletal muscle outlined by Schmidt and Parrish (1971) are common to both the folded and stretched muscles in this study. These include progressive myofibrillar shrinkage and degradation, thin filament disintegration and thick filament coagulation. However, in the semitendinosus and sartorius muscles of the turkey the application of heat causes no further decrease in sarcomere length when the unheated values are 2.4μ and 2.1μ respectively (Table 1). It could not be determined from the light microscope if the decrease in the longer sarcomeres of the stretched muscles was due to a sliding of the actin and myosin filaments. If this is the cause, it is interesting to speculate why the sarcomeres in the folded muscles do not change when heated. Since the myosin filaments are 1.6μ long (Wilkie, 1969), the minimum theoretical undamaged sarcomere length is 1.6μ. Recent evidence on chicken muscle showed that the maximum shortening in undamaged sarcomeres was 1.3μ (Hagopian, 1970). Therefore, all the sarcomeres in the muscles in this study had the potential to shorten to between 1.3 and 1.6μ. Obviously the heat changes outlined above prevent this, but the question remains as to why there should be a differential effect within the same muscle which was either folded or stretched.

The fiber diameter distributions were also normal (Fig. 3a–e) confirming similar measurements on unheated turkey muscle by Varadarajulu and Cunningham (1971). These observations add another species to the list compiled by Hegarty and Hooper (1971) which indicate that the fiber distribution of unheated skeletal muscle from adult animals is monophasic rather than biphasic.

Photomicrographs from the light microscope showing the appearance of the sarcomeres subjected to the different stretch tensions and heat treatments are shown in Fig. 3a–e. Because of the wide degree of overlap between the different treatments a certain amount of subjectivity had to be introduced in the selection of these photomicrographs. Therefore, those from the stretched muscles are representative of fibers with long sarcomeres within this treatment. Clearly defined fibers and A bands were obtained

at all heat treatments for both folded and stretched muscles. This is in agreement with Schmidt and Parrish (1971) who found that on heating bovine skeletal muscle at various temperature intervals to 90°C, the principal banding features of the sarcomeres could be identified regardless of temperature. The cracks in heated fibers referred to by Paul (1966) in rabbit muscle and by Paul et al., (1970) in beef muscle were rarely seen in the heated turkey muscles.

It is difficult to explain why the stretched muscles did not give lower shear force values. All previous evidence indicates that the longer the sarcomere length in the unheated muscle the lower the shear force value when cooked. Interesting exceptions in turkey muscles have been reported. Welbourn et al., (1968) found no significant correlations between shear values and sarcomere length. However, Ma et al., (1971) have pointed out that these correlations may not be valid since the sarcomere length was determined on myofibrils separated 3 hr postmortem in the absence of a calcium chelator. On the basis of rigor studies on turkey muscle, Ma et al., (1971) concluded that this procedure probably stimulates contraction in the sarcomeres because the muscle is probably not in rigor mortis. Varadarajulu and Cunningham (1971) reported no significant correlation between tenderness and either sarcomere length or fiber diameter in turkey muscles. The authors state that this may be due to the fact that fiber measurements were made on prerigor meat, whereas sensory evaluation was done on rigor samples. In the present study the difference in sarcomere length between the folded and stretched heated muscles would suggest that the stretched muscles should be more tender (except the rigor stretched semitendinosus). Possible changes in the molecular structure of collagen in stretched muscle could also contribute to increased tenderness Kruggel and Field (1971).

A further variable between the folded and stretched muscles in this investigation was the time taken for the internal temperature to reach 82°C. The entire muscles were heated, therefore the thinner stretched muscles attained this temperature about 2–3 min sooner than the folded controls. In order to check if this temperature differential alters tenderness, 30 strips of varying dimensions were taken from the breast muscle and heated in a similar manner. The time taken to attain an internal temperature of 82°C ranged from 8–25 min. However, no significant relationship was obtained between shear force values and duration of heating. It was therefore presumed that the same effect occurred in the semitendinosus and sartorius muscles.

Sarcomere length varies along a muscle (Williams and Goldspink, 1971), and because of the relationship between sarcomere length and shear force values, variations may occur within the five shears that were performed on each muscle in this investigation. Sarcomere length decreases near the tendon, therefore about 2 cm from each tendon was discarded. No significant location effect on shear force values could be detected.

Though rigor skeletal muscle is regarded as being inextensible by most authors, reference must be made to the rigor extensibility studies of Wang et al. (1956), Hostetler and Cover (1961), Buck and Black (1967) and Stanley et al. (1971). Wang et al. (1956) found that the overall length of single fibers from unheated and heated beef muscles could be increased by stretching. Hostetler and Cover (1961), in a similar type of study on heated beef muscle fibers, confirmed these observations. Recent evidence by Stanley et al. (1971) showed that thin strips of unheated rigor beef and rabbit muscle could be extended. None of these studies investigated changes or damage to the structure of the fiber as seen under the microscope. Furthermore, the studies of Hostetler and Cover (1961) and possibly those of Stanley et al. (1971) were performed on aged beef muscle (7 days and "commercially obtained," respectively). Goll (1968) states that a considerable lengthening of contracted sarcomeres has been reported to occur 2–3 days postmortem. This is thought to be the result of a considerable weakening or partial dissociation of the actin-myosin interaction, and also to the degradation of the sarcomere at the level of the Z line due to proteolytic activity. Because of these weakenings in the muscle ultrastructure, it may be easier to stretch muscle that has passed through the resolution of rigor phase. However, Wang et al. (1956) found that the extensibility of both the unheated and heated beef muscle fibers decreased when aged for 2–4 wk. Confirmation of this observation was made by Davey and Dickson (1970) in extensibility studies on entire beef muscles. A weakening of sarcomere linkages occurred largely between thin filaments and Z lines and no ultrastructural extension occurred when weight was applied to muscle 90 hr postmortem. The authors refer to unpublished work which showed that unaged meat (30 hr postmortem) yields on forcible stretching largely through a withdrawal of the thin filaments from between the myosin rods of the A band. This observation was confirmed by electron microscope studies on some of the rigor stretched semitendinosus muscles used in the present study. Therefore, the lack of extensibility in aged meat is probably due to the breakdown of the sarcomere at the junction of the thin filament and Z line. This breakdown in the structural components of the sarcomere may counteract its potential to extend due to the weakening of the actin-myosin interaction. Goll (1968) states that the increased fragility of postmortem muscle strips has prevented the direct testing by macroscopic extensibility measurements the theory of a weakened actin-myosin interaction in postmortem muscle. Stanley et al. (1971) also used 24 hr postmortem rabbit muscle stored at 0–5°C. Henderson et al. (1970) showed that some breaks in the Z line begin to appear when this muscle is held for this time and temperature. The extension in the present study was performed on muscle immediately after the muscle had entered rigor and electron micrographs show negligible ultra-structure degradation (unpublished observations). The positive extension in the heated rigor muscle fibers reported by Hostetler and Cover (1961) are not comparable to the observations reported here for the muscles stretched prior to heating. Thermal denaturation of the myofibrillar proteins, and changes in the elasticity (Hoeve and Willis, 1963) and isometric tension (Mohr and Bendall, 1969) of thermally denatured connective tissue may account for some of these changes. The extensibility studies of Buck and Black (1967) are not comparable to the observations of Wang et al. (1956), Hostetler and Cover (1961), Stanley et al. (1971) and those of the present investigation because the uncooked fibers were fixed in neutral formalin prior to extension. Formalin creates additive compounds with proteins, and can produce bridging and polymerization (McManus and Mowry, 1960). This may alter the extensibility properties of fibers.

CONCLUSIONS

RESULTS of this investigation on unaged, rigor semitendinosus and sartorius muscles from adult turkeys indicates: (1) unheated muscle can be extended in situ in the rigor state; (2) the degree of rigor extensibility is significantly less than when the same muscle is stretched immediately postmortem; (3) prerigor stretching produced a significant decrease in fiber diameter compared to folded controls; (4) heat treatment caused a significant decrease in sarcomere length in the stretched muscles, but no significant decrease was obtained in the folded controls; (5) shear force values were either nonsignificant between the folded and stretched muscles or the stretched muscles gave significantly higher values; (6) application of heat caused significant decreases in the fiber diameter of both the folded and the stretched muscles (except for the prerigor stretched sartorius); (7) sarcomere length and fiber diameter distribution curves were normal for

all treatments in both muscles; and (8) light microscopy showed no damage due to stretching the unheated muscles; the major structural features of the muscle were still intact after heating to 82°C.

REFERENCES

Abbott, B.C. and Baskin, R.J. 1962. Volume changes in frog muscle during contraction. J. Physiol. 161: 379.

Bendall, J.R. and Voyle, C.A. 1967. A study of the histological changes in the growing muscles of beef animals. J. Food Technol. 2: 259.

Buck, W.M. and Black, D.L. 1967. The effect of stretch-tension during rigor on certain physical characteristics of bovine muscle. J. Food Sci. 32: 539.

Davey, C.L. and Dickson, M.R. 1970. Studies in meat tenderness. 8. Ultra-structural changes in meat during aging. J. Food Sci. 35: 56.

Forrest, J.C., Haugh, C.G. and Allen, C.E. 1969. The myotron: An instrument for the study of physical properties of muscle under controlled conditions. American Society of Agricultural Engineers, St. Joseph, Mich., Paper No. 69-876.

George, J.C. and Berger, A.J. 1966. "Avian Myology." Academic Press, New York and London.

Goll, D.E. 1968. The resoluction of rigor mortis. In "Proceedings 21st Annual Reciprocal Meats Conference," 21: 16. National Live Stock & Meat Board, Chicago.

Goll, D.E. 1970. In "The Biochemistry and Physiology of Muscle as a Food," ed. Briskey, E.J., Cassens, R.G. and Marsh, B.B., Vol 2, p. 822. The University of Wisconsin Press, Madison.

Hagopian, M. 1970. Contraction bands at short sarcomere length in chick muscle. J. Cell Biol. 47: 790.

Hamm, R. 1966. Heating of muscle systems. In "The Biochemistry and Physiology of Muscle as Food." ed. Briskey, E.J., Cassens, R.G. and Trautman, J.C., Vol 1, p. 363. The University of Wisconsin Press, Madison.

Hegarty, P.V.J. 1972. Molecular and macroscopic extensibility of rigor skeletal muscle due to stretch tension. Life Sci. 11 (Part 2): 155.

Hegarty, P.V.J. and Naude, R.T. 1970. The accuracy of measurement of individual skeletal muscle fibres separated by a rapid technique. Lab. Pract. 19: 161.

Hegarty, P.V.J. and Hooper, A.C. 1971. Sarcomere length and fibre diameter distributions in four different mouse skeletal muscles. J. Anat. 110: 249.

Henderson, D.W., Goll, D.E. and Stromer, M.H. 1970. A comparison of shortening and Z line degradation in post-mortem bovine, procine and rabbit muscle. Am. J. Anat. 128: 117.

Herlihy, E., Hegarty, P.V.J. and Heffron, J.J.A. 1972. Ultrastructural evidence of positive extension in mouse skeletal muscle stretched in rigor mortis. Life Sci. (in press).

Herring, H.K., Cassens, R.G., Suess, G.G., Brungardt, V.H. and Briskey, E.J. 1967. Tenderness and associated characteristics of stretched and contracted bovine muscle. J. Food Sci. 32: 317.

Hoeve, C.A.M. and Willis, Y.A. 1963. Elasticity of the fibrous muscle proteins. Biochemistry 2: 279.

Hostetler, R.L. and Cover, S. 1961. Relationship of extensibility of muscle fibers to tenderness of beef. J. Food Sci. 26: 535.

Hostetler, R.L. and Landmann, W.A. 1968. Photomicrographic studies of dynamic changes in muscle fiber fragments. 1. Effect of various heat treatments on length, width and birefringence. J. Food Sci. 33: 468.

Kruggel, W.G. and Field, R.A. 1971. Soluble intramuscular collagen characteristics from stretched and aged muscle. J. Food Sci. 36: 1114.

Locker, R.H. 1959. Striation patterns of ox muscle in rigor mortis. J. Biophys. Biochem. Cytol. 6: 419.

Ma, R.T-I., Addis, P.B. and Allen, E. 1971. Response to electrical stimulation and post-mortem changes in turkey pectoralis major muscle. J. Food Sci. 36: 125.

McManus, J.F.A., and Mowry, R.W. 1960. "Staining Methods: Histologic and Histochemical," p. 10. Paul B. Hoeber, Inc., New York.

Mohr, V., and Bendall, J.R. 1969. Constitution and physical chemical properties of intramuscular connective tissue. Nature 223: 404.

Paul, P.C. 1966. Storage- and heat-induced changes in the macroscopic appearance of rabbit muscle. J. Food Sci. 30: 960.

Paul, P.C., Mandigo, R.W. and Arthaud, V.H. 1970. Textural and histologic differences among three muscles in the same cut of beef. J. Food Sci. 35: 505.

Schmidt, J.G. and Parrish, F.C. Jr. 1971. Molecular properties of post-mortem muscle. 10. Effect of internal temperature and carcass maturity on structure of bovine longissimus. J. Food Sci. 36: 110.

Smith, G.C., Arango, R.C. and Carpenter, Z.L. 1971. Effects of physical and mechanical treatments on the tenderness of the beef longissimus. J. Food Sci. 36: 445.

Stanley, D.W., Pearson, G.P. and Coxworth, V.E. 1971. Evaluation of certain physical properties of meat using a universal testing machine. J. Food Sci. 36: 256.

Steel, R.G.D. and Torrie, J.H. 1960. "Principles and Procedures of Statistics." McGraw-Hill Book Co., Inc., New York.

Varadarajulu, P. and Cunningham, F.E. 1971. A histological study of turkey meat as related to sensory characteristics. Poultry Sci. 50: 1144.

Wang, H., Doty, D.M., Beard, F.J., Pierce, J.C. and Hankins, O.G. 1956. Extensibility of single beef muscle fibers. J. Anim. Sci. 15: 97.

Welbourn, J.L., Harrington, R.B. and Stadelman, W.J. 1968. Relationships among shear values, sarcomere lengths and cooling procedures in turkeys. J. Food Sci. 33: 450.

Wilkie, D.R. 1969. "Muscle." Institute of Biology's Studies in Biology, No. 11, p. 20. Edward Arnold (Publishers) Ltd., London.

Williams, P.A. and Goldspink, G. 1971. Longitudinal growth of striated muscle fibres. J. Cell Sci. 9: 751.

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METABOLISM AND HISTOCHEMISTRY OF SKELETAL MUSCLE FROM STRESS-SUSCEPTIBLE PIGS

INTRODUCTION

SKELETAL MUSCLE of stress-susceptible pigs (Forrest et al. 1968 and Judge et al. 1968), when obtained either through regular biopsy or excision simultaneous with exsanguination, has smaller amounts of creatine phosphate (CP), adenosine triphosphate (ATP) and glycogen and larger amounts of lactic acid than muscle from stress-resistant pigs (Lister et al. 1970 and Sair et al. 1970). A high level of lactic acid and a low level of CP and ATP at the time of exsanguination, leads to the rapid development of pale, soft, exudative (PSE) characteristics in the muscle (Lister et al. 1970; Sair et al. 1970 and Kastenschmidt et al. 1968). The muscles of stress-susceptible animals are also particularly sensitive to anoxia (Lister et al. 1970) and when the animals are incubated and forced to breathe nitrogen, large quantities of lactic acid accumulate. Additionally, these muscles are sensitive to excision anoxia and exsanguination anoxia during which time they accumulate large quantities of lactic acid. Through the development of a cryobiopsy technique, Teeter et al. (1969) observed that if the samples were frozen in the living animal and removed as frozen biopsy cores, the metabolite levels in muscle of stress-susceptible pigs were quite similar with those of the stress-resistant pigs.

Sair et al. (1970) also demonstrated that if stress-susceptible animals were anesthetized with Mg^{++}, metabolite levels were similar to levels in the same muscles of untreated stress-resistant pigs.

To further understand the etiology of PSE, in vitro metabolism of intact muscle fiber strips from muscle of stress-resistant and stress-susceptible pigs was studied. Magnesium anesthesia was used to obtain samples from stress-susceptible and stress-resistant pigs which had approximately similar metabolite levels; thereafter we could study the in vitro metabolism in Warburg flasks in the presence of O_2 or N_2 without the effect of widely differing levels of metabolites.

In preliminary histochemical studies, we (Sair et al., 1970) observed that the fibers of stress-susceptible Poland China pigs were larger and had a higher percentage of their fiber area staining with red fiber characteristics than the muscles of stress-resistant Chester White pigs. Previous histochemical studies (Sair et al., 1970) were difficult to evaluate because of sample isolation procedures and therefore further work was done to clarify and confirm this point. In this study we were able to extend the histochemical phase to include stress-susceptible and stress-resistant pigs of each breed, and thereby evaluate the characteristics of the fibers independent of breed. By clamping fiber bundles during excision we were also able to bring further clarity to the differences in sizes of fibers from stress-susceptible and stress-resistant animals and to evaluate whether differences in fiber size could contribute to the development of PSE.

MATERIALS & METHODS

In vitro metabolism

Animal treatment and sample source. Ten stress-susceptible (previously established strain; Sair et al. 1970) Poland China and ten stress-resistant (previously established strain; Sair et al. 1970) Chester White pigs were intravenously injected with 0.20–0.25 ml/kg body weight of a 50% solution of magnesium sulfate. Approximately one-half of this dose was injected to produce narcosis and the remainder was infused over a 20-min period in order to maintain this condition. Six control (without Mg^{++}) animals of each group were also utilized in this study. The animals were then exsanguinated and samples of the longissimus muscle were taken at death, 1 and 3 hr post-mortem for CP, ATP and lactic acid analyses and at death for in vitro studies.

Metabolism studies. Muscle fiber strips of 1 mm^2 cross section and about 2.5–3.5 cm in length were dissected, with as little damage as possible, along fascicular planes of the longis-

Table 1 —Oxygen consumption of muscle from stress-resistant and stress-susceptible pigs

	μmoles oxygen/g tissue/2 hr
Stress-susceptible[a]	8.30 ± 0.65
Stress-resistant[a]	13.43 ± 0.84
Stress-susceptible (Mg treated)[b]	13.01 ± 0.74
Stress-resistant (Mg treated)[b]	12.00 ± 0.14

[a]Six animals each group
[b]Ten animals each group

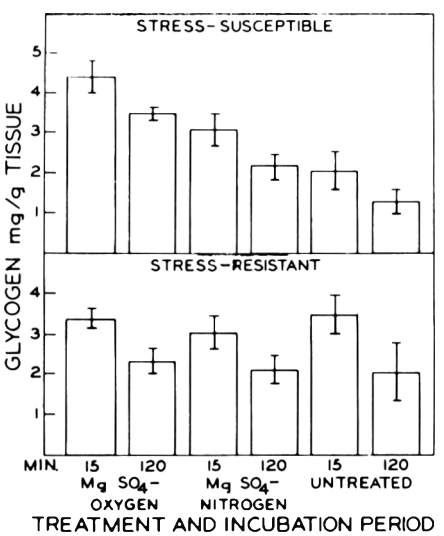

Fig. 1—The amount of glycogen in muscle from Poland China and Chester White pigs incubated in vitro. One standard error of the mean is given on the bars. Results expressed as mg glycogen (glucose equivalents)/g tissue.

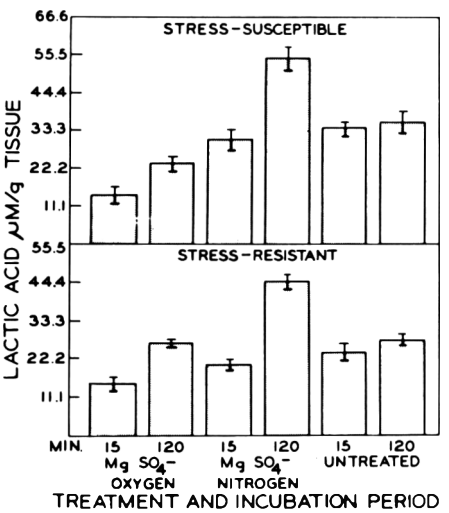

Fig. 2—Amount of lactic acid efflux from muscle of Poland China and Chester White pigs incubated in vitro. One standard error of the mean is given on the bars. μmoles/g tissue lactic acid.

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simus muscle. Excised tissues were held at room temperature in a modified Krebs-Ringer (Beatty et al., 1966) solution of pH 7.4 and aerated with 95% O_2 if the incubations were to be carried out in an oxygen atmosphere or 100% N_2 if the incubations were to be carried out under a nitrogen atmosphere. After the dissections were completed, the muscle tissues were drained on filter paper and 200–250 mg of tissue were added to Warburg flasks containing 3 ml of modified Krebs-Ringer solution buffered with glycyl glycine at pH 7.4 and containing 100 mg percent of glucose. The flasks were then attached to a Gilson Differential Respirometer, gassed with either O_2 or N_2 for 5 min, equilibrated for a further 15 min at 37°C and then incubated for 2 hr. In experiments in which O_2 consumption was measured, 0.2 ml of 10% KOH was added to the center well. At the end of the equilibration period 0.1 ml of 18N H_2SO_4 was added to the remaining flasks. All of the flasks were shaken for an additional 30 min after the addition of the acid. Oxygen (O_2) consumption was determined by a direct digital readout apparatus in ml of oxygen using the Gilson Differential Respirometer.

The muscle fiber strips from the 15 min and 2 hr incubations were digested in 0.5 ml of 30% KOH for 20 min. The glycogen in the KOH digest was determined by the method of Roe (1955). Aliquots of the media were taken for the estimation of lactic acid by the method of Barker and Summerson (1941).

Histochemical characterization

Animal selection. Four stress-susceptible and four stress-resistant Poland China pigs which developed PSE and normal muscle characteristics, respectively, and four stress-susceptible and four stress-resistant Chester White pigs, which developed PSE and normal muscle characteristics, respectively, were used in this study. All animals were similar in age and weight.

Sample treatment. Two samples of the longissimus muscle were removed immediately after exsanguination. One sample was excised directly, without any attempt to prevent contraction, and trimmed to a cube shape of about 0.5 cm per side. The samples were frozen in isopentane cooled with liquid nitrogen and subsequently equilibrated to −20°C in the cryostat. The second sample was restrained during excision so as to prevent contraction. Two hemostats were mounted in a metal bar so that they were separated by approximately 3.7 cm. A muscle strip was clamped in situ and then dissected loose so that a strip of about 0.5 cm per side was obtained. The clamped strip was then immersed in the freezing solution, equilibrated to −20°C and then an appropriate sample was dissected from the center of the strip. Serial sections, 10μ thick, were mounted on slides and air dried prior to incubation. Amylophosphorylase and reduced diphosphopyridine nucleotide tetrazolium reductase (DPNH-TR) reactions as described by Engel and Brooke (1967) were used in these studies. Photomicrographs of representative areas of the serial sections were made and enlarged so that fiber areas could be evaluated quantitatively with a Zeiss Particle Size Analyzer.

RESULTS & DISCUSSION

Oxygen uptake

The results for oxygen uptake of isolated fiber strips are given in Table 1. Preparations from stress-susceptible animals utilized oxygen at the level of 8.30 μmoles/g 2 hr compared to 13.43 μmoles/g 2 hr for muscle of stress-resistant animals. This indicated that the muscle of stress-susceptible animals had a lower ability for aerobic metabolism or some damage had occurred during the isolation procedure that lowered the ability for oxygen consumption. Support for the latter point was furnished by treating both the stress-susceptible and stress-resistant animals with $MgSO_4$ before the muscle preparations were made. Results in Table 1 show that when the stress-resistant and stress-susceptible animals were treated with $MgSO_4$, then oxygen consumption values of the muscle were quite similar. The value for oxygen consumption by muscle of stress-resistant animals changed little due to $MgSO_4$ treatment but there was a large increase in ability of muscle from stress-susceptible animals to utilize oxygen. We used our system in trial experiments on rat muscle and found oxygen consumption values to compare well with literature values.

Table 2 —Levels of ATP, CP and lactic acid in muscle from stress-susceptible and stress-resistant pigs treated with $MgSO_4$

Hours post-mortem		Control (12)[f] ATP	CP[c]	LA[d]	$MgSO_4$[e] (20)[f] ATP	CP	LA
		Stress-susceptible					
0	$\bar{x}$[a]	3.85*	4.23**	32.40**	4.54	14.89**	12.01
	$s_{\bar{x}}$[b]	.62	.77	2.32	.35	1.10	2.02
1	$\bar{x}$	2.02*	1.85	47.21*	3.23	8.31**	21.31**
	$s_{\bar{x}}$	.23	.43	3.85	.20	.75	4.75
3	$\bar{x}$	–	–	62.70*	2.58*	1.32	40.60
	$s_{\bar{x}}$	–	–	6.85	.67	.23	6.88
		Stress-resistant					
0	$\bar{x}$	3.12	1.10	47.67	3.50	13.80	15.07
	$s_{\bar{x}}$	.32	.40	3.22	.77	1.74	2.17
1	$\bar{x}$	.60	.10	79.93	3.00	3.86	47.23
	$s_{\bar{x}}$	.40	.07	4.58	.24	.86	6.34
3	$\bar{x}$	–	–	86.72	1.07	.28	70.23
	$s_{\bar{x}}$	–	–	2.51	.28	.10	5.72

[a]Mean value (μmoles/g)
[b]Standard error of mean
[c]Creatine phosphate
[d]Lactic acid
[e]Injected intravenously with $MgSO_4$ as described by Sair et al. (1970).
[f]Number of observations
*Significant ($P < .05$)
**Significant ($P < .01$) difference (stress-susceptible, strain of Poland China, vs. stress-resistant, strain of Chester White pigs, for control and $MgSO_4$ treatment)

Table 3—Fiber size[a] of dark and white fibers from restrained and unrestrained muscle

		Dark fibers Unrestrained	Dark fibers Restrained	White fibers Unrestrained	White fibers Restrained
Chester White					
Stress-resistant	$\bar{x}$[b]	2.54††	2.30†	4.13	3.19
	$s_{\bar{x}}$[c]	.33	.21	.11	.56
Stress-susceptible	$\bar{x}$	2.74††	2.29††	4.64†	4.76†
	$s_{\bar{x}}$	.48	.38	.45	.77
Poland China					
Stress-resistant	$\bar{x}$	3.78*	3.10*	4.80*	3.81**
	$s_{\bar{x}}$	.08	.63	.37	.05
Stress-susceptible	$\bar{x}$	4.60	4.23	6.03	6.44
	$s_{\bar{x}}$	.49	.39	.47	.71

[a]($mm^2 \times 10^{-3}$)
[b]Mean value
[c]Standard error of mean
*Significant ($P < .05$) difference between normal and PSE Poland China pigs
**Significant ($P < .01$) difference
†Significant ($P < .05$) difference between normal Chester White and normal Poland China pigs or between PSE Chester White and PSE Poland China pigs
††Significant ($P < .01$) difference

Effect of O_2 and N_2 on glycogen and lactic acid

Figure 1 shows the effect of an oxygen or nitrogen incubation atmosphere on glycogen levels in longissimus muscle of stress-susceptible and stress-resistant pigs that were treated with magnesium as well as from untreated controls. The glycogen levels were similar at corresponding sampling periods for the fibers from stress-resistant pigs regardless of whether the fiber strips were from Mg^{++}-treated pigs and incubated in a N_2 or O_2 atmosphere or from untreated control pigs. In contrast, however, the glycogen levels in the fibers from the stress-susceptible Poland China pigs varied greatly between treatments. The 15 min glycogen values were about 2 mg/g in muscle from untreated stress-susceptible pigs, 3 mg/g in N_2-incubated fibers from Mg^{++}-treated pigs, while the values were 4.5 mg/g in O_2-incubated fibers from Mg^{++}-treated pigs.

If we consider the 15 min glycogen value from the O_2-incubated muscle of the Mg^{++}-treated pigs as the in vivo resting level, then the following possibility becomes evident. There appears to be a much faster utilization of glycogen in the muscles of stress-susceptible than in stress-resistant pigs, i.e., there was significantly ($P < .05$) less glycogen in the muscle of the untreated stress-susceptible pigs than in the untreated stress-resistant pigs, but in the O_2-incubated fibers from Mg^{++}-treated pigs there was significantly more glycogen in the stress-susceptible pigs.

Figure 2 shows values of lactic acid in μmoles/g tissue in the incubation media for fibers from stress-susceptible and stress-resistant pigs. There was no difference in lactic acid production at the 15 min and 2 hr time period between the O_2-incubated muscles from the stress-susceptible and stress-resistant pigs which had been Mg^{++}-treated. The level of lactic acid was significantly higher ($P < .05$) in the N_2-incubated fibers from the stress-susceptible pigs treated with Mg^{++}, both at the 15 min and 2 hr time periods. The 15 min values for the untreated stress-susceptible pigs were higher than the untreated stress-resistant pigs but neither group of untreated pigs appeared to produce lactic acid over the 2 hr time period. The reason for this may be that the 15 min time period was actually close to 1 hr after the death of the pig for it required 20–30 min to dissect the samples and 20–30 min to set up the flasks on the respirometer and complete the equilibration. From the data on biochemical intermediates of excised muscle samples after death (Table 2) it is seen that most of the lactic acid production in the untreated pigs took place by 1 hr after death while lactic acid production in the Mg^{++}-treated pigs continued for 3 hr after death.

The important point from these data is that, when incubations were conducted

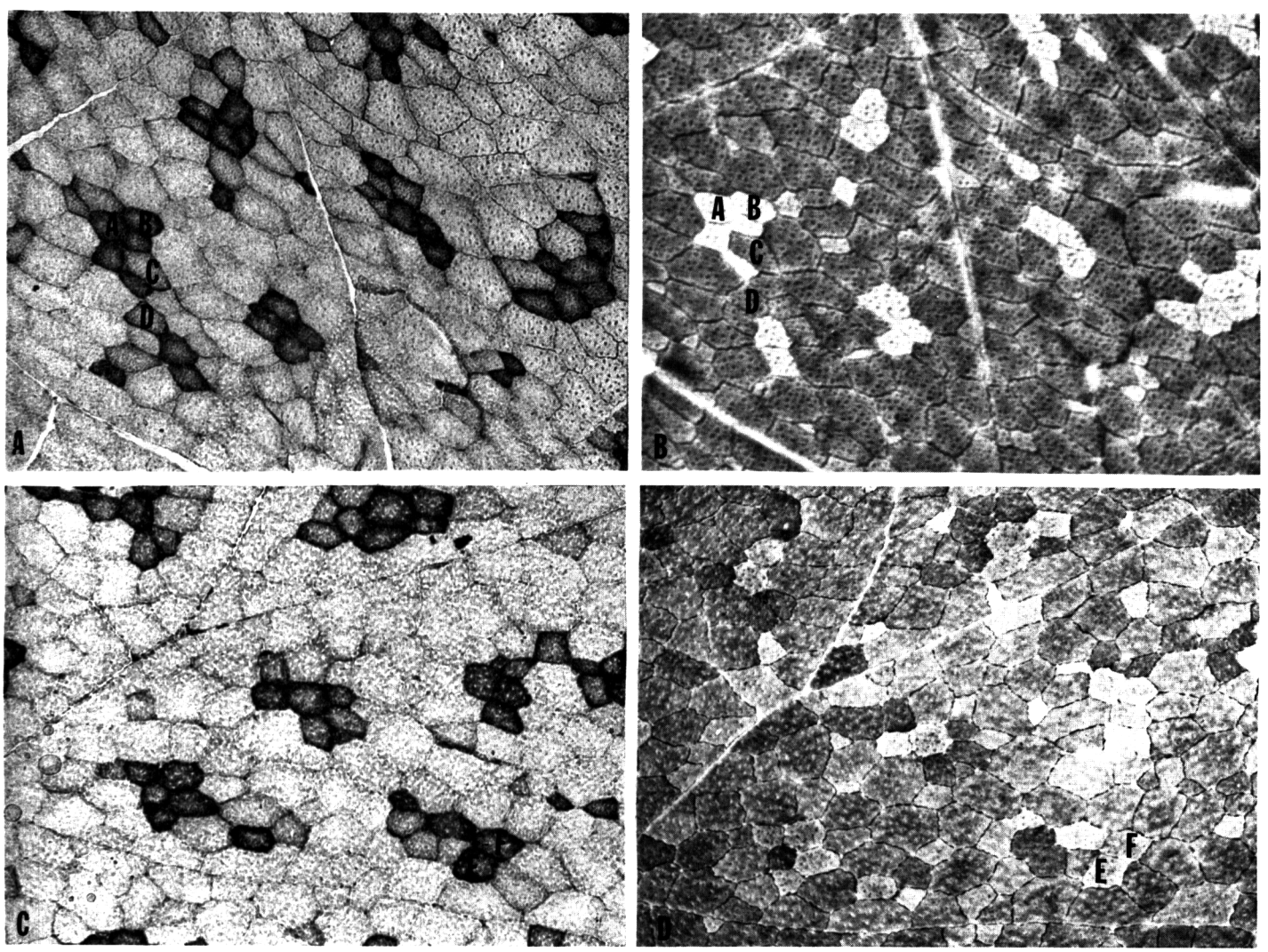

Fig. 3–Sections of longissimus muscle from normal and PSE Chester White pigs reacted for phosphorylase and DPNH-TR. (A) Normal Chester White–DPNH-TR; (B) Normal Chester White–phosphorylase; (C) PSE Chester White–DPNH-TR; (D) PSE Chester White–phosphorylase. (Magnification 138X)

under the anoxic conditions of a nitrogen atmosphere, then the muscle from the stress-susceptible pigs produced more lactic acid than did the muscle from the stress-resistant pigs under conditions where both types of muscle had similar initial CP and ATP levels.

Histochemical evaluation

The sizes of the dark (red plus intermediate) and white fibers in restrained and unrestrained muscle from stress-resistant and stress-susceptible pigs are shown in Table 3. In Poland China pigs, area of both dark and white fibers was larger in stress-susceptible than in stress-resistant animals, but in Chester White pigs, area of dark and white fibers did not differ between stress-susceptible and stress-resistant animals. There was a trend for the restrained muscle samples to have somewhat smaller fibers than the unrestrained samples. The only significant difference, however, was that the white fibers from restrained samples of stress-resistant Poland Chinas were smaller ($P < .05$) than corresponding fibers in unrestrained muscle samples.

The effects of DPNH-TR and amylophosphorylase staining for red, white and intermediate fibers in muscles from stress-resistant and stress-susceptible Chester White and Poland China pigs are seen in serial section (Figs. 3 and 4). Muscle from stress-resistant Chester White pigs showed that the same fibers which reacted strongly for DPNH-TR (red fibers A, B; Fig. 3A) reacted negatively for phosphorylase (red fibers A, B; Fig. 3B), while intermediate fibers (fibers C, D; Fig. 3A) which reacted positively for DPNH-TR (but not as positive as for red fibers) also were positive for phosphorylase (fibers C, D; Fig. 3B). Fibers from stress-susceptible Chester White pigs which reacted positively for DPNH-TR (fibers E, F; Fig. 3C) also reacted, to some degree, positively for phosphorylase (fibers E, F; Fig. 3D). Apparently there are few red fibers and many intermediate fibers (positive reaction for both DPNH-TR and phosphorylase) in muscle from stress-susceptible Chester White pigs.

Fibers in Figures 4A and 4B are from stress-resistant Poland China pigs. As was the case for fibers from stress-resistant Chester White pigs, fibers which reacted strongly for DPNH-TR (red fibers G, H; Fig. 4A) reacted negatively for phosphorylase (red fibers G, H; Fig. 4B) while fibers that reacted with DPNH-TR, but not as strongly as with red fibers, also reacted positively with phosphorylase and are intermediate fibers (fibers I, J; Fig. 4A and 4B). Figures 4C and 4D are from muscle of stress-susceptible Poland China pigs and show the same pattern as was seen in PSE muscle from stress-susceptible Chester White pigs in that there are

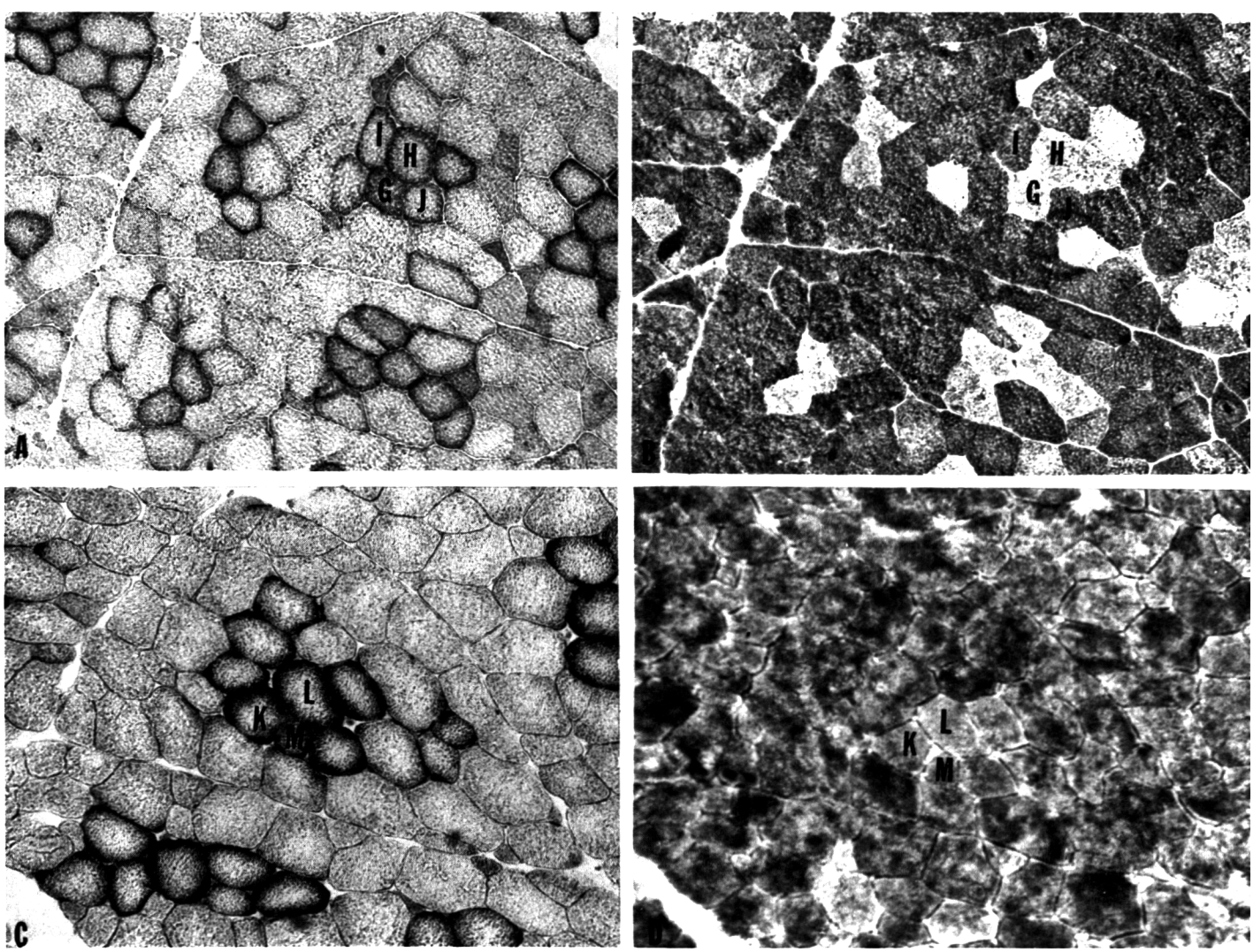

Fig. 4–Sections of longissimus muscle from normal and PSE Poland China pigs reacted for phosphorylase and DPNH-TR; (A) Normal Poland China–DPNH-TR; (B) Normal Poland China–phosphorylase; (C) PSE Poland China–DPNH-TR; (D) PSE Poland China–phosphorylase. (Magnification 138X)

many intermediate fibers. They reacted positively for DPNH-TR (fibers K, L; Fig. 4C) and positively for phosphorylase (fibers K, L; Fig. 4D). The uniformity of the positive reaction for phosphorylase (Fig. 4D) in muscle that develops the PSE condition in the Poland China pig is even more apparent than in muscles from Chester White pigs which develop the PSE condition (Fig. 3D).

The data indicate that size per se does not predispose muscle fibers to postmortem anoxia and the development of PSE characteristics, but rather that a high glycolytic potential seems to be the chief factor predisposing fibers to the PSE condition.

Hence, stress-susceptible animals have large numbers of intermediate and white fibers, both of which stain positively for phosphorylase indicating their glycolytic capacity. Padykula (1952) categorized fibers according to their metabolic function, as revealed by strong or weak reactions for succinic dehydrogenase activity and Dubowitz and Pearse (1960) showed, histochemically, that an inverse relationship existed between mitochondria and glycolytic enzymes. Since stress-susceptible pigs have larger areas of intermediate fibers and less red fibers than do stress-resistant pigs we would expect, according to the work of Padykula (1952) and Dubowitz and Pearse (1960), a greater glycolytic capacity. High levels of lactic acid at death and 1 hr after death in muscles of stress-susceptible pigs are in line with the high glycolytic capacity found histochemically.

Fiber size appears to be related to the PSE condition in that large fibers will contribute to the severity of the PSE condition provided that the basic requirement of a high glycolytic capacity is met.

REFERENCES

Barker, S.B. and Summerson, W.H. 1941. The colorimetric determination of lactic acid in biological material. J. Biol. Chem. 138: 535.

Beatty, C.H. and Bocek, R.M. 1970. Biochemistry of red and white muscle. In "Physiology and Biochemistry of Muscle as a Food," ed. Briskey, E.J., Cassens, R.G. and Marsh, B.B., Vol 2, p. 155. University of Wisconsin Press, Madison.

Beatty, C.H., Peterson, R.D., Basinger, G.M. and Bocek, R.M. 1966. Major metabolic pathways for carbohydrate metabolism of voluntary skeletal muscle. Am. J. Physiol. 210: 404.

Dubowitz, V. and Pearse, A.G.E. 1960. Reciprocal relationship of phosphorylase and oxidative enzymes in skeletal muscle. Nature 185: 701.

Engel, W.K. and Brooke, M.H. 1967. Muscle biopsy as a clinical diagnostic aid. In "Neurological Diagnostic Techniques," ed. Thomas, C.C., p. 1. Springfield, Ill.

Forrest, J.C., Will, J.A., Schmidt, G.R., Judge, M.D. and Briskey, E.J. 1968. Homeostasis in animals (Sus domesticus) during exposure to a warm environment. J. Appl. Physiol. 24: 33.

Judge, M.D., Briskey, E.J., Cassens, R.G., Forrest, J.C. and Meyer, R.K. 1968. Adrenal and thyroid function in stress-susceptible pigs (Sus domesticus). Am. J. Physiol. 214: 146.

Kastenschmidt, L.L., Hoekstra, W.G. and Briskey, E.J. 1968. Glycolytic intermediates and co-factors in "fast" and "slow-glycolyzing" muscles of the pig. J. Food Sci. 33: 151.

Lister, D., Sair, R.A., Will, J.A., Schmidt, G.R., Cassens, R.G., Hoekstra, W.G. and Briskey, E.J. 1970. Metabolism of striated muscle of stress-susceptible pigs breathing oxygen or nitrogen. Am. J. Physiol. 218: 102.

Padykula, H.A. 1952. The localization of succinic dehydrogenase in tissue sections of the rat. Am. J. Anat. 91: 107.

Roe, J.H. 1955. The determination of sugar in blood and spinal fluid with anthrone reagent. J. Biol. Chem. 212: 335.

Sair, R.A., Lister, D., Moody, W.G., Cassens, R.G., Hoekstra, W.G. and Briskey, E.J. 1970. Action of curare and magnesium on striated muscle of stress-susceptible pigs. Am. J. Physiol. 218: 108.

Teeter, C., Carr, S.C., Tsai, R. and Briskey, E.J. 1969. A cryobiopsy technique for assessing metabolic levels in skeletal muscle (33790). Proc. Soc. Exp. Biol. Med. 131: 5.

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PRODUCTION AND BINDING OF MALONALDEHYDE DURING STORAGE OF COOKED PORK

INTRODUCTION

MANY INVESTIGATORS have made use of malonaldehyde (MA) as a measure of lipid oxidation. MA can be used to follow oxidative deterioration of food products, but the relationship between oxidative food deterioration and MA is not simple. Kwon et al. (1965) showed that with moist foods the volatility of MA depends upon the pH and that MA can interact with food proteins. The interaction with other food constituents can account for the fact that TBA values do not decline for complex foods after prolonged storage as they do for pure lipids undergoing oxidation (Kwon and Olcott, 1966a). There are several studies showing the interaction between MA and protein (Buttkus, 1967; Crawford et al., 1967). Chio and Tappel (1969a, b) have shown that one mole of MA interacts with two moles of amino acid and can cross link proteins.

The fact that acid and heat are needed to produce MA from food products has been interpreted to mean that the MA is bound in some way. The evidence for interaction between proteins and MA makes proteins the most likely food constituent responsible for the binding.

This paper describes changes in the MA present in water extracts of cooked pork during refrigerated storage of the pork.

EXPERIMENTAL

Materials

Purified MA was prepared by acid hydrolysis of the distilled acetal (Kwon and Watts, 1963).

Pork and beef were purchased as ground meat from a local market. For cooked samples, the meat was sealed in a No. 2 can with a thermometer inserted. The cans were heated in a boiling water bath until the internal temperature reached 70°C and then held for 5 min. After heating, the cans were immediately cooled in an ice bath to 3°C.

Cooked and raw 10g samples were weighed into petri dishes and spread evenly over the surface. The samples were stored with covers at 3°C in a refrigerator.

Methods

Analysis of MA was done by the acid distillation procedure of Tarladgis et al. (1960) for all meat samples.

The fractions collected from Sephadex column chromatography of water extracts from pork samples were analyzed for MA by reacting with TBA reagent for 20 hr at room temperature (Tarladgis et al., 1964; Kwon and Olcott. 1966b).

Water extracts of meat samples were prepared by mixing 10g of meat with 50 ml of glass-distilled water at room temperature for 1 min in a Waring Blendor and stirring the slurry for 15 min at 3°C. The meat slurry was centrifuged at 31,500G for 40 min, and the supernate was filtered using Whatman No. 2 paper to remove fat and suspended particles. This filtrate was used as the sample to be applied to the Sephadex column.

Ultraviolet spectra of column fractions were obtained on a Beckman DK 2A spectrophotometer.

Analysis of column fractions for protein was done by the Folin-Lowry method (Lowry et al., 1951) as modified by Goll et al. (1964).

A Sephadex G-10 column was prepared by soaking the Sephadex gel overnight and gravity packing the gel into a 1.5 × 92 cm column. After this initial packing, phosphate buffer (0.1M, pH 6.9 + 0.1M NaCl) was pumped through the column for several hours to insure complete and even packing and to equilibrate the gel with buffer. Final column length was 86 cm.

The void volume of the column was determined by blue dextran elution. The column was also standardized by applying and eluting pure MA.

Fractions were eluted with phosphate buffer pumped at a rate of 1 ml per min and 2 ml fractions were collected.

RESULTS & DISCUSSION

THE DEVELOPMENT OF MA in raw and cooked pork samples is compared in Figure 1. Also shown are raw and cooked beef samples. Since the raw samples showed no increase in TBA number over a 26-day period, we decided to work with only the cooked samples to learn more about MA binding. Furthermore, since cooked pork had higher TBA numbers than cooked beef, we concentrated our efforts on the cooked pork samples. Note that the TBA numbers do not decline after reaching a maximum as they do when purified fatty acids are oxidized.

The lack of increase in TBA number with raw meat samples is in contrast with results of Keskinel et al. (1964) but agrees with the results of Witte et al. (1970). Two important factors bearing on oxidation of raw samples are: (1) the degree of subdivision and exposure to oxygen of the sample; and (2) the bacterial growth in the sample. Keskinel et al. (1964) used well subdivided and spread samples with probably very few microorganisms because freshly ground meat samples were used. Hence, there was little chance for anaerobic conditions in

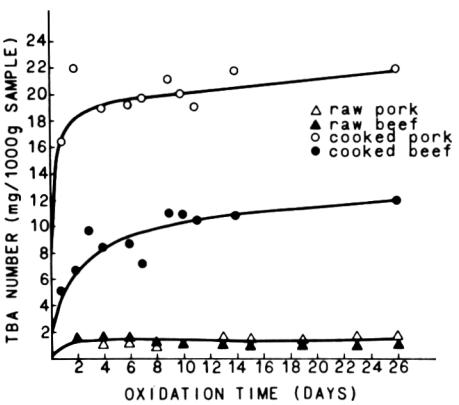

Fig. 1—The progressive development and maintenance of MA in cooked and raw samples of both beef and pork.

Table 1—MA in successive water and KCl extracts of cooked pork[a]

Extraction solution	μg MA extracted
50 ml H_2O	133
20 ml H_2O	36
20 ml H_2O	19
20 ml H_2O	12
20 ml H_2O	6
20 ml H_2O	5
20 ml H_2O	4
20 ml 0.6M KCl	8
20 ml 0.6M KCl	4
20 ml 0.6M KCl	3

[a]Cooked pork sample was 20g. A molar absorbancy coefficient of 7.6×10^4 cm mole^{-1} liter^{-1} at 532 nm was used to calculate the amount of MA extracted and reacted with TBA

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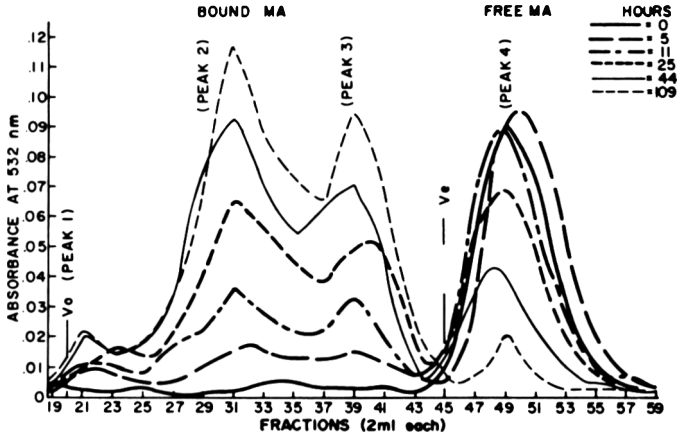

Fig. 2–Measured changes in MA of cooked pork extracts with time of storage of the meat and with elution volumes from Sephadex G-10 chromatography. V_O is the void volume and V_e is the elution volume of a purified malonaldehyde sample.

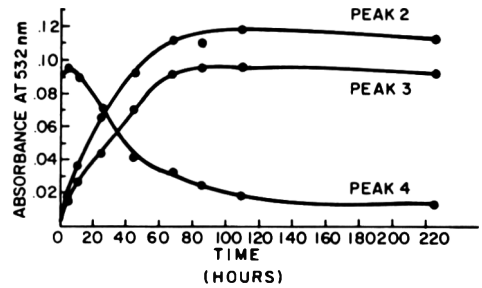

Fig. 3–A direct comparison of the changing absorbancies of peaks 2, 3 and 4 with time of storage of the cooked pork samples.

the samples and oxidation could take place. In the study by Witte et al. (1970) samples of 50g were used and the exposure to air was less than when 10g samples were spread out. In the present study, ground meat was purchased from a local market and even though good contact with air was achieved, it is possible that bacterial growth prevented the oxidation from occurring.

To study the compounds in cooked pork that bind MA, we made water extracts of the pork samples and fractionated the extracts on a Sephadex G-10 column. The extractions normally were done with 50 ml of water for each 10g sample, but initially successive extractions were done to determine the efficiency of the extraction. Table 1 shows an initial 50 ml water extraction followed by extractions with 20 ml of water and finally with 20 ml of 0.6M KCl. The results show most of the MA removed by the first extraction. Furthermore, the extractions with 0.6M KCl for removal of salt-soluble proteins contained no appreciable MA.

Approximately 130 μg of MA was extracted from a 20g sample of cooked pork by a single 50 ml extraction, or 65 μg MA per 10g sample. Figure 1 shows a TBA number of approximately 20 for an oxidized cooked pork sample or approximately 200 μg MA per 10g sample. Hence, the MA extracted by water is about 1/3 of the total MA present. Only 3 ml of the 50 ml extract was fractionated on G-10 Sephadex, but that 3 ml is representative of the changes taking place in approximately 1/3 of the MA in the sample.

In an attempt to concentrate the extract, it was lyophilized, but subsequent column chromatography showed no free MA in the lyophilized sample. Since MA is volatile at low pH, we did an experiment to measure the loss of free MA by lyophilization. The results for both a meat extract and a pure MA sample are shown in Table 2. Loss of the MA from meat extract was greater than from purified MA but was appreciable in both samples. The greater loss from the meat sample could be due to its lower pH of 6 compared to pH 7 for the purified sample. We found that lowering the pH of a purified MA sample to 3 and lyophilizing resulted in 97% loss of the sample.

Further experiments were done by placing water extracts (3 ml) directly on the column without prior concentration. The fractionation of cooked pork extract is shown in Figure 2 for several samples of extract taken after increasing storage times. There were four elution peaks showing reactivity with TBA to produce an absorption band at 532 nm. Peak 1 was located at the void volume of the column and increased with time but had very little pigment associated with it.

Table 2–Loss of MA during lyophilization[a]

Water extract from cooked pork				Malonaldehyde			
Sample vol	Absorbance at 532 nm[b] Observed	Expected[c]	% Lost[d]	Sample vol	Absorbance at 532 nm[b] Observed	Expected[c]	% Lost[d]
110 ml	0.67	0.67	–	100 ml	0.38	0.38	–
40	1.50	1.84	18.5	50	0.61	0.76	19.2
16	3.06	4.54	32.6	16	1.82	2.34	22.2
2.5	10.35	29.50	65.0	2.5	8.40	14.00	44.0

[a]Pure malonaldehyde was made by hydrolysis of the diacetal.
[b]Reacted with TBA by heating for 35 min in a boiling water bath.
[c]Expected absorbance = initial absorbance $\left\{\frac{\text{initial vol}}{\text{sample vol}}\right\}$.
[d]% Malonaldehyde lost = $\left\{\frac{\text{expected} - \text{observed}}{\text{expected}}\right\} \times 100$.

Table 3–Acid and base ultraviolet absorbancy ratios of Sephadex G-10 fractions compared to pure inosine.

	Ratios	Inosine[a]	Peak A 28	Peak B Fraction 40	Peak C 52
Acid (pH 3)	250 nm/260 nm	1.68	1.44	1.01	1.65
	280 nm/260 nm	0.25	0.23	0.16	0.19
Base (pH 11)	250 nm/260 nm	1.05	1.09	1.08	1.12
	280 nm/260 nm	0.18	0.22	0.02	0.13

[a]According to Beaven et al. (1955).

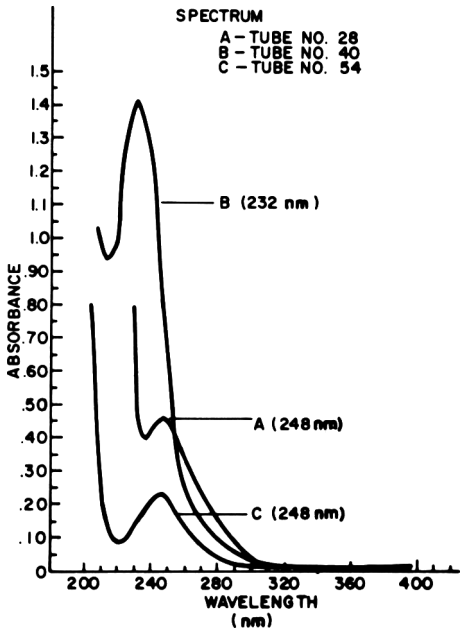

Fig. 4—Ultraviolet absorption spectra of representative fractions from elution peaks A, B and C of Figure 5.

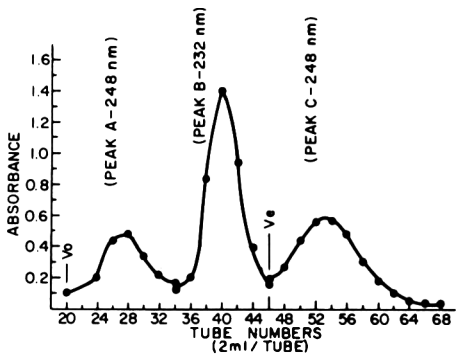

Fig. 5—Elution pattern from Sephadex G-10 chromatography of cooked pork extracts. Peaks A, B and C were identified by their ultraviolet absorbancy as shown in Figure 4. V_O is the void volume and V_e is the elution volume of a purified malonaldehyde sample.

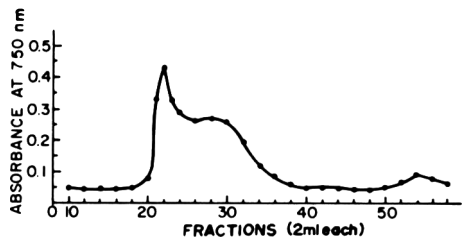

Fig. 6—Relative amounts of protein in the cooked pork extract. Fractions resolved by Sephadex G-10 chromatography. Absorbance of 0.4 equals 0.06 mg protein/ml.

Peak 4 was located close to the elution volume of free MA and decreased with storage time. Peaks 2 and 3 were between the void volume and free MA (indicating some binding to increase the molecular weight), and both peaks increased with storage time. The patterns of increase and decrease for peaks 2, 3 and 4 are shown in Figure 3. These data indicate that free MA is decreasing with storage time and some sort of bound MA, that still reacts with TBA in the presence of acid, is being produced. In the water extract of cooked pork, the binding is not by a protein, except for a very small amount in peak 1.

From Figure 3 the decrease in peak 4 occurs simultaneously with the increase in peaks 2 and 3, but based on absorbancies, the total MA in peaks 2 and 3 is more than could have been derived from peak 4 alone.

As alternate fractions were being analyzed for TBA reactive material, the other fractions were studied for their ultraviolet absorption spectra. Prominent ultraviolet absorption peaks were found in three elution peaks labeled A, B and C. The absorption spectra are shown in Figure 4 and the elution pattern in Figure 5. Elution peaks A and C absorbed at 248 nm while elution peak B absorbed at 232 nm. There was no noticeable change with time of the absorbancy peaks in Figure 5 as had been seen for the TBA reactive compounds in Figure 2. The changes in Figure 2 are of the order of 0.1 absorbancy unit which may not be appreciable in the UV range particularly when compared to a fairly high existing absorbancy.

Alternate fractions were analyzed for protein after studying their ultraviolet spectra. Figure 6 shows the protein content of various fractions with the most protein being present in the fraction that coincides with the void volume. However, fractions 26, 28 and 30 have considerable protein and these fractions coincide with peak 2 (Fig. 2) and with peak A (Fig. 5). Subsequent fractions show very little protein present. Since pure inosine eluted in the same fractions as those showing protein (26, 28 and 30), there is doubt whether this is really protein.

Elution peaks A and C do not coincide directly with elution peaks 2 and 4 but they fall in the same general region (A with 2 and C with 4). The absorbancy at 248 nm suggests the presence of inosine or inosine breakdown products both of which are found in meat extracts. A sample of pure inosine eluted from the Sephadex column in the same position as peak A (Fig. 2). Following Beaven et al. (1955), we treated pure inosine samples and chromatography fractions 28, 40 and 52 with acid and base and recorded ultraviolet absorbancies at 250, 260 and 280 nm. The results shown in Table 3 indicate that both peak A (fraction 28) and peak C (fraction 52) contain inosine. Since the pure inosine eluted with peak A, peak C may be a breakdown product of inosine such as xanthine or hypoxanthine.

Elution peak B of Figure 5 coincided well with elution peak 3 of Figure 2. The absorbancy maximum of peak B was at 232 nm, typical of conjugated dienes formed during lipid oxidation, but the relation between the material absorbing at 232 nm and binding of MA is not known.

The most likely compounds that might interact with MA in cooked meat and still be water soluble would be free amino aicds. Chio and Tappel (1969a) give spectral data on 1-amino-3-imino propane derivatives resulting from reaction of 1 mole of MA with two moles of amino acid. The spectra do not correspond with those shown in Figure 5, so we conclude that the higher molecular weight derivatives of MA are not due to amino acid reactions.

This analysis of water extracts from cooked pork has shown that free MA is present initially, but decreases with storage. The free MA is converted to a bound form which is evident in two separate peaks on G-10 Sephadex. The compounds reacting with MA are not known, but they do not have the spectra of amino acid derivatives. The binding of MA to protein reported by Buttkus (1967), Crawford et al. (1967) and Kwon et al. (1965) was not evident because we analyzed only the water extractable MA from cooked meat. This water extract contained approximately 1/3 of the total MA in the sample.

REFERENCES

Beaven, G.H., Holiday, E.R. and Johnson, E.A. 1955. Optical properties of nucleic acids and their components. In "Nucleic Acids," ed. Chargaff, E. and Davidson, J.N. p. 493. Academic Press, New York, N.Y.

Buttkus, H. 1967. The reaction of myosin with malonaldehyde. J. Food Sci. 32: 432.

Chio, K.S. and Tappel, A.L. 1969a. Synthesis and characterization of the flourescent products derived from malonaldehyde and amino acids. Biochemistry 8: 2821.

Chio, K.S. and Tappel, A.L. 1969b. Inactivation of ribonuclease and other enzymes by peroxidizing lipids and by malonaldehyde. Biochemistry 8: 2827.

Crawford, D.L., Yu, T.C. and Sinnhuber, R.O. 1967. Reaction of malonaldehyde with protein. J. Food Sci. 32: 332.

Goll, D.E., Hoekstra, W.G. and Bray, R.W. 1964. Age associated changes in bovine muscle connective tissue. 1. Rate of hydrolysis by collagenase. J. Food Sci. 29: 608.

Keskinel, A., Ayres, J.C. and Snyder, H.E. 1964. Determination of oxidative changes in raw meats by the 2-thiobarbituric acid method. Food Technol. 18: 101.

Kwon, T.W., Menzel, D.B. and Olcott, H.S. 1965. Reactivity of malonaldehyde with food constituents. J. Food Sci. 30: 808.

Kwon, T.W. and Olcott, H.S. 1966a. Thiobar-

bituric-acid-reactive substances from autoxidized or ultraviolet irradiated unsaturated fatty esters and squalene. J. Food Sci. 31: 552.

Kwon, T.W. and Olcott, H.S. 1966b. Malonaldehyde from autoxidation of methyl linolenate. Nature 210: 214.

Kwon, T.W. and Watts, B.M. 1963. Determination of malonaldehyde by ultraviolet spectrophotometry. J. Food Sci. 28: 627.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. 1951. Protein estimation with the Folin phenol reagent. J. Biol. Chem. 193: 265.

Tarladgis, B.C., Watts, B.M., Younathan, M.T. and Dugan, L.R., Jr. 1960. A distillation method for the quantitative determination of malonaldehyde in rancid foods. J. Am. Oil Chem. Soc. 37: 44.

Tarladgis, B.G., Pearson, A.M. and Dugan, L.R. Jr. 1964. Chemistry of the 2-thiobarbituric acid test for determination of oxidative rancidity in foods. 2. Formation of the TBA-malonaldehyde complex without acid-heat treatment. J. Sci. Food Ag. 9: 602.

Witte, V.C., Krause, G.F. and Bailey, M.E. 1970. A new extraction method for determining 2-thiobarbituric acid values of pork and beef during storage. J. Food Sci. 35: 582.

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EFFECT OF SODIUM NITRITE CONCENTRATION ON N-NITROSODIMETHYLAMINE FORMATION IN FRANKFURTERS

INTRODUCTION

UNTIL 1926 nitrate salts were used in the United States in the fixing of color of meat products by the "corning" or curing process. Haldane (1901) demonstrated that this function takes place through the reduction of nitrate to nitrite. As a result of the work of Kerr et al. (1926), the Department of Agriculture permitted the use of sodium nitrite in meat curing. In addition to color fixation, nitrite serves to develop the characteristic flavor of the cured meat products (Brooks et al., 1940; Cho and Bratzler, 1970) and preserve them against bacterial spoilage (Silliker et al., 1958). In general cured meat products have enjoyed a long period of safe consumption. Concern over the use of food additives as potential public health hazards has increased in recent years. With it, the use of sodium nitrite has been questioned. One of the main reasons for this is that sodium nitrite, under acidic conditions, reacts with many amines, particularly secondary amines to form N-nitrosamines which may be tumorigenic or carcinogenic (Druckery et al., 1967).

The presence in meat products of free amines or amine precursors such as proteins, amino acids, phospholipids and other compounds poses a situation in which they may possibly react with nitrite to form N-nitrosamines. There have been reports of finding a nitrosamine in cured meat products (Möhler and Mayrhofer, 1968; Ender and Ceh, 1968; Freimuth and Gläser, 1970). The methods employed may have lacked the necessary sensitivity and selectivity to give positive identification. Other workers (Fazio et al., 1971; Fiddler et al., 1971; Telling et al., 1971) have not confirmed the presence of nitrosamines in various cured meat products at a level greater than 25 ppb. However, recently the presence of dimethylnitrosamine (DMNA) in three frankfurter samples, one of which contained 80 ppb, has been demonstrated (Wasserman et al., 1972). Studies to date indicate that DMNA occurrence is random and no adequate explanation is available. This could be due to a number of variables which are involved in the preparation of cured meat products e.g., the age and condition of the meat, the concentration of nitrite and the type and amounts of other ingredients used, the actual processing conditions, and subsequent time and temperature of storage. In view of the lack of information available on the effect of these processing factors on nitrosamine formation, a study was undertaken to determine the effect of nitrite concentration on DMNA formation in frankfurters containing sugar and salt, but without nitrate, ascorbate and delta-gluconolactone. The latter three ingredients are sometimes used commercially.

EXPERIMENTAL

Frankfurter preparation

Fresh beef and pork were trimmed to desired fat levels, 5 and 17% respectively, and ground through a 3/4-in. plate. The beef, pork and pork fat were stored under vacuum in Cryovac bags at 0°F. The day prior to processing, the frozen portions were removed from the freezer and allowed to thaw. The meat was ground through a 5/8-in. plate, then a 3/16-in. plate. A formulation based on the results of analyses of raw materials was used to produce frankfurters containing 9–10% added moisture and 30% fat. The weights (kg) of the components of the emulsion were: lean beef–3.934; lean pork–2.514; pork fat–3.552; ice–2.287; salt–0.251; and sugar–0.198. Nitrate, ascorbate and delta-gluconolactone were omitted from the formulation to reduce the number of variables that may effect nitrosamine formation. The ingredients were comminuted in a high-speed Stephan Universal Schnellkuter Type USF25 chopper equipped with a thermocouple and a sealable Plexiglass plate. Under 110 mm Hg vacuum, the initial temperature of the mixture was about 35°F. The chopping was continued up to a temperature of 60°F, taking approximately 5 min. The amount of sodium nitrite required was weighed, dissolved in 15 ml distilled water, and mixed thoroughly with 1.274 kg emulsion (equivalent to 1 kg of meat) using a Hobart Model N-50 mixer, to give the following levels of sodium nitrite added in ppm with respect to meat (lean plus fat): 150, 750, 1050, 1500 and 2500.

The emulsion was then stuffed into size 23 Nojax frankfurter casings using a screw-type stainless steel sausage stuffer having a capacity of 500g. The franks were linked using a Model MF Ty Linking Machine and cooked in a Dry-Sys Smokehouse using a 90-min commercial program of increasing heat and controlled humidity: 1/2 hr dry bulb (DB) 130°F, wet bulb (WB) 0; 1/2 hr DB 150°F, WB 130°F; 1/2 hr DB 170°F, WB 150°F. A light smoke was generated in a Mepaco apparatus and introduced into the smokehouse for the entire period. After the franks had reached an internal temperature of 160°F (about 2 hr), one batch containing different levels of added sodium nitrite was removed and the remainder was heated and smoked for an additional 2 hr (DB 170°F, WB 150°F). These frankfurters had an average internal temperature of 160°F. After removal from the smokehouse, the frankfurters were immersed in an ice-water bath for 3 min, dried, weighed and stored overnight at 36°F. A sample of the franks processed normally for approximately 2 hr at the 150 ppm level was analyzed according to AOAC procedures and found to contain 53.40% moisture, 29.42% fat and 14.42% protein. The remaining franks were then vacuum packaged, frozen and kept in a freezer at 5°F until analyses were carried out.

When needed, the frankfurters containing different levels of sodium nitrite were thawed, ground, mixed and an aliquot taken for analyses. The residual nitrite was determined by the AOAC method; the average loss in sodium nitrite in the 2-hr and 4-hr processed frankfurters was 48.6% and 55.4% respectively.

Table 1—The effect of sodium nitrite concentration on dimethylnitrosamine formation in frankfurters

	Processing time			
	2 Hr		4 Hr	
$NaNO_2$ added mg/kg meat	$NaNO_2$ residual mg/kg	DMNA μg/kg[a]	$NaNO_2$ residual mg/kg	DMNA μg/kg[a]
150	67	trace	53	trace
750	361	3	310	8
1050	574	8	473	12
1500	811	10	724	14
2500	1386	19 MS	1345	19 MS

[a]Corrected using recovery of sample with 20 μg/kg added; MS-confirmed by mass spectrometry.

Analytical procedures

The methodology employed for the determination of DMNA in frankfurters is a modification of the procedure described by Howard et al. (1970). The method is as follows: the ground frankfurter samples are subjected to digestion with methanolic potassium hydroxide, followed by distillation from aqueous alkaline solution. The distillate is extracted with methylene chloride. The combined extracts are washed with base and dried over anhydrous sodium sulfate, concentrated and subjected to column chromatography using Florisil acidified with hydrochloric acid. After the column is washed with hexane, the sample is eluted with methylene chloride, concentrated and the DMNA present determined by GLC. The average recovery of DMNA in an aliquot of the same sample with 20 ppb added was 76%.

Gas-liquid chromatography

Varian Aerograph Model 1740-1 gas chromatograph equipped with two 9 ft × 1/8 in. OD stainless steel columns packed with 15% Carbowax 20M-TPA on 60 80 Gas-Chrom P, was conditioned overnight at 180°C, and installed for on-column injection. The standard flame ionization detector was modified for use as an alkali flame ionization detector by the use of a potassuim chloride coated coil as described by Howard et al. (1970). The flow conditions used were: helium 58, hydrogen 45 and air 188 ml/min. Helium flow was monitored continuously with Matheson Model LF-100 mass flowmeter. 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 190 and 250°C, respectively. Column temperature was 115° isothermal for routine analyses involving only DMNA.

GLC-mass spectrometric analysis

The analytical procedure described above, which used 25g of frankfurter, was repeated as needed to provide sufficient DMNA for confirmation of identity by mass spectrometry. An identical gas-liquid chromatograph equipped with a flame ionization detector and column was used as for the determination of DMNA. A column temperature of 115°C was used with injector port and detector temperatures of 200° and 230°, respectively. Helium, at a flow rate of 25 ml/min, was used as the carrier gas. The hydrogen and air flow rates were 40 and 335 ml/min., respectively.

The column effluent was split approximately 1:1 and passed into a DuPont Model 21-492 mass spectrometer equipped with a jet-type separator via an inlet line heated at 200°C. The mass spectra were obtained at an ionizing voltage of 70 ev and an ion source temperature of 200°C using a CEC model 5-124A recording oscillograph with a linear scan rate of 20 sec/decade.

RESULTS & DISCUSSION

THE PROCESSING study was performed three times under the same 2- and 4-hr processing conditions covering a wide range of sodium nitrite concentrations added to frankfurter emulsion. Analyses of the frankfurters for DMNA yielded similar values for all of the studies. Representative data from one of these experiments is shown in Table 1.

Ideally the identification of DMNA in natural products should be unambiguous. Where very small amounts of DMNA are encountered, confirmation may be accomplished by mass spectrometry. In the present study, however, DMNA concentrations of less than 10 μg/kg in frankfurter samples prepared as described were insufficient for confirmation. Treating larger amounts of frankfurter and/or concentrating multiple extracts to obtain sufficient material resulted in the presence of artifacts which interfered with mass spectral interpretation. Therefore, a minimum level of DMNA 10 μg/kg was considered significant. When DMNA was not characterized by mass spectrometry, identity was based only on its GLC retention time. In the discussion of the results, the term "apparent" DMNA is used to indicate levels at which mass spectral confirmation could not be made.

The U.S. Code of Federal Regulations (1971) limits the quantity of sodium nitrite that may be added to comminuted meat products, including frankfurters, to 156 mg/kg (1/4 oz per 100 lb chopped meat). At levels of up to about five times this permissible concentration no significant amount of DMNA was observed when the normal 2-hr processing procedure was used; however, a DMNA concentration bordering on significance, i.e., 8 μg/kg meat, was obtained on heating an additional 2 hr. Concentrations of apparent or confirmed DMNA of 10 μg/kg and greater were found in frankfurters made with levels of sodium nitrite of 1500 mg/kg or higher, regardless of processing time. In addition to 2500 mg sodium nitrite per kg meat, levels of 3075 and 4250 mg/kg were also studied in another series of the same experiment, and concentrations of DMNA up to 60 μg/kg were found for the 4-hr processed frankfurters and confirmed readily by mass spectrometry. Sodium nitrite concentrations between 150 and 750 ppm sodium nitrite, for both the 2- and 4-hr processing times, gave negligible amounts of DMNA.

For most of the levels of added nitrite at which apparent or confirmed DMNA could be demonstrated, there was an increase in DMNA concentration when the frankfurters were treated an additional 2 hr. The extra heating period is not normally used commercially; however, its use here represents an extreme in experimental conditions.

Residual nitrite analyses showed that about one-half of the sodium nitrite added was still present after processing. Commercially prepared frankfurters made with 156 mg/kg sodium nitrite usually have residual nitrite levels ranging from 10–30% of the added material. In previous studies, we have observed that more nitrite is lost when processing in combination with ascorbate and nitrate, and the concentration of nitrite continues to decrease upon storage. Therefore the significance of the higher values found in this study cannot be evaluated at this time.

CONCLUSIONS

FRANKFURTERS conforming to U.S. Federal Regulations contain a concentration of sodium nitrite insufficient to produce, under the processing conditions described in this paper, amounts of DMNA measurable by our procedures. However, under laboratory conditions DMNA can be formed in a meat product. Concentrations of sodium nitrite up to about ten times the legal limit may lead to significant nitrosamine formation. This study does not explain why DMNA can be isolated from some commercial cured meat products containing the permissible level of sodium nitrite. Many variables have been suggested or can be conceived as contributing to DMNA formation in the products, e.g., localized high concentrations of nitrite in emulsions due to inadequate homogenization during processing, the effect of other cure ingredients, storage conditions, etc. Further work is needed to establish the contributions, if any, of these variables.

REFERENCES

Brooks, J., Haines, R.B., Moran, T. and Pace, J. 1940. The function of nitrate, nitrite and bacteria in the curing of bacon and hams. Dept. Sci. & Indust. Res., Food Invest. Spec. Rept. No. 49, (Great Britain).

Cho, I.C. and Bratzler, L.J. 1970. Effect of sodium nitrite on flavor of cured pork. J. Food Sci. 35: 668.

Code of Federal Regulations. 1971. Animal and Animal Products, Chapter III Section 318.7, Rev Jan. 1, Title 9.

Druckrey, H., Preussmann, R., Ivankovic, S., Schmal, D., Afkham, J., Blum, G., Memmel, H.D., Muller, M., Petropoules, P. and Schneider, H. 1967. Organotropic carcinogenic effects of 65 different N-nitroso compounds on BD rats. Z. Krebsforsch. 69: 103.

Ender, F. and Ceh, L. 1968. Occurrence of nitrosamines in foodstuffs for human and animal consumption. Food Cosmet. Toxicol. 6: 569.

Fazio, T., White, R.H. and Howard, J.W. 1971. Analysis of nitrite- and/or nitrate-processed meats for N-nitrosodimethylamine. J. Assoc. Offic. Anal. Chem. 54: 1157.

Fazio, T., Damico, J.N., Howard, J.W., White, R.H. and Watts, J.O. 1971. Gas chromatographic determination and mass spectrometric confirmation of N-nitrosodimethylamine in smoke-processed marine fish. J. Agr. Food Chem. 19: 250.

Fiddler, W., Doerr, R.C., Ertel, J.R. and Wasserman, A.E. 1971. Gas-liquid chromatographic determination of N-nitrosodimethylamine in ham. J. Assoc. Offic. Anal. Chem. 54: 1160.

Freimuth, U. and Gläser, E. 1970. The occurrence of nitrosamines in foods. Die Nahrung 14: 357.

Haldane, J. 1901. The red colour of salted meat. J. Hyg. 1: 115.

Howard, J.W., Fazio, T. and Watts, J.O. 1970. Extraction and gas chromatographic determination of N-nitrosodimethylamine in smoked fish. Application to smoked nitrite-treated chub. J. Assoc. Offic. Anal. Chem. 53: 269.

Kerr, R.H., Marsh, C.T.N., Schroeder, W.F. and Boyer, E.A. 1926. The use of sodium nitrite in the curing of meat. J. Agr. Res. 33: 541.

Möhler, K. and Mayrhofer, O.L. 1968. Detec-

tion and determination of nitrosamines in foodstuffs. Z. Lebensm.-Unters. Forsch. 135: 313.

Silliker, J.H., Greenberg, R.A. and Schack, W.R. 1958. Effect of individual curing ingredients on the shelf-stability of canned comminuted meats. Food Technol. 12: 551.

Telling, G.M., Bryce, T.A. and Althorpe, J. 1971. Use of vacuum distillation and gas chromatography-mass spectrometry for determination of low levels of volatile nitrosamines in meat products. J. Agr. Food Chem. 19: 937.

Wasserman, A.E., Fiddler, W. and Kushnir, I. 1972. Unpublished results.

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Reference to brand names or firm name does not constitute endorsement by the U.S. Dept. of Agric. over others of a similar nature not mentioned.

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FLAVOR SENSITIVITY OF SELECTED ANIMAL PROTEIN FOODS TO GAMMA RADIATION

INTRODUCTION

BECAUSE SUBJECTIVE evaluation methods are usually used, various terms have been used to describe the human response to the irradiation odor and flavor, such as burnt, metallic, bitter, cured meat, reminiscent of cress, cheesy, goaty, wet dog, wet grain, acrylic or unappetizing (Huber and Brasch, 1953; Mehrlich, 1966; Batzer et al., 1959).

Merritt et al., (1967) concluded that the irradiation odor in raw meat is a characteristic property, is the same for beef, pork, lamb and other meats, and varies only in intensity among the different meats.

Reports by Hannan and Thornley (1957), Huber and Brasch (1953) and Coleby (1959) reveal that beef is the most sensitive to irradiation, followed by lamb, veal, chicken and pork. Some threshold doses of certain meats have been determined: 100 Krad for beef, 250 Krad for lean pork and chicken (Hannan and Thornley, 1957; Coleby, 1959; and Thornley, 1957) and 500 Krad for trout (Graikoski et al., 1957).

The objectives of this study were to determine the relative sensitivities to ^{60}Co radiation of raw protein foods derived from animals of different biological classification and to determine the threshold doses of foods studied.

MATERIALS & METHODS

THE ANIMAL PROTEIN foods were selected to cover animal species as varied as possible, but still keeping in mind the possibility of the tasters' acceptance as foods. The animals selected can be placed into the phylla of Chordata (subphyllum Vertebrate) and Arthropoda. They were beef, swine, lamb, deer, hippopotamus, rabbit, horse, beaver, whale, bear, elephant and opossum (mammals), chicken, turkey (birds), turtle (reptile), frog (amphibian), halibut and trout (fishes), and shrimp and lobster (crustaceans). The foods were supplied by Czimer Foods, Inc. in Chicago or by Michigan State University food stores in East Lansing. All the samples were kept frozen until shortly before use. The histories of the foods prior to receipt were unknown.

Preparation of samples

Samples in the frozen state were cut into steaks (except shrimp, lobster and frog leg) about 1 in. thick and about 0.5–0.75 lb for each cut. Each portion was packed and vacuum sealed in a gas-impermeable pouch consisting of Mylar polyester base with a thin coat of polyvinylidine chloride (Saran) applied to the outer surface of a heavier coat of polyethylene as a sealant, supplied by International Kenfield.

Irradiation process

The source of radiation used was the ^{60}Co irradiator in the Food Science Building at Michigan State University, East Lansing. The dose rate in the center well was about two Mrad/hr at the height of 15.2 cm. The temperature in the irradiation chamber was kept at 40–50°F. The sample temperature prior to irradiation was about the same; after removal from the source it was about 60°F.

The raw samples were irradiated in the previously described plastic bags. One sample of each food was irradiated at each of the following doses: 10, 50, 100, 500, 1000, 2000, 3000, 4000 and 5000 Krad (±10%). Two nonirradiated samples were used as controls. The irradiated samples were served as soon as possible or kept at 40°F for not more than 2 days.

Cooking method

Samples were cooked in the plastic bags in boiling water for 30 min. This method assured a well-done degree of cook, no burning and dehydration, and also minimum leaching by the cook water. The cooked samples were cut into pieces suitable for the panel (ca. 10g), and were kept warm over warm water during the time of sample presentation (as long as 30 min).

Taste testing method

Just prior to the actual taste test, the panel members were given two samples, one not irradiated and one irradiated at either 500 or 3000 Krad. The flavor intensity score value for the irradiated sample was arrived at through agreement among the panel members. Then the other samples were presented in individual booths in the panel room, disguised under coded numbers and concealed from color difference as much as possible by using blue-colored fluorscent light. The samples were divided evenly number-wise for two sessions. The first session employed samples with doses of 50,500, 2000 and 4000 Krad with one nonirradiated control. The second session had doses of 10, 100, 1000, 3000 and 5000 Krad with one nonirradiated control. The irradiated flavor intensity was recorded by score numbers of one to five on score sheets. The word description of the score numbers is as follows:

1 = No irradiation flavor
2 = Slight irradiation flavor
3 = Moderate irradiation flavor
4 = Strong irradiation flavor
5 = Very strong irradiation flavor

Statistical method

The Analysis of Variance of Split-plot design and the Duncan's new multiple test were used (Steel and Torrie, 1960).

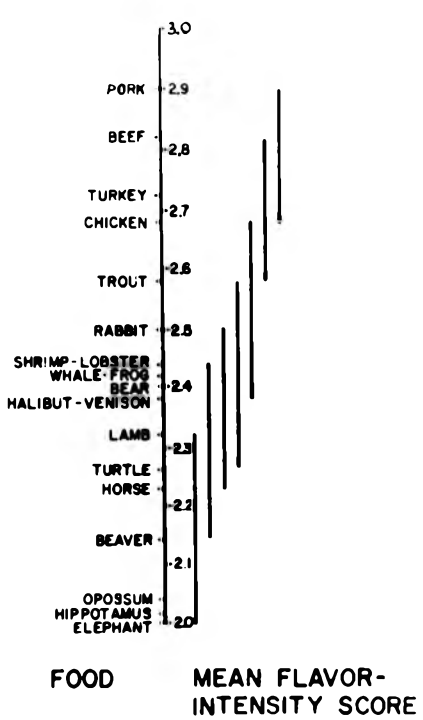

Fig. 1—Mean scores for each food for all radiation doses employed. Means joined by same line are not significantly different from each other.

RESULTS & DISCUSSION

A SIGNIFICANT DIFFERENCE of flavor intensity among the protein foods studied was observed. In Figure 1 the means of scores for each food for all radiation doses employed are shown.

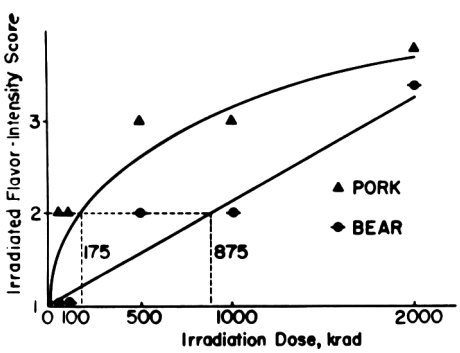

Fig. 2—Determination of threshold dose for pork and bear meat.

Table 1—The threshold dose for each animal protein food investigated, determined at flavor intensity score of 2.0 (slight irradiation flavor)

Animal food	Threshold dose Krad
Turkey	150
Pork	175
Beef	250
Chicken	250
Lobster	250
Shrimp	250
Rabbit	350
Frog	400
Whale	400
Trout	450
Turtle	450
Halibut	500
Opossum	500
Hippopotamus	525
Beaver	550
Lamb	625
Venison	625
Elephant	650
Horse	650
Bear	875

Those which are not covered by the same vertical line are significantly different in irradiation flavor intensity; means covered by the same line are not significantly different.

Figure 1 indicates there is no evidence of a relationship between flavor sensitivity to irradiation of a food and the biological classification of animal from which it is derived. It appears that foods derived from the more intensively domesticated animals (swine, beef, turkey and chicken) are among the most sensitive to irradiation. All sea foods and amphibious foods fall in the middle of the flavor intensity scale as one group. The foods least sensitive to irradiation are those derived from the less domesticated or unusual food animals.

Two possible explanations for these findings are: (1) The mode of living, environmental condition, physical activities, the variety of feeds or maybe the psychological condition (stress) of the animal may have some effects on the physical or chemical nature of the derived foods which will contribute to the development of irradiated flavor; and (2) It is possible that lack of familiarity of the panel members with certain of the animal foods may have affected their judgments of the irradiated flavor. However, there is no evidence that either of these explanations is correct.

Determination of threshold dose

By plotting each food flavor intensity score vs. dose, and noting the dose corresponding to a 2.0 score (which corresponds to "slight irradiation flavor"), a dose value was assigned as the threshold dose. This is illustrated in Figure 2 for pork and bear meat representing low and high threshold dose values.

The threshold levels so determined for all protein foods investigated are listed in Table 1. As expected, the threshold dose is higher for the less sensitive animal foods. The order of sensitivity to radiation based on the threshold dose value is somewhat different form that determined by the mean score for all doses employed (Fig. 1). This difference is probably due to the nonlinear relationship between dose and flavor score. The figure of 250 Krad for beef is higher than 100 Krad of Hannan and Thornley (1957). This difference may be due to the variation of sample score which corresponds to the threshold dose or to cooking method. Threshold doses of 250 Krad for lean pork (Hannan and Thornley, 1957), 250 Krad for chicken (Coleby, 1959; Thornley, 1957), 500 Krad for trout (Graikoski et al., 1967) are very closely related to the figures given in Table 1.

REFERENCES

Batzer, O.F., Sliwinski, R.A., Chang, L., Pih, K., Fox, J.B. Jr., Doty, D.M., Pearson, A.M. and Spooner, M.E. 1959. Some factors influencing radiation induced chemical changes in raw beef. Food Technol. 9: 501.

Coleby, B. 1959. The effect of irradiation on the quality of meat and poultry. Intl. J. of Appl. Radiation & Isopes 6: 115.

Graikoski, J.T., Kazanas, N., Watz, J., DuCharme, S., Emerson, J.A. and Seagran, H.L. 1967. Irradiation preservation of freshwater fish. Bureau of Comm. Fisheries, Ann Arbor.

Hannan, R.S. and Thornley, M.J. 1957. Radiation processing of foods. A commentary on present research. Food Mfg. 12: 559.

Huber, W. and Brasch, A. 1953 Effect of processing conditions on organoleptic changes in foodstuffs sterilized with high intensity electrons. Food Technol. 3: 109.

Mehrlich, F.P. 1966. The United States Army food irradiation programme. Proceedings of Symposium in Karlsruhe, p. 695. International Atomic Energy Agency, Vienna.

Merritt, C. Jr., Angelini, P. and McAdoo, D.J. 1967. Volatile compounds induced by irradiation in basic food substances. In "Radiation Preservation of Foods." Advances in Chemistry Series 65, American Chemical Society, Washington, D.C.

Steel, R.G.D. and Torrie, J.H. 1960. "Principles and Procedures of Statistics." McGraw-Hill Book Co., New York.

Thornley, M.J. 1957. Preservation of chicken meat: Observation on the use of cathode and gamma rays. International Institute of Refrigeration.

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DEVELOPMENT OF A MATHEMATICAL MODEL FOR OXIDATION OF POTATO CHIPS AS A FUNCTION OF OXYGEN PRESSURE, EXTENT OF OXIDATION AND EQUILIBRIUM RELATIVE HUMIDITY

INTRODUCTION

OPTIMAL DESIGN of protective packaging requires knowledge of factors affecting rates of food deterioration and of the interrelationships between these factors. We have investigated the deterioration of potato chips as a representative food undergoing oxidative deterioration. In a previous paper (Quast and Karel, 1972) we presented results showing the dependence of oxidation rates of potato chips on oxygen pressure, equilibrium relative humidity and extent of oxidation. These results were presented as plots of the dependent variable (oxidation rate) versus the three independent variables.

An alternative approach to storage behavior prediction is the development of a mathematical model to fit all of the experimental data on oxidation rate as a function of the three important variables: oxygen pressure, extent of oxidation and equilibrium relative humidity. Such modeling is difficult because: (1) The complexities of the system necessitate extensive experimental data to be collected and fitted; (2) The system at the boundaries of the region of interest often shows large deviations from behavior predicted on the basis of more typical portions of the region; (3) Variability of the results requires replicate measurements. All these facets of the problem dictate that statistical techniques be used and that the model be based, as much as possible, on information available from theoretical analysis of oxidation kinetics.

The general procedure in this paper is that of reducing the data to a representative set, and building the model by a combination of simple analytical models suggested by the previous work and of various pair-wise relations, by least squares fitting to the reduced data set, and comparing the relative error sums of squares. Once the general form of the complete model is determined, the full data set is used to determine, by least squares fitting, its final form and the specific values of the constants involved.

The use of mathematical models was applied by Simon (1969) and Karel and Labuza (1969) to several deteriorative reactions of foods. More recently Mizrahi et al. (1970a, b) developed an empirical model for the browning rate of dehydrated cabbage as a function of moisture content.

We describe here a mathematical model which we developed for potato chips, but we hope that the form of the equation is general and applicable to similar food products with appropriate constants evaluated for each.

The approach reported here had as its main purpose a simple and fairly general equation which can be used for package design and simulation, but it may also help to give additional insight into the kinetics of food product oxidation.

EXPERIMENTAL

THE RATES OF oxygen uptake (RATE) of potato chips at 37°C were determined in triplicate at the following values of the independent variables:

- Oxygen partial pressure (PO2): 0.0055, 0.035, 0.072, 0.122, 0.21 atm;
- Equilibrium relative humidity (RH): 0.1, 11.0, 20.0, 32.0, 40.0;
- Extent of oxidation (EXT): at 5 to 15 different extents (μl O_2 STP/g), depending on the RH.

The technique for obtaining these results has been described by Quast and Karel (1972). In all cases the samples entered a rapid oxidation period after an extent of oxidation of 1200 μl O_2 STP/g was reached which was the point above which the product became unacceptably rancid. Therefore this extent was defined as the critical extent and the model was not applied beyond this value. We also assumed that the rate of oxidation was zero at PO2 = 0.0. In this manner a total of 843 experimental rates were available for building a mathematical model.

RESULTS & DISCUSSION

Development of a reduced set of experimental results

A larger number of results was available initially and at high RH since the rates were lowest under these conditions due to the nature of the experimental procedure. Also, when the experimental rates were plotted against extent at each of the combinations of the other independent variables (PO2 and RH), we found that the triplicate results agreed fairly well. Some of these plots are shown in a previous publication (Quast and Karel, 1972). Since the dependent variable (RATE) was available at irregular and variable intervals of the independent variable (EXT) and since the average number of intervals for this variable was 11, we obtained a reduced but representative set of experimental results at five equally spaced intervals of the variable (EXT). This was done by interpolating RATE from the RATE versus EXT plots of all the 843 experimental values. The rates were read at these extents: 0.0, 300.0, 600.0, 900.0 and 1200.0. This procedure reduced the number of experimental results and therefore made the computer calculations faster and cheaper. As one computer card contained each set of experimental results consisting of a combination of the independent variables and the corresponding value of the dependent variable (RATE), we used a total of 150 cards instead of 843 for preliminary investigations. The reduced set of 150 cases is shown in Table 1 and was used for crossplotting RATE versus the other independent variables (Quast and Karel, 1972) and for preliminary least squares fitting of several models.

Criteria for model building

The criteria and approaches for model building have been discussed by Box and Hunter (1962) and Himmelblau (1970). In this paper we decided on the form of the model according to these criteria:

a. Close agreement with the models derived from postulated reaction mechanisms;
b. Simplicity of the model (usually as few constants as possible);
c. Low error mean square between the experimental values and those predicted by the model (s^2);
d. The model should have no discontinuities in the range of practical values of the independent variables;
e. The model should satisfy conditions at the extrema of some of the independent variables;
f. The deviations of the predicted values from the experimental values should be reasonably uniform over the whole range of values of the independent variables;
g. The model should be simple from a computational point of view; for instance, a linear model is simpler than a nonlinear model.

Even with these criteria, hundreds of different models of three independent

variables are possible, the advantages and limitations of which are not obvious and depend largely on their ultimate use.

Computations

The forms of the models were based on the lipid oxidation kinetics, on previous experience and on different cross-plots and linearizations. Starting with approximate values of the constants, least squares fitting was used to estimate the best values of these constants with the aid of several programs on the IBM S/360 computer of the M.I.T. Information Processing Center. The results for different models were compared and some models eliminated by criteria discussed previously, and promising models studied for further refinements or simplifications.

Two basic types of least squares fitting were used. For linear models, stepwise linear regression programs were employed. For nonlinear models, we used nonlinear least squares fitting (Hartley, 1961; Jennrich and Sampson, 1968) embodied in the BMD-X85 program (Dixon, 1969). The nonlinear models in general require more computation time and frequently do not converge. Approximate initial values of the constants must be obtained from plots. However, such models often give significantly better fits and may also be required from theoretical considerations of the kinetics.

Models of one independent variable

First we considered models in which the dependent variable (RATE) was studied as a function of only one of the independent variables at a time, at each of the different combinations of the remaining independent variables. These relatively simple models were chosen based on theoretical considerations and on the shape of the different plots. Only the reduced set of 150 cases was used in these calculations.

Considerations based on the free radical mechanism of lipid oxidation led to this equation (Karel, 1960; Lundberg, 1961):

$$\text{RATE} = \frac{\text{PO2}}{\text{P1} + \text{P2} \cdot \text{PO2}} \qquad (1)$$

where P1, P2 are constants.

Observation of our experimental results (by plotting P1/PO2 versus 1/RATE) showed excellent agreement with Eq. (1), with an average mean square error (s^2) of 0.010. This equation also has the important extremum property that the RATE is zero in the absence of oxygen (PO2 = 0.0) which is a physical requirement.

Inspection of plots of the experimental results immediately suggests a linear relationship between RATE and EXT, especially during the initial stages. At higher values of EXT the RATE versus EXT plots show increasing slopes which suggest a polynomial. We first tried the model

$$\text{RATE} = \text{P1} + \text{P2} \cdot \text{EXT} \qquad (2)$$

where P1 and P2 are constants. We also tried a second degree polynomial. The value of s^2 was 0.014 for the first degree and 0.002 for the second degree polynomial (Quast, 1972). Addition of the term EXT^2 will result in improved accuracy if a more complicated model can be tolerated. Kinetic considerations also justify relations involving $\text{EXT}^{1/2}$ if initiation occurred by monomolecular decomposition of hydroperoxides. However, this model gave a poorer fit than Eq. (2).

There is no simple kinetic model based on theory of lipid oxidation to explain the effect of moisture content and RH on the rate of oxidation. Hydrogen bonding of hydroperoxides with water and metal catalyst inactivation by the water are some of the important mechanisms (Maloney et al., 1966). The relative intensity of these factors and the effects of other mechanisms are quite variable from product to product. However, the general shape of the RATE versus RH plot is quite typical (Quast and Karel, 1972). Usually the RATE decreases very rapidly when RH is low and slower in the range of 10–40% RH. At higher RH, the rate may increase again (Heidelbaugh et al., 1971).

We tested first and second degree polynomials in RH as possible models. The overall average s^2's were 0.10 and 0.053, respectively, which is quite poor.

We tested the model

$$\text{RATE} = \text{P1} + \frac{\text{P2}}{\text{RH}^{1/2}} \qquad (3)$$

and obtained a value of s^2 equal to 0.016. We also tested Eq. (3) with the term RH in the place of $\text{RH}^{1/2}$. The error mean square was higher. Later work with models of two independent variables confirmed that models containing the term $1/\text{RH}^{1/2}$ gave lower s^2 than those containing 1/RH.

We considered Eq. (3) a more useful model for the present study than those derived on the basis of kinetics. It must be noted here that Eq. (3) does have limitations at the extremum of RH = 0.0 where RATE would become infinite. Clearly, this equation cannot be used close to this value of the independent

Table 1—Reduced set of 150 cases

PO2 (atm)	EXT μlO_2 STP / g	RATE $\left\{\frac{\mu lO_2\ STP}{g \cdot hr}\right\}$ RH = 0.1	RH = 11.0	RH = 20.0	RH = 32.0	RH = 40.0
0	0	0	0	0	0	0
	300	0	0	0	0	0
	600	0	0	0	0	0
	900	0	0	0	0	0
	1200	0	0	0	0	0
0.0055	0	0.56	0.10	0.15	0.08	0.10
	300	0.60	0.30	0.30	0.30	0.24
	600	0.62	0.44	0.44	0.50	0.40
	900	0.65	0.60	0.58	0.72	0.56
	1200	0.70	0.72	0.72	0.90	0.70
0.035	0	1.10	0.20	0.16	0.08	0.10
	300	1.10	0.40	0.42	0.34	0.28
	600	1.10	0.56	0.70	0.58	0.48
	900	1.10	0.72	0.98	0.84	0.72
	1200	1.10	1.00	1.24	1.15	0.94
0.072	0	1.40	0.20	0.10	0.10	0.10
	300	1.36	0.40	0.48	0.38	0.30
	600	1.32	0.62	0.86	0.64	0.50
	900	1.28	0.84	1.24	0.95	0.78
	1200	1.30	1.20	1.64	1.30	1.12
0.122	0	1.90	0.20	0.16	0.10	0.12
	300	1.56	0.44	0.48	0.44	0.32
	600	1.32	0.64	0.84	0.80	0.52
	900	1.32	0.95	1.20	1.15	0.80
	1200	1.70	1.70	1.64	1.50	1.20
0.21	0	2.70	0.26	0.16	0.15	0.16
	300	1.90	0.48	0.50	0.50	0.34
	600	1.50	0.64	0.88	0.85	0.58
	900	1.48	0.84	1.20	1.20	0.84
	1200	2.00	1.54	1.72	1.60	1.30

Table 2–Models of two independent variables

Model (RATE =)	Average s^2	Equation no.
$P1 + P2 \cdot EXT + P3 \cdot RH + P4 \cdot EXT \cdot RH$	≃0.080	(4)
$P1 + P2 \cdot EXT + \frac{P3}{RH^{1/2}} + \frac{P4 \cdot EXT}{RH^{1/2}}$	0.021	(5)
$\frac{EXT \cdot PO2}{(P1 + P2 \cdot PO2)}$	0.153	(6)
$\frac{(P3 + EXT) \cdot PO2}{(P1 + P2 \cdot PO2)}$	0.029	(7)
$\frac{(P3 + EXT + P4 \cdot EXT^2) \cdot PO2}{(P1 + P2 \cdot PO2)}$	0.013	(8)
$\frac{PO2}{(P3 + P4 \cdot PO2)} + \frac{EXT \cdot PO2}{(P1 + P2 \cdot PO2)}$	0.019	(9)
$\frac{(EXT)^{P3} \cdot PO2}{(P1 + P2 \cdot PO2)}$	0.137	(10)
$P1 \cdot PO2^{P2} \cdot (P3 \cdot P4^{PO2})^{RH}$	0.020	(11)

variable. From a practical point of view RH = 0.0 is unattainable and is of no importance for food products. The relative errors in establishing and determining very low values of RH are very large. In our work, the lowest value of RH was 0.1 and a small error in RH in this range can result in a large error in RATE. Consequently, Eq. (3) and others developed later hold only for $0.1 < RH < 40$. The maximum value of RH = 40 was chosen because above this value the product was unacceptable due to excessive moisture content at any extent of oxidation (Quast and Karel, 1972).

Models of two independent variables

Based partially on the knowledge gained from the development of models of one independent variable, we proceeded to models with two independent variables. The use of products of single-variable models has been discussed by Wedekind (1971).

The models tested, together with the average s^2 over the different values of the independent variable not included in the model are summarized in Table 2. Model (4) is the product of first degree polynomials in EXT and RH. It gave the high s^2 that could be expected from the poor fit of the first degree polynomial in RH, since the products of good one-variable models are in general more likely to result in good two-variable models than the products of poor one-variable models. Models (6) through (11) are products of the different one-variable models. Eq. (10) gave slightly better results than Eq. (6). The exponent P3 in model (10) increased with RH from 0.5 at RH = 0.1 to 0.9 at RH = 11 and 1.0 at RH = 40. However, the introduction of a term independent of EXT is necessary to decrease s^2 drastically. Model (8) is better than model (5) from the point of view of low error mean square, with the same number of constants. However, the equations contain different independent variables. Subsequent incorporation of the last independent variable in Eq. (5) is easy while the introduction of RH into Eq. (8) is difficult since no good relations could be found to represent the constants as a function of RH. The large increase in s^2 when RH is introduced into Eq. (8) requires a number of additional constants making the resultant model cumbersome and probably less general.

Since Eq. (5) contains the essence of the kinetics for the independent variable EXT and since it contains RH in a form similar to Eq. (3), it will be discussed in more detail. This equation is actually linear in parameters, and therefore can be easily fitted by stepwise linear regression. We obtained Eq. (5) by generating 19

Table 3–Models of three independent variables

Model (RATE =)	s^2	Equation No.
$P1 + P2 \cdot PO2 \cdot EXT + \frac{P3 \cdot PO2}{RH^{1/2}} + P4 \cdot EXT \cdot PO2 + \frac{P5 \cdot PO2 \cdot EXT}{RH^{1/2}} + P6 \cdot EXT^2 + \frac{P7 \cdot PO2 \cdot EXT^2}{RH^{1/2}}$	0.046	(12)
$\left\{P1 + EXT + \frac{P2}{RH^{1/2}} + \frac{P3 \cdot EXT}{RH^{1/2}}\right\} \cdot \frac{PO2}{P4 + P5 \cdot PO2}$	0.026	(13)
$\left\{P1 + EXT + \frac{P2}{RH^{P3}} + \frac{P4 \cdot EXT}{RH^{P5}}\right\} \cdot \frac{PO2}{P6 + P7 \cdot PO2}$	0.026	(14)
$\frac{PO2}{(P1 + P2 \cdot PO2)} + \frac{PO2 \cdot EXT}{(P3 + P4 \cdot PO2)} + \frac{PO2}{RH^{1/2}(P5 + P6 \cdot PO2)} + \frac{PO2 \cdot EXT}{RH^{1/2}(P7 + P8 \cdot PO2)}$	0.018	(15)
$\frac{(EXT + P7 + P8 \cdot P9^{RH}) \cdot PO2}{P1 + P2 \cdot P3^{RH} + (P4 + P5 \cdot RH + P6 \cdot RH^2) \cdot PO2}$	0.027	(16)

different functions of EXT and RH, including EXT, RH, $EXT^{1/2}$, EXT^2, 1/RH, $1/RH^{1/2}$, $1/RH^2$ and various products thereof. Next we applied stepwise linear regression of RATE versus these functions at each of the values of PO2 and found the terms that could explain most of the variation (Quast, 1972). Eq. (5) was thus obtained and was able to explain between 90% (at PO2 = 0.21) and 96% (at PO2 = 0.0055) of the variation. $EXT/RH^{1/2}$ is an interaction term which incorporates into the model the fact that at high EXT the RATE becomes essentially independent of RH.

Models of three independent variables

The simplest empirical models are polynomials and, for several independent variables, products of polynomials. As a term of comparison, we investigated a linear model containing the terms PO2, EXT, RH, their squares and all products of these. We then obtained an equation by stepwise multiple linear regression. Even a 9-constant model gave the unacceptably high value of $s^2 \cong 0.5$. Next we generated 23 different terms consisting of different functions of the independent variables and products thereof. The 7-constant model thus obtained is Eq. (12) in Table 3. Almost no reduction in s^2 could be obtained by addition of other terms. Aside from poor fit, the linear model does not satisfy the important extremum condition of RATE = 0.0 at PO2 = 0.0.

Observation of the RATE versus PO2 plots showed that the curves have very similar shapes at all EXT and RH. It appeared that multiplication of Eq. (1) by a factor which is a function of EXT and RH could represent well the experimental results. We chose Eq. (5) as such a factor to obtain Eq. (13). Note that upon multiplication one of the constants becomes redundant. This 5-constant nonlinear model was a very significant improvement over the 7-constant linear model (12). Next we investigated whether the addition of two constants in the form of exponents of RH could improve the model. Although P3 and P5 in Eq. (14) both changed, this did not bring about a significant reduction in s^2.

Finally, we made the constants of Eq. (1) independent for each of the terms of Eq. (5), thus obtaining Eq. (15) which has eight constants and a lower s^2 than the 5-constant model (13). There is no clearcut criterion for determining which model is better. Certainly model (13) is simpler and probably more general while model (15) represents more closely the particular set of experimental results under consideration.

While investigating alternative methods for obtaining a useful model of three independent variables we obtained the 9-constant model (16) of Table 3. In this case we attempted to correlate the values of the constants of Eq. (7) with RH. This was no easy task and resulted in a complex model with higher s^2 than Eq. (13).

Table 4–Values of the constants for the model (13):

$$RATE = \left\{P1 + EXT + \frac{P2}{RH^{1/2}} + \frac{P3 \cdot EXT}{RH^{1/2}}\right\} \cdot \frac{PO2}{(P4 + P5 \cdot PO2)}$$

Experiment	Values of the constants[a] P1	P2	P3	P4	P5	No. of cases	s^2
Reduced set of 150 cases	5.8 (30.5)	463.6 (21.1)	−0.378 (0.02)	7.02 (0.86)	817.1 (36.1)	150	0.026
Experiment 1	0.000 (0.000)	508.1 (26.6)	−0.428 (0.03)	6.71 (0.89)	843.1 (24.3)	286	0.027
Experiment 2	0.000 (0.000)	437.8 (23.8)	−0.350 (0.03)	6.82 (0.88)	807.7 (22.7)	283	0.026
Experiment 3	0.000 (0.000)	411.9 (21.9)	−0.402 (0.03)	5.16 (0.69)	829.5 (21.9)	274	0.024

[a]The number in parentheses under the constant value is the standard deviation of constant.

Table 5–Values of the constants of model (17):

$$RATE = \left\{EXT + \frac{P1}{RH^{1/2}} + \frac{P2 \cdot EXT}{RH^{1/2}}\right\} \cdot \frac{PO2}{(P3 + P4 \cdot PO2)}$$

calculated by least squares fitting of 843 experimental cases

Constant	Value	Standard deviation
P1	445.24	14.05
P2	−0.39111	0.01889
P3	6.1924	0.4838
P4	818.36	13.30

$s^2 = 0.027$ $s = 0.16$

Application of Eq. (13) to all the experimental results

We recall that the modeling described so far was all done on the reduced set of 150 cases to facilitate computations. We have assumed that these "smoothed experimental results" would represent well the whole set of triplicate experimental results. We therefore applied Eq. (13) to each of the individual experimental sets. The results are summarized in Table 4. On the basis of the similarity of the error mean squares, the values of the constants and their standard deviations, we concluded that the reduced set of 150 cases represented well each of the individual experiments of the triplicate. There is one small difference which results in further simplification of Eq. (13). Even for the reduced set, the value of P1 is very small compared with the other terms and with its standard deviation. With the actual experimental results P1 becomes zero and can therefore be eliminated. Eq. (17) of Table 5 results. Taking all the triplicate experimental results together, the best estimate of the constants of Eq. (17) are shown in Table 5 together with their standard deviations.

From the triplicate experimental results, the mean square due to pure error (s_e^2) could also be calculated. For this the values of the dependent variable at the same values of the independent variables were compared. Unfortunately, the RATE was not available at exactly the same values of EXT, due to the nature of the experiment. Thus the value of s_e^2 = 0.020 which was obtained is actually slightly larger than that due to pure error. This value is similar to $s^2 = 0.027$ between the model (17) and the experimental results.

Finally we applied Eq. (17) to a duplicate set of experimental results which were obtained previously from a completely different sample of potato chips at the same temperature. This experiment did not include RH = 20. The constants were quite different from those shown in Table 5 but the model represented the experimental results satisfactorily with $s^2 = 0.030$.

Possibility of predictions with a small number of experimental results

In order to provide for practical application of the model, it is necessary to

Table 6–Values of the constants of model (17):

$$RATE = \left\{EXT + \frac{P1}{RH^{1/2}} + \frac{P2 \cdot EXT}{RH^{1/2}}\right\} \cdot \frac{PO2}{(P3 + P4 \cdot PO2)}$$

estimated from 27 experimental results at PO2: 0.0055, 0.035, 0.21, RH: 0.1, 11., 40., EXT: 0., 600., 1200.

	Experiment 1		Experiment 2	
Constant	Value of constant	Standard deviation	Value of constant	Standard deviation
P1	408.	52.	413.	54.
P2	0.343	0.06	0.285	0.07
P3	10.7	2.3	7.9	1.9
P4	680.	57.	899.	76.
	$s^2 = 0.040$		$s^2 = 0.030$	

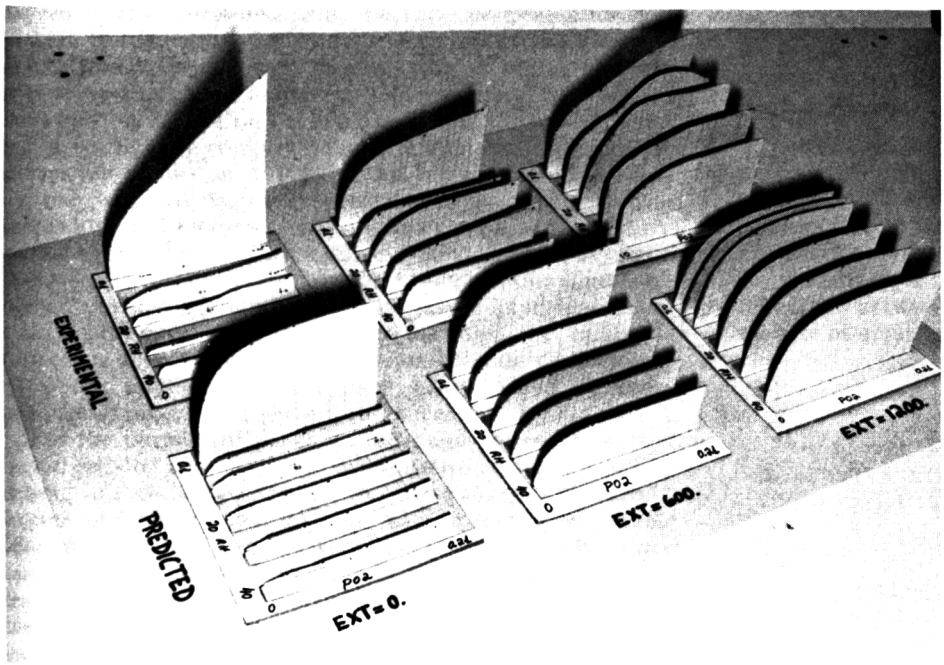

Fig. 1–Three-dimensional representation of experimental (from reduced set of 150 cases) and predicted rates of oxidation of potato chips at 37°C. (Ordinate represents RATE.)

obtain the numerical value of its constants from a simple experiment providing much less data than those used in model development. One such experiment would involve the determination of RATE at only three values of each of the independent variables. To test this possibility, we chose 3 X 3 X 3 = 27 experimental cases for two of the triplicate experiments. The choice of the values of the independent variables is not arbitrary. For EXT, we used equal intervals because of the linearity with respect to this variable. Since RATE is a strong function of PO2 and RH at low values of these variables, we chose smaller intervals in this range. The results are summarized in Table 6. It can be seen that the constants are similar to those from all the experimental results (Table 5) although their standard deviations are much larger. The error mean squares are only slightly larger than for the model with constants evaluated from all the experimental results. This suggests means for improving the experimental design and at the same time reduce the amount of experimentation.

In Figure 1 we have represented the reduced set of experimental rates and the predicted rates [Eq. (17)] at selected values of the independent variables. The ordinate in this model represents the RATE. The experimental and predicted values agree reasonably well.

CONCLUSIONS

A MIXED APPROACH to model building, including theoretical considerations of reaction kinetics as well as empirical data fitting resulted in a simple and potentially useful relation for the rate of oxidation of potato chips as a function of oxygen partial pressure, extent of oxidation and equilibrium relative humidity.

Further, we found that a small number of experimental results could be used to evaluate the equation constants once the form of the model was established.

Two important questions remain. The first is whether the model can be applied at higher temperatures simply by using an Arrhenius temperature correction factor. If this is possible, the applicability of this investigation would be increased by permitting rapid experiments at high temperatures to be used to evaluate the constants of the equation, and this equation could be used for package simulation and storage life prediction at room temperatures. The second question refers to the effect of "history of oxidation" or the particular path in the space of the three independent variables along which a particular combination of these three variables is reached. If there is a path effect, then the rate will be different for different paths even at the same values of the independent variables. If this effect is very large, then it is virtually impossible to build meaningful models. In the present case, the path effect could be responsible for some of the differences between the experimental and predicted results. This subject is being studied presently.

The mathematical model developed in this paper is specifically for one product, potato chips. However, it should generally be applicable to other food products which undergo deterioration by autoxidation of lipids. Verification requires further experimentation and of course a specific set of constants to be determined for each such food product.

APPENDIX

Symbol	Definition	Units
RATE	Rate of oxidation	$\mu l\ O_2$ STP/g·hr
EXT	Extent of oxidation	$\mu l\ O_2$ STP/g
RH	Equilibrium relative humidity (%)	–
PO2	Oxygen partial pressure	atm
P1, . . . PN	Constants	–
s^2	Error mean square	–
s_e^2	Mean square due to pure error	–

REFERENCES

Box, G.E.P. and Hunter, W.G. 1962. A useful method for model-building. Technometrics 4: 301.

Dixon, W.J., ed. 1969. "Biomedical Computer Programs, X-Series Supplement." University of California Press, Los Angeles.

Hartley, H.O. 1961. The modified Gauss-Newton method for fitting of nonlinear

regression functions by least squares. Technometrics 3: 269.
Heidelbaugh, N.D., Yeh, C.P. and Karel, M. 1971. Effects of model system composition on autoxidation of methyl linoleate. Agr. Food Chem. 19: 140.
Himmelblau, D.M. 1970. "Process Analysis by Statistical Methods." John Wiley & Sons, N.Y.
Jennrich, R.I. and Sampson, P.F. 1968. Application of stepwise regression to nonlinear estimation. Technometrics 10: 63.
Karel, M. 1960. Some effects of water and of oxygen on the rates of reactions of food components. Ph.D. thesis, M.I.T., Cambridge, Mass.
Karel, M. and Labuza, T.P. 1969. Optimization of protective packaging of space foods. USAF School of Aerospace Medicine, Contract No. F41-609-68-C-0015, Final Report.
Lundberg, W.O., ed. 1961. "Autoxidation and Antioxidants." Vol 1. Wiley Interscience, N.Y.
Maloney, J.F., Labuza, T.P., Wallace, D.H. and Karel, M. 1966. Autoxidation of methyl linoleate in freeze-dried model systems. 1. Effect of water on the autocatalyzed oxidation. J. Food Sci. 31: 878
Mizrahi, S., Labuza, T.P. and Karel, M. 1970a. Computer-aided prediction of extent of browning in dehydrated cabbage. J. Food Sci. 35: 799.
Mizrahi, S., Labuza, T.P. and Karel, M. 1970b. Feasibility of accelerated tests for browning in dehydrated cabbage. J. Food Sci. 35: 804.
Quast, D.G. 1972. Mathematical models for oxidation of dry foods and application to storage stability studies. Sc.D. thesis, M.I.T., Cambridge, Mass.
Quast, D.G. and Karel, M. 1972. Effects of environmental factors on the oxidation of potato chips. J. Food Sci. 37: 584.
Simon, I.B. 1969. Computer predictions of food storage stability. M.S. thesis, M.I.T., Cambridge, Mass.
Wedekind, G.L. 1971. Application of multifactor experimental design plans in parametric studies of theoretical problems. Simulation 16: 147.

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COMPUTER SIMULATION OF STORAGE LIFE OF FOODS UNDERGOING SPOILAGE BY TWO INTERACTING MECHANISMS

INTRODUCTION

PREDICTION OF "shelf life" of food products stored under different environmental conditions is an important problem of the food industry. Shelf life depends on a large number of factors such as temperature, equilibrium relative humidity, oxygen partial pressure, light, package permeabilities and package configuration. Some of these factors remain constant during storage while others change, such as the equilibrium relative humidity and oxygen partial pressure. The effect of these variable factors on the deterioration of the food product can be sufficiently complex to require numerical methods for storage life prediction. It is also necessary to determine which are the particular factors responsible for the eventual unacceptability of the product. In some cases, spoilage may clearly be due to one specific deteriorative process such as enzymatic or nonenzymatic browning; in others, two or more simultaneously occurring deteriorative mechanisms are important. Interactions introduce complexity. For instance, the carbonyls formed in lipid oxidation can increase the rate of browning while the rate of oxidation may be decreased by some compounds produced in the browning reaction.

For the purpose of package design and optimization, it is important to consider the deteriorative reactions which depend on the characteristics of the package. Water and oxygen are transmitted through many packages and their concentrations inside the package change continuously. Water can cause spoilage due to caking in products such as soluble tea (Karel and Labuza, 1969) or due to loss of crispness as in the case of potato chips (Kaghan, 1968). Indirectly, the increase in moisture content can decrease the rate of oxidation and increase the rate of browning. Similarly the change in oxygen concentration will directly affect the rate of oxidation of lipids, pigments, vitamins and proteins. Indirectly, it can affect the rate of browning via the intermediates formed in lipid oxidation.

Some of the early work on mathematical models of storage prediction of foods in permeable packages was done by Jurin and Karel (1963), Karel and Go (1964), and Veeraju and Karel (1967). These authors studied the storage of fruits in packages permeable to oxygen and carbon dioxide. The concentration of these gases was also a function of the respiration of the fruits, and the storage behavior could be predicated by analytical techniques.

Analytical and numerical techniques were used by Karel and Labuza (1969) and by Simon et al. (1971) to predict storage life of several food products in which only one deterioration reaction occurred. Mizrahi et al. (1970) successfully predicted the extent of browning of dehydrated cabbage using numerical methods. In this product, the rate of browning was only a function of moisture content, but this function was complicated by the fact that water was one of the products of browning reactions.

Dürichen and Heiss (1970) applied mathematical techniques to the analysis of storage behavior of roasted coffee in flexible packages. In this case, the problem was the release of CO_2 from the freshly roasted beans and the transfer of gases through the film. Rapid release of the CO_2 associated with low permeability of the film to this gas can result in rupture of the package.

In the present paper, we investigated the storage life of potato chips used as a model of a dry food which deteriorates by two mechanisms simultaneously: namely, by oxidation due to atmospheric oxygen, and by textural changes due to pick-up of water. However, the general approach outlined can be applied to any number of deteriorative reactions, with or without interactions.

Development of the mathematical model

Let us consider the case of a dry food product packaged in a permeable container. For this system, oxygen and water are transferred through the package and the former can cause spoilage due to oxidation of the product while the latter can cause deterioration due to gain or loss of moisture. The rate of accumulation of oxygen in the package headspace is the difference between the rate of transfer into the package and the rate of uptake by the food product:

$$\frac{d\left(\frac{VO2}{V}\right)}{dt} = \frac{d(PO2/P)}{dt} =$$

$$\frac{T \cdot A \cdot KO2}{TO \cdot V \cdot X}(PO2O - PO2) - \frac{T \cdot w \cdot RATE}{TO \cdot V \cdot 1000} \qquad (1)$$

where

VO2 = volume of oxygen (cm^3)
V = total headspace volume (cm^3)
t = time (hr)
PO2 = oxygen partial pressure inside package (atm)
P = total pressure (atm)
T = temperature (°K)
A = area of package film (m^2)
KO2 = oxygen permeability ($cm^3 O_2$ STP·mil)/(m^2·hr·atm)
TO = reference temperature (273°K)
X = thickness of film (mil)
PO2O = outside oxygen partial pressure (atm)
w = weight of product (g)
RATE = rate of oxidation (expressed as O_2 consumption per unit time) $\frac{\mu l\ O_2\ STP}{g \cdot hr}$

The rate of oxygen uptake has to be determined for each product and can be a function of oxygen partial pressure, water activity and other variables. In the case of potato chips at 37°C, the rate of oxidation was a function of three variables and a suitable model was developed for this product (Quast et al., 1972):

$$RATE = \frac{d(EXT)}{dt} =$$

$$\left|EXT + \frac{P1 + P2 \cdot EXT}{RH^{1/2}}\right| \cdot \left|\frac{PO2}{P3 + P4 \cdot PO2}\right| \qquad (2)$$

where EXT = extent of oxidation ($\mu l\ O_2$ STP)/g
P1, P2, P3, P4 = empirical constants
RH = equilibrium relative humidity

The rate of change of moisture content of the product is a function of the rate of moisture transfer through the packaging film:

$$\frac{d(m)}{dt} = \frac{A \cdot KW \cdot PWS}{X \cdot w} (ao - ai) \quad (3)$$

where

m = moisture content of the product (g/g solids)
KW = water vapor permeability (g·mil)/(m^2·hr·mm Hg)
PWS = pressure of saturated water vapor
ao = water activity outside of package
ai = water activity inside of package

This equation can only be solved if a suitable relation between the moisture content and the water activity is known. In the case of potato chips, several models were fitted to experimental results. It was found that over the range of interest the Kuhn isotherm gave the best fit:

$$m \cdot 100 = \frac{P5}{\ln(ai)} + P6 \quad (4)$$

where P5 and P6 are empirical constants estimated as: P5 = −3.87 and P6 = −0.51 by least squares fitting.
It is convenient to define dimensionless variables:

$$Y1 \equiv \frac{PO2}{PO2O}$$

$$Y2 \equiv \frac{EXT}{EMAX}$$

$$Y3 \equiv \frac{RH}{RHMAX}$$

where

EMAX = maximum allowable extent of oxidation (μl O_2 STP)/g
RHMAX = maximum allowable equilibrium relative humidity of the product.

Substituting these values and Eq. (4) into the differential Eq. (1) to (3), and noting that RH = ai·100 and RHO = ao·100, we obtain:

$$\frac{d(Y1)}{dt} = \frac{T \cdot A \cdot KO2}{TO \cdot V \cdot X} (1 - Y1) - \frac{T \cdot w \cdot EMAX}{TO \cdot V \cdot PO2O \cdot 1000} \cdot \frac{d(Y2)}{dt} \quad (5)$$

$$\frac{d(Y2)}{dt} = \frac{\left| Y2 \cdot EMAX + \frac{P1 + P2 \cdot Y2 \cdot EMAX}{(Y3 \cdot RHMAX)^{1/2}} \right| \cdot \left| \frac{Y1 \cdot PO2O}{P3 + P4 \cdot Y1 \cdot PO2O} \right|}{EMAX} \quad (6)$$

$$\frac{d(Y3)}{dt} = \frac{Y3 \cdot RHMAX}{-P5} \left| \ln \frac{Y3 \cdot RHMAX}{100} \right|^2 \cdot \frac{A \cdot KW \cdot PWS}{X \cdot w} \left(\frac{RHO}{RHMAX} - Y3 \right) \quad (7)$$

To obtain Y1, Y2 and Y3 as a function of time, the differential Eq. (5) to (7) have to be solved simultaneously. This is difficult to do analytically due to the complexity of the equations. However, with the aid of computers these equations can be solved numerically with a high degree of accuracy.

It must be mentioned here that in the above treatment it was assumed that the total pressure is one atmosphere and that the headspace volume does not change. Actually, small changes in volume do occur under certain conditions. Another assumption in writing Eq. (1) and (3) is that even as the driving forces for oxygen and for water transfer change with time, the transfer always occurs at the steady state rate corresponding to the driving force prevailing at the given instant of time. Experience has shown that this assumption is satisfactory for the packaging problems discussed here.

EXPERIMENTAL

THE TECHNIQUE for obtaining Eq. (2) has been described previously (Quast et al., 1972; Quast and Karel, 1972b). For the same potato chips, package experiments were made to investigate the feasibility of predicting the storage life by solving Eq. (5) to (7) with appropriate parameter values. In packages of known characteristics and containing different weights of potato chips, oxygen concentration was determined as a function of time by a technique described by Quast and Karel (1972a). The rate of transfer of oxygen into the package can then be calculated:

$$\frac{d(VO2)}{dt} = \frac{T \cdot A \cdot KO2}{TO \cdot X} (PO2O - PO2) \quad (8)$$

where

$$PO2 = f(t). \quad (9)$$

The partial pressure PO2 was determined experimentally. The total volume of oxygen transferred into the package can be determined by expanding and integrating Eq. (8) and (9) between t = 0 and t = t.

$$VO2_t = \frac{T \cdot A \cdot KO2}{TO \cdot X} \cdot PO2O \cdot t - \frac{T \cdot A \cdot KO2}{TO \cdot X} \int_0^t f(t) \cdot dt \quad (10)$$

where $VO2_t$ = volume of oxygen transferred into package from time 0 to time t

where the integral $\int_0^t f(t) \cdot dt$ is simply the area under the curve of the plot of PO2 versus t. If there is no product consuming oxygen inside the package, the calculated volume of oxygen would be present in the package headspace. However, if there is an oxidizing sample inside the package, then the experimental results of the oxygen concentration (PO2) can be used to determine the actual volume of oxygen inside the package ($VO2_p$).

$$VO2_p = PO2 \cdot V \quad (11)$$

The difference between the transferred volume and the volume present at any time represents the volume of oxygen taken up by the product. Then

$$EXT = \frac{VO2_t - VO2_p}{w} \cdot \frac{TO}{T} \cdot 1000 \quad (12)$$

The moisture content of the product as a function of time was determined by weight gain of the packages since the weight gain due to oxygen uptake is much smaller, and does not result in a significant error in the present experiment. From these results, the dimensionless variables Y1, Y2, Y3 could be obtained as a function of time for all the experiments. The calculations, including the numerical integration of Eq. (10), were done on the IBM/360 computer at the M.I.T. Computation Center.

The oxygen permeability of the packages was determined by measuring the oxygen concentrations inside empty packages which initially contained only nitrogen. By plotting PO2O/(PO2O − PO2) versus time, the permeability was calculated from the slope. The water vapor per-

Table 1–Experimental condition for three packaging situations

	Case 1	Case 2	Case 3
RHO	40	40	0.1
w	10	50	10
V	371.4	337.0	371.4

meability was determined by weight gain of packages containing calcium sulfate desiccant. The permeabilities of the packaging film used (ACLAR 33C; brand name of Allied Chemical Corp.) were not affected by the water activity.

For computer simulation of storage behavior, a main program and two subroutines were used. The main program was used mainly for input and output. The integration of Eq. (5) to (7) was done with the aid of a fourth order Runge-Kutta subroutine which used a subroutine containing the derivative functions. Accurate results could usually be obtained with intervals of 20 hr. In some cases, intervals of 50 hr could be used while in others, intervals of 5 hr had to be employed to avoid instability.

In our calculations, we considered the maximum allowable equilibrium relative humidity (RHMAX) of potato chips to be 32 which corresponds to a moisture content of 3%, the limit for commercial acceptability (Kaghan, 1968). In previous work (Quast and Karel, 1972b), we found that the potato chips become very rancid and enter a rapid oxidation period after an extent of 1200 μl O_2 STP/g is reached. We therefore used EMAX = 1200 in our calculations.

RESULTS & DISCUSSION

Experimental packages

The following packaging parameters and conditions were constant as determined experimentally for all situations:

KO2	= 9.1	T	= 310
KW	= 0.0010	EMAX	= 1200
X	= 1.0	PO2O	= 0.21
A	= 0.0123	RHMAX	= 32

Initial conditions

Y1 = 0.0 (i.e., inert gas packaging)
Y2 = 0.0 (i.e., start with unoxidized product)
Y3 = 0.003

The value of Y3 = 0.003 corresponds to an initial equilibrium relative humidity (RH) of 0.1. This is very low, and can be considered the lowest value of practical importance. Note that the rate of oxidation as given by Eq. (2) becomes infinite for RH = 0.0, and this model does not hold for RH<0.1 (or Y3<0.003, if RHMAX = 32).

The different experimental parameters of three different experimental packages are summarized in Table 1. The experimental results in triplicate are shown in Figures 1 to 3. It can be seen that for these particular package designs, spoilage occurred due to excessive oxidation rather than excessive moisture. The package in case no. 1 appears to be better designed than in case no. 2 where the product is over-protected against moisture while under-protected against oxygen. It can also be seen that the product in case no. 2 has a longer storage life for the same permeability and film area. This is due to the larger amount of product inside the package which serves as the "sink" for the spoilage inducers (H_2O and O_2) transferred into the package.

In case no. 3, the outside RH was equal to the initial inside RH of the product. Consequently, the RH of the product did not change with time since no moisture transfer could take place. Spoilage could only be due to oxidation and occurred after a short time because of the high rate of oxidation at low RH. Case no. 3 represents a very poor packaging condition.

It can be seen that in all cases, for the particular initial conditions of the experiments, the oxygen concentration increased initially and then decreased to a very low level. As expected, the rapid decrease of the oxygen concentration usually coincided with the reaching of EXT/EMAX = 1, when the period of rapid oxidation of the potato chips starts.

Package simulation

The three experimental cases were simulated on the computer by solving Eq. (5) to (7) simultaneously with the same package parameters and up to the time when either Y2 or Y3 reached a value of unity. Beyond this point, the product was considered spoiled and also Eq. (6) does not hold for Y2>1. The following values were used for the constants in Eq.

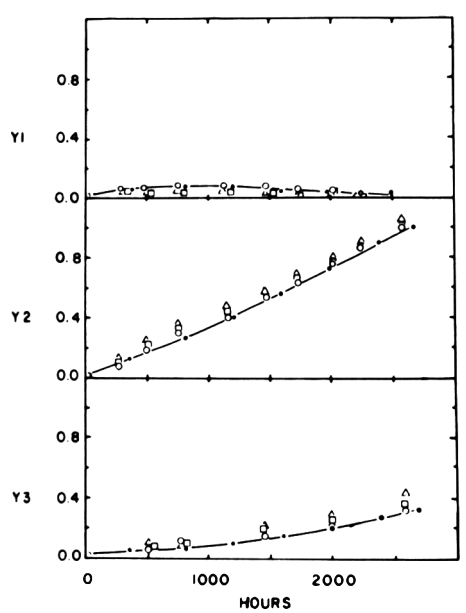

Fig. 1–Predicted and experimental changes in oxygen pressure, relative humidity and extent of oxidation in potato chip packages (RHO = 40; w = 10; V = 371.4). Lines represent predicted changes; open circles, squares, and triangles represent experimental results of triplicate experiments.

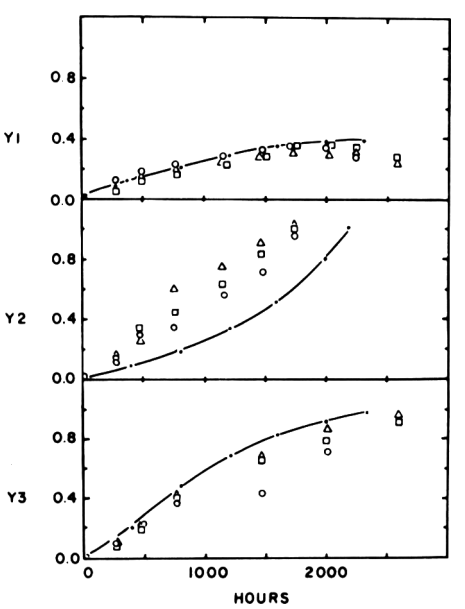

Fig. 2–Predicted and experimental changes in oxygen pressure, relative humidity and extent of oxidation in potato chip packages (RHO = 40; w = 50; V = 337.0). Lines represent predicted changes; open circles, squares, and triangles represent experimental results of triplicate experiments.

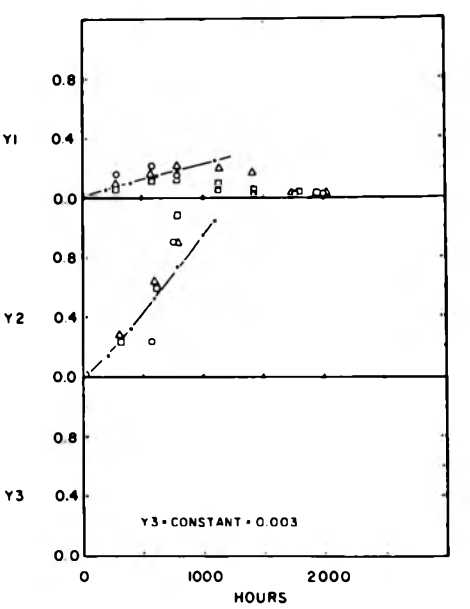

Fig. 3–Predicted and experimental changes in oxygen pressure, relative humidity and extent of oxidation in potato chip packages (RHO = 0.1; w = 10; V = 371.4). Lines represent predicted changes; open circles, squares, and triangles represent experimental results of triplicate experiments.

Table 2–Comparison of simulated and experimental value of maximum storage life and the values of Y3 and Y1 at that time[a]

	Maximum storage life (hr)		Y3		Y1	
Case no.	Experimental	Predicted	Experimental	Predicted	Experimental	Predicted
1	1750	2200	0.65	0.90	0.30	0.35
2	2600	2700	0.35	0.30	0.01	0.02
3	850	1050	0.003	0.003	0.15	0.25

[a]In cases 1–3, Y2 = 1 before Y3 = 1.

(6) (Quast et al., 1972): P1 = 445.24; P2 = –0.39111; P3 = 6.1924; P4 = 818.36.

The results of the computer simulations are shown as lines in Figures 1 to 3. The open circles, triangles and squares show experimental points obtained in triplicate experiments. It can be seen that for most of the cases there is reasonable agreement between the experimental and predicted values of the three variables. The largest deviation occurred for the variable Y2 in case no. 1 (Figure 1). Note that the predicted values of the variable Y1, representing the oxygen concentration in the headspace, are only slightly higher than the experimental values. However, since the headspace volume is very large compared to the sample weight, this small difference in Y1 reflects itself as a large difference in Y2. The larger differences between the predicted and experimental values of Y3 for case no. 1 can also be attributed in part to the small sample size which results in small weight changes due to moisture uptake.

In all three cases, spoilage occurred due to excessive oxidation of the product and therefore the storage life was limited by this factor. This is, of course, due to the low RHO chosen in the present study and does not preclude the possibility of water-caused spoilage under other storage conditions. The maximum experimental and predicted storage lives for the three cases are summarized in Table 2. It can be seen that over the wide range of packaging conditions represented by the three cases, the predictions agree with the experimental results sufficiently to be of practical value. The advantage of the mathematical model lies in the insight gained from simulating the package behavior for a variety of initial conditions, outside conditions, package sizes and shapes and packaging films. In a future paper we will discuss means by which optimal packaging conditions can be calculated, such that spoilage due to moisture and oxygen uptake occur at the same time.

Effect of the history or path of oxidation

The history of oxidation of a product is here referred to as the path in the space of PO2 and RH (and other variables) along which a particular extent of oxidation is reached. This path can be quite different depending on the packaging parameters and outside conditions. It is conceivable that the rate of oxidation at the same values of the independent variables (PO2, EXT, RH in the present case) be different if this point was reached by different paths. Mizrahi et al. (1970) found that this effect was negligible for nonenzymatic browning of dehydrated cabbage which was a function only of the moisture content. If, for potato chips, there is a significant effect of the path, then Eq. (6) does not hold and accurate predictions with any mathematical model are virtually impossible.

To investigate the effect of history on the rate of oxidation of potato chips, we followed the oxidation at several constant values of RH and at a constant PO2 of 0.21. We determined the rates at certain extents of oxidation. In a second experiment, we determined the rates of oxidation of samples at the same constant values of RH, but with cyclically changing PO2 through the following values: PO2 = 0.0055, 0.035, 0.072, 0.12, 0.21. Finally, we determined the rates of oxidation of these samples at the same RH, EXT, and PO2 (0.21) as in the previous case. Because of the changes in PO2, the average PO2 in the second set of samples was lower and the time to reach the same extent was longer. In these experiments, the paths along the RH direction were identical but the paths along the PO2 direction were different. The results are summarized in Table 3. It can be seen that the rates for the cyclical path are consistently higher, especially at low RH.

In another set of experiments, we oxidized samples at constant PO2 = 0.21 and constant RH = 0.1 up to certain extents and then changed the RH to 11. Results were also obtained by oxidizing samples at constant RH of 11. The results are summarized in Table 4. The samples oxidized at RH = 0.1 reached the same EXT as those at RH = 11 in a shorter time. The rate obtained at EXT = 500 for constant RH = 11 is slightly higher than the rate with RH = 0.1. The effect of the change in path along RH appears to be smaller than the effect of the path along PO2.

It must be noted that the paths investigated here were quite simple and arbitrary, involving one variable at a time. These paths were quite irregular in that they involved step changes in the independent variables. In real packages, these changes are slow and follow smooth lines and usually involve simultaneous changes in RH and PO2. Therefore the path effects in real

Table 3–Effect of history of oxidation on the rate of oxygen uptake of potato chips at 37°C

		Rate (μl O_2 STP/g·hr) at PO2 = 0.21	
RH	EXT	For constant PO2 = 0.21	For cyclical change of PO2
0.1	500	0.91	1.60
0.1	1000	0.91	1.60
20	500	0.60	0.75
20	1000	1.00	1.30
40	500	0.42	0.50
40	1000	0.93	1.00

Table 4–Effect of history of oxidation on the rate of oxygen uptake of potato chips

	Rate (μl O_2 STP/g·hr) measured at PO2 = 0.21 and RH = 11	
EXT	RH = 0.1 except during measurement period	Constant RH = 11
500	0.64	0.75
1000	0.96	0.95

packages are likely to be substantially smaller but could still account for some differences between the predicted and experimental results.

CONCLUSIONS

WE HAVE SHOWN in this paper that numerical techniques can be applied to predict the storage life of a dry food product undergoing spoilage by two mechanisms simultaneously, with interactions between the mechanisms.

With the aid of a set of differential equations describing the system, it is possible to predict the storage life of the product for any package size and configuration as well as for different initial conditions and different environmental conditions.

Furthermore, because of the ease of simulating the system on the computer, it is possible to apply it to package design and optimization. This will be the subject of a future paper. Likewise, the approach described here can be extended to any number of interacting or independent deteriorative mechanisms for foods in flexible packages.

APPENDIX

Symbol	Definition	Units
ao	water activity outside of package (same as RHO/100)	–
ai	water activity inside package (same as RH/100)	–
A	area of package	m^2
EXT	extent of oxidation	$\mu l\ O_2 STP/g$
EXT/EMAX	dimensionless extent of oxidation (same as Y2)	–
EMAX	Maximum extent of oxidation (usually 1200)	$\mu l\ O_2 STP/g$
KO2	oxygen permeability of packaging film	$\frac{cm^3 O_2 STP \cdot mil}{m^2 \cdot hr \cdot atm}$
KW	water vapor permeability of packaging film	$\frac{g \cdot mil}{m^2 \cdot hr \cdot mm\ Hg}$
m	moisture content of the product	$g\ H_2O/g$ solids
p	total pressure	atm
PO2	oxygen partial pressure inside the package	atm
PO2O	oxygen partial pressure outside the package	atm
PO2/PO2O	dimensionless oxygen concentration inside the package (same as Y1)	–
P1, P2 . . . Pn	constants	–
PWS	pressure of saturated water vapor at experimental temperature	mm Hg
RATE	rate of oxidation [same as d(EXT)/dt]	$\frac{\mu l\ O_2 STP}{g \cdot hr}$
RH	equilibrium relative humidity inside the package (same as ai × 100)	–
RHMAX	maximum allowable equilibrium relative humidity of product	–
RHO	equilibrium relative humidity outside the package (same as ao × 100)	–
RH/RHMAX	dimensionless equilibrium relative humidity (same as Y3)	–
t	time	hr
T	temperature	°K
TO	reference temperature	273°K
V	headspace volume of the package	cm^3
VO2	volume of oxygen at temperature T	cm^3
$VO2_t$	volume of oxygen transferred into package	cm^3
$VO2_p$	volume of oxygen present in the package headspace	cm^3
w	weight of product in package	g
X	thickness of the package film	mil
Y1	dimensionless oxygen concentration inside package (same as PO2/PO2O)	–
Y2	dimensionless extent of oxidation (same as EXT/EMAX)	–
Y3	dimensionless equilibrium relative humidity (same as RH/RHMAX)	–

REFERENCES

Dürichen, K. and Heiss, R. 1970. Physikalische Überlegungen zur Verpackung von Röstkaffee in Weichpackungen. Verpackungs Rundschau Nr. 5: 35.

Jurin, V. and Karel, M. 1963. Studies on control of respiration of McIntosh apples by packaging methods. Food Technol. 17: 104.

Kaghan, W.S. 1968. Olin two-side coated glassine. Preprint of paper presented at The Packaging Institute, 30th Annual Packaging Forum, N.Y.

Karel, M. and Go, J. 1964. Control of respiratory gases. Modern Pkg. 37(6): 123.

Karel, M. and Labuza, T.P. 1969. Optimization of protective packaging of space foods. USAF School of Aerospace Medicine, Contract No. F 41-609-68-C-0015, Final Report, M.I.T. Cambridge, Mass.

Labuza, T.P., Tannenbaum, S.R. and Karel, M. 1969. Water content and stability of low-moisture and intermediate-moisture foods. Food Technol. 24(5): 35.

Mizrahi, S., Labuza, T.P. and Karel, M. 1970. Computer-aided predictions of extent of browning in dehydrated cabbage. J. Food Sci. 35: 799.

Quast, D.G. and Karel, M. 1972a. Technique for determining oxygen concentration inside packages. J. Food Sci. 37: 490.

Quast, D.G. and Karel, M. 1972b. Effects of environmental factors on the oxidation of potato chips. J. Food Sci. 37: 584.

Quast, D.G., Karel, M. and Rand, W.M. 1972. Development of a mathematical model for the rate of oxidation of potato chips as a function of oxygen partial pressure, extent of oxidation, and equilibrium relative humidity. J. Food Sci. 37: 673.

Simon, I.B., Labuza, T.P. and Karel, M. 1971. Computer-aided predictions of food storage stability: Oxidative deterioration of a shrimp product. J. Food Sci. 36: 280.

Veeraju, P. and Karel, M. 1967. Control of atmosphere inside a fruit container. Modern Packaging 40(2): 168.

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GAS CHROMATOGRAPHIC DETERMINATION OF VOLATILE NITROSAMINES IN FOODS

INTRODUCTION

THE ROLE OF N-nitroso compounds in the etiology of human cancer has not been established, but these compounds are a cause of increasing concern as potential environmental carcinogens (Lijinsky and Epstein, 1970). Since nitrosamine concentrations in the nanogram per gram range may be formed by reaction of naturally occurring amines and added nitrites in fish (Sen et al., 1970; Fazio et al., 1971a), it is essential that conditions under which formation occurs by investigated. Rates and mechanisms of formation and destruction of nitrosamines in foods must be evaluated in order to eliminate any potential public health hazard from this source in processed foods. Screening methods are required for application in epidemiological studies of the relationship between exposure of human populations to nitrosamines and cancer risk.

Systematic investigation of nitrosamine formation during processing, storage and cooking of foods is limited by the analytical methods currently available. Wasserman (1971) has recently reviewed the literature and reported a survey of analytical procedures. Nitrosamines are ordinarily removed from water and nonvolatile materials by distillation and extraction, though not necessarily in that order (Fazio et al., 1971a, b; Sen, 1971). Concentration is required because of the very small quantities (ng/g) which must be detected. Clean-up is a critical step because most foods contain relatively large quantities of volatile material which may interfere with nitrosamine detection during gas chromatographic (GC) analysis. Methods having sufficient specificity are limited to those using mass spectrometry (MS) for confirmation of identity of volatile nitrosamines (Fazio et al., 1971a; Telling et al., 1971), after quantitative estimation by GC. The available procedures are time consuming, and the number of steps and extreme care required to minimize losses limit the number of samples which can be examined simultaneously.

Our investigations were undertaken to determine the extent to which analytical procedures for determination of volatile nitrosamines could be simplified without loss of sensitivity and specificity. Distillation and extraction were combined into a continuous process using a technique which allows large volumes of water to be distilled from a food sample and the distillate effectively extracted with large volumes of solvent without dilution of the extract. Some factors influencing recovery of volatile nitrosamines from foods were examined.

A sensitive, specific GC detector was desirable for efficient screening and routine quantitative determination of nitrosamines in food extracts. The detector described by Rhoades and Johnson (1970) was evaluated.

Confirmation of identity by MS is essential to establish the presence of nitrosamines unequivocally. Problems associated with adequate sample clean-up for combined GCMS were studied.

A chromatographic solvent stripping technique which permits GC analysis of dilute solutions was evaluated.

EXPERIMENTAL

CAUTION: Many nitrosamines are highly potent carcinogens. In the work reported below, nitrosamine solutions, spiked samples, and concentrates were handled in efficient fume hoods. Gloves were worn and precautions taken to avoid spills, skin contact and inhalation. Gas chromatographs used for nitrosamine determination should be equipped with destructive detectors or be vented to a hood.

Reagents

Pesticide grade dicholoromethane (DCM) and chromatoquality pentane were obtained from Matheson, Coleman and Bell, Norwood, Ohio. All other reagents were of analytical grade.

Distillation-extraction of nitrosamines from aqueous solutions

Nitrosamines were recovered from aqueous solutions (200 ml) containing 10–50 ng/ml dimethylnitrosamine (DMN), 10–50 ng/ml diethyl nitrosamine (DEN), 0.1g/ml NaCl, and 0.2g/ml K_2CO_3. Approximately 25 ml of DCM were used as the extracting solvent in the distillation-extraction apparatus described by Likens and Nickerson (1964). The vapor tubes above the aqueous solution were wrapped with heating tape and the distillation rate of each liquid was adjusted to approximately 1 ml/min. Distillation times ranged from 1–5 hr after which extracts were dried with anhydrous Na_2SO_4 and concentrated in a nitrogen stream to 1 ml. In later studies, a Kuderna-Danish apparatus was used for initial concentration; 50-μl aliquots were analyzed by GC.

Distillation-extraction of food materials

A number of foods was examined to determine the nature and amounts of interfering compounds. We examined smoked fish, canned chopped ham, bologna, summer sausage, canned "potted meat," "old fashioned loaf," corned beef spread, Vienna sausage and frankfurters. A 100-g food sample was homogenized with 100 ml distilled H_2O, 20g NaCl and 4g K_2CO_3 and then subjected to distillation-extraction for 4 hr, concentration in a nitrogen stream and GC analysis. If necessary, 1 ml concentrates were treated by a simple clean-up procedure involving a single stage extraction with an equal volume of 0.01N HCl in Na_2SO_4-saturated distilled H_2O.

Further modification of the procedure for samples of canned corned beef was necessary to remove compounds interfering with GC determinations. 100g of sample were homogenized with 200 ml of H_2O, 100g NaCl, 10g K_2CO_3 and after distillation-extraction, concentrated using the Kuderna-Danish apparatus. The concentrate was acid extracted and then subjected to a liquid column chromatography clean-up step using both 0.05–0.2 mm chromatographic grade silica gel (E. Merck) and alumina (Fisher Scientific Co.) packed columns. Each column was packed with 5g of material and rinsed with 30 ml of pentane. The concentrated corned beef extract (1 ml) was transferred onto the alumina column which was then eluted successively with 20 ml of pentane and 25 ml of 25% DCM in pentane. The fraction which would have contained nitrosamines was eluted with 15 ml of 30% diethyl ether in DCM which was

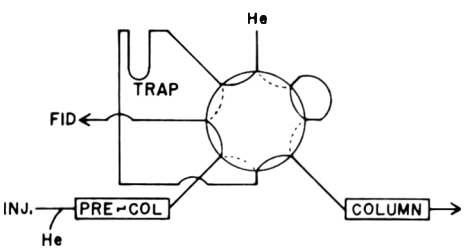

Fig. 1—Gas chromatographic solvent stripping system. Precolumn, 5.3 mm ID x 46 cm 20% Carbowax 20M on 80/100 mesh Chromosorb G. Solid lines indicate valve ports connected during solvent stripping and during analysis. Broken lines indicate ports connected during sample trapping. Valve is model 2012 (Carle Instrument Co., Fullerton, Calif.).

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then concentrated to 1 ml in a nitrogen stream. This concentrate was then treated identically on the silica gel column. The silica-gel column clean-up technique was adapted from the procedure described by Fazio et al. (1971b). This chromatographic clean-up was developed using both DCM solutions of nitrosamines and corned beef extracts.

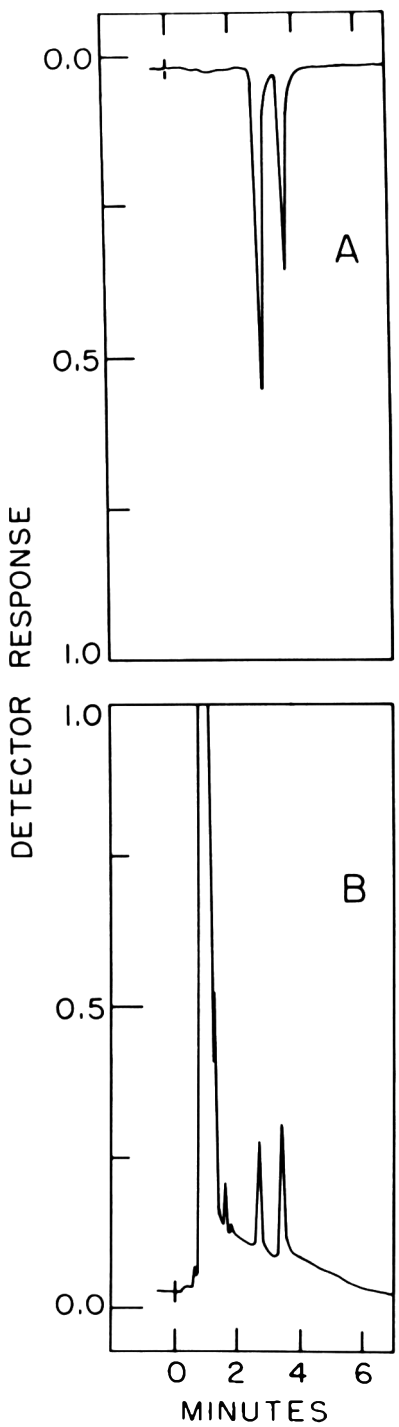

Fig. 2–Chromatograms of 50 µl of a 1 ng/µl solution of DMN and DEN. The retention times of DMN and DEN are 2.9 and 3.7 min, respectively, in the CECD chromatogram (A) and 2.8 and 3.5 min in the FID chromatogram (B).

GC analysis

The chromatographic system used is shown schematically in Figure 1. 50–100 µl aliquots of solution were injected into a 5.3-mm ID x 46-cm long precolumn packed with 20% Carbowax 20M on 80/100 mesh Chromosorb W. After elution of solvent (1.5 min for DCM) to the atmosphere (solid lines), the effluent from the precolumn was diverted by a valve (Carle model 2012) to the trap (broken lines) which was cooled with liquid nitrogen (−196°C). Trapping was continued for a period sufficient to collect all of the nitrosamines of interest (7.0 min for DEN, 15 min for nitrosopyrrolidine). The valve was then returned to its original position (solid lines) and the trap immediately heated to approximately 90°C. This procedure rapidly transferred the nitrosamines from the trap to the analytical column. An identical solvent stripping system was installed on the combined GCMS instrument.

As a safety precaution, material eluted from the columns was transferred either to a fume hood or to destructive detectors.

Analytical columns used with the solvent stripping system included a 2.3-mm ID x 3.2-m long column packed with 3% Carbowax 4000 on 80/100 mesh Chromosorb G, a 0.5-mm ID x 160-m long open tubular column coated with Carbowax 20M, and a 0.5-mm ID x 15.2-m long SCOT column coated with Carbowax 1540 (Perkin-Elmer Corp., Norwalk, Conn.). The packed column and precolumn were installed in an F & M model 810 GC equipped with a flame ionization detector (FID) and a Coulson electrolytic conductivity detector (CECD). Helium flow rates for both columns were 35 ml per min. Injector and FID temperatures were 220 and 270°C, respectively. Column temperature was 140°C for both columns. The Carbowax 1540 SCOT column and the Carbowax 20M open tubular column were used in the inlet chromatograph of the GCMS system (Issenberg et al., 1969).

Detection of nitrosamines: comparison of the CECD with the FID

Sensitivity and selectivity of the CECD (Tracor, Inc., Model C321, Austin, Texas) were compared with the FID for the determination of some alkyl and some heterocyclic nitrosamines. The CECD was connected to the GC oven by a 0.75-mm ID heated transfer line, maintained at 125°C. The detector was operated in the pyrolytic mode as described by Rhoades and Johnson (1970) and an auxillary carrier flow of 95 ml per min was used. In these studies, small volumes (1 µl) of DCM solutions containing approximately 1 µg of each nitrosamine were injected onto the Carbowax 4000 analytical column which was directly connected to either the CECD or the FID. Peak areas were measured by using a digital integrator (Problematics, Inc., model CDA100A, Waltham, Mass.).

RESULTS & DISCUSSION

THE DISTILLATION-EXTRACTION apparatus has a number of theoretical advantages. Most volatile alkyl nitrosamines have partition ratios that favor extraction with DCM (Nunn and du Plessis, 1971). The combined distillation-extraction technique permits continuous recycling of the aqueous and organic phases without dilution of the extract. A number of distillation-extractions can be performed simultaneously because each apparatus requires a minimum amount of attention. Early use of this method with food homogenates was encumbered by severe foaming problems. This problem was controlled when the homogenate was distilled from a large (2-liter) flask with careful stirring. It was also found helpful to heat the vapor tubes above the homogenate and solvent flasks.

Table 1–Relative response of the Coulson electrolytic conductivity detector (CECD), operated in the pyrolytic mode, and the flame ionization detector (FID) to selected nitrosamines

Nitrosamine	Retention time[a] (min)	Peak area relative to dimethylnitrosamine	
		CECD	FID
Dimethylnitrosamine	2.1	1.0	1.0
Methylethylnitrosamine	2.4	0.81	1.5
Diethylnitrosamine	2.6	0.53	1.8
Methylpropylnitrosamine	3.2	0.60	1.7
Dipropylnitrosamine	4.6	0.29	2.1
Diallylnitrosamine	4.8	0.28	2.3
Methylbutylnitrosamine	4.6	0.53	1.8
Ethylbutylnitrosamine	5.1	0.46	2.1
Propylbutylnitrosamine	6.4	0.22	2.2
Dibutylnitrosamine	9.3	0.18	2.3
Nitrosopyrrolidine	12.0	0.12	1.6
Ethylbenzylnitrosamine	58.7	0.42	2.3
Ethylphenylnitrosamine	9.7	0.0	0.61
Butylphenylnitrosamine	19.5	0.0	0.54
Nitrosomorpholine	15.2	0.03	0.43

[a]Column: 2.3 mm ID x 3.2m packed with 3% Carbowax 4000 on 80/100 mesh Chromosorb G; helium flow rate 35 ml/min; temperature 140°C

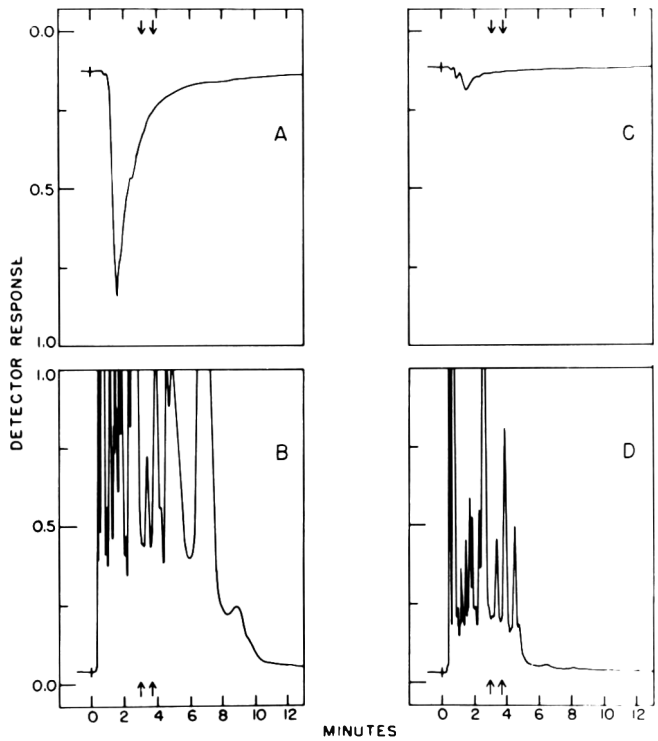

Fig. 3–Chromatograms of 50 µl of concentrate prepared from 100g of smoked fish. Arrows indicate retention times of DMN and DEN. (A) CECD, untreated concentrate; (B) FID, untreated concentrate; (C) CECD, concentrate extracted with 0.01N HCl; (D) FID, concentrate extracted with 0.01N HCl.

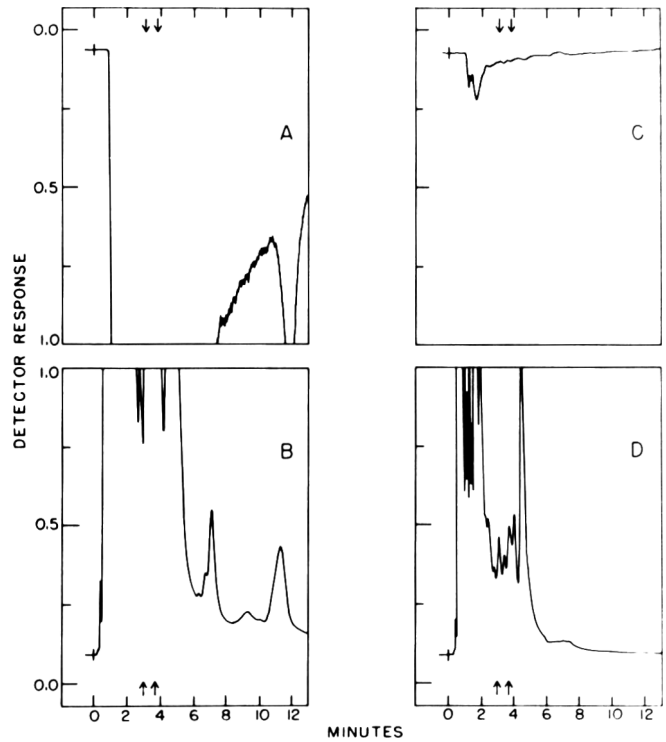

Fig. 4–Chromatograms of 50 µl of concentrate prepared from 100g of canned "potted meat." Arrows indicate retention times of DMN and DEN. (A) CECD, untreated concentrate; (B) FID, untreated concentrate; (C) CECD, concentrate extracted with 0.01N HCl; (D) FID, concentrate extracted with 0.01N HCl.

Recoveries of DMN and DEN from water solutions in initial studies were about 30 and 40%, respectively. Part of this loss was found to be associated with concentration of the large volume of organic extract in a nitrogen stream. This step was replaced by concentration in a Kuderna-Danish apparatus to approximately 5 ml and final concentration to 1 ml in a nitrogen stream. Recovery was also increased by adding a condenser to the top of the distillation-extraction apparatus and by cooling all condensers with ethylene glycol chilled to 0–5°C. With these modifications, recoveries for 10 ng/g aqueous solutions of DMN and DEN were approximately 60 and 70%, respectively, with a distillation-extraction time of 4 hr.

The solvent stripping system permits injection of large volumes (50–100 µl) of dilute solutions without solvent damage to high efficiency GC columns. The precolumn is a high capacity column with efficiency adequate for separating nitrosamines of interest from the solvent (DCM). The system has been used successfully with support-coated open tubular, capillary and conventional packed columns. Considerably less than 1 µl of solvent reaches the analytical column for a 50-µl injection. This technique has proven particularly useful in analysis of dilute solutions by GCMS, where injection of large volumes of solvent must be avoided.

The CECD, operated in the pyrolytic mode, exhibited sensitivity and selectivity necessary to determine small quantities of volatile nitrosamines in food extract concentrates. Figure 2 compares response of the CECD and the FID to a sample containing 50 ng each of DMN and DEN. Levels of approximately 10 ng can be measured routinely with the CECD. Table 1 indicates that under the appropriate analytical conditions the response to nitrosopyrrolidine would be approximately one-tenth that to DMN. With satisfactory recovery from food homogenates, a 50-µl aliquot of a concentrate representing 10 ng/g nitrosopyrrolidine in the original sample would produce an easily measured peak in the chromatogram. It would probably be necessary to

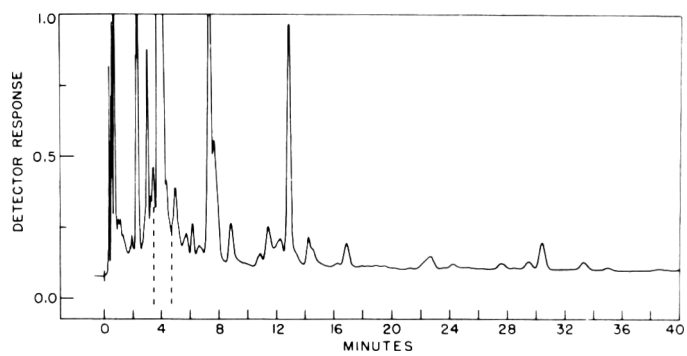

Fig. 5–Total ion current chromatogram of 50 µl of concentrate prepared from chopped ham to which 66 ng/g DMN and DEN were added. Broken lines indicate retention times of DMN and DEN.

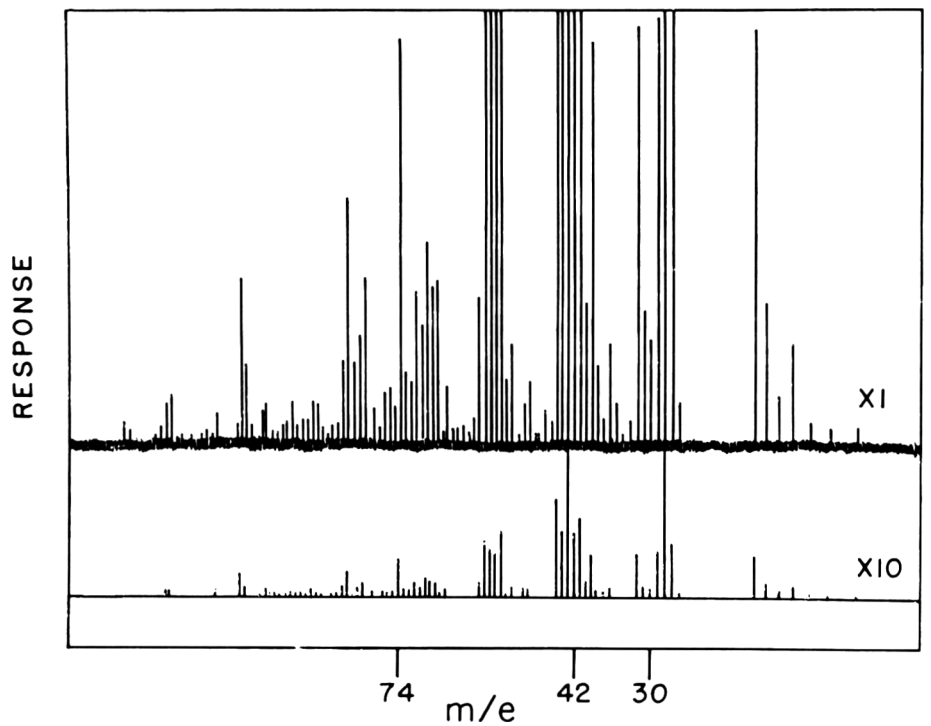

Fig. 6–Mass spectrum recorded at the retention time of DMN from concentrate prepared from canned chopped ham to which 66 ng/g DMN were added.

use high efficiency columns or temperature programming with packed columns to provide GC peaks of sufficient sharpness for routine determination of nitrosopyrrolidine.

The complexity of concentrates prepared from foods by the distillation-extraction procedure is illustrated by Figure 3. A 50-μl sample of a concentrated distillate from smoked fish was analyzed using a splitter to divide the column effluent equally between the CECD (3A) and FID (3B). The effect of a single contact extraction with 0.01N HCl on the CECD and FID chromatograms is shown in Figures 3C and 3D. The times at which DMN and DEN, if present, would elute are indicated on these chromatograms. Figure 4 shows chromatograms of a similarly prepared concentrate of a 100-g sample of "potted meat." In this case, the efficacy of the acid extraction clean-up is clearly shown. Removal of basic compounds from extracts of smoked fish, canned chopped ham, bologna, summer sausage, canned "potted meat," "old fashioned loaf," corned beef spread, Vienna sausage and frankfurters provided sufficient clean-up for specific detection of alkyl nitrosamines with the CECD. GC analysis of DCM solutions of nitrosamines before and after clean-up by acid extraction showed that no DMN or DEN was lost to the aqueous phase. Neither DMN nor DEN was detected at the 10-ng/g level in the limited number of commercial food products examined.

In Figures 3 and 4, CECD sensitivity is such that 50 ng of DMN, corresponding to 100% recovery of 10 ng/g in the original sample would yield a peak amplitude of approximately 30% of full scale. The detection limit is approximately 2 ng/g.

The most difficult interference problem encountered was with a concentrate from distillation-extraction of a sample of canned corned beef. The simple acid extraction was not sufficient to remove interferences detected using both the FID and the CECD. GCMS analysis demonstrated that the chromatographic peaks were not nitrosamines. The inclusion of a column chromatography step using both silica gel and alumina columns produced a concentrate which permitted quantitative determination of alkyl nitrosamines added to the sample. Recovery of DMN and DEN through the column chromatography procedure was greater than 80%.

Figure 5 shows the total ion current chromatogram recorded during GCMS analysis of a concentrate prepared from canned chopped ham to which 66 ng/g DMN and DEN were added. For this analysis, the precolumn was operated at 130°C and the Carbowax 1540 SCOT analytical column was operated at 100°C. A mass spectrum, recorded at the retention time of DMN, is shown in Figure 6. The spectrum indicates that DMN is indeed present, even though the major spectral peaks arise from a mixture of interfering compounds that constitute the GC peak. Repetitive scanning of spectra and plotting of specific ion peaks during elution of GC peaks provides stronger evidence for confirmation of the presence of DMN. Figure 7 shows a series of such plots recorded during elution of the DMN added to the sample. The peaks at m/e 30, 42 and 74 are major peaks in the DMN spectrum. These all attain maximum intensity at the retention time of DMN, while the other more intense peaks (m/e 75 and 71) reach maximum values earlier. Identification of the nitrosamine is limited, in this case, by the extent of

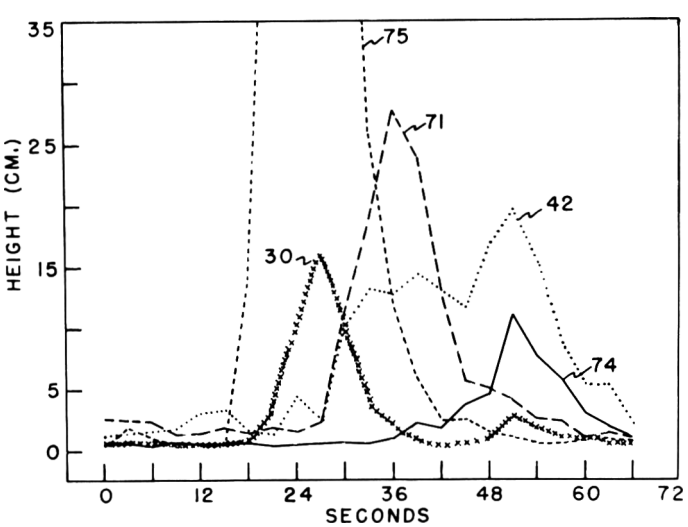

Fig. 7–Plot of individual mass peaks appearing around the retention time of DMN from concentrate prepared from canned chopped ham to which 66 ng/g DMN were added. The peaks at m/e 30, 42 and 74 are major peaks in the DMN spectrum.

separation from interfering compounds. If these data were recorded with an unspiked sample, identification of DMN would have to be considered tentative. Improved separation is required during the GCMS confirmation stage. Open tubular columns may prove helpful for this purpose in the future. Multiple column GC clean-up is feasible and may be necessary if low resolution MS is used for confirmation of identity. Less clean-up is required when high resolution MS is employed (Telling et al., 1971).

The need for unambiguous confirmation of identity of chromatographic peaks tentatively identified as nitrosamines must be emphasized. The approach described provides flexibility so that foods containing no interfering compounds may be examined rapidly and easily. Clean-up steps, consisting of simple extraction, liquid chromatography, or preparative GC may be added as required by the nature of the materials present. A standard analytical method appropriate for all volatile nitrosamines and all foods is not yet available.

REFERENCES

Fazio, T., Damico, J.N., Howard, J.W., White, R.H. and Watts, J.O. 1971a. Gas chromatographic determination and mass spectrometric confirmation of N-nitrosodimethylamine in smoke-processed marine fish. J. Agr. Food Chem. 19: 250.

Fazio, T., Howard, J.W. and White, R. 1971b. Multidetection method for analysis of volatile nitrosamines in foods. Presented at the International Agency for Research on Cancer and the Deutsches Krebsforschungszentrum Joint Meeting on the Analysis and Formation of Nitrosamines, Heidelberg, October.

Issenberg, P., Kobayashi, A. and Mysliwy, T.J. 1969. Combined gas chromatography-mass spectrometry in flavor research: Methods and applications. J. Agr. Food Chem. 17: 1377.

Lijinsky, W. and Epstein, S.S. 1970. Nitrosamines as environmental carcinogens. Nature 225: 21.

Likens, S.T. and Nickerson, G.B. 1964. Detection of certain hop oil constituents in brewing products. Proc. Am. Soc. Brewing Chemists, p. 5.

Nunn, J.R. and du Plessis, L.S. 1971. N-nitrosamine analysis. Part 1. Estimation of low molecular weight alkyl N-nitrosamines. Presented at the International Agency for Research on Cancer and the Deutsches Krebsforschungszentrum Joint Meeting on the Analysis and Formation of Nitrosamines, Heidelberg, October.

Rhoades, J.W. and Johnson, D.E. 1970. Gas chromatography and selective detection of N-nitrosamines. J. Chromatog. Sci. 8: 616.

Sen, N.P. 1971. Multidetection methods for determining volatile nitrosamines in foods. Presented at the International Agency for Research on Cancer and the Deutsches Krebsforschungszentrum Joint Meeting on the Analysis and Formation of Nitrosamines, Heidelberg, October.

Sen, N.P., Smith, D.C., Schwinghamer, L. and Howsam, B. 1970. Formation of nitrosamines in nitrite treated fish. Can. Inst. Food Technol. J. 3: 66.

Telling, G.M., Bryce, T.A. and Althorpe, J. 1971. Use of vacuum distillation and gas chromatography-mass spectrometry for determination of low levels of volatile nitrosamines in meat products. J. Agr. Food Chem. 19: 937.

Wasserman, A.E. 1971. A survey of analytical procedures for N-nitrosamines. Presented at the International Agency for Research on Cancer and the Deutsches Krebsforschungszentrum Joint Meeting on the Analysis and Formation of Nitrosamines, Heidelberg, October.

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CHEMISTRY OF THIAMINE DEGRADATION: 4-Methyl-5-(β-Hydroxyethyl) Thiazole from Thermally Degraded Thiamine

INTRODUCTION

THE HEAT SENSITIVITY of thiamine was recognized almost immediately after thiamine was discovered. Considerable information about the thermal destruction of thiamine during cooking, processing and storage of foods has been published (Rice and Beuk, 1945; Farrer and Morrison, 1949; Bendix et al., 1951; and Hermann and Tunger, 1966). However, most of this information is concerned with the loss of thiamine during the treatment of a particular food under specific conditions. Only recently have the reaction products of thiamine degradation been identified.

Based upon a selective microbial assay, Obermeyer and Chen (1945) reported initial cleavage of thiamine to its pyrimidine and thiazole moieties under conditions encountered in bread baking. Arnold et al. (1969) identified hydrogen sulfide, 2-methyl furan, 2-methyl thiophene, and 2-methyl-4,5 dihydrothiophene from heated solutions of thiamine in phosphate buffer (pH 6.7). Recently, Morfee and Liska (1972) identified elemental sulfur as a major degradation product from slightly acidic or basic buffered thiamine solutions which were heated at 121°C for 40 min. These workers also detected a "compound D" having a molecular weight of about 145. In our work on the heat degradation of thiamine, a major degradation product, 4-methyl-5-(β-hydroxyethyl) thiazole, which has properties comparable to "compound D" has been identified.

EXPERIMENTAL

U.S.P. GRADE thiamine hydrochloride was used in preparing solutions containing 10 mg/ml thiamine. These solutions were brought to pH 5.0, 6.0 and 7.0 with 1N NaOH or with 0.1M phosphate buffer.

Thiamine solutions (10 ml each) were placed in screw-cap vials sealed with Teflon-lined caps. Duplicate samples of thiamine solutions were heated under the following conditions: (1) in sealed tubes in a boiling water bath for 30 min; (2) in tubes open to the air in the boiling water bath for 30 min; (3) autoclaved in sealed tubes for 30 min at 121°C.

The separation of the degradation products of heated thiamine by thin layer chromatography (TLC) was accomplished by the method of Waring et al. (1968). Heated thiamine solutions were spotted on pre-coated silica gel TLC plates containing fluorescent indicator (No. 6060 Chromatogram Sheet, Eastman Kodak Co.). The solvent mixture consisting of acetonitrile/H_2O/formic acid: 40/10/sufficient formic acid to adjust pH to 2.54, was allowed to equilibrate for 15 min in covered chromatography jars before placing chromatogram sheets in the jar for development. Detection of compounds was accomplished in an ultraviolet viewing box using short wavelength (254 nm) for revealing absorbing compounds, and 366 nm for detecting fluorescent compounds.

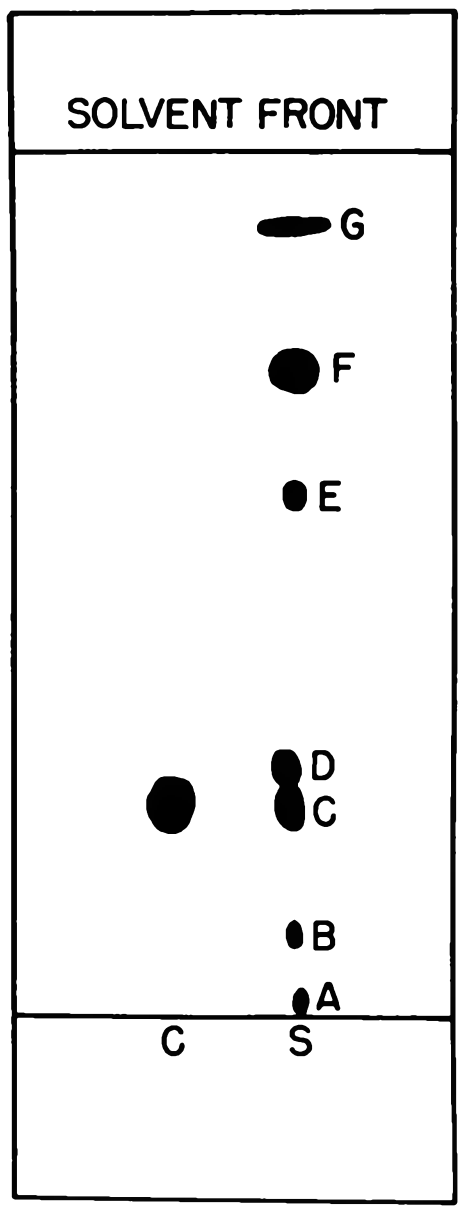


Fig. 1—Thin layer chromatogram of heated thiamine sample pH 6.0. (C) unheated control; (S) heated sample. Solvent system: acetonitrile/H_2O/formic acid: 40/10/sufficient formic acid to adjust pH to 2.54.

Heated thiamine solutions were extracted twice with 50 ml diethyl ether. The ether extracts were concentrated to dryness on a rotary evaporator. During concentration, temperature of the water bath was maintained at 20°C. Purification of the compound responsible for spot F (Fig. 1), which appeared to be a major degradation product was accomplished by diluting the dried extract to 1 ml with deionized distilled water and subjecting it to gel filtration chromatography on a 2.5 cm x 45 cm Sephadex column. Sephadex G-10 gel was prepared by allowing the gel to swell in deionized distilled water for 6 hr, and deaerating the resulting gel in a vacuum desiccator. 6-ml fractions were collected from the gel filtration column in 10 ml tubes on a fraction collector equipped with 254 nm UV monitor (Instrumentation Specialties Co., Inc.). All fractions were chromatographed on thin layer plates as previously described. Fractions possessing the R_f value of spot F were pooled and extracted twice with 100 ml diethyl ether. The ether solution was dried with 25g sodium sulfate. Ether was evaporated on a rotary evaporator at 20°C. A light yellow viscous liquid thus obtained was checked for purity by TLC and gas liquid chromatography (GLC). A Varian Aerograph, Model 1200, gas-chromatograph with a flame ionization detector was employed. The column was 1.5 m x 3.2 mm od stainless steel packed with 6% SE 30 on Chromosorb G. The column temperature was maintained at 85°C.

An infrared (IR) spectrum of this compound was obtained with a Beckman IR 5A spectrophotometer. A TMS derivative of compound F was prepared according to the procedure of Horning et al. (1967). Mass spectra of compound F and its TMS derivative was obtained on an Atlas CH-4 mass spectrometer equipped with a Honeywell 1508 Visicorder oscillograph. The nuclear magnetic resonance spectrum (NMR) of the compound was recorded in $CDCl_3$ on a Varian Associates' XL-100 NMR spectrometer. The UV spectrum of the compound was recorded with a Beckman spectrophotometer.

RESULTS & DISCUSSION

A TYPICAL thin layer chromatogram of the heated thiamine sample as seen under

short wave ultraviolet light is shown in Figure 1. Under the aforementioned experimental conditions, different heat treatments and pH conditions gave similar patterns on thin layer chromatograms, although the relative proportions of degradation products changed with different heating conditions. Spot C was undegraded thiamine as determined by a control thiamine sample. Spot F was very intense, indicating that this compound is a major degradation product. The R_f value of spot F was compared with thiamine related compounds and found to be equal to that of authentic 4-methyl-5-(β-hydroxyethyl) thiazole (Merck and Co., Inc.). Two fluorescent spots were also observed by TLC, but their concentration appeared to be extremely low. The intensity of the fluorescence of these spots increased when pH 7.0 thiamine solutions were extensively heated. Spot G was most likely elemental sulfur (Morfee and Liska, 1972) and some other relatively nonpolar compounds.

The separation of ether soluble degradation products of heated thiamine on Sephadex G-10 gave a fraction which was eluted just after the void volume. This fraction gave a single spot in TLC with the R_f value of 4-methyl-5-(β-hydroxyethyl) thiazole and a single peak by GLC. To check for possible modification or rearrangement of this compound as it eluted from the GLC column, an IR spectrum of a sample trapped from the GLC effluent was obtained. This spectrum was identical to the IR spectrum of the authentic 4-methyl-5-(β-hydroxyethyl) thiazole sample.

The UV spectrum of the compound from spot F in water was in agreement with the reported UV spectrum of 4-methyl-5-(β-hydroxyethyl) thiazole (Makino and Imai, 1936). When the UV spectrum of this compound was recorded in carbon tetrachloride, a shift of the absorption maximum by about 10 nm towards lower wavelength was noted. Since the extinction coefficient remained unchanged, this shift was considered to be a solvent effect.

The IR spectrum of the compound F when recorded as a thin film (Fig. 2) resembled closely the infrared spectrum of 4-methyl-5-(β-hydroxyethyl) thiazole reported by Neal (1968). When the IR spectrum of the compound was recorded in carbon tetrachloride (Fig. 3) using two identical IR cells in a double beam mode, the broad OH peak near 3300 cm^{-1} decreased considerably, likely due to the free OH group in dilute solution (Colthup et al., 1964). The position of the C-O stretching band shifted from 1040 cm^{-1} to 1105 cm^{-1}, and two additional peaks between 1400 and 1500 cm^{-1} appeared, possibly due to ring strain. Based on these considerations, compound F in nonpolar

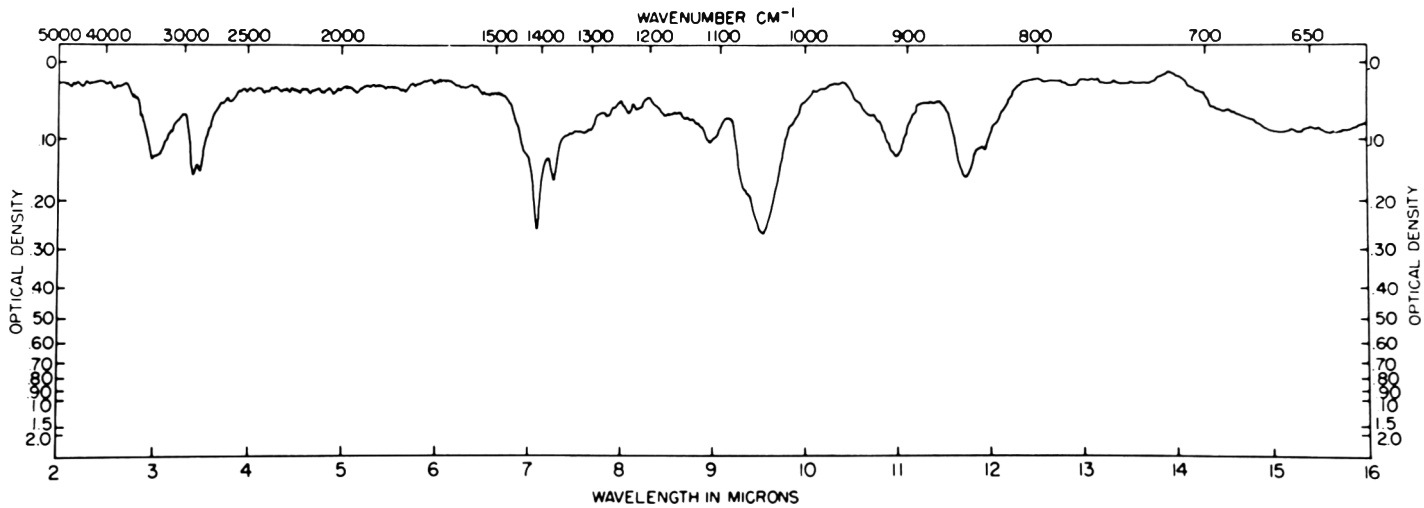


Fig. 2—Infrared spectrum of compound F (thin film).

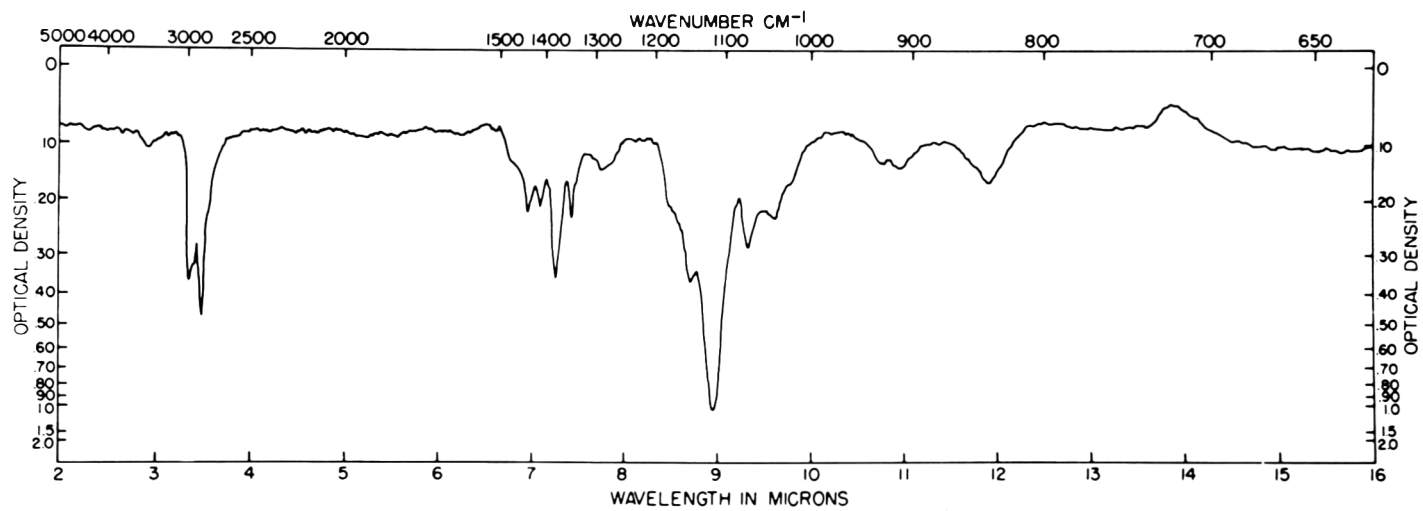


Fig. 3—Infrared spectrum of compound F (CCl_4 double beam mode).

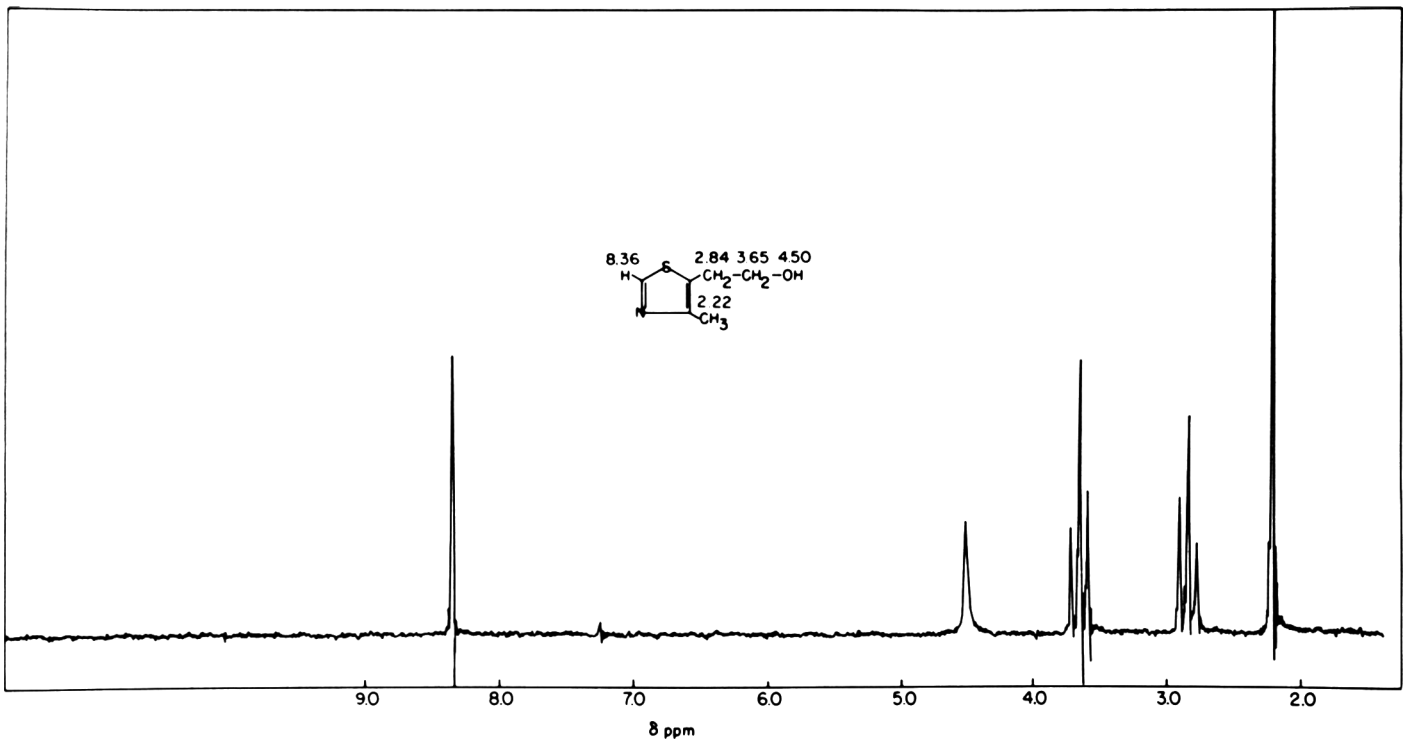
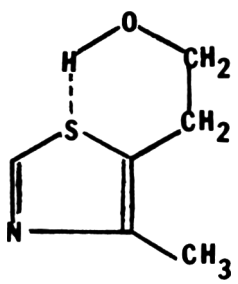


Fig. 4—NMR spectrum of compound F.

solvents may exist in an internally hydrogen bonded form, as shown:



The NMR spectrum of this compound has not been reported previously. Asahi (1968) published the NMR spectrum of 3,4-dimethyl-5-(β-hydroxyethyl) thiazolium iodide in D_2O. The chemical shifts of various groups of 4-methyl-5-(β-hydroxyethyl) thiazole and the corresponding chemical shifts in the NMR spectrum of 3,4-dimethyl-5-(β-hydroxyethyl) thiazolium iodide are reasonably close. The chemical shifts of the different groups in this compound are recorded in the NMR spectrum (Fig. 4).

The mass spectrum of the compound isolated from heated thiamine solution is shown in Figure 5. This spectrum is in close agreement to the mass spectrum of 4-methyl-5-(β-hydroxyethyl) thiazole previously reported by Shima et al. (1969). The mass spectrum of the TMS derivative exhibited a fragmentation pattern similar to that reported for the TMS derivative of 4-methyl-5-(β-hydroxyethyl) thiazole by Amos and Neal (1970).

In pure form, 4-methyl-5-(β-hydroxyethyl) thiazole appeared as an oily, viscous liquid. It did not have the sharp, disagreeable or pungent odor as described by Morfee and Liska (1972) for their compound D isolated from heated thiamine solutions in which the pH was adjusted with 1N NaOH. However, when phosphate buffer was used, our fraction F did have a pungent odor. Apparently the latter fraction contained a pungent smelling contaminant, which may be closely related to 4-methyl-5-(β-hydroxyethyl) thiazole. This contaminant could not be separated by any of the usual techniques. Possibly this compound existed in very minute concentrations. This contaminant was also present in the authentic sample obtained from Merck and Co., as this sample had a sharp, disagreeable odor.

4-Methyl-5-(β-hydroxyethyl) thiazole appears to be a major degradation product of thiamine heated under slightly acidic conditions. The UV, infrared, NMR and mass spectra of both authentic and isolated sample were identical. To date, this compound has not been isolated from any food product. Lack of standard IR, NMR and mass spectral data on this compound may be a reason. Furthermore, degradation and/or complexing with other compounds is also a possibility.

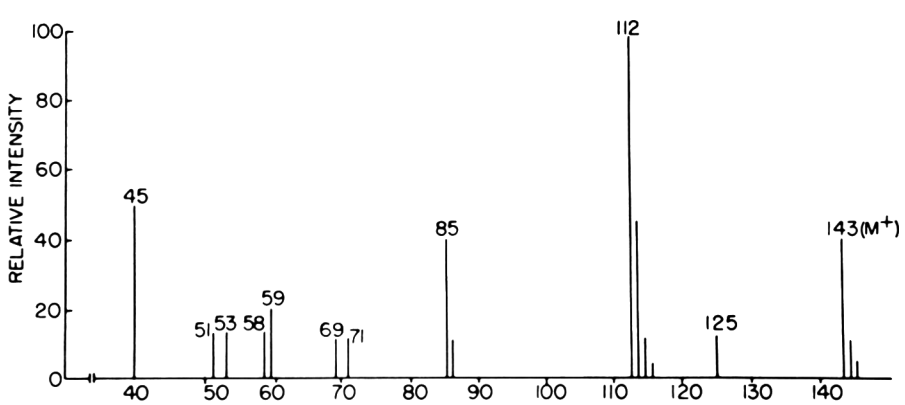


Fig. 5—Mass spectrum of compound F.

REFERENCES

Amos, W.H. and Neal, R.A. 1970. Gas chromatography–mass spectrometry of the trimethylsilyl derivatives of various thiamine metabolites. Analytical Biochem. 36: 332.

Arnold, R.G., Libbey, L.M. and Lindsay, R.C. 1969. Volatile flavor compounds produced by heat degradation of thiamine. J. Agr. & Food Chem. 17: 390.

Asahi, Y. 1968. NMR spectra of compounds related to thiamine. Annual Report Takeda Res. Labs. (Japan) 27: 58.

Bendix, G.H., Heberlein, D.G., Ptak, L.R. and Clifcorn, L.E. 1951. Factors influencing the stability of thiamine during heat sterilization. Food Res. 16: 494.

Colthup, N.B., Daly, L.H. and Wiberley, S.E. 1964. "Introduction to Infrared and Raman Spectroscopy," p. 189. Academic Press, New York.

Farrer, K.T.H. and Morrison, P.G. 1949. The thermal destruction of Vitamin B. 6. The effect of temperature and oxygen on the rate of destruction of aneurin. Australian J. Exptl. Biol. Med. Sci. 27: 517.

Hermann, J. and Tunger, L. 1966. Thermal destruction of thiamine in relation to moisture content of food, with special reference to flour products. Nahrung 10(8): 705. [Chem. Abs. 66: 75031g.]

Horning, M.G., Boucher, E.A. and Moss, A.M. 1967. The study of urinary acids and related compounds by gas phase analytical methods. J. Gas Chromatog. 5: 297.

Makino, K. and Imai, T. 1936. Chemistry of antineuretic vitamin. Z. Physiol. Chem. 239: 1.

Morfee, T.D. and Liska, B.J. 1972. Elemental sulfur: Degradation product of thiamine in model systems. J. Dairy Sci. 55: 124.

Neal, R.A. 1968. Bacterial metabolism of thiamine. 1. The isolation and characterization of the initial intermediates in the oxidation of thiamine. J. Biol. Chem. 243: 4634.

Obermeyer, H.G. and Chen, L. 1945. Biological estimation of the thiazole and pyrimidine moieties of Vitamin B_1. J. Biol. Chem. 159: 117.

Rice, E.E. and Beuk, J.F. 1945. Reaction rates of decomposition of thiamine in pork at various cooking temperatures. Food Res. 10: 99.

Shima, T., Nishikawa, M., Ouchida, A. and Asahi, Y. 1969. Mass spectra of thiamine and related compounds. Shitsuryo Bunseki. 17: 767.

Waring, P.P., Wayne, C.G. and Ziporin, Z.Z. 1968. The use of thin layer chromatography to separate thiamine and related compounds as well as N-methylnicotinamide and related compounds. Anal. Chem. 24: 185.

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CRYSTALLIZATION IN SOLUTIONS SUPERSATURATED WITH SUCROSE AND LACTOSE

INTRODUCTION

LACTOSE, naturally present in most milk products, is proving useful in an ever-increasing number of food products (Webb and Johnson, 1965) because of its unique physical-chemical properties. Being less sweet than most other commercial sugars, it can be added to many foods to increase osmotic pressure or viscosity or to improve texture without making the food excessively sweet. Also with increasing frequency, new food products contain a mixture of sugars. In candy, lactose can change the crystallization habits of other sugars present (Webb, 1966), thereby improving body, texture, chewiness, or shelf-life.

There are many reports on various aspects of sucrose or lactose crystallization from solutions, but few on mixtures of these sugars. Nor is there much information concerning the influence of one sugar on the crystal habit of the other.

EXPERIMENTAL

THE MATERIALS used were USP-grade α-lactose hydrate and commercial sucrose. The standard was a 75% sucrose solution against which were compared solutions with lactose replacing 10, 20, 30, 40 and 50% of the sucrose (Table 1).

The calculated amounts of lactose and water were weighed into a stainless-steel container and dissolved by heating. The calculated amount of sucrose was then added, and the mixture was heated to dissolve the sugar. The samples were stored at room temperature (approximately 27°C). Samples were drawn at regular intervals from each batch and centrifuged at 27,000 × G for 10 min. The clarified supernatant liquid was then analyzed for lactose and sucrose by a polarimetric method (Kendrew and Moelwyn-Hughes, 1940).

Final analyses of total solids, refractive index, and lactose and sucrose contents of the liquid portion were made after 10 days of storage at room temperature. The sediment of crystals was removed by carefully draining off as much of the supernatant liquid as possible and then washing the crystals several times with ice water on a Buchner funnel. The washed crystals were dried at 100°C in vacuum oven and analyzed for lactose and sucrose content by the polarimetric method.

After crystallization was complete, the apparent viscosities of the solutions were measured at room temperature (27°C) with a Brookfield Viscometer, model LVT, using a No. 3 spindle at 3, 6, 12, 30 and 60 rpm. Since the crystals tended to settle out, the samples were mixed thoroughly to disperse the contents uniformly before the final reading. Container size and solution height were the same for all samples during viscosity measurements.

The growth habits of the crystals in all solutions were observed under the microscope at 10× magnification and photomicrographs were prepared.

Rate of crystallization, crystal habit, viscosity and compositions of the liquid and solid portions at equilibrium were studied as influenced by lactose seeding, sucrose seeding, seeding with a mixture of lactose-sucrose and without seeding. 1g of seed was used per 604.8g batch (Table 1) after cooling to 30°C. In most cases, crystallization was allowed to proceed undisturbed, i.e., without agitation.

After undisturbed storage at room temperature for more than 2 months, the hardness of the crystalline mass in each sample was measured by depth of penetration with a "Precision" Junior Penetrometer. Penetration time was 10–15 sec, and the weight of the test rod, including rod and cone, was 127.50g.

RESULTS & DISCUSSION

Rate of crystallization

It is recognized that the rate constant tends to increase as supersaturation is increased (Hook, 1944), though Michaels and Kreveld (1966) observed that the growth rate of single lactose crystals increased at a power of the supersaturation greater than one. This power was different for different faces of the crystals. Twieg and Nickerson (1968) showed that the overall rate constant for lactose crystallization from solutions was not proportional to the supersaturation, but increased with some power of supersaturation greater than one.

Figures 1 to 4 show changes in composition of the liquid portions of the solutions with time as a result of crystallization. On cursory examination, the line drawings appear similar. A more critical inspection, however, reveals that crystallization rate was influenced by changes in sucrose-lactose balance.

Figures 1 to 4 show that the sucrose concentration decreased with time in all solutions containing at least 70% sucrose. The cause was crystallization of the sucrose from the supersaturated solutions. Crystallization rate was influenced by seeding regimen and lactose concentration as well as by sucrose concentration. As expected, seeding crystals gave more rapid and uniform crystallization.

Since the 70% sucrose solution was close to the saturation level, crystallization of sucrose was slight. In the 50 and 60% sucrose solutions, sucrose concentration did not change (Fig. 1 to 4) in storage even with seeding. Thus, the sucrose solubility had not been exceeded.

On the other hand, lactose solubility was exceeded even at the 10% level and subsequent crystallization reduced the soluble lactose to a nearly constant level, usually within a 48 hr period. As the concentration of lactose was increased in the range of 10–50%, the rate of lactose crystallization increased greatly. At the 10% replacement level of sucrose by lactose, the lactose was slow to crystallize and to establish equilibrium, even when the solution was seeded (all Fig. 1–4). At the high replacement levels of 40 and 50%, crystallization was rapid, as shown by the steep drop in lactose concentration in these solutions during the first 24 hr. Solutions containing intermediate levels of lactose required intermediate time for complete crystallization (Fig. 1).

Table 1—Solutions used in lactose crystallization studies

	Solution	Sucrose (g)	Lactose[a] (g)	Water (g)
I.	All sucrose	453.60 (75%S)	–	151.20
II.	90% sucrose (10%L)	408.24 (67.5%S)	45.36	151.20
III.	80% sucrose (20%L)	362.88 (60%S)	90.72	151.20
IV.	70% sucrose (30%L)	317.52 (52.5%S)	136.08	151.20
V.	60% sucrose (40%L)	272.16 (45.0%S)	181.44	151.20
VI.	50% sucrose (50%L)	226.80 (37.5%S)	226.80	151.20

[a]Alpha lactose hydrate

[1]Current address: North American Lab. Co., Inc., Indianapolis, Ind.

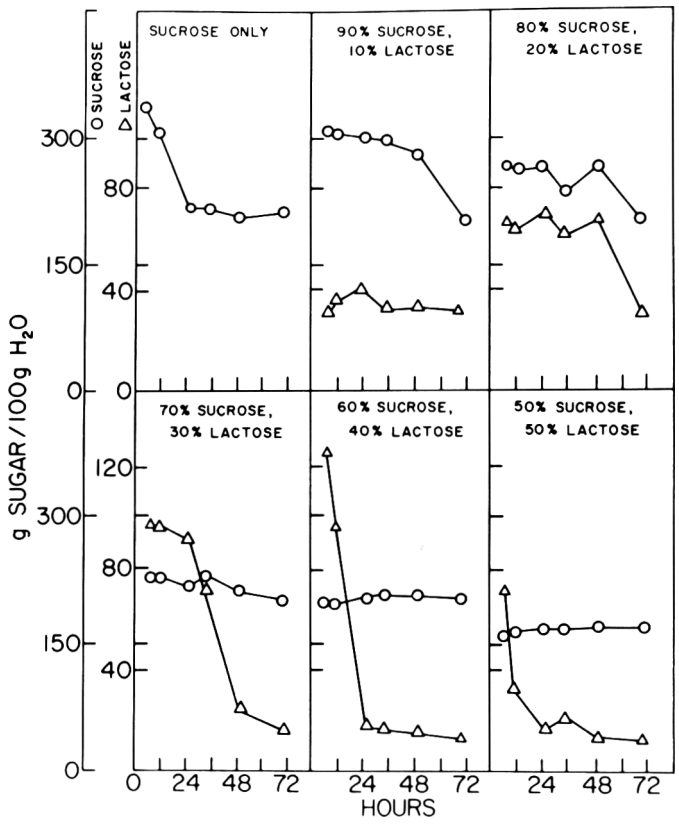

Fig. 1—Effect of replacing sucrose with lactose on changes in solution composition during normal crystallization.

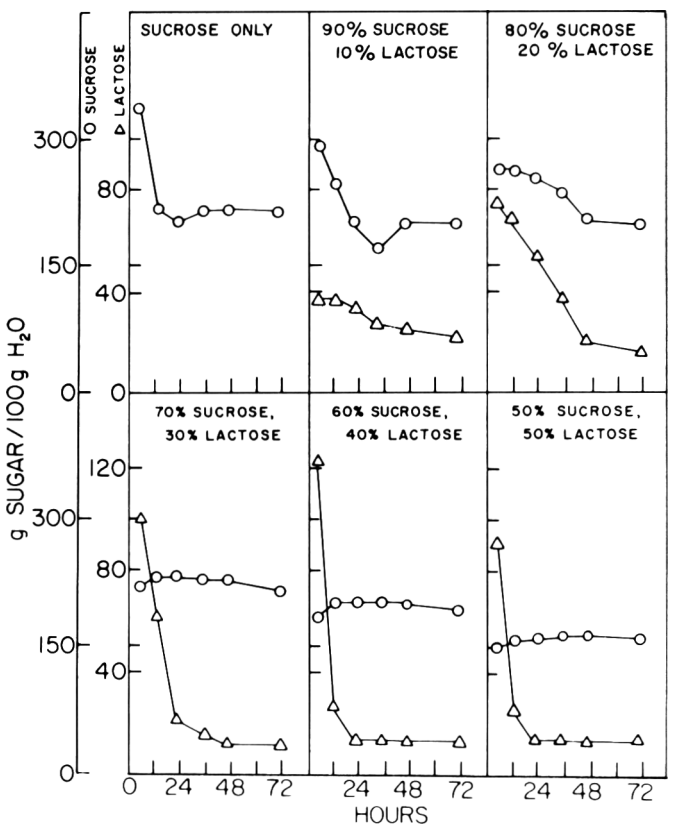

Fig. 2—Effect of replacing sucrose with lactose on changes in solution composition following seeding with lactose crystals.

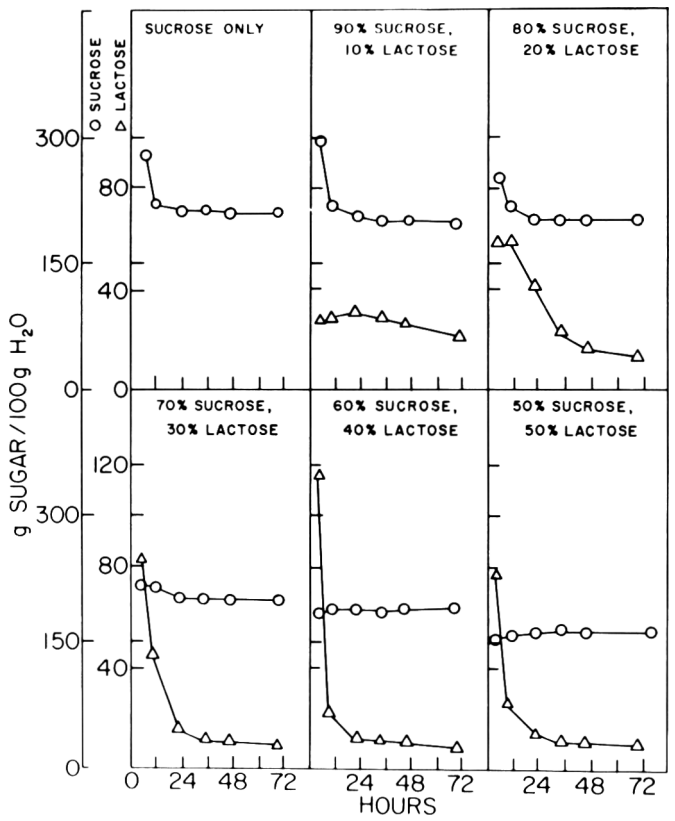

Fig. 3—Effect of replacing sucrose with lactose on changes in solution composition following seeding with sucrose crystals.

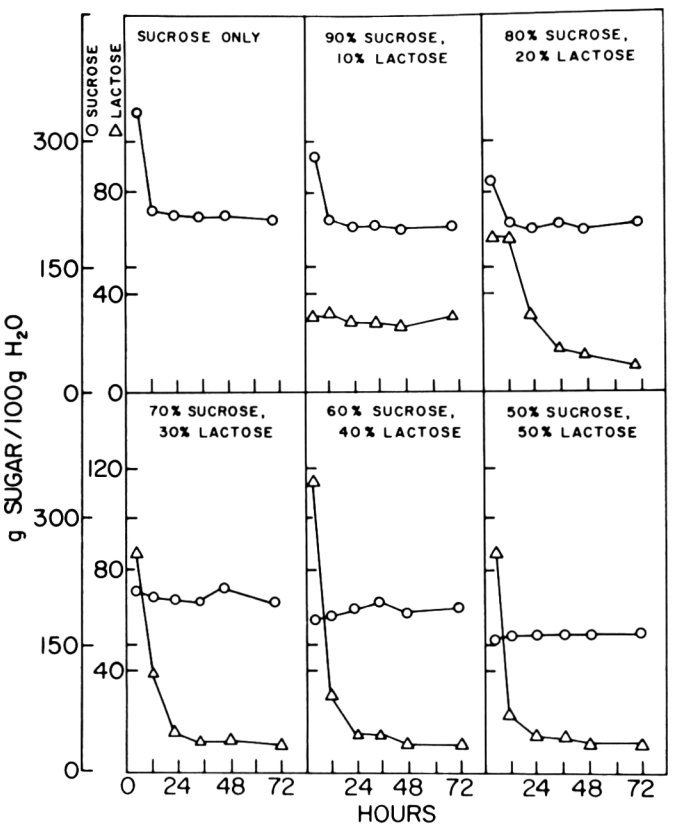

Fig. 4—Effect of replacing sucrose with lactose on changes in solution composition following seeding with a mixture of lactose and sucrose crystals.

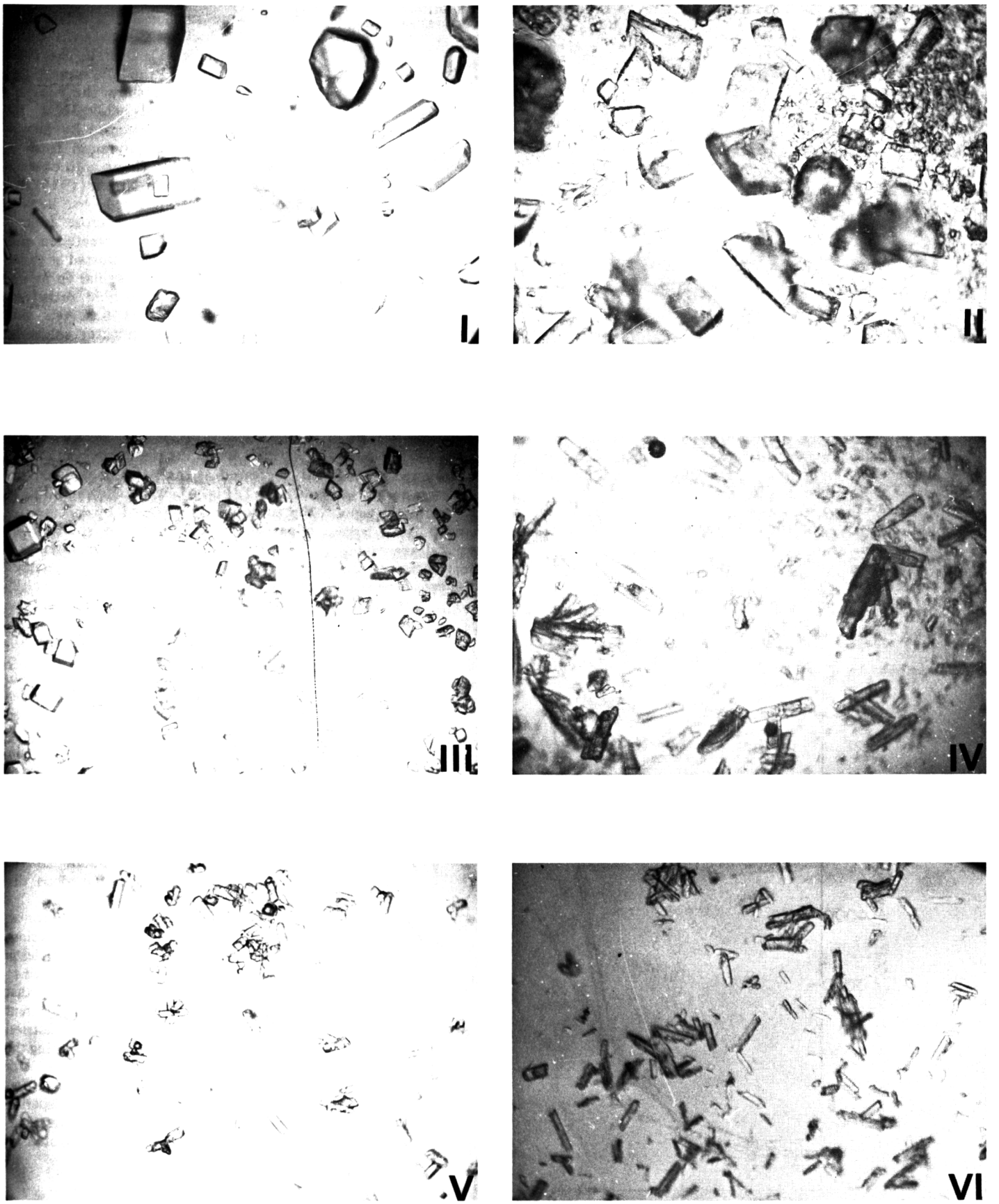

Fig. 5–Photomicrographs showing effect of replacing portions of sucrose with lactose on the crystal habit (no seeding). I. 100% sucrose; II. 90% sucrose, 10% lactose; III. 80% sucrose, 20% lactose; IV 70% sucrose, 30% lactose; V. 60% sucrose, 40% lactose; VI. 50% sucrose, 50% lactose.

Table 2–Average compositions of liquid and crystalline portions of lactose-sucrose solutions after complete crystallization (10 days, 27°C)

Solution		Liquid portion			Crystalline portion	
		% sucrose (g/100g soln)	Sucrose (g/100g H_2O)	Lactose (g/100g H_2O)	Sucrose (%)	Lactose (%)
I.	All sucrose	67.0	206.9		99.9	–
II.	10% lactose	62.4	197.0	15.0	89.2	10.8
III.	20% lactose	63.8	200.0	13.4	62.7	37.3
IV.	30% lactose	64.4	201.9	11.5	44.9	55.1
V.	40% lactose	63.9	195.8	10.5	34.4	65.6
VI.	50% lactose	59.6	164.7	10.6	18.2	81.8

Seeding speeded the crystallization of both sugars in solution, particularly at the intermediate concentrations (Fig. 2, 3, 4, vs. Fig. 1). At the high concentrations, spontaneous nucleation undoubtedly occurred, initiating rapid crystallization. Crystallization of the mixture of lactose and sucrose was much smoother and more rapid with (Fig. 4) seeding than when normal development of nuclei was relied upon (Fig. 1). Affected especially by seeding, therefore, were the crystallization curves for solutions with low precipitation pressure (supersaturation).

Also, differences in rates of change in the liquid portions were shown to depend upon whether the solutions were unseeded or were seeded with lactose, sucrose, or both. After 10 days, however, seeding had little influence on the final composition of the liquid and solid phases (Table 2).

Crystal habit

The modification of the lactose crystal form became more pronounced as the sucrose concentration increased, and, similarly, sucrose crystal habit was modified as the lactose concentration was increased. Sucrose crystals normally grow relatively large and tend to form large masses of intergrown crystal aggregates. Hence, the crystalline portion from solutions containing only sucrose became very hard. As the proportion of lactose was increased, both lactose and sucrose crystals were smaller and had less tendency to interlock, as shown in the photomicrographs (Fig. 5). Overall, the 100% sucrose solution and the 90% sucrose plus 10% lactose solution show the fully developed sucrose crystals, while 30, 40 and 50% replacement of sucrose by lactose shows the prism and pyramid form of lactose crystals. This agrees with observations of Hunziker and Nissen (1934) that lactose crystals appeared to become shorter and broader as sucrose concentration was increased in sweetened condensed milk. The crystal started as rhomboid rather than the triangular form, and built up from the rhomboid form into truncated pyramids, as observed with crystals from lactose-in-water solutions.

Thus, mixing of lactose and sucrose modified the crystal-forming habits of both sugars in the solutions.

Viscosity

The viscosity of the lactose-sucrose solutions, including the crystalline material, increased as the percentage of lactose increased (Fig. 6). When 20% and 30% of the sucrose was replaced by lactose, the apparent viscosity of the solution increased 1.6–1.8 times in the absence of seeding. Viscosity also increased as crystals developed through seeding, especially when 40% of the sucrose was replaced with lactose and the solution then seeded with lactose. When lactose replaced 20% and 30% of the sucrose, the viscosity resulting from crystallization was about the same whether the solution was seeded with lactose or with sucrose. Moreover, the solutions with 40% and 50% replacement of sucrose by lactose showed non-Newtonian behavior as a result of the seeding. Table 2 shows that the liquid portions of the solutions with 20, 30 and 40% replacement were surprisingly similar in composition. Therefore the differences in viscosity can be attributed to the differences in the crystalline mass dispersed in the solutions.

It is recognized that non-Newtonian flow results from mechanical obstructions and that the size and shape of particles in solution determine the force required to move them. It is also recognized that different rates of flow result in different degrees of alignment of asymmetrical particles, with the alignment affecting the force required to maintain the flow. With the diversity of crystal sizes and differences in composition of the dispersed phase in the solutions, it is understandable that non-Newtonian flow would be observed.

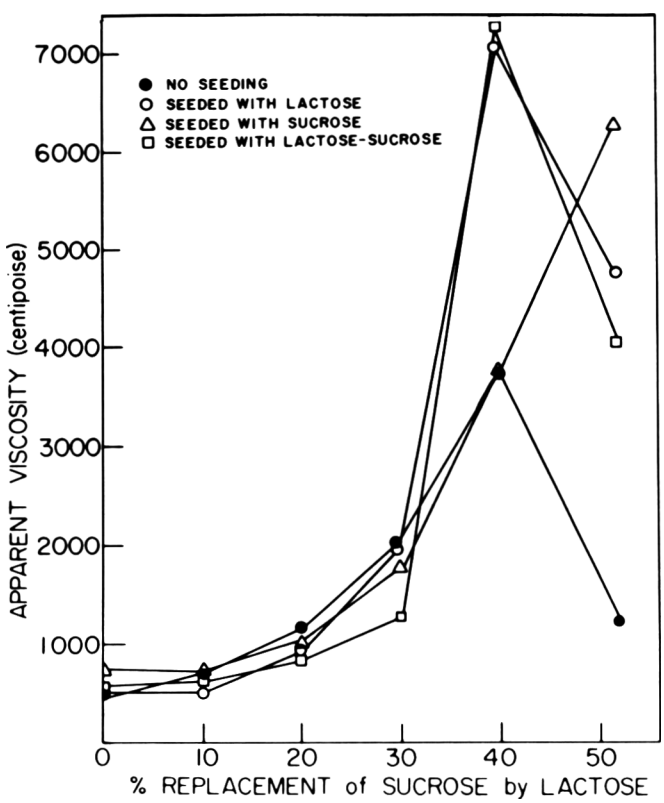

Fig. 6–Effect of solution composition on viscosity following crystallization.

Composition of crystalline mass

Analysis of the crystalline mass showed that as the percentage of lactose in the solutions increased, the proportion of lactose in the crystalline mass also

Table 3–Effect of composition on firmness of the crystalline mass

	Composition of solution					
		% Replacement of sucrose by lactose				
Seeding regimen	100% Sucrose	10	20	30	40	50
	Depth of penetration (mm)					
Without	11.5	13.5	16.5	21.5	29.5	38.0
Lactose alone	12.0	13.0	17.2	23.5	33.8	>39
Sucrose alone	13.0	14.5	19.5	25.5	36.5	>39
Lactose-Sucrose mix	12.0	14.0	17.5	23.0	35.0	>39

increased and the proportion of sucrose decreased (Table 2), as would be expected from their relative supersaturations.

Lactose is less soluble than sucrose and is further reduced in solubility in the presence of high concentrations of sucrose (Nickerson and Moore, 1972). Thus the composition of the crystalline portion separating from the experimental solutions changed rapidly as increasing amounts of lactose were substituted for sucrose. At 10% substitution the composition of the crystalline material was roughly in proportion to the relative amounts of sugar solids in the solution, i.e., 90% sucrose and 10% lactose. The proportion of lactose in the crystalline material increased more rapidly than the proportion of lactose substituted for sucrose, however, so that over 4 times as much lactose as sucrose crystallized from the solution containing equal quantities of sucrose and lactose (Table 2).

Hardness of crystalline mass

As the percentage of lactose increased, the hardness of the crystalline mass decreased (depth of penetration increased). Seeding with lactose, sucrose, or a mixture of both decreased hardness slightly. This shows that the type and number of crystals as influenced by variations in seeding does play a role in determining the firmness of the crystallizing mass. This is minor, however, compared with the influence of composition, i.e., the extent of the substitution of lactose for sucrose. Replacing sucrose with lactose was most effective in reducing the hardness of the crystalline material from the solutions, as shown in Table 3. The crystalline mass was harder when the solutions were not seeded than when they were seeded. Among the seeding procedures, the use of sucrose was most effective in softening the crystalline material, as it produced large numbers of small crystals and reduced the interlocking of the sucrose crystals.

The data developed should be useful where products resulting from the crystallization of sucrose are harder than desired. Obvious examples would be certain candy and confectionery products. A partial substitution of lactose for sucrose, with appropriate seeding, appears to offer considerable promise for improvement of these qualities.

REFERENCES

Hook, A. Van. 1944. Kinetics of sucrose crystallization from pure sucrose solutions. Ind. & Eng. Chem. 39: 1042.

Hunziker, O.F. and Nissen, B.H. 1934. Lactose solubility and lactose crystal formation. 2. Lactose crystal formation. J. Dairy Sci. 17: 139.

Kendrew, J.C. and Moelwyn-Hughes, E.A. 1940. The kinetics of mutarotation in solution. Proc. Roy. Soc. (London) A176: 352.

Michaels, A.S. and Kreveld, A. van. 1966. Influences of additives on growth rates in lactose crystals. Neth. Milk & Dairy J. 20: 163.

Nickerson, T.A. and Moore, E.E. 1972. Solubility interrelations of lactose and sucrose. J. Food Sci. 37: 60.

Twieg, W.C. and Nickerson, T.A. 1968. Kinetics of lactose crystallization. J. Dairy Sci. 51: 1720.

Webb, B.H. 1966. Whey—A low-cost dairy product for use in candy. J. Dairy Sci. 49: 1310.

Webb, B.H. and Johnson, A.H. 1965. "Fundamentals of Dairy Chemistry," Chapter 6. Avi Publishing Co., Westport, Conn.

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DIRECT ENZYMATIC CONVERSION OF LACTOSE TO ACID: GLUCOSE OXIDASE AND HEXOSE OXIDASE

INTRODUCTION

CURRENT BIOLOGICAL methods for the formation of acid in milk are primarily accomplished by the production of lactic acid. Sufficient acid must be formed in milk under quiescent conditions in order to overcome the buffer capacity and lower the pH to 4.5–5.0, where milk coagulates. The behavior of microorganisms utilized for this purpose is not always predictable, since the amount of acid produced varies with each strain and problems can occur due to contamination by other microorganisms and phage.

Alternative approaches to the acidification of milk, which would simplify the process, have met with some success. Deane and Hammond (1960) coagulated skim milk by the addition of lactones and lactides. These acidogens, when dissolved, slowly hydrolyzed to their acid form. McNurlin and Ernstrom (1962) added concentrated lactic or hydrochloric acid to skim milk at 40°F, until a pH of 4.6 was obtained. The milk remained liquid until warmed uniformly without agitation to 70–80°F, where the milk coagulated.

The direct enzymatic conversion of lactose to an acid might also provide a practical and efficient alternative for the acidification of milk. Several oxidoreductases are capable of converting a sugar substrate in the presence of oxygen to a lactone, with subsequent hydrolysis to the corresponding aldobionic acid. Enzymes of this general class, which are specific for glucose or lactose, would appear capable of acidifying milk in a manner similar to that employed by Deane and Hammond (1960). However, the use of an enzyme would eliminate the necessity of adding an acidogen to the milk, since acid would be formed from the sugar already present.

The capability of several bacteria and molds to synthesize an enzyme for the direct conversion of glucose to gluconic acid has been known for some time (Bentley, 1963). Underkofler (1961) discussed the enzyme involved in this conversion, glucose oxidase (EC 1.1.3.4.) and the commercial applications which have been developed. He briefly mentioned the possibility of acidifying milk with this enzyme, and noted that glucose would have to be present, as well as a supply of oxygen from aeration or hydrogen peroxide. This specificity of glucose oxidase puts certain limitations on its use in milk, since either glucose or a second enzyme, lactase (beta-galactosidase) (EC 3.2.1.23) would have to be added. The simplest approach would be to add an enzyme which could utilize lactose directly.

Enzymes which can oxidize lactose, glucose, and galactose have been isolated and studied, but no reports are available on their application for milk acidification. Bean and Hassid (1956) found a hexose oxidase (EC 1.1.3.5) in the marine red alga, *Iridophycus flaccidum*, and demonstrated that the enzyme was capable of oxidizing glucose, galactose, and lactose, as well as maltose and cellobiose to their corresponding aldobionic acids. Hauge (1960a, b) isolated a glucose dehydrogenase (EC 1.1.99) from *Bacterium anitratum* which reacted with a number of aldose sugars, and utilized oxygen and 2,6-dichlorophenolindophenol (DIP) as hydrogen acceptors in the crude form. The purified enzyme did not react with oxygen and functioned independently of NAD or NADP. Nishizuka and Hayaishi (1962) separated a lactose dehydrogenase (EC 1.1.99) from lactose-adapted cells of *Pseudomonas graveolens*. The crude extract of this enzyme also utilized both oxygen and DIP as hydrogen acceptors. However, following partial purification lactose dehydrogenase reacted only with DIP or methylene blue, and did not utilize oxygen.

The two most promising enzymes for milk acidification, with respect to availability and mechanism, would appear to be glucose oxidase and hexose oxidase. The purpose of this study was to examine the direct enzymatic conversion of sugar in milk to an aldobionic acid catalyzed by these two enzymes, and establish whether this reaction would have merit for the acidification of milk.

EXPERIMENTAL

Glucose oxidase

Samples were provided by Dawes Labs., Chicago, Ill. (13,000 units/g); Fermco Labs., Chicago, Ill. (750 units/ml); and Miles Chemical Co., Elkhart, Ind. (750 units/ml). An additional sample was obtained from Nutritional Biochemicals Corp., Cleveland, Ohio (1500 units/g).

Lactase

The preparation used was from Nutritional Biochemicals Corp., Cleveland, Ohio.

Catalase

A sample of purified catalase containing

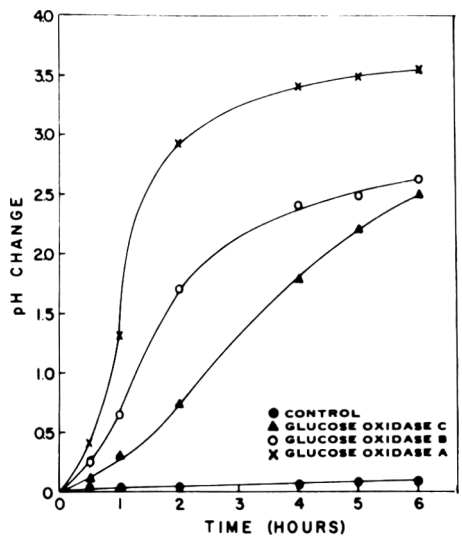

Fig. 1 –Activity of glucose oxidase at 30°C in 0.04M phosphate-citrate buffer at pH 7.0 containing 2.5% glucose (enzyme conc 75 units/ml).

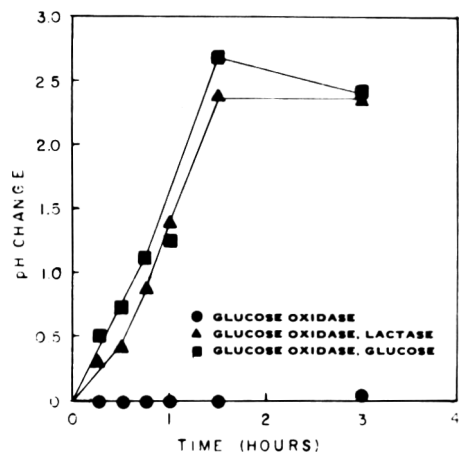

Fig. 2 –Formation of acid in skim milk by glucose oxidase at 24°C (enzyme conc 130 units/ml).

1600 units/ml was provided by Fermco Labs., Chicago, Ill.

Hexose oxidase

The enzyme was prepared from the marine red alga, *Iridophycus flaccidum*, according to the procedure of Bean and Hassid (1956). Samples of the alga were harvested along the California coast, shipped frozen, and stored at −20°C until utilized. A portion of the frozen material (305g) was blended with 200 ml of cold distilled water, agitated gently with a magnetic stirrer for 1 hr, and then kept overnight at 2°C. The tissue was removed by filtration through cheesecloth, and the red extract was dialyzed against tap water overnight. Barium chloride (60g) was dissolved in 350 ml of the dialyzed extract, and the solution was frozen at −20°C. After thawing, the extract was centrifuged at 10,000 × G for 10 min at 0°C. The supernatant was cooled to −2°C, and cold methanol (−20°C) was slowly added in three steps until a 1:1 mixture was formed. After each of the first two methanol additions, the solution was centrifuged at 2500 × G for 10 min at −5°C. Following the third addition of methanol, the solution was centrifuged at 16,000 × G for 20 min at −5°C. The final precipitate was recovered, dissolved in 10 ml of distilled water, and dialyzed against distilled water at 2°C.

Enzymatic acidification

Acid-forming activity of glucose oxidase and hexose oxidase was determined with a substrate consisting of 0.04M phosphate-citrate buffer at pH 6.5–7.0 containing glucose, or reconstituted skim milk containing the appropriate sugar, either glucose or lactose. The general procedure was to temper the substrate at a selected temperature in a water bath. The oxidase enzyme solution was added, and the reaction was followed by pH measurements at regular intervals as determined with a Beckman Expandomatic pH meter equipped with an expanded scale and temperature compensator All results were expressed as the change in pH from the zero time reading. The data presented are usually typical of several trials.

RESULTS

Glucose oxidase

In order to establish the feasibility of enzymatic conversion of sugar for acid formation, the reaction with glucose oxidase was studied initially. Figure 1 shows a comparison of the rate of acid formation in buffered glucose solutions with different enzyme preparations. A gradual change in pH occurred with time for each glucose oxidase, indicating the conversion to gluconic acid. However, Sample A appeared to be the most active enzyme under these conditions, and this preparation of glucose oxidase was utilized in all subsequent studies.

The action of glucose oxidase in reconstitited skim milk is illustrated in Figure 2. The substrate was prepared by rehydrating 9% nonfat dry milk solids (NDM) in distilled water. Separate portions were modified by adding 5% glucose to one, while another contained 0.25% of the enzyme lactase which was incorporated into the milk just prior to the start of the experiment. Glucose oxidase was added to both of these solutions, as well as a sample of the original milk substrate. The pH changed rapidly in the milk samples containing added glucose or lactase when compared to the control of milk plus glucose oxidase. During this experiment, it was noted that local coagulation occurred at the surface of the samples containing added glucose or lactase when the pH approached 5.0. Apparently the enzyme used up available oxygen in the milk, and then continued to react only at the surface.

Commercial glucose oxidase preparations normally contain catalase in order to decompose hydrogen peroxide formed during the reaction with glucose (Underkofler, 1961). The addition of hydrogen peroxide to the reaction mixture, in the presence of catalase, would supply oxygen throughout the system and avoid local coagulation, as suggested by Underkofler (1961). The effect of hydrogen peroxide on the action of glucose oxidase is shown in Figure 3. In all cases, the substrate was 2.5% glucose dissolved in 0.04M phosphate-citrate buffer at pH 7.0. In Figure 3A, it is evident that the rate of reaction increased with increasing concentrations of hydrogen peroxide from 0–0.2%. Since the reaction was quite rapid at the higher concentrations of hydrogen peroxide, the experiment was repeated and the amount of glucose oxidase was reduced from 50 to 10 units per ml of the solution. The results presented in Figure 3B demonstrate that a decrease in the rate of reaction occurred with the reduction in enzyme concentration. However, the change in pH at 0.1 and 0.2% hydrogen peroxide was still rapid and essentially complete in 12 min.

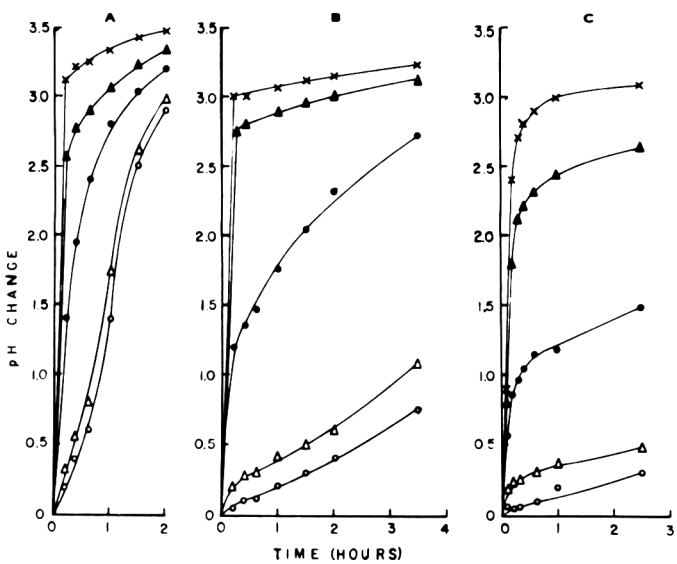

Fig. 3.–Effect of hydrogen peroxide on the formation of acid at 35°C by glucose oxidase. Substrate: 0.04M phosphate-citrate buffer at pH 7.0 containing 2.5% glucose; Glucose oxidase conc: A-50 units/ml, B-10 units/ml and C-1 unit/ml; Hydrogen peroxide conc: ○ 0%, △ 0.01%, ● 0.05%, ▲ 0.10% and × 0.20%.

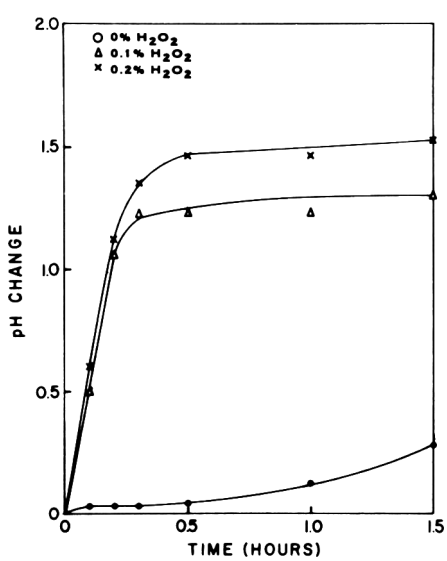

Fig. 4 –Effect of hydrogen peroxide on the formation of acid at 35°C by glucose oxidase in skim milk containing 2.5% glucose (enzyme conc 1 unit/ml).

A third experiment with hydrogen peroxide was conducted with a further reduction in the glucose oxidase concentration to 1 unit per ml of the reaction mixture, as shown in Figure 3C. This level of enzyme concentration made possible an accurate determination of the reaction at all levels of hydrogen peroxide, and it appeared that each reaction rate decreased as the supply of hydrogen peroxide was exhausted by the catalase.

The effect of hydrogen peroxide on the action of glucose oxidase in milk is presented in Figure 4. Reconstituted skim milk containing 9% NDM, 2.5% glucose, and 1 unit/ml of glucose oxidase was incubated at 35°C. Hydrogen peroxide concentrations of 0.1 and 0.2% were compared with a sample containing only enzyme. A smooth uniform gel was formed in the sample containing 0.2% hydrogen peroxide after 30 min.

Hexose Oxidase

The level of enzymatic activity in 1 ml of the partially purified hexose oxidase solution was assayed in 10 ml of a 3% glucose substrate dissolved in 0.04M phosphate-citrate buffer at pH 6.5, and compared against two different glucose oxidase preparations which contained 11 units/ml, as shown in Figure 5. There was definite acid production catalyzed by the solution derived from *Iridophycus flaccidum*, confirming the presence of oxidase activity. The reaction rates of the two glucose oxidase samples were linear over the first 5 hr and essentially identical, demonstrating the reproducibility of the method. From this data, one unit of enzyme activity was defined as the amount of enzyme which produced a pH change of 0.02 units per hour at 30°C under the above conditions.

The hexose oxidase solution was examined for its effect on skim milk. Figure 6 shows the formation of acid by hexose oxidase in reconstituted skim milk containing 9% NDM. A volume of 10 ml of milk, containing a crystal of thymol, was placed in a 50 ml beaker and 1 ml of the enzyme solution containing 1 unit was added to start the reaction. The enzyme was the only addition to one sample of milk, while a second also contained 3% added glucose, and a third contained 0.25% lactase. All samples were held at 30°C and evaluated against a milk control. Hexose oxidase catalyzed a very slow pH change in all samples. The most rapid reaction occurred in the sample containing lactase, while the slowest change in pH was produced when lactose alone was the substrate.

In an attempt to increase the rate of enzyme activity, catalase and hydrogen peroxide were introduced into the reaction mixture. A solution of catalase and hexose oxidase was prepared, which contained 1 unit of oxidase activity and 200 units of catalase per ml. Three reaction samples were established by combining 2 ml of the enzyme mixture with 20 ml of reconstituted skim milk containing 9% NDM and a crystal of thymol. The enzyme mixture was the only addition to one sample of milk while 0.1% hydrogen peroxide was added to the second, and the third contained 0.1% hydrogen peroxide and 0.125% lactase. A fourth sample of milk was maintained as a control. All four samples were incubated at 30°C, and the results are presented in Figure 7. The rate of acid formation increased slightly in the presence of hydrogen peroxide when lactose was the substrate. However, when glucose and galactose were both available in the presence of hydrogen peroxide, there was a marked increase in the rate of reaction and in the amount of acid produced, as was indicated in Figure 6. Part of this increase may be due to the presence of additional catalase in the lactase preparation which was used. However, it is significant that a smooth uniform gel was formed within 18 hr by hexose oxidase, in combination with catalase and lactase under the above conditions.

DISCUSSION

UNDER THE appropriate conditions, both glucose oxidase and hexose oxidase can catalyze the conversion of sugar in milk to an acid for coagulation and gel formation. The reaction appears to be highly reproducible, as shown in Figure 5. This comparison of separate preparations of glucose oxidase at identical levels of activity illustrates the dependability of the method. A source of oxygen was shown to be an essential factor in order to provide a uniform rate of reaction in milk. The combination of hydrogen peroxide and catalase does fulfill this requirement and provides an even oxygen distribution under the quiescent conditions necessary for gel formation. Also, the level of hydrogen peroxide and catalase may provide a method for controlling the enzymatic conversion of sugar to acid. This could be a particularly important factor in the potential application of glucose oxidase for milk acidification, since Underkofler (1961) has reported

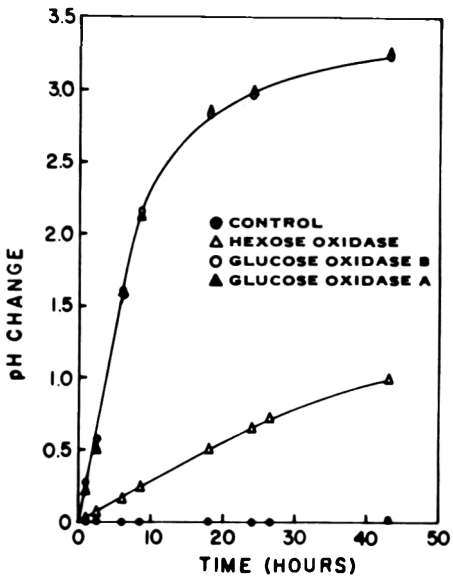

Fig. 5 —A comparison of the activity of hexose oxidase with glucose oxidase at 30°C in 0.04M phosphate-citrate buffer at pH 6.5 containing 3.0% glucose.

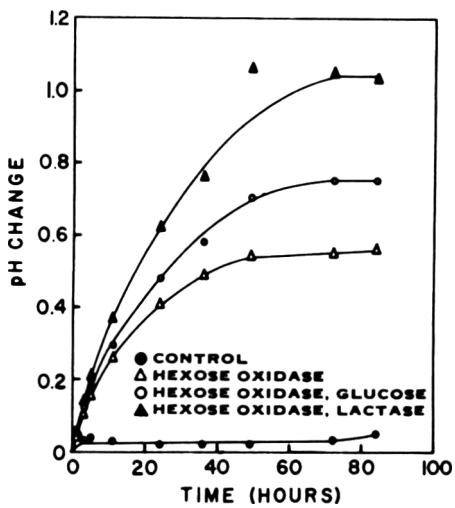

Fig. 6 —Formation of acid in skim milk at 30°C by hexose oxidase (enzyme conc 0.1 unit/ml).

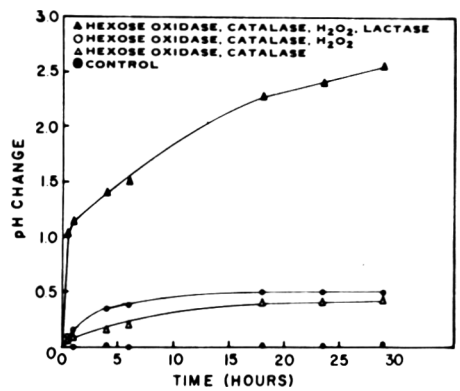

Fig. 7 —Effect of hydrogen peroxide and catalase on the formation of acid in skim milk at 30°C by hexose oxidase (enzyme conc 0.1 unit/ml).

that this enzyme has high thermal stability at pH 5.0. Thus, it would be very difficult to stop the glucose oxidase acidification reaction merely by raising the temperature.

The narrow specificity of glucose oxidase, as well as its thermal stability, might limit the potential use of this enzyme for milk acidification. Either glucose or a second enzyme, lactase, must be present to assure that a substrate is available. A less complicated approach would be to employ an enzyme which could utilize lactose directly. Hexose oxidase was thought to be one of the more promising enzymes for the direct conversion of lactose to lactobionic acid. While this enzyme did show some reaction with lactose, it was far more reactive when the monosaccharide sub-units of lactose were available as substrate. Hexose oxidase was easily extracted from *Iridophycus flaccidum*, and could have potential as a by-product from the industrial processing of seaweed.

REFERENCES

Bean, R.C. and Hassid, W.Z. 1956. Carbohydrate oxidase from a red alga, Iridophycus flaccidum. J. Biol. Chem. 218: 425.

Bentley, R. 1963. Glucose oxidase. In "The Enzymes," ed. Boyer, P.D., Lardy, H. and Myrback, K. Vol 7, p. 567. Academic Press, New York.

Deane, D.D. and Hammond, E.G. 1960. Coagulation of milk for cheese making by ester hydrolysis. J. Dairy Sci. 43: 1421.

Hauge, J.G. 1960a. Purification and properties of glucose dehydrogenase and cytochrome b from Bacterium anitratum. Biochem. Biophys. Acta 45: 250.

Hauge, J.G. 1960b. Kinetics and specificity of glucose dehydrogenase from Bacterium anitratum. Biochem. Biophys. Acta 45: 263.

McNurlin, T.F. and Ernstrom, C.A. 1962. Formation of curd by direct addition of acid to skim milk. J. Dairy Sci. 45: 647.

Nishizuka, Y. and Hayaishi, O. 1962. Enzymic formation of lactobionic acid from lactose. J. Biol. Chem. 237: 2721.

Underkofler, L.A. 1961. Glucose oxidase: Production, properties, present and potential applications. In "Production and Applications of Enzyme Preparations in Food Manufacture," Society of Chemical Industry Monograph No. 11, p. 72. Macmillan Co., New York.

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INHIBITION OF o-DIPHENOL OXIDASE BY DICHLORODIFLUOROMETHANE

INTRODUCTION

THERE ARE MANY instances in food processing where enzyme activity must be controlled if the food product is to be accepted by the consumer. Heat often has been used for this purpose but it sometimes has detrimental effects on product quality, especially with fruits and fruit juices. A nonthermal method to inhibit enzymes, therefore, would be advantageous in many situations. Treatment of foods with recoverable lipophilic chemicals is a possible nonthermal means to control enzyme activity in foods, but little is known about the effectiveness of this method. The potential for success does, however, appear good considering results of several studies which have shown that relatively inert, lipophilic chemicals, such as saturated hydrocarbons and halogenated hydrocarbons, can bind to and alter the structure of proteins (including enzymes) in aqueous solutions (Balasubramanian and Wetlaufer, 1966; Inoue and Timasheff, 1968; Mohammadzadeh-K. et al., 1967, 1969a, b; Timasheff and Inoue, 1968; Wishnia, 1962; and Wishnia and Pinder, 1964, 1966). Furthermore, Lund et al. (1969) reported that liquefied CCl_3F, $CHCl_2F$, or C_3H_8 caused a substantial and irreversible decrease in invertase activity. $CHCl_2F$ was the most effective of the three compounds, probably because it is more soluble in water than are the other two compounds. Intimate contact between CCl_3F and invertase (effectively achieved by decomposing the clathrate hydrate of CCl_3F) also increased the extent of inhibition. Lund et al. (1969) theorized that CCl_3F interacted with nonpolar portions of the invertase molecule and impaired its catalytic capability by interfering with normal intramolecular bonding. The above findings provide ample justification to further study the effects of relatively inert, lipophilic chemicals on enzyme activity. The hope is that this approach will prove successful for controlling enzyme activity in fluid foods such as fruit juices.

Reported here are the effects of dichlorodifluoromethane on the activity of o-diphenol oxidase (tyrosinase, polyphenol oxidase, catecholase) in a simple system. o-Diphenol oxidase was chosen because it catalyzes undesirable oxidative browning of many fruits and vegetables. Dichlorodifluoromethane (fluorocarbon-12) was selected because it is similar to compounds previously shown to bind to and alter the structure of proteins, is reasonable in cost, is unlikely to cause undue damage to the quality attributes of food, and has approval of the U.S. Food and Drug Administration as a direct contact food freezant (Federal Register, 1967).

EXPERIMENTAL

CHEMICALS used in this study included: (1) L-tyrosine (0.001 molar, Nutritional Biochemicals Co., 99+% pure); (2) o-diphenol oxidase (1.10.3.1; polyphenol oxidase, mushroom source, Worthington Biochemical Corp., Grade II, 500 units per mg, where one unit produces 0.001 Δ absorbance per min at 280 nm when present in 3 ml buffer at pH 6.5 and 25°C); (3) phosphate buffer (ACS grade, pH 7.2, ionic strength 0.05, 0.012 molar); (4) acetate buffer (ACS grade, pH 4.6, ionic strength 0.1, 0.2 molar); (5) dichlorodifluoromethane (f-12, food grade, DuPont); and (6) nitrogen (Airco).

The apparatus utilized included: (1) colorimeter (Spectronic-20, Bausch and Lomb); (2) buret, high-pressure; (3) aerosol cans (double epoxy lined, 202 x 206 and 202 x 303, Continental Can Co., Inc.); (4) aerosol valves (stainless steel, Avoset Co.); and (5) shaker (horizontal, mechanical, Model No. 6000, Eberbach Corp.).

o-Diphenol oxidase (ODO) catalyzes two oxidative steps in the conversion of tyrosine to melanin (Lerner and Fitzpatrick, 1950). The activity of ODO was determined from the rate of dopachrome formation. Dopachrome is an intermediate in the oxidative conversion of tyrosine to melanin and its formation was assessed by measuring the increase in absorbance at 470 nm (Boscan et al., 1962).

The general procedure used in this study was as follows. To each 202 x 206 aerosol can (97 ml capacity) was added 12.5 ml of a buffered enzyme solution consisting of a ratio of 2.8 ml water to 9.8 ml phosphate buffer containing either 0.28 or 0.467 mg enzyme. A 10-ml portion of the L-tyrosine solution was added, and this moment was recorded as "zero time." The can was immediately sealed and a known amount of f-12 was added through the can valve with the aid of a high-pressure buret. This filling procedure was used to avoid loss of oxygen from the headspace of the can. Samples were reacted for the desired time while being agitated at room temperature, then the can valve was dislodged and f-12 was allowed to vaporize. The reaction was immediately stopped by adding 6.27 ml of 0.2 molar acetate buffer (Behnke et al., 1968). ODO activity was determined by measuring absorbance (470 nm) of both treated and control samples (devoid of f-12) at zero time and after various reaction times.

Some experiments involved larger aerosol cans (202 x 303; 142 ml capacity) and in these instances the reaction mixture was increased in direct proportion to the can volume (the greater enzyme concentration was always used in the larger cans).

In all instances concentrations were such that reactions were zero-order with respect to substrate.

RESULTS & DISCUSSION

THE FIRST EXPERIMENT was conducted simply to determine if the activity

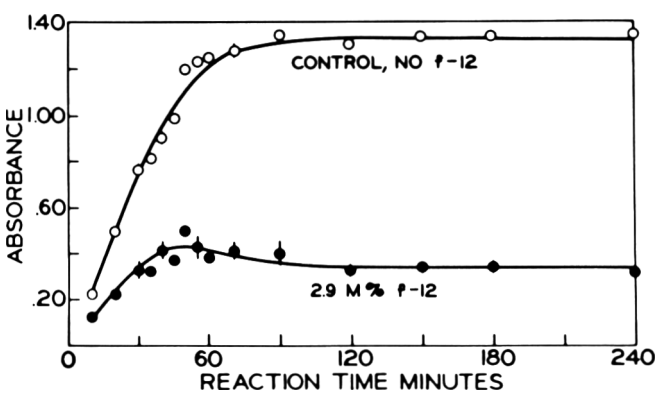

Fig. 1—Activity of o-diphenol oxidase as influenced by 2.9 mole % f-12 at room temperature and 180 cpm agitation. (Means of duplicate determinations with ranges indicated by size of the symbols.)

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of ODO could be inhibited significantly by f-12. Duplicate samples containing 2.9 mole % f-12 (moles f-12 ÷ [moles f-12 + (grams of all constituents except f-12/18g)]) and the ODO-L-tyrosine solution (0.667 mg enzyme per sample) were placed in 202 x 303 cans and reacted for 10–240 min. These samples and suitable controls were agitated at 180 cycles per min (cpm) throughout the reaction period. As shown in Figure 1, both treated and control samples showed increasing absorbance up to about 60 min, with relatively constant absorbance thereafter. (Eventual inactivation of ODO will occur even in control samples since this enzyme is susceptible to "reaction inactivation," Nelson and Dawson, 1944.) It is clearly evident that ODO was substantially less active in the presence of 2.9 mole % f-12 than in its absence. This finding was encouraging and justified investigation of factors influencing the effect of f-12 on the activity of ODO.

An additional study (results not shown) indicated that the inhibitory effect of f-12 on ODO occurs regardless of whether tyrosine is added before or after treatment with f-12. This suggests that f-12 interacts in some way with the enzyme.

Investigated next was the influence of various levels of f-12 on the activity of ODO. Samples containing 0.667 mg enzyme and various amounts of f-12 (0, 1.00 or 2.41 mole %) were placed in 202 x 303 cans and the reactions were monitored for 25 min while the samples were agitated at 180 cpm. The results shown in Figure 2 indicate ODO was increasingly inhibited as the level of f-12 was increased from 1.0 to 2.41 mole %. A concentration of 2.41 mole % f-12 is sufficient to condense a small amount of f-12. From the time course of the reaction it is also apparent that total inhibition of ODO (which would result in a horizontal line) was not achieved even after a 25 min treatment.

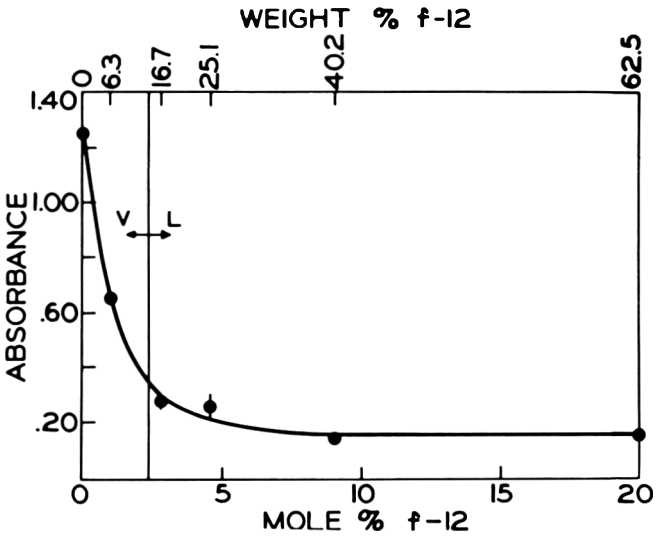

Fig. 3—Activity of o-diphenol oxidase as influenced by various concentrations of f-12 during a 60-min reaction period. (Room temperature; 180 cpm; means of triplicate determinations with ranges indicated by the size of the symbols.) V = f-12 present only as vapor in the headspace; L = f-12 present both as liquid and vapor in headspace.

Shown in Figure 3 are additional data concerning the effect of f-12 concentration on the activity of ODO. This study involved a longer reaction period and a broader range of f-12 concentrations than used in previous tests. Samples containing 0.467 mg ODO and various levels of f-12 were placed in 202 x 206 cans and agitated at 180 cpm during a fixed reaction period of 60 min. The activity of ODO declined greatly as the level of f-12 was increased from 0 to about 3 mole %. The increased effectiveness achieved as the level of f-12 was increased from 0 to 2.41 mole % was expected since each increment of increase within this range results in a greater amount of dissolved f-12. However, beyond about 2.41 mole %, condensation occurs and both the pressure and amount of dissolved f-12 remain constant. The slight increase in effectiveness that occurred as the concentration of f-12 was raised above 2.41 mole % is probably attributable to more rapid attainment of saturation.

Agitation is another important factor influencing inhibition of ODO by f-12. Samples containing 0.28 mg ODO were placed in 202 x 206 cans and reacted 60 min with the rate of agitation ranging from 0–300 cpm. It is evident from data in Figure 4 that absorbance of the control

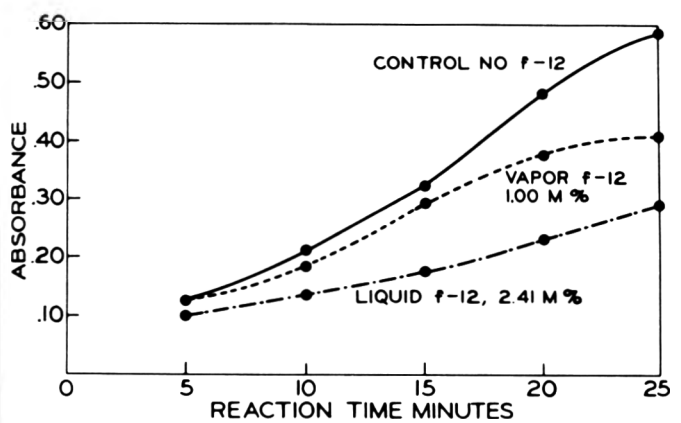

Fig. 2—Activity of o-diphenol oxidase as influenced by the amount of f-12 and the reaction time. (Room temperature; 180 cpm; means of duplicate determinations.)

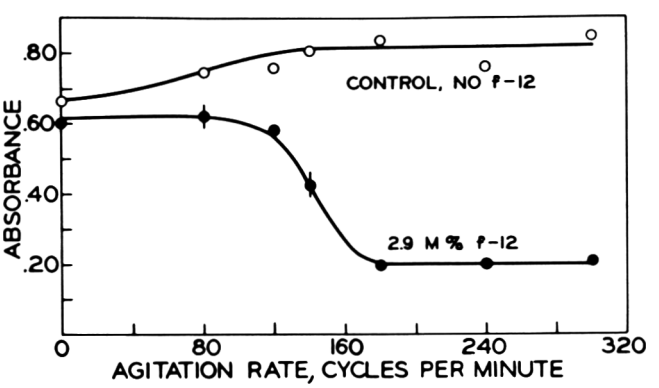

Fig. 4—Effect of intensity of agitation on the ability of f-12 to inhibit o-diphenol oxidase. (Room temperature; 60-min reaction period; single determinations for controls; means of duplicates for treated samples with ranges indicated by size of symbols.)

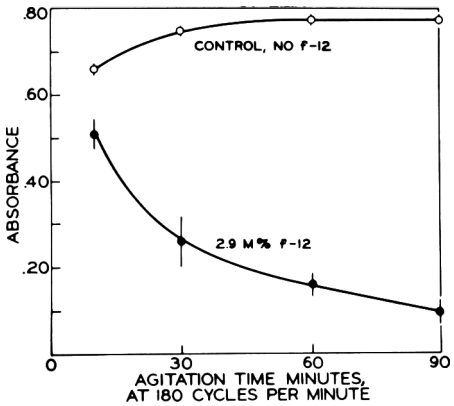

Fig. 5—Effect of time of agitation at 180 cpm on the ability of f-12 to inhibit o-diphenol oxidase. (Room temperature; 90 min reaction period; means of triplicate determinations with ranges indicated by size of symbols.)

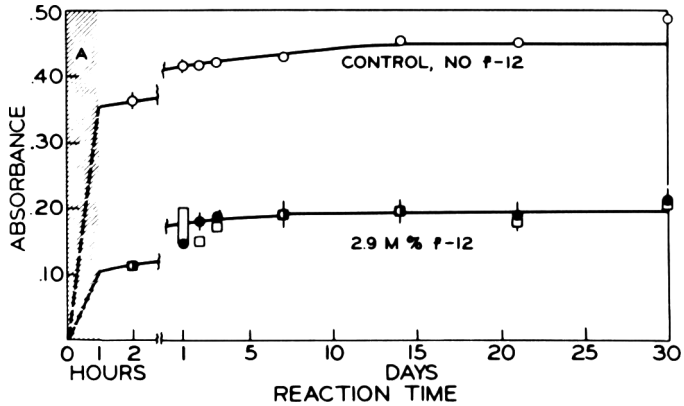

Fig. 6—Permanence of the inhibitory effect of f-12 on o-diphenol oxidase. (Room temperature; 180 cpm during first hour and quiescent thereafter; means of triplicate determinations with ranges indicated by size of symbols.) A = agitation period; □ = exposed to 2.9 mole % f-12 during first hour of reaction period; ● = exposed to 2.9 mole % f-12 for the entire reaction period.

samples increased slightly as the rate of agitation was increased from 0–140 cpm, and remained relatively constant thereafter. The slight increase in activity associated with increasing agitation up to 140 cpm was probably caused by improved contact between enzyme and substrate. Absorbance of the treated samples containing 2.9 mole % f-12 remained essentially constant as the agitation rate was increased from 0 to 120 cpm, decreased abruptly as the agitation rate was increased to about 180 cpm, and remained constant with further increases in the rate of agitation. Thus, the inhibitory effect of f-12 on ODO was greatly enhanced by vigorous agitation, with 180–300 cpm producing maximum inhibition under the conditions employed. It is not clear from these data whether agitation increases the effectiveness of a given amount of f-12 or merely hastens establishment of equilibrium conditions. These results, nevertheless, provided a reasonable basis for selecting an agitation rate of 180 cpm in the following studies.

The inhibitory effect of f-12 on ODO is also influenced by the duration of agitation at 180 cpm (Fig. 5). In this study samples containing 0.28 mg of ODO were placed in 202 x 206 cans and reacted for 90 min. Each sample was agitated at 180 cpm for the time indicated in Figure 5, held quiescently for the remainder of the 90-min reaction period, then analyzed following release of f-12 and addition of acetate buffer. In control samples absorbance increased somewhat as time of agitation was increased from 10 to 60 min. In samples containing 2.9 mole % f-12, absorbance decreased as time of agitation was increased, especially between 0–60 min. During the period studied, the greatest difference between control and treated samples occurred after 90 min agitation. From the trends in Figure 5, it is likely that a longer reaction period would have resulted in a still greater difference.

Although it has been demonstrated that f-12 can substantially inhibit the activity of ODO, it was also important to know the permanence of this inhibition. Samples containing 0.667 mg of ODO were placed in 202 x 303 cans and reacted for 60 min at room temperature and 180 cpm. Treated samples contained 2.9 mole % f-12. After agitation for 1 hr, one-half of the treated samples were opened and f-12 was permitted to vaporize. All samples were then allowed to stand quiescently at room temperature for various reaction times up to 30 days. Absorbance of dopachrome was measured at the conclusion of the desired reaction times and the data are shown in Figure 6. After the 1-hr agitation-reaction period, absorbance of the treated samples was markedly less than that of corresponding controls, and the difference remained essentially constant during the 30-day period. Furthermore, at any given reaction time, treated samples in which f-12 had been released exhibited essentially the same absorbance values as treated samples in which f-12 was present. This indicates: (1) that slow vaporization of f-12 following the initial 1-hr treatment period did not, in itself, inhibit ODO; and (2) that continued presence of f-12, with the possible exception of that amount which failed to vaporize (bound to solutes) is not necessary for continuance of the original inhibitory effect.

A final experiment was done to help determine how completely ODO can be inhibited by f-12, and to assess whether the inhibitory action of f-12 can be reversed when the chemical is removed by flushing with nitrogen. Treated samples containing 0.28 mg ODO, 2.9 mole % f-12 and no tyrosine were placed in 202 x 206 aerosol cans and agitated for 1 hr at 180 cpm. Control samples devoid of f-12 and tyrosine were handled in the same fashion. Following agitation for 1 hr, one-half of both the treated and control samples were flushed with nitrogen gas for 30 min. The remaining sam-

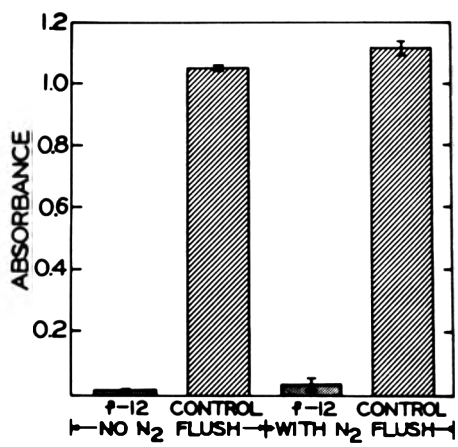

Fig. 7—Effect of nitrogen flushing on the permanence of the inhibitory effect of f-12 on o-diphenol oxidase. (Room temperature; samples devoid of tyrosine were exposed to f-12 for 60 min at room temperature and 180 cpm agitation; f-12 was released and one-half of both the treated and control samples were flushed with nitrogen for 30 min; tyrosine was added to all samples and they were allowed to react for 60 min at room temperature and 180 cpm. Results are means of triplicate determinations with ranges indicated at tops of bars.)

ples were opened and permitted to stand quiescently during this period. Tyrosine was then added to all samples and they were allowed to react for 60 min at room temperature and 180 cpm. It is evident from the data in Figure 7 that inhibition of enzyme activity in the treated samples was not reversible regardless of whether f-12 was simply released quiescently or was thoroughly flushed away with nitrogen. (It should be noted, however that both techniques for removal of f-12 probably were incapable of removing any f-12 that was tightly bound to ODO). Furthermore, it is apparent that inhibition of the reaction was almost totally complete.

Although it is reasonable to conclude that inhibition of ODO activity is irreversible, this does not necessarily mean that the inhibitory effect of f-12 is irreversible. This point is unclear because of the nature of ODO. As mentioned earlier, ODO is susceptible to "reaction inactivation." Thus, even if the inhibitory action of f-12 was in fact reversible, the enzyme would, in this instance, be incapable of renewed activity beyond a reaction time of one day (absorbance values of control samples remained essentially constant after one day, suggesting that the enzyme is inactive at this point).

The means by which f-12 inhibits ODO is unknown at this point. The mechanisms could be chemical, physical or a combination of both. If a carbon-halogen bond of f-12 should cleave by homolytic fission, the products would be a neutral organic radical and a neutral halogen atom. The sulfhydryl groups of ODO would then be subject to oxidation or alkylation by the organic radical and the enzyme could be irreversibly inactivated (Holaday, 1970). Although this mechanism cannot be dismissed, its likelihood appears somewhat remote considering that f-12: (1) contains two fluoride atoms which tend to inhibit homolytic fission; (2) has been assigned by Underwriters Lab. to group 6 (least toxic class for potentially hazardous gases and vapors; no evidence of injury to test animals after exposure to air containing at least 20 vol % f-12 for a period of 2 hr; Clayton, 1968); (3) has been assigned by the American Conference of Governmental Industrial Hygienists a Threshold Limit Value of 1000 ppm (maximum level for continuous safe exposure; CO_2 with a Threshold Limit Value of 5000, is the only rated gas with a higher value than f-12; Clayton, 1968); and (4) underwent thorough screening before being approved as a direct contact food freezant.

Although halogenated hydrocarbons can be chemically degraded in vivo to yield toxic products, it is highly unlikely that ODO could catalyze degradation of f-12 (Cohen, 1971).

A physical interaction between f-12 and ODO is likely to occur and this could result in diminished activity of ODO. Presumably the f-12 molecule would associate with hydrophobic regions of the enzyme, thereby altering its conformation and reducing its activity. As mentioned in the introduction, various studies involving interactions of aqueous solutions of proteins and enzymes with hydrocarbons and halogenated hydrocarbons, provide ample evidence that this type of mechanism could occur.

Knowledge of the amount of f-12 remaining in the reaction mixture following flushing with nitrogen would assist in determining the mechanism of enzyme inactivation. This information will be gathered in a future study.

REFERENCES

Balasubramanian, D. and Wetlaufer, D.B. 1966. Reversible alteration of the structure of globular proteins by anesthetic agents. Proc. Nat. Acad. Sci. (US) 55: 762.

Behnke, J.R., Fennema, O. and Powrie, W.D. 1969. Enzyme-catalyzed reactions as influenced by inert gases at high pressure. J. Food Sci. 34: 370.

Boscan, L.A., Powrie, W.D. and Fennema, O. 1962. Darkening of red beets, Beta vulgaris. J. Food Sci. 27: 574.

Clayton, J.W. Jr., 1968. Fluorocarbon toxicity and biological action. In "Toxicity of Anesthetics," ed., Fink, B.R. p. 77. Williams and Wilkins, Baltimore, Md.

Cohen, E.N. 1971. Metabolism of the volatile anesthetics. Anesthesiology 35: 193.

Federal Register. 1967. Document 67-10535, 32: 174.

Holaday, D.A. 1970. Potential toxicity of metabolic degradation products of volatile, fluorinated anesthetics. In "Complications of Anesthesia," ed. Saidman, L.J. and Moya, F., p. 219. C.C. Thomas, Springfield, Ill.

Inoue, H. and Timasheff, S.N. 1968. The interaction of β-lactoglobulin with solvent compounds in mixed water-aqueous solvent systems. J. Amer. Chem. Soc. 90: 1890.

Lerner, A.B. and Fitzpatrick, T.B. 1950. Biochemistry of melanin formation. Physiol. Rev. 30: 91.

Lund, D.B., Fennema, O. and Powrie, W.D. 1969. Effect of gas hydrates and hydrate formers on invertase activity. Arch. Biochem. Biophys. 129: 181.

Mohammadzadeh-K., A., Fenney, R.E. and Smith, L.M. 1969a. Hydrophobic binding of hydrocarbons by proteins. 1. Relationship of hydrocarbon structure. Biochem. Biophys. Acta 194: 246.

Mohammadzadeh-K., A., Smith, L.M. and Feeney, R.E. 1969b. Hydrophobic binding of hydrocarbons by proteins. 2. Relationship of protein structure. Biochem. Biophys. Acta 194: 256.

Mohammadzadeh-K., A., Feeney, R.E., Samuels, R.B. and Smith, L.M. 1967. Solubility of alkanes in protein solutions. Biochem. Biophys. Acta 147: 583.

Nelson, J.M. and Dawson, C.R. 1944. Tyrosinase. In "Advances in Enzymology," ed. Ford, F.F. and Werkman, C.H., Vol 4, p. 99. Interscience Publ., Inc., New York.

Timasheff, S.N. and Inoue, H. 1968. Preferential binding of solvent compounds to proteins in mixed water-organic solvent systems. Biochemistry 7: 2501.

Wishnia, A. 1962. The solubility of hydrocarbon gases in protein solutions. Proc. Nat. Acad. Sci. (US) 48: 2200.

Wishnia, A. and Pinder, T. 1964. Hydrophobic interactions in proteins: conformational changes in bovine serum albumin below pH 5. Biochemistry 3: 1377.

Wishnia, A. and Pinder, T.W. Jr. 1966. Hydrophobic interactions in proteins. The alkane binding site of β-lactoglobulin A and B. Biochemistry 5: 1534.

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LOW-TEMPERATURE DESTRUCTION OF Trichinella spiralis USING LIQUID NITROGEN AND LIQUID CARBON DIOXIDE

INTRODUCTION

THE INCIDENCE of the parasite, *Trichinella spiralis*, in swine has declined in the past several decades. However, the incidence of 1.25 infected pigs per 1,000 reported for the period 1966 to 1970 by Zimmermann and Zinter (1971) still represents the potential of 40 million exposures for humans consuming pork not treated to destroy the trichinae. Because some pork products are designed to be eaten without complete cooking, or may inadvertently be undercooked, it is necessary to provide a rapid and economical means for destroying the trichinae.

Historically, this was accomplished by holding the meat products in freezer storage for various periods (USDA, 1965). Holding times for pieces not exceeding 6 in. in thickness or stored in containers not exceeding 6 in. in depth were specified as 20 days at 5°F, 10 days at −10°F and 6 days at −20°F. For pieces or containers exceeding 6 in. in thickness or depth but not exceeding 27 in., the recommended holding times were 30 days at 5°F, 20 days at −10°F and 12 days at −20°F. These time and temperature relationships were based on studies by Ransom et al. (1920). The problems and economic costs associated with the relatively long-term holding of pork products in freezer storage to insure trichinae destruction represent a serious limitation to this particular procedure.

Augustine (1952) stated that trichinae could be destroyed by lowering the temperature of the meat product immediately to −35°C. Gould and Kaasa (1949) concluded that a temperature of −37°C would destroy trichinae in 2 min. At the time these studies were conducted, however, mechanical refrigeration was the only commercially available form of freezing. To attain these temperatures with conventional, mechanical freezing techniques would have been highly impractical.

With the advent of cryogenic materials, such as liquid nitrogen and liquid carbon dioxide, the attainment of the temperatures indicated became, not only feasible, but practical. This study was instituted to determine the effectiveness of these freezing materials for the destruction of trichinae and the minimum temperatures required to render the product safe for consumption without further treatment.

EXPERIMENTAL

THIS STUDY was conducted by using pork patties made from trichina-infected pork. The trichina-infected pork was obtained from pigs used in another experiment. The experimental pigs were orally infected 1 to 4 months before slaughter with a dose of approximately 300,000 trichinae per pig. The pigs were slaughtered and eviscerated according to conventional procedures. After chilling in a 2°C cooler for a 24-hr period, the carcasses were cut, and the loins, bellies and trimmings boned out and ground through a 19 mm plate. This product was then mixed in a Leland mixer to insure uniform distribution of fat and lean and reground through a 4.8 mm plate. On second grinding, the product was extruded through a Hobart Model 61 patty machine into patties approximately 88 mm square and 9 mm thick. Each patty weighed approximately 110g.

A random sample of the meat mixture was examined for the initial number of trichinae per gram of tissue by the artificial digestion–Baermann technique (Zimmermann et al., 1961). These initial counts are shown in Table 1.

Thermocouples were inserted in the center of three of each lot of 10 patties, and the patties were subsequently run through the freezing tunnel. In trials 1, 2 and 3, the patties were frozen with liquid nitrogen spray in a modified Heath freezer tunnel. In trial 4 the patties were frozen with liquid CO_2 in a Certified Multideck freezer. The dwell times in the tunnel were varied to produce equilibrated end-point temperatures that approached predetermined levels.

After freezing, the patties were stacked side by side in an insulated container so that the patties containing the thermocouples were located in the center and approximately equi-

Table 1–Results of rat feeding trials number showing positive and negative digestion results

Final equilibrated temp (°C)	Trial no. 1	2	3	4
−47	0+ 20−			
−39		0+ 10−		0+ 20−
−29	0+ 20−	0+ 20−		0+ 20−
−28			1+ 19−	
−25	1+ 19−	8+ 12−		
−23			2+ 18−	0+ 20−
−20		10+ 10−		
−17				13+ 7−
−14			17+ 3−	
−12	14+ 6−	18+ 2−		
−10				8+ 2−
Control (+3)	20+ (20 Died)	20+ (15 Died)	5+ (4 Died)	10+
Initial counts trichinae/g	685	2993	3120	353

distant between the center and each end of the stack. The patties were left in the insulated container until the patty temperature equilibrated. This equilibrated temperature was determined as the point at which the temperature at the three locations remained stable for approximately 10 min. It required approximately 45 minutes for the patties to reach the equilibration.

After equilibration, the patties were thawed at room temperature, and, to determine the presence of viable trichinae, 15-g samples were fed to albino rats. Duplicate samples from each patty were fed to each of two rats, with the exception of the control and −39°C in trial 2 and the control and −10°C in trial 4. In these instances, only one sample was fed. For the control in trial 3, only 5 patties were selected, and a single sample was fed to each of 5 rats. The rats were maintained for 30–35 days then sacrificed, skinned and eviscerated. The rat carcasses were examined for trichina viability by the artificial digestion–Baermann technique (Zimmermann et al., 1961), and the number of trichinae per gram in the eviscerated rat carcass was determined. The presence of even one trichina was classified as a positive result. Rats that died during the feeding period were not examined for trichina viability because it was assumed that the high level of trichina infection was the cause of death.

RESULTS & DISCUSSION

THE RESULTS of the four trials are shown in Table 1. No positive samples were found when the patties were frozen to a final equilibrated temperature of −29°C or lower. In trial 3, one positive sample was found at −28°C and positive samples were found at −25°C in both trials 1 and 2. With LCO_2 freezing, there were no positive samples found at the −23°C level. From these results, it was concluded that, if a product attained a final equilibrated temperature of −29°C, it would be rendered free of trichinae and that subsequent freezer holding would be unnecessary.

A processor, therefore, need only assure that temperatures lower than −29°C be attained to render the product free from the danger of trichinae. The USDA (1970) proposed that refrigeration to a temperature of −30°F (−34.4°C) would be accepted as rendering pork and pork products immediately trichina free. The results of this experiment indicate that the treatment prescribed should insure an adequate margin of safety. With modern cryogenic freezing equipment, the attainment of this recommended temperature should be relatively simple and economical and free the processor from the problems associated with the long-term freezer storage of pork to destroy trichinae.

REFERENCES

Augustine, D.L. 1952. Low temperature treatment of pork. Proc. First Nat. Conf. on Trichinosis. p. 45.

Gould, S.E. and Kaasa, L.J. 1949. Low temperature treatment of pork. Effect of certain low temperatures on the viability of trichina larvae. Am. J. Hygiene 49: 17.

Ransom, B.H., Schwartz, B. and Raffensperger, H.B. 1920. Effects of pork-curing processes on trichinae. USDA Bull. 880, p. 37.

USDA. 1965. Regulations governing the meat inspection of the United States Department of Agriculture. Consumer & Marketing Service, Meat Inspection Division, USDA, p. 243.

USDA. 1970. Prescribed treatment of pork and products containing pork to destroy trichinae. Federal Register 35: 15593.

Zimmermann, W.J., Schwarte, L.H. and Biester, H.E. 1961. On occurrence of Trichinella spiralis on pork sausage available in Iowa (1953—60). J. Parisitol. 47: 429.

Zimmermann, W.J. and Zinter, D.E. 1971. The prevalence of trichiniosis in swine in the United States, 1966—70. HSMHA Health Reports 86: 937.

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PHYSIOLOGICAL AND CHEMICAL STUDIES OF CHILLING INJURY IN PEPPER FRUITS

INTRODUCTION

IT IS WELL KNOWN that some vegetables and fruits of subtropical and tropical origin suffer physiological injury during cold storage (0–10°C) and their keeping quality deteriorates. Pepper fruits are easily subject to chilling injury at a temperature lower than 7°C which seems to disturb normal metabolism and causes a gradual weakening of tissues and eventual death of peppers. Lacy and McColloch (1962) published a study of chilling injury in Bell peppers, indicating that sheet pitting occurred on fruits stored at 32°F, but not on those at 40°F.

We conducted a study on the physiological effects of low temperature on peppers, and in this paper report physiological, biochemical and chemical changes in pepper fruits exposed to low temperature.

MATERIALS & METHODS

Materials

Pepper fruits (cv. Sakigakemidori and Kyomidori) were obtained from Sennan Agriculture cooperative, Osaka prefecture. All fruits were dipped in a solution of 0.5% sodium dehydroacetate for a few minutes prior to storage to reduce infection by microoganisms. Fruits were stored in darkness in rooms held at 1°C, 6°C, 18°C and 20°C.

Methods

The respiratory rate of pepper fruits was determined by the method of Claypool and Keefer (1942), extraction and determination of α-keto acids by the method described by Isherwood and Cruickshank (1954), and the organic acids by the method described by Bulen et al. (1952). Extraction and identification of polyphenols in pepper seeds were conducted by the method of Nilo and Luh (1968) and shikimic acid content by the micro-colorimetric method described by Yoshida and Hasegawa (1957).

For the extraction and assay of phenylalanine ammonia-lyase (PAL) and tyrosine ammonia-lyase (TAL) of pepper seeds, usually 5g of pepper seeds were ground with a blender in 50 ml of acetone previously chilled to −25°C. The homogenate was filtrated through filter paper and the residue washed three times with cold acetone and dried completely in a vacuum.

PAL activity was determined spectrophotometrically, by measuring the conversion of L-phenylalanine to trans-cinnamic acid as described by Koukol and Conn (1961). The mixture for the assay consisted of 500 μmoles of sodium borate buffer (pH 8.8), 30.2 μmoles L-phenylalanine, 0.1g acetone powder (total vol 5.0 ml). After incubation at 40°C for 1 hr, the increase in absorbance at 290 mμ was measured against a reference from which only L-phenylalanine had been omitted. PAL activity was expressed as mμ moles of trans-cinnamic acid formed per 0.1g acetone powder per hour. TAL activity was determined by measuring the amount of p-coumaric acid formed from L-tyrosine by the method of Neish (1961).

RESULTS & DISCUSSIONS

Symptoms of pepper fruits stored at 1°C, 6°C and 18°C

Pepper fruits (cv. Sakigakemidori) were stored at 1°C, 6°C and 18°C in darkness. While typical surface pitting was not observed in peppers stored at 1°C for 7 days, there was discoloration of the calyces and browning of the seeds. A similar symptom also appeared on the calyces of peppers at 6°C after 14 days, while those stored at 18°C ripened normally.

The quality of the fruits stored at 1°C for 2 wk or 6°C for 3 wk or longer deteriorated rapidly when they were transferred to 18°C.

CO_2 production of pepper fruits stored at 1°C and 18°C

As shown in Figure 1, the carbon dioxide production of pepper fruits increased slightly up to 7 days' storage at 18°C, decreased sharply to the 18th day and then increased. On the contrary, the rate of carbon dioxide production of peppers stored at 1°C increased gradually throughout the storage periods. A similar tendency in respiration at low temperature was reported by Iwata et al. (1969) in the case of chilling injury in Natsudaidai fruits.

CO_2 production after transfer to 18°C from 1°C

Eaks and Morris (1956) and Murata (1969) showed that fruits sensitive to chilling injury usually produce a rather large flush of CO_2 at the warmer temperature after exposure to low temperatures. As shown in Figure 2, peppers transferred to 18°C after exposure to 1°C for various periods showed a spur of carbon dioxide production reaching to a far higher level than that of 18°C-stored fruits.

It is suggested that the stimulated CO_2 production of chilled fruits after transfer from low-temperature storage to warmer temperature may show an abnormality of the intermediate metabolism.

Changes of α-keto and organic acids

Hulme et al. (1964) showed that oxaloacetic acid in Cox's orange pippin apple increased during cold storage and that low temperature injury was preceded by the accumulation of oxaloacetic acid. Judging from these results, they suggested that low temperature injury might be caused by an interference in the operation of the Krebs cycle in apple tissues. Table 1 shows the changes of α-ketoglutaric acid content of peppers during low

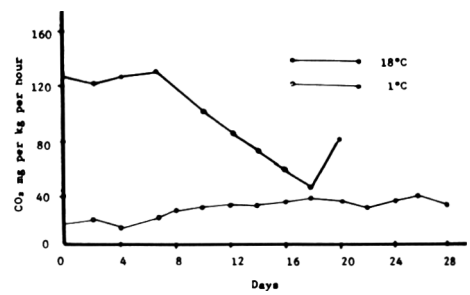

Fig. 1—Carbon dioxide production by peppers stored at 18°C and 1°C.

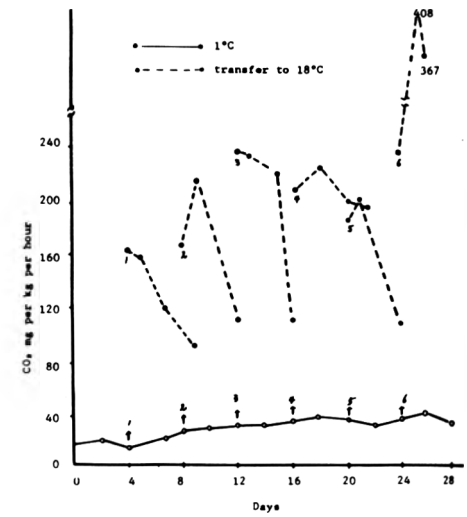

Fig. 2—Carbon dioxide production by peppers stored at 18°C after exposure to 1°C. (↑: Days of transferring to 18°C).

Table 1–Changes of α-ketoglutaric acid content of peppers during low temperature storage and after transfer to 18°C

Storage temp	Wk of storage	Days after transfer to 18°C: 0	3	7
		(mg acid/100g fresh wt)		
	Initial	0.91		
6°C	2	2.14	3.23	10.35
	4	2.59	–	6.28
1°C	2	3.04	3.49	4.86
	4	3.37	–	10.35

Table 2–The changes of pyruvic acid content of peppers during low temperature storage and after transfer to 18°C

Storage temp	Wk of Storage	Days after transfer to 18°: 0	3	7
		(mg acid/100g fresh wt)		
	Initial	1.90		
6°C	2	2.38	6.18	5.23
	4	8.55	–	10.90
1°C	2	2.06	2.07	5.08
	4	11.20	–	17.00

temperature storage (1°C and 6°C) and after transfer to 18°C. The content of α-ketoglutaric acid in peppers before storage was 0.91 mg per 100g fresh weight. During cold storage, α-ketoglutaric acid increased by two to three times the initial amount, showing a more rapid increase after transfer to 18°C from 1°C and 6°C.

The changes of pyruvic acid content of peppers during low temperature storage (1°C and 6°C) and after transfer to 18°C are shown in Table 2. The changes of pyruvic acid content also showed a similar tendency to that of α-ketoglutaric acid.

As the oxaloacetic acid was difficult to separate by paper chromatography, the change is not shown in this paper; however, it is suggested that the change of oxaloacetic acid may be similar to that of the former two acids. Murata (1969) also reported that α-keto acids are accumulated in banana suffering chilling injury. As shown in Table 1 and 2, the α-keto acid content of peppers was accumulated during cold storage; a greater accumulation of α-keto acids after transfer to 18°C from 1°C and 6°C may be due to the reduction of the enzyme activity concerning the release of keto acids in the Krebs cycle. In a study of mitochondria of animal tissues, Tyler (1960) reported that oxaloacetate might be an important rate-regulator of oxidative metabolism through the Krebs cycle. Giving attention to this rate-regulator effect of oxaloacetate in the Krebs cycle, we are now investigating the changes in and the role of oxaloacetate in peppers by the method of thin layer chromatography. Details of this work will be reported elsewhere.

Table 3 shows the changes of organic acids contents of peppers stored at 1°C and 18°C. Fumaric acid, succinic acid and malic acid were detected in peppers, with malic acid the most abundant. Since fumaric and succinic acids were difficult to separate from each other completely, the contents are shown as the sum of the two, expressed as succinic acid. Fumaric + succinic acid and citric acid increased to some extent during storage of 18°C. The contents of malic acid and citric acid were found to increase markedly during cold storage.

The changes of organic acids after transfer to 18°C are shown in Table 4. After transfer to 18°C from 1°C, the content of malic acid decreased quickly but fumaric + succinic acid increased rapidly. Uritani (1968) demonstrated that malate dehydrogenase is susceptible to removal from mitochondria in chilled sweet potatoes. It is considered that the malic acid accumulation in peppers during cold storage may have been caused by removal of malate dehydrogenase from mitochondria.

Production of browning substances in pepper seeds by chilling injury

The surface color of pepper seeds becomes brown at an early period of cold storage and eventually turns black when the storage period is prolonged. It is suggested that in severely chilled pepper seeds, polyphenolic substances may be oxidized by the catalyzation of polyphenol oxidase.

Figure 3 shows a two-dimensional paper chromatogram of the polyphenolic compounds in the ethanol extract of pepper seeds before storage. Two blue color spots were detected when the chromatogram was sprayed with $FeCl_3$ + $K_3Fe(CN)_6$ reagent. Table 5 lists the R_f values and color reactions of the polyphenolic compounds extracted from

Table 3–Changes of organic acid content of peppers stored at 1°C and 18°C

Storage temp	Wk of storage	Fumaric acid + Succinic acid	Malic acid	Citric acid
		(mg acid/100g fresh wt)		
	Initial	6.0	67.0	trace
18°C	2	12.2	34.8	trace
	4	47.6	29.4	28.6
1°C	2	7.0	71.6	trace
	4	10.6	104.2	28.8

Table 4– The changes of organic acid after transfer to 18°C

Low temp storage periods	Days after transfer to 18°C	Fumaric acid + Succinic acid	Malic acid	Citric acid
		(mg acid/100g fresh wt)		
1°C-2 wk	0	7.0	71.6	trace
	3	38.2	46.4	30.0
	7	22.2	50.0	31.6
1°C-4 wk	0	10.6	104.2	28.0
	3	22.4	76.6	19.8
	7	27.4	25.6	99.2

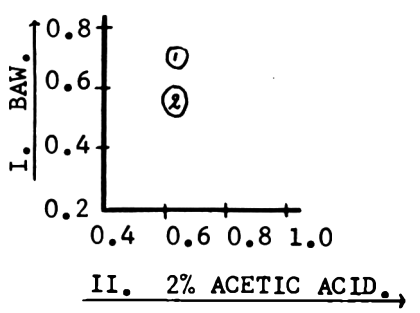

Fig. 3–Two-dimensional chromatogram of polyphenolic compounds in pepper seeds before storage. [Papers were chromatographed with BAW (n-butanol-acetic acid-water, 4:1:5 v/v) as the first dimension for 20 hr and with 2% ACOH (2% acetic acid) as the second dimension for 4 hr at 20°C.]

Table 5–R_f values, maximum absorption peak and color characteristics of polyphenolic compounds extracted from pepper seeds compared with authentic chlorogenic acid

Spot no	R_f values at 20°C BAW (4:1:5)	2% ACOH	λ max(mμ)	Color under[a] UV	UV+NH_3	Visible light+NH_3	Color with[a] $FeCl_3$ + $K_3Fe(CN)_6$	DPNA	Hoepfner
1	0.70	0.64	328	Bfl	YGfl	Ysl	B	T	R
2	0.56	0.63	320	Bfl	YGfl	Ysl	B	T	R
Authentic chlorogenic acid	0.69	0.65	328	Bfl	YGfl	Ysl	B	T	R

[a]B=blue, G=green, R=red, T=tan, Y=yellow, fl=fluorescence, sl=slight.

pepper seeds on a two-dimensional chromatogram examined under ultraviolet reaction with or without ammonia vapor; under visible light with ammonia vapor; and when sprayed on the $FeCl_3$ + $K_3Fe(CN)_6$, diazotized p-nitroaniline (DPNA) and Hoepfner reagents; and the maximum absorption peak of spot 1, spot 2 and authentic chlorogenic acid in 90% ethanol.

Figure 4 shows the absorption spectra in 90% ethanol of spot 1 (A) and spot 2 (B) isolated from pepper seeds and authentic chlorogenic acid (C). The absorption spectrum of spot 1 in 90% ethanol matches that of authentic chlorogenic acid, with a maximum absorption peak at 328 mμ. To prove further that spots 1 and 2 were chlorogenic acid or its isomers, acid hydrolyses of these compounds were done with 2N HCl. The ether extract of the acid hydrosates of spot 1 and spot 2 contained caffeic acid, and the aqueous hydrochloric acid layer of spot 1 and spot 2 contained quinic acid.

Judging from the results of the R_f values, color reactions, absorption spectra and acid hydrolyses of spot 1, spot 2 and authentic chlorogenic acid, spot 1 was identified as chlorogenic acid. Spot 2 coincided with the color reactions of authentic chlorogenic acid except for the R_f value and absorption spectrum, and contained caffeic acid and quinic acid in the products of the acid hydrolysis. Thus, spot 2 was supposed to be an isomer or a derivative of chlorogenic acid.

Spot 1 examined under ultraviolet light was cut off with scissors. After being cut into small pieces, the chlorogenic acid contained was extracted with 5 ml of 80% ethanol, and determined by measuring the light absorbance at 328 mμ with a Beckman DU-type spectrophotometer. Results are shown in Table 6. The content of chlorogenic acid before storage was 62.6 mg per 100g fresh weight. The content in seeds of peppers stored at 1°C increased, reaching a maximum at 7 days' storage and decreasing rapidly during subsequent storage periods. The changes of chlorogenic acid content in seeds of peppers stored at 20°C showed the same tendency as those of cold storage, but the amount of the chlorogenic acid at 7 days in 20°C-stored fruits was less than half that in 1°C-stored fruits.

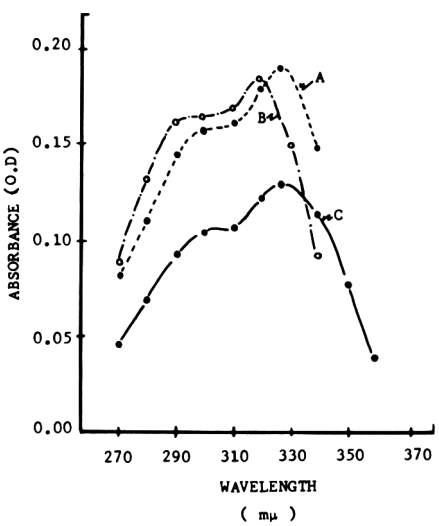

Fig. 4–Absorption spectra of Spot 1 and Spot 2 isolated from pepper seeds and authentic chlorogenic acid. [A: Spot 1 (●- - -●); B: Spot 2 (○—·—·—○); C: Authentic chlorogenic acid (●——●)].

Table 6–Changes of chlorogenic acid content of pepper seeds stored at 1°C and 20°C

Temp	Days in storage 0	7	14	21	28
	(mg acid/100g fresh wt)				
1°C	62.6	144.0	40.9	22.8	4.6
20°C		70.5	37.9	26.6	24.0

Table 7–Changes of shikimic acid content of pepper seeds stored at 1°C and 20°C

Temp	Days in storage 0	1	4	7	14
	(mg acid/100g fresh wt)				
1°C	7.3	17.0	42.0	2.8	6.8
20°C		14.5	18.0	10.0	7.5

Changes of shikimic acid content in pepper seeds stored at 1°C and 20°C

It has become clear in recent years that shikimic acid plays an important role as a precursor of aromatic acid, such as chlorogenic acid, via the phenylalanine or tyrosine pathway. The changes of shikimic acid content of pepper seeds were determined in relation to the accumulation of chlorogenic acid. As shown in Table 7, the shikimic acid content before storage was 7.3 mg per 100g fresh weight. In cold storage the content increased rapidly reaching a maximum of 42.0 mg per 100g fresh weight after 4 days; it then began to decrease rapidly. The content in 20°C-stored fruits showed a similar trend but was much less pronounced. From the results it was supposed that the shikimic acid pathway might be activated in cold storage to produce phenylalanine or tyrosine as a good precursor of phenylpropanoids.

Table 8–Changes of phenylalanine ammonia-lyase activity of pepper seeds stored at 6°C and 20°C

Temp	Days in storage 0	4	7
6°C	173[a]	280	160	65
20°C		60	35	24

[a]trans-cinnamic acid (mμM/0.1g acetone powder/hr)

Table 9–Changes of tyrosine ammonia-lyase activity of pepper seeds stored at 6°C and 20°C

Temperature	Days in storage 0	2	4	7
6°C	16[a]	8	14	11
20°C		8	25	12

[a]para-coumaric acid (mμM/0.1 g acetone powder/hr)

Table 10—Comparison of phenylalanine ammonia-lyase and tyrosine ammonia-lyase activities of pepper seeds stored at 6°C and 20°C

	Days in storage			
Temp	0	2	4	7
6°C	10.8[a]	35.0	11.4	5.9
20°C		7.5	1.4	2.0

[a]cinnamic acid/coumaric acid

Changes of PAL and TAL activities of pepper seeds stored at 6°C and 20°C

Table 8 shows PAL activity in enzyme preparation from pepper seeds stored at 6°C and 20°C. PAL activity in the seeds of pepper fruits stored at 6°C showed a rapid increase up to 2 days, and then a sharp decrease. The activity in 20°C-stored fruits decreased slightly during storage, with values being much lower than those of cold storage.

Table 9 shows the changes of TAL activity which was fairly low in comparison with PAL activity throughout the storage periods and a consistent difference was not found between samples stored at 6°C and 20°C.

Comparison of PAL and TAL activities of pepper seeds stored at 6°C and 20°C

Table 10 compares PAL and TAL activities of seeds stored at 6°C and 20°C. The ratio of cinnamic acid/coumaric acid formed before storage was 10.8. In the 6°C-stored fruit the ratio increased rapidly to a maximum value of 35.0 after 2 days in cold storage, then decreased sharply. The ratio of 20°C-stored fruits decreased consistently up to 7 days' storage.

Thus it is suggested that PAL in pepper seeds is activated at low temperature and phenylpropanoids are produced rapidly.

CONCLUSIONS

THE FIRST STEP of physiological injury of pepper fruits during low temperature storage is observed as a change of respiration before the symptoms of chilling injury become visible: the rapid increase of CO_2 production occurs when fruits are transferred to room temperature after low temperature storage. This abnormal increase of CO_2 production results in changes of the intermediate metabolism. The accumulation of α-keto acids (Table 1 and 2) during low temperature storage and after transfer to room temperature, the accumulation of malic acid (Table 3) during low temperature storage and the decrease of malic acid (Table 4) after transfer to room temperature were observed. These metabolite changes indicate occurrence of chilling injury.

The browning of pepper seeds is also a typical symptom of chilling injury. The main browning substance of seeds was identified as chlorogenic acid (Table 5, Fig. 4) which increased in fruits stored at 1°C, reached a maximum after 7 days, and then decreased rapidly during storage. The shikimic acid pathway is known as the important pathway of the biosynthesis of aromatic compounds. The content of shikimic acid also increased rapidly in low temperature storage (Table 7). PAL known as a key enzyme of phenylpropanoids showed the peak after 2 days of storage at 6°C. From these results it was emphasized that the shikimic acid pathway and PAL in pepper seeds were activated in low temperature storage, phenylpropanoids were produced rapidly, and that those propanoids might become the cause of the browning of pepper seeds as the oxidation substrate.

REFERENCES

Bulen, W.A., Varner, J.E. and Burrell, R.C. 1952. Separation of organic acids from plant tissues. Anal. Chem. 24: 187.

Claypool, L.L. and Keefer, R.M. 1942. A colorimetric method for CO_2 determination in respiration studies. Proc. Amer. Soc. Hort. Sci. 40: 177.

Eaks, I.L. and Morris, L.L. 1956. Respiration of cucumber fruit associated with physiological injury at chilling temperatures. Plant Physiol. 31: 308.

Hulme, A.C., Smith, W.H. and Wooltorton, L.S.C. 1964. Biochemical changes associated with development of low temperature breakdown in apples. J. Sci. Food Agric. 15: 303.

Isherwood, F.A. and Cruickshank, D.H. 1954. Chromatographic separation and analysis of mixtures of pyruvic, oxaloacetic acid and alpha-ketoglutaric acid. Nature 173: 121.

Iwata, T., Nakagawa, K. and Ogata, K. 1969. Physiological studies of chilling injury in Natsudaidai. J. Jap. Soc. Hort. Sci. 38: 93.

Koukol, J. and Conn, E.E. 1961. The metabolism of aromatic compounds in higher plants. 4. Purification and properties of the phenylalanine deaminase of Hordeum vulgare. J. Biol. Chem. 236: 2692.

Lacy, P. and McColloch, L.P. 1962. Chilling injury and Alternaria rot of Bell peppers. USDA Mktg. Res. Rpt. 536.

Murata, T. 1969. Physiological and biochemical studies of chilling injury in banana. Physiol. Planta. 22: 401.

Neish, A.C. 1961. Formation of m- and p-coumaric acid by enzymatic deamination of the corresponding isomers of tyrosine. Phytochemistry 1: 1.

Nilo, R. and Luh, B.S. 1968. Polyphenolic compounds in canned tomato pastes. J. Food Sci. 33: 358.

Tyler, B.D. 1960. Effect of citric acid-cycle intermediates on oxaloacetate utilization and succinate oxidation. Biochem. J. 76: 293.

Uritani, I. 1968. The chilling injury of fruits and vegetables. J. Food Sci. & Technol. (Symposium) p. 9.

Yoshida, S. and Hasegawa, S. 1957. A microcolorimetric method for the determination of shikimic acid. Arch. Biochem. Biophys. 70: 377.

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ACID-SOLUBLE NUCLEOTIDES OF JUICE VESICLES OF CITRUS FRUIT

INTRODUCTION

ACID-SOLUBLE nucleotides have been isolated and identified from both fruit and vegetables (Luh and Chen, 1969). These compounds are known to be essential intermediates in numerous metabolic reactions, especially carbohydrate and organic acid metabolism (Hassid, 1967). Citrus fruit in particular are characterized by their capacity to synthesize and accumulate sugars in some cases and organic acids in others (Sinclair, 1961). Concentrations of specific nucleotides in fruit at various stages of growth and development could possibly serve as an important regulatory mechanism in carbohydrate and organic acid metabolism. Buslig (1970) has demonstrated that a change in the level of acidity in citrus fruit is correlated with a shift in an ATP/ADP (adenosine diphosphate/adenosine triphosphate) ratio.

Not only are nucleotides essential for metabolism, but they have also been found to modify flavor. Studies by Kuninaka et al., (1964) and Kuminaka (1960) have shown that some of the nucleotides normally found in plant tissues will modify flavor. 5′-GMP (guanosine monophosphate) has the strongest modifying influence on flavor of the nucleotides isolated from plant tissue. Little is known of the interaction between nucleotides or between nucleotides and other flavor modifying compounds. That an interaction can occur is evident from the observation that 5′-AMP (adenosine monophosphate) has little or no effect on flavor, but together with glutamic acid has a taste-improving effect (Kuninaka, 1960).

A knowledge of the types and concentration of nucleotides in citrus is needed for an understanding of their possible role in the quality and flavor of fresh fruit and processed citrus products.

This is a report of the tentative identification and semiquantitation of acid-soluble nucleotides in citrus fruit at various stages of maturity.

EXPERIMENTAL

Material

Fruit of "Orlando" tangelo and "Hamlin" and "Pineapple" sweet oranges were harvested at various intervals after the fruit had attained commercial maturity. The fruit were frozen with dry ice immediately after harvest and the segments separated from the rind before they were pulverized. Care was taken to insure that the frozed fruit and the subsequently separated samples remained at or below −10°C until after the first step of the extraction procedure.

Extraction

The extraction procedure followed was similar to that described by Selvendran and Isherwood (1967). Frozen fruit segments (40g) were macerated in cold 0.6N perchloric acid using a Sorvall omni-mixer operated at 16,000 rpm for 3 min. The ground mixture was then steeped for 3 hr at 4°C before filtering the extract through a column (3 cm × 9 cm) of polyvinylpolypyrrolidone (Polyclar AT). The column was immediately washed with 2 bed-volumes of 0.6N perchloric acid. The filtrates and washings were collected in a vacuum flask that was immersed in an alcohol-dry ice mixture, pH adjusted to 6.0 with 15% KOH, allowed to stand for 3 hr at 4°C, and then filtered through a bed of celite on filter paper to remove the precipitated $KClO_4$ salts. The extract was then treated with activated charcoal (Darco G6) for 12 hr at 4°C to selectively absorb the nucleotides, leaving in solution many UV-absorbing nonnucleotide compounds present in the extract. After removing the residue by filtration and washing with cold H_2O, the nucleotides were eluted from the charcoal with a hot ethanol:water:ammonuim hydroxide (50:49:1 v/v) solution. The filtrate was reduced to 5 ml under vacuum.

Anion-exchange chromatography

Extract, prepared by the foregoing procedure, was added to the top of a Dowex AG 1-X8 resin (formate form, 200–400 mesh, Bio-Rad Laboratories) and washed onto the column with several bed-volumes of distilled water before the anions (nucleotides) were separated by gradient elution using a 2-phase formic acid:ammonium formate system. The first phase was a gradient between H_2O (250 ml) and 4N formic acid (250 ml); the second phase was a gradient between 4N formic acid (250 ml) and 2N ammonium formate (250 ml). The effluent, 1 ml/min, was continuously monitored at 260 nm using a Gilford Auto-Analyzer (model 2000).

Identification and quantitation

Identification was made by comparing the retention time from an exchange column with standards fractionated by the same procedure and from elution sequences reported by others (Brown, 1962; Brown and Nordin, 1969; Selvendran and Isherwood, 1967). Also, further proof of identity was obtained, where possible, by comparing the absorption spectra of the unknown to a standard. The quantity of each was determined by measuring the area under the curve and by comparing it to a standard nucleotide having approximately the same retention time.

Determination of ATP concentration

Concentration of ATP in the fruit segments was estimated by using the firefly, luciferin-luciferase system described by Buslig and Attaway (1969). Each batch of firefly extract was standardized with known amounts of ATP.

RESULTS & DISCUSSION

THE EXTRACTION PROCEDURE described herein was found to be adequate for extraction of acid-soluble nucleotides from citrus fruit segments. The elution sequences of both the 5′ mononucleotide standards and the acid-soluble nucleotides from Hamlin orange harvested at early

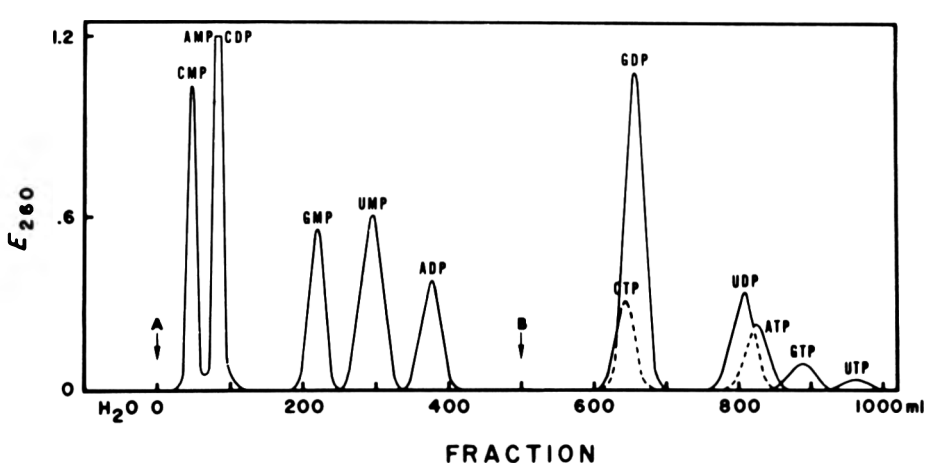

Fig. 1—Anion-exchange chromatogram of the 5′ mononucleotides separated on a Dowex 1 × 8 (formate form) ion-exchanger in a formate system: (A) water to 4N formic acid; (B) 4N formic acid to 1.6N ammonuim formate.

Table 1—Estimated quantity of the acid-soluble nucleotide fraction in mg/100g pulp of citrus fruit at commercial maturity

Fraction	Harvest date—1970							Identification
	Orlando tangelo		Hamlin orange		Pineapple orange			
	11–25	12–12	11–25	12–22	11–25	12–22	1–17	
a	1.15	0.40	2.75	1.56	2.45	1.32	3.16	CMP-NAD[b]
b	0.06	0.77	0.03	–	0.50	–	–	AMP
c	–[a]	–	0.56	0.03	0.03	0.15	0.12	CDP
d	–	0.03	0.09	–	–	–	–	GMP
e	–	–	–	–	0.12	0.20	0.08	–
f	0.10	–	0.08	–	–	–	0.12	UMP
g	–	0.33	–	–	–	–	–	ADP
m	1.13	0.93	0.63	0.25	0.70	0.83	0.60	–
n	0.13	0.11	0.07	0.01	0.09	0.07	0.13	CTP-GDP[b]
o	0.05	–	0.23	–	0.03	0.04	–	UDP-ATP[b]
Total	2.62	2.57	4.44	1.85	5.77	2.61	3.65	

[a]Not detected
[b]Tentative identification

maturity are shown in Figures 1 and 2, respectively. The profile for Hamlin will demonstrate a typical elution pattern obtained. However, the quantities of each fraction and the number of fractions differ with the variety of citrus examined.

The acid-soluble nucleotide fractions separated from the fruits of three varieties harvested at various stages of commercial maturity are presented in Table 1. The quantity and tentative identification of the various fractions are also indicated. Specific identification of several fractions was not possible because of incomplete separation of components (Fig. 1) and the gradual elution of other UV-absorbing compounds from the extract at the beginning of the gradient (Fig. 2). However, peaks b, c, d, f, and g were identified as AMP, CDP (cytidine diphosphate), GMP, UMP (uridine monophosphate) and ADP, respectively. Peaks e and g are not shown in Figure 2, however they were found in one or more of the varieties (Table 1). Identity of these components were confirmed by spectrophotometric scans from 200–320 nm at pH of 2, 7 and 11. Spectra and ratios at 250/260 and 280/260 were matched to standards. Peaks a, n, and o are thought to consist of at least two nucleotide compounds of CMP-NAD (cystidine monophosphate-nicotinamide-adenine dinucleotide), CTP-GDP (cystidine triphosphate-guanosine diphosphate) and UDP-ATP (uridine diphosphate), respectively. NAD was not used as a standard but has been reported by others to elute from a formate column at approximately the same position as CMP (Brown, 1962).

Fractions a, m and n were present in all fruit analyzed. These three fractions comprised approximately 80–90% of the total nucleotide concentration throughout the period studied in all three varieties. Fraction m was not identified but indications were that it was a sugar nucleotide.

Because of its metabolic significance, ATP was quantitated further in citrus fruit pulp. Analysis of this nucleotide was made by the luciferin-luciferase system (Buslig and Attaway, 1969). The ATP concentration in mg/100g pulp in fruit ranged from 2.06–2.58 for Orlando tangelo, 0.94–1.99 for Hamlin and 0.55–1.33 for Pineapple orange (Table 2). These data are in about the same range as those reported by Buslig (1970).

Quantitative estimates of the individual acid-soluble nucleotides in citrus fruit pulp indicate that these compounds are in abundance at commercial fruit maturity. These data are presented in Table 1. The total quantity in mg/100g of pulp at maturity was 2.62–2.57 mg for Orlando tangelo, 4.44–1.85 mg for Hamlin and 2.61–5.77 mg for Pineapple orange. Luh and Chen (1969) found that the primary nucleotides in tomato fruits were the phosphorylated derivatives, 5′ cytidylic, 5′ adenylic and 5′ uridylic acids, comprising approximately 13 mg/100g of fruit. The nucleotides tentatively identified have been shown to be essential in the interconversion of sugars in plant tissue (Hassid, 1967). These compounds can be visualized as having an important function in the synthesis of sugars in citrus juice vesicles since it is a tissue adapted for sugar synthesis and storage (Sinclair, 1961).

Studies by Schinneller (1972) indicate that both 5′ GMP, and 5′ ADP will modify the flavor of specific compounds present in citrus juice, particularly octanal. Significantly, less than 10 ppm was

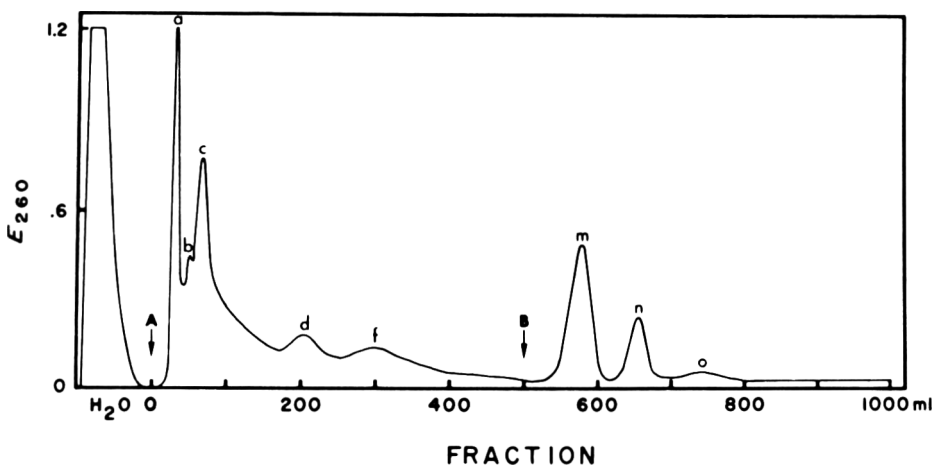

Fig. 2—Anion-exchange chromatogram of the acid-soluble nucleotides of Hamlin orange fruit pulp harvested 11-25-70. Separation of the extract was on Dowex 1 (formate form) ion-exchanger in a formate system: (A) water to 4N formic acid; (B) 4N formic acid to 1.6N ammonium formate.

Table 2—Concentration of ATP in citrus fruit pulp at commercial maturity

Harvest date	ATP mg/100g pulp
Orlando tangelo	
11-25-70	2.06
12-12-70	2.58
Hamlin orange	
11-25-70	1.37
12-12-70	1.99
12-22-70	0.94
Pineapple orange	
11-25-70	1.33
12-22-70	0.52
1-17-71	0.55

required to cause an enhancement in the detection of octanal. Shown by the present data, tissue concentrations of nucleotides in the juice vesicles of citrus are at a level where they could modify flavor. Also, Luh and Chen (1969) reported isolating several nucleotides from other fruits and vegetables and have suggested that these compounds in combination with others may be related to the taste appeal of these products.

Organoleptic tests have demonstrated that the 5′ monophosphate derivatives of purines generally have a greater effect on modifying flavor than either the 5′ monophosphate derivatives of pyridines, or the nucleosides of both purines or pyrimidines (Kuninaka et al., 1960). There are some exceptions to this generality, yet it is noteworthy that the more stable phosphate esters are the better modifiers of flavor. Relating this to the observations that ripening of fruits generally result in degradation of the di- and tri-phosphate nucleotides, and monophosphate nucleotides to a lesser extent, it follows that the kinds of nucleosides and nucleotides and tissue concentrations is very dependent on the metabolic state of the organ. Also, the method of processing fruits would alter the nucleotide content. Thus, a part of the flavor modification of citrus fruits with handling and storage and with processing could possibly result from chemical changes among nucleotides.

REFERENCES

Brown, E.G. 1962. The acid-soluble nucleotides of mature pea seeds. Biochem. J. 85: 633.

Brown, J. and Nordin, P. 1969. Soluble nucleotides from the immature fruit of tomato. J. Ag. Food Chem. 17: 341.

Buslig, S.B. 1970. Biochemical basis of acidity in citrus fruits. Doctoral Dissertation, Univ. of Fla., Gainesville.

Buslig, S.B. and Attaway, J.A. 1969. A study of acidity levels and adenosine triphosphate concentration in various citrus fruits. Proc. Fla. State Hort. Soc. 82: 206.

Hassid, W.Z. 1967. Transformation of sugars in plants. Ann. Rev. Plt. Physiol. 18: 253.

Kuninaka, A. 1960. Studies on taste of ribonucleic acid derivatives. J. Agric. Chem. Soc. (Japan) 34: 489.

Kuninaka, A., Kibi, M. and Sakaguchi, K. 1964. History and development of flavor nucleotides. Food Technol. 18: 287.

Luh, B.S. and Chen, K.C. 1969. Acid-soluble ribonucleotides in fruits and vegetables. Fruchtsaft Industrie 14(4): 146.

Schinneller, D.J. 1972. Flavor and aroma thresholds of selected aldehydes and interaction with 5′-nucleotides associated with citrus juice. M.S. thesis, Univ. of Fla., Gainesville.

Selvendran, R.R. and Isherwood, F.A. 1967. Identification of guanosine diphosphate derivatives of D-xylose, D-mannose, D-glucose and D-galactose in mature strawberry leaves. Biochem. J. 105: 723.

Sinclair, W.B. 1961. Principal juice constituents. In "The Orange," ed. Sinclair, W.B., p. 131. Univ. of Calif. Press, Berkeley.

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EFFECT OF GAMMA RADIATION ON WHEAT PROTEINS

INTRODUCTION

NUTRITIONAL and other wholesomeness aspects of Co_{60} gamma ray-disinfestation of stored wheat have been extensively studied (WHO, 1970). Subtle changes in the susceptibility of amylolysis of starch in irradiated wheat have been reported by Ananthaswamy et al. (1970). Although at dose levels of 20–200 Krads, irradiation does not alter the total protein content or the protein efficiency of wheat (Aravindakshan et al., 1970) and damage to gluten in terms of molecular fragmentation is insignificant, some loss in its colloidal properties with resulting lower sedimentation value has been reported (Milner, 1961). Degradation of gluten by X-rays in presence of water is characterized by changes in viscosity (Barron and Finkelstein, 1952).

The present studies report on the radiosensitivity and enzymic susceptibility of wheat proteins in situ and of isolated gluten. Degradation products of wheat proteins were characterized by gel filtration. Lysine availability as well as total and free amino acids in control and irradiated wheat samples were also determined.

MATERIALS & METHODS

SAMPLES of hard red winter variety wheat, *Triticum aestivum*, obtained from the Food Corp. of India, as well as isolated gluten and gliadin samples were made into 25g packets, heat-sealed in polythene pouches and irradiated at 25°C in a cobalt-60 gamma cell 220 (Atomic Energy of Canada Ltd.) at dose levels of 20, 40, 60 and 200 Krad and at 1 Mrad, with a dose rate of 25 Krad per minute.

Gluten, gliadin and glutenin, purchased from Nutritional Biochemical Corp., U.S.A., were used as standards for identification of breakdown products of wheat proteins. Sephadex G-200 was obtained from Pharmacia Fine Chemicals, Uppsala, Sweden.

Isolation of gluten and gliadin

Well-kneaded dough, prepared from defatted wheat flour, was washed with distilled water to expel out starch and other nongluten materials. The resulting gluten ball was dispersed in 0.01M acetic acid and centrifuged at 20,000 × G for 1 hr. To inactivate proteolytic enzymes, the supernatant was heated at 95°C for 5 min, cooled and lyophilized (Olcott et al., 1943). The gluten samples thus prepared contained 85% protein and only traces of carbohydrate. Glutenin was further separated from gluten by treatment with 70% ethanol at pH 6.5 and allowing the solution to stand at 4°C overnight. Gliadin, retained in solution, was recovered by removing alcohol under vacuum. Suspensions of glutenin and gliadin were made separately in 0.01M acetic acid and resulting solutions lyophilized.

Autolysis

2.5g wheat flour (60 mesh) was suspended in 50 ml 0.1M citrate buffer pH 5.5 and incubated under toluene at 40°C with frequent shaking. Reaction was stopped at zero time (control) or after 5½ hr by addition of 20% trichloroacetic acid (TCA). Tyrosine liberated was estimated in the supernatant colorimetrically (Udenfriend et al., 1952).

Enzymic susceptibility of wheat proteins

To determine the susceptibility of wheat proteins to proteolytic enzymes, 10 ml of isolated gluten or gliadin (0.5%, pH 4.5), from unirradiated or irradiated (20–200 Krads) wheat samples, was incubated under toluene with 5 mg papain, activated with reduced glutathione (El-Miligi and Vakar, 1964), at 30°C for 10 min. with frequent shaking. The reaction was stopped by 5 ml of 20% TCA, and tyrosine estimated in the supernatant.

UV absorption spectra

Gluten (2 mg) was dissolved in ethanol acetic acid (1:1) mixture (3 ml) and its UV absorption maximum determined between 240 and 320 nm wavelengths in a Zeiss-Ikon Recording spectrophotometer. The optical density at 276 nm was measured in a Beckman DB spectrophotometer.

Separation of wheat proteins by gel filtration

Wheat flour (8g) was suspended in 70 ml AUC solvent (0.1M acetic acid, 3M urea and 0.01M cetyltrimethyl ammonium bromide) and kept at 25°C for 1 hr with frequent shaking. The suspension was centrifuged at 105,000 × G for 30 min. 2 ml of the supernatant (25 mg protein) was applied on a Sephadex G-200 column (2.2 × 55 cm) and eluted with AUC solvent (Meredith and Wren, 1966). 2.5 ml fractions were collected using LKB automatic fraction collector. E_{280} of each fraction was measured in a 1 cm cell against solvent in a Beckman DB spectrophotometer. The area of each peak occupied by individual proteins was measured by planimeter. The position of each protein peak on the column was ascertained by employing authentic samples.

Amino acid analyses

Wheat flour or isolated gluten (25–30 mg protein) samples were hydrolyzed with 6N HCl in vacuo for 24 hr at 110°C ± 1°C and individual amino acids determined in the filtrate. Serine and threonine were lost up to 8–10% during acid hydrolysis. Necessary corrections were made for this loss in the final calculations.

Table 1–Autolysis in irradiated wheat[a]

Dose level (Krads)	Initial free tyrosine mg/g wheat flour	% Increase over control	Tyrosine liberated after 5½ hr digestion μg/g wheat flour	% Increase over control
0	145	–	304	–
20	153	5.5	322	6.2
40	153	5.5	375	28.3
60	153	5.5	381	30.2
200	164	13.1	396	31.4

[a]Values are averages of three experiments. For details of autolytic procedures and determination of tyrosine in autolysate, see text under "Methods."

Table 2–Susceptibility of irradiated gluten and gliadin to papain action

	Gluten			Gliadin		
Dose level (Krads)	0 min	10 min	% Increase over control	0 min	10 min	% Increase over control
	(mg tyrosine liberated/g)			(mg tyrosine liberated/g)		
0	13.8	24.8	–	3.2	8.7	–
20	14.2	25.8	5.4	3.6	9.6	9.0
40	14.6	26.9	12.0	4.0	10.4	18.0
60	15.2	28.2	18.1	4.2	11.3	29.1
200	15.2	28.5	20.9	4.4	11.8	34.5

[a]Values are averages of three experiments. Tyrosine released by papain action was carried out as described under "Methods" and % increase in irradiated samples over corresponding controls after 10 minutes' incubation calculated.

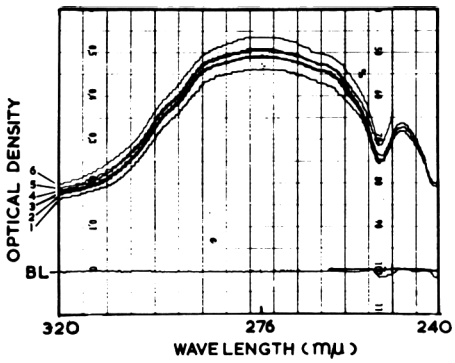

Fig. 1–UV absorption spectra of irradiated gluten samples. Isolated gluten, irradiated at 0.02–1 Mrad dose levels, was dissolved in 1:1 ethanol–0.1M acetic acid solution (2 mg/3 ml). Absorption between 240–320 nm was measured in a Zeiss-Ikon Recording Spectrophotometer. 1–unirradiated; 2–0.02 Mrad; 3–0.04 Mrad; 4–0.06 Mrad; 5–0.2 Mrad; 6–1 Mrad; BL–base line (solvent).

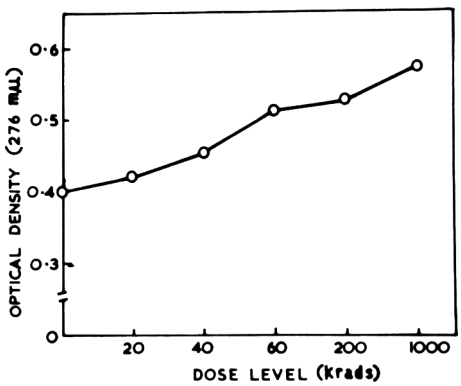

Fig. 2–Spectrophotometer readings of irradiated gluten. Optical density of isolated and irradiated (0.02–1 Mrad) gluten (2 mg/3 ml) was measured at 276 nm in a Beckman DU Spectrophotometer.

To estimate free amino acids, 5g wheat flour was extracted thrice with 70% ethanol. The combined extracts were passed through Dowex-50 H^+ column and eluted with 2N NH_4OH.

The hydrolysate or eluate was dried by evacuation on a rotary evaporator at 60°C. The residue was dissolved in 10 ml 0.2M citrate buffer pH 2.2. Aliquots were analyzed for amino acids with Beckman Unichrome automatic amino acid analyzer. Tryptophan was estimated by the colorimetric method described by Spies and Chambers (1949).

Total nitrogen

Nitrogen was estimated by the micro Kjeldahl method (Hawk et al., 1954). A factor of 5.7 was used to convert nitrogen to protein values.

Available lysine

The amounts of available lysine in wheat samples were determined with 1-fluoro-2, 4-dinitrobenzene (FDNB), essentially according to the method developed by Carpenter (1960). The extinction was read at 435 nm and DNP-lysine was used as a reference.

Table 3–Effect of radiation treatment on amino acids composition of wheat[a]

Amino acid	Control	Mrad 0.02 (g/16g nitrogen)	0.2	1.0
1 Aspartic acid	4.62	4.51	4.58	4.49
2 Threonine	2.97	3.02	2.91	2.88
3 Serine	5.32	5.10	5.28	5.41
4 Glutamic acid	31.60	32.90	34.22	32.30
5 Proline	11.07	12.30	11.38	11.00
6 Glycine	3.57	3.41	3.45	3.42
7 Alanine	3.03	2.93	2.95	2.71
8 ½ Cystine	1.02	0.98	1.00	1.04
9 Valine	4.55	4.62	4.42	4.48
10 Methionine	1.73	1.69	1.75	1.68
11 Isoleucine	4.10	4.25	4.01	4.08
12 Leucine	6.83	7.20	6.98	7.08
13 Tyrosine	3.25	3.31	3.18	3.20
14 Phenylalanine	4.74	4.85	4.79	4.82
15 Lysine	2.42	2.38	2.45	2.38
16 Histidine	1.91	1.88	1.95	1.93
17 Arginine	4.59	4.41	4.38	4.39
18 Tryptophan	1.02	0.98	0.92	0.95

[a]Values are averages of three experiments. Acid hydrolysate of wheat flour was obtained and concentrated as described in "Methods." The residue was taken into 20 ml of 0.2M citrate buffer and filtered; aliquots were analyzed for amino acids. Corrections were made in the values for serine and threonine to account for losses during acid hydrolysis.

RESULTS

Autolysis in irradiated wheat

Free tyrosine values (145 μg/g) in control wheat sample were increased by 5.5–13.1% (Table 1) in irradiated wheat flour (20–200 Krads) though no difference was observed in samples irradiated at 20–60 Krads. Autolysis or self-digestion of irradiated wheat flour for 5½ hr also resulted in 6–32% increase in tyrosine values, this being rapid initially up to 40 Krads.

Results on the initial tyrosine levels and tyrosine liberated after papain digestion from isolated proteins of unirradiated and irradiated wheat samples are presented in Table 2. Enzymatic reaction was linear only up to 10 min under the experimental conditions employed. Free tyrosine values increased from 2–9% with gluten and from 12–38% with gliadin on radiation treatment compared to their respective controls. Significantly more tyrosine was liberated by papain action from irradiated gluten and gliadin samples.

Spectral characteristics of irradiated gluten

No difference was observed in the general pattern of the UV absorption spectra, obtained from gluten samples irradiated at different dose levels (Fig. 1). There was no shift in the UV absorption maximum, which was at 276 nm. However, an increase in optical density, particularly in the region of the aromatic amino acids (276 nm), in irradiated samples was observed (Fig. 2). The optical density increased from 0.4 in control to 0.57 in irradiated (1 Mrad) samples.

Elution pattern of irradiated wheat proteins on Sephadex G-200

Figure 3 represents the distribution of wheat proteins according to their molecular size, resolved by gel filtration. The elution curve obtained with the control sample showed four main components viz., glutenin (47%), gliadin (32%), albumin (17%) and nonproteins (4%), representing the profile of protein distribution according to their molecular weights. Though the same pattern was obtained with irradiated wheat (1 Mrad), a shift in the molecular weight distribution to lower values was observed. About 17% lower protein values were obtained in glutenin peak with concomitant increase in nonprotein peaks.

Amino acid profiles of irradiated wheat

Analyses of total amino acids of control and irradiated (0.02 to 1 Mrad) wheat samples (Table 3) as well as of gluten (Table 4) revealed no appreciable changes due to radiation treatment. Irradiation at 1 Mrad did not reduce cystine or methionine content in the samples. However, an overall increase of

Table 4–Effect of radiation treatment on amino acids composition of isolated gluten[a]

Amino acid	Control	Mrad 0.02 (g/16g nitrogen)	0.2	1.0
1 Aspartic acid	3.42	3.37	3.45	3.41
2 Threonine	2.37	2.39	2.41	2.38
3 Serine	5.08	5.17	5.20	5.18
4 Glutamic acid	36.69	36.62	35.97	36.49
5 Proline	12.84	13.19	12.64	13.04
6 Glycine	3.29	3.21	3.21	3.22
7 Alanine	2.39	2.41	2.36	2.40
8 ½ Cystine	1.20	1.18	1.22	1.24
9 Valine	4.25	4.28	4.26	4.24
10 Methionine	1.85	1.83	1.82	1.82
11 Isoleucine	4.12	4.11	4.17	4.11
12 Leucine	6.64	6.67	6.54	6.53
13 Tyrosine	2.94	3.01	3.01	3.02
14 Phenylalanine	5.14	5.08	5.11	5.10
15 Lysine	1.60	1.59	1.60	1.63
16 Histidine	2.13	2.14	2.19	2.19
17 Arginine	3.56	3.55	3.51	3.50
18 Tryptophan	1.04	1.04	1.05	1.03

[a]Values are averages of three experiments. Experimental details as given in Table 3.

Table 5–Effect of irradiation on the free amino acid contents of wheat[a]

Amino acid	Control	Mrad 0.02 (mg/g nitrogen)	0.2	1.0
1 Aspartic acid	4.03	3.97	3.97	4.01
2 Threonine	0.37	0.35	0.39	0.39
3 Serine	3.47	3.51	3.35	3.28
4 Glutamic acid	3.04	2.88	2.77	2.90
5 Proline	0.44	0.45	0.47	0.46
6 Glycine	0.84	0.83	0.86	0.87
7 Alanine	1.92	2.11	2.36	2.60
8 Valine	0.76	0.78	0.82	0.84
9 Methionine	0.33	0.34	0.34	0.37
10 Isoleucine	0.52	0.56	0.53	0.64
11 Leucine	0.62	0.64	0.66	0.65
12 Tyrosine	0.48	0.49	0.54	0.60
13 Phenylalanine	0.47	0.49	0.50	0.51
14 Lysine	0.46	0.45	0.45	0.47
15 Histidine	0.22	0.21	0.23	0.21
16 Arginine	1.73	1.79	1.77	1.80

[a]Values are mean of three independent determinations. Samples of wheat flour were extracted thrice with 70% ethanol. Extracts were passed through cation exchanger. Amino acids were analyzed in the eluate.

about 8.4% in free amino acid levels in wheat irradiated at 1 Mrad was observed (Table 5). This could be attributed to appreciable release of isoleucine, tyrosine, valine and alanine amounting to 23–35% higher values in their free form compared to control. Similarly, no significant differences in available lysine content in control and irradiated samples were observed (Table 6).

DISCUSSION

THERE ARE REPORTS to show that degradation (Deschreider, 1966), specific or nonspecific amino acid damage (Patten and Gordy, 1964), hydrogen bond disruption (Williams and Hunt, 1963) and cleavage of intermolecular disulfide bonds (Doguchi, 1969) take place in proteins irradiated at high dose levels (5–10 Mrads). The potential effects of free radicals, sulphydryl (-SH) and disulfide (-S-S-) groups in irradiated proteins have been stressed (Lee, 1962). The chemical nature of damage in irradiated proteins has also been explained as due to changes in molecular configuration resulting in condensation or random polymerization (Alexander et al., 1960). The present work indicates fragmentation of wheat proteins to low molecular entities even at low dose levels (0.02–1 Mrad) as revealed by increased free tyrosine values in irradiated flour (Table 1) and in isolated wheat proteins (Table 2). Results on autolysis of wheat flour, under the experimental conditions adopted, showed significant increase in release of free tyrosine (Table 1). Similarly, when isolated gluten and gliadin were subjected to protease action, significantly more tyrosine was liberated from irradiated samples. The observed changes may be due to altered susceptibility of wheat proteins and proteolytic enzymes on irradiation and/or to changes in enzymatic properties. It was further noticed that gliadin was more susceptible to enzymic hydrolysis than gluten. This may be explained by the fact that gliadin is a much simpler protein molecule, having a molecular weight of approximately 40,000 compared to 2–3 millions for gluten (Jones et al., 1961).

The increases in the absorption spectrum at 276 nm (Fig. 1) and in optical density (Fig. 2) of irradiated gluten could be correlated with the observed increase in free tyrosine content (Table 2). A similar increase in the UV absorption at the aromatic maximum has been reported in egg albumin (Alexander et al., 1956). Barron and Finklestein (1952) postulated that the nature of the UV spectrum depends mainly on tryptophan:tyrosine ratio of protein.

Total amino acid profiles of wheat (Table 3) and of isolated wheat gluten

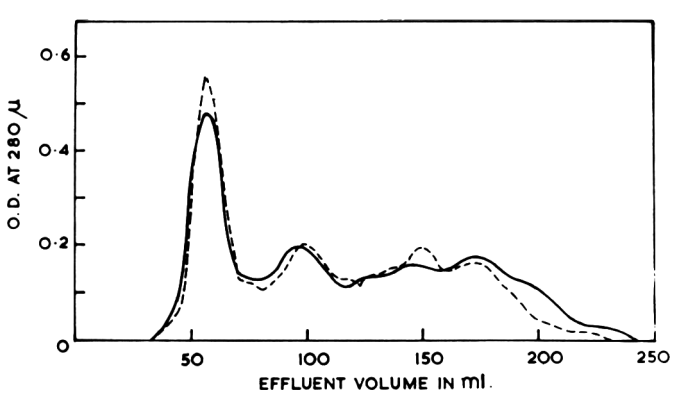

Fig. 3–Elution pattern of irradiated wheat proteins on Sephadex G-200. 2 ml (25 mg protein) of wheat flour extract (8 g in 70 ml AUC solvent, centrifuged at 105,000 × G for 30 min) was applied on the column (0.5 × 50 cm). 2.5 ml fractions were collected and O.D. was measured in a Beckman DB Spectrophotometer at 280 nm. - - - control: —— irradiated. Peaks 1, 2, 3 and 4 represent the four main components of wheat proteins namely glutenin, gliadin, albumin and nonprotein nitrogen, respectively.

Table 6–Available lysine content of irradiated wheat and gluten[a]

Treatment (Mrad)	Wheat flour g/16 g nitrogen	Gluten
0	1.96	1.42
0.02	1.88	1.39
0.20	1.94	1.42
1.00	1.90	1.44

[a]8 ml of 8% $NaHCO_3$ and 0.3 ml FDNB diluted in 12 ml ethanol were added to 300 mg samples and shaken continuously for 2 hr. This was followed by refluxing for 16 hr with 8N HCl and extraction with ethyl ether. Color in the water phase was read at 435 nm in a Baush and Lomb colorimeter. Results are averages of three experiments.

(Table 4) did not reveal any appreciable changes up to 1 Mrad. Moran et al. (1968) did not observe any differences in amino acid composition of wheat bran irradiated at 5 Mrad. However, amino acid composition of gluten irradiated at 5 Mrad indicated small losses (10%) of leucine, isoleucine and methionine (Kennedy, 1965). Considerable reduction in cysteine content of wheat irradiated at 10 Mrad (Doguchi, 1969) has been reported. Available lysine content (Table 6), employing the procedure outlined, showed no noticeable differences in wheat or gluten samples irradiated up to 1 Mrad. No change in true digestibility (99%) or in biological value (42%) was observed when gluten, irradiated at 2.8 Mrads, was fed to animals (Metta and Johnson, 1959).

The changes in the elution diagram of wheat proteins obtained by gel filtration are shown in Figure 3. The first peak is composed mainly of heterogenous glutenin components (Wright et al., 1964; Beckwith et al., 1966), which are diminished by 17% with concomitant increase in gliadin and albumin peaks in irradiated sample. This suggests that wheat proteins could be degraded to lower molecular weight peptides and amino acids due to radiation treatment. Woychick et al. (1964) have suggested that glutenin molecules are formed primarily by intermolecular disulfide bonding of gliadin components. Since disulfide bonds contribute to the unique viscoelastic properties of wheat proteins (Beckwith et al., 1965), the observed changes in glutenin and gliadin proportions in irradiated wheat (Fig. 3) may influence its rheological properties. Unpublished data from this laboratory (Rao et al., 1971) have shown that irradiation of wheat in the dose range 20–75 Krad, needed for wheat disinfestation which has also been approved by WHO (1970), resulted in better water absorption capacity, stability and blending value of the dough. The total loaf-volume in yeast-leavened bread was increased up to 8% with irradiated wheat samples compared to control and judged better with respect to cell structure, crumb color and acceptability. Some adverse effects on baking and rheological qualities have, however, been reported with wheat flour, irradiated at very high (1 Mrad) dose levels (Milner, 1961). Sosedov and Vakar (1961) postulated the possibility of producing desirable changes in the baking properties of wheat flour by considering the initial qualities of gluten and regulating the moisture content accordingly before irradiation.

REFERENCES

Alexander, P., Fox, M., Stacy, K.A. and Rosen, D. 1956. Comparison of some direct and indirect effect of ionizing radiations in protein. Nature 178: 846.

Alexander, P., Hamilton, L.D.G. and Stacy, K.A. 1960. Irradiation of proteins in the solid state. 1. Aggregation and disorganization of secondary structure in bovine serum albumin. Rad. Res. 12: 510.

Ananthaswamy, H.N., Vakil, U.K. and Sreenivasan, A. 1970. Susceptibility to amylolysis of gamma-irradiated wheat. J. Food Sci. 35: 792.

Aravindakshan, M., Vakil, U.K. and Sreenivasan, A. 1970. Studies on wholesomeness testing of gamma irradiated wheat. B.A.R.C. Report No. 455, A.E.C. Government of India.

Barron, E.S.G. and Finkelstein, P. 1952. Studies on the mechanism of action of ionizing radiations. 10. Effect of X-rays on some physico-chemical properties of wheat. Arch. Biochem. Biophys. 41: 212.

Beckwith, A.C., Wall, J.S. and Jordan, R.W. 1965. Reversible reduction and reoxidation of disulfide bonds of wheat gliadin. Arch. Biochem. Biophys. 112: 16.

Beckwith, A.C., Nielsen, H.C., Wall, J.S. and Huebner, F.R. 1966. Isolation and characterization of a high molecular weight protein from wheat gliadin. Cereal Chem. 43: 14.

Carpenter, K.J. 1960. The estimation of available lysine in animal protein foods. Biochem. J. 77: 604.

Deschreider, A.R. 1966. Action of the gamma rays on the constituent elements of the wheat flour. IAEA/SM-73/4, Karlsruhe, Germany, 6–10 June.

Doguchi, M. 1969. Effects of gamma irradiation on wheat gluten. Agr. Biol. Chem. 33: 1769.

El-Miligi, A.K. and Vakar, A.B. 1964. Effect of conditions of cultivation on wheat gluten. Dokalady Biochem. 155: 91.

Hawk, P.B., Oser, B.L. and Summerson, W.H. 1954. "Practical Physiological Chemistry," 13th ed. p. 881. McGraw Hill Book Co., N.Y.

Jones, R.W., Babcock, G.E., Taylor, N.W. and Senti, F.R. 1961. Molecular weights of wheat gluten fractions. Arch. Biochem. Biophys. 94: 483.

Kennedy, T.S. 1965. Nutritional value of foods treated with gamma irradiation. 1. Effects of some B-complex vitamins in egg and wheat. J. Sci. Food Agri. 16: 433.

Lee, C.C. 1962. Electron para magnetic resonance (EPR) and baking studies on gamma irradiated flour. Cereal Chem. 39: 147.

Meredith, O.B. and Wren, J.J. 1966. Determination of molecular weight distribution in wheat flour proteins by extraction and gel filtration in a dissociating medium. Cereal Chem. 43: 169.

Metta, V.C. and Johnson, B.C. 1959. Biological value of gamma irradiated corn protein and wheat gluten. J. Agr. Food Chem. 7: 131.

Milner, M. 1961. Technological effect of gamma irradiation on wheat. In "Proceedings of the Fifth International Congress of Biochemistry," Moscow, 10–16 August, Vol. 8, p. 108. Pergamon Press.

Moran, E.T. Jr., Summer, J.D. and Bayley, H.S. 1968. Effect of Cobalt-60 gamma irradiation on the utilization of energy, protein and phosphorous from wheat bran by the chicken. Cereal Chem. 45: 469.

Olcott, H.S., Sapirstein, L.A. and Blish, M.J. 1943. Stability of wheat gluten dispersions towards reducing agents in the presence and absence of a gluten proteinase. Cereal Chem. 20: 87.

Patten, R.A. and Gordy, W. 1964. Further studies of radiation effects on the proteins and their constituents. Rad. Res. 22: 29.

Rao, S.R., Vakil, U.K. and Sreenivasan, A. 1971. Rheological properties and baking qualities of irradiated wheat. Unpublished data.

Sosedov, N.I. and Vakar, A.B. 1961. Effect of gamma-rays on biochemical properties of wheat. In "Proceedings of the Fifth International Congress of Biochemistry," Moscow, 10–16 August, Vol. 8, p. 133. Pergamon Press.

Spies, J.R. and Chambers, D.C. 1949. Chemical determination of tryptophan in proteins. Anal. Chem. 21: 1249.

Udenfriend, S. and Cooper, J.R. 1952. Chemical estimations of tyrosine and tyramine. J. Biol. Chem. 196: 227.

Williams, J.F. and Hunt, J.W. 1963. Molecular lesions produced in ribonuclease by gamma rays. Nature 200: 779.

WHO. 1970. Wholesomeness of irradiated food with special reference to wheat, potatoes and onions. World Health Organization Technical Report, Series No. 451, of a Joint FAO/IAEA/WHO Expert Committee.

Woychik, J.H., Huebner, F.R. and Dimler, R.J. 1964. Reduction and starch gel electrophoresis of wheat gliadin and glutenin. Arch. Biochem. Biophys. 105: 151.

Wright, W.B., Brown, P.J. and Bell, A.V. 1964. Fractionation of flour proteins by means of gel filtration on Sephadex G-100. J. Sci. Food Agri. 15: 56.

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STABILIZATION OF CALCIUM SENSITIVE PLANT PROTEINS BY κ-CARRAGEENAN

INTRODUCTION

POLYSACCHARIDE GUMS constitute an important group of hydrocolloid additives in modern food formulations. In addition to thickening ability, food gums impart other desirable physicochemical characteristics to food systems (Farkas and Glicksman, 1967). The mechanisms of their action on foods are not completely understood. It is evident from current information (Rees, 1969) that the effects of the polysaccharides must be interpreted on the basis of interaction properties.

Bernfeld (1966) reviewed the specific interactions between β-lipoproteins and polysaccharides at the neutral pH ranges. Hansen (1968) demonstrated that similar interaction between sulfated polygalactans of the carrageenan family and caseins prevented precipitation of the latter by ionic calcium. Further study of these complexes indicated that κ-carrageenan was more effective than other polysaccharides and its protein stabilizing ability was related to its chemical structure and molecular weight (Lin and Hansen, 1970).

In solution, κ-carrageenan forms double helices by interstrand H-bonding (Anderson et al., 1969). Junction zones may originate from the aggregation of several double helices resulting from the cationic interaction of the sulfate groups (SO_3^-) located outside the helix core (Rees, 1969). Electron microscopy suggested that the protein free areas of the casein-carrageenan complexes corresponded to the double helix junction zones of low protein reactivity (Chakraborty and Hansen, 1971). The casein-calcium-κ-carrageenan interaction was mainly intensified in the random areas of polysaccharide strands. On the basis of these morphological features, an interrelationship could be observed between casein stabilization and double helix junction zone formation by different carrageenans.

The present study was undertaken to determine if κ-carrageenan would similarly stabilize calcium sensitive proteins other than caseins, and if the morphology of such interacted particles would also be consistent with the double helix junction zone model. Plant protein isolates were selected for this purpose because of their future application potentials in synthetic foods.

EXPERIMENTAL

PROTEIN ISOLATES were obtained from various sources as dried powder prepared from protein solutions at pH 7. The soy protein isolate (Promine-D) was from Central Soya (Chicago, Ill.). Protein isolates from peanut, coconut and glandless cottonseed were obtained from the Food Protein R&D Center of Texas A&M University. The soy and peanut proteins were the fractions precipitated at pH 4.6 and 4.5, respectively. The glandless cottonseed protein was the isolate-I of Lawhon and Cater (1971) precipitated at pH 4.0. The coconut protein was the isolate extracted at pH 7.0 with 1M NaCl (Samson et al., 1971) and dialyzed against an infinite volume of water prior to concentration of the isolate.

Protein solutions (0.5%) were prepared by dispersing calculated amounts of the isolates in distilled water, tempering the solution momentarily at 95°C and discarding the sediment after centrifuging for 5 min at 3000 × G. The clear supernatant was stored at 4.5°C until used.

The sodium salt of κ-carrageenan (RENJ 6187, Marine Colloids Inc., Rockland, Me.) was dissolved in water to yield a stabilizer concentration of 0.05 to 0.1%.

The stability test of Zittle (1965) was performed after incorporating various amounts of carrageenan solution in 0.15% protein solutions. The solution of $CaCl_2$ (0.1M) was rapidly added to the mixture and its final concentration in the mixture was 0.01M. The mixture was incubated at room temperature for 15 min and the supernatant was collected by centrifugation at 3000 × G for 5 min. Stability was expressed in terms of protein concentration in the supernatant, calculated as percent of total protein initially present in the mixture. Coconut, peanut and soy protein concentrations in mixtures and supernatants were measured spectrophotometrically at 280 nm, using a few drops of 5N NaOH to eliminate turbidity. Glandless cottonseed protein was determined by the micro-Kjeldahl technique (AOAC, 1970).

For electron microscopy, the κ-carrageenan stabilized protein complexes were prepared at a stabilizer/protein ratio of 0.2 and the supernatant fractions were collected by centrifuging the freshly prepared complexes at 3000 × G for 5 min. Fixation was achieved by mixing 1 volume of the complex with 3 volumes of glutaraldehyde solution (3% in 0.1M phosphate buffer at pH 7.2), and by 12–18 hr incubation at 0–5°C. This preparation was diluted five times with glass distilled water and air dried as a thin film on parloidon-coated #300 mesh, copper grids. The preparations were stained for 15 min with an aqueous solution of $KMnO_4$ (1%), and washed with dilute citric acid and glass distilled water (Chakraborty, 1970). Stained preparations were air dried prior to examination with a Hitachi HS-8A Electron Microscope. Normal precautions were taken for avoiding external contamination by handling the samples in dust-free atmosphere during various preparatory steps. Examination of blank grids containing no sample, but carried through the fixation, staining and air-drying steps, showed that these procedures did not contribute to the structural features observed in the complex particles.

RESULTS & DISCUSSION

THE PLANT PROTEIN isolates used in this investigation were soluble in water at pH 7.0 and retained only 10–25% of their initial solubility when ionic calcium was added in the absence of carrageenan (Fig. 1). Addition of κ-carrageenan up to a stabilizer/protein ratio of 0.10 to 0.15 caused very little improvement in stability. However, at a stabilizer protein ratio of 0.2, the peanut and coconut protein isolates were completely stabilized and the stability of the glandless cottonseed and soy protein isolates was increased to 86 and 70% of their initial stability, respectively. Higher carrageenan concentrations might improve the stability of soy and glandless cottonseed proteins; however, a stabilizer/protein ratio higher than 0.2 was not attempted in the present study. For any carrageenan protein ratio,

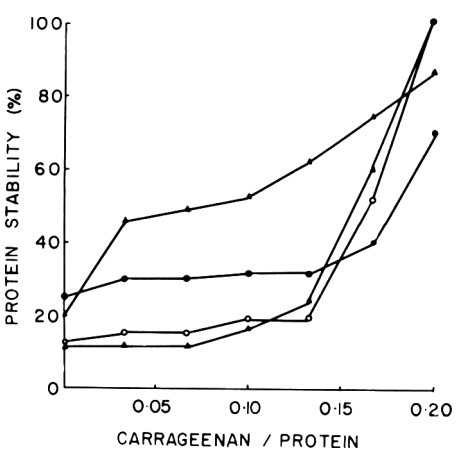

Fig. 1—Stabilization of plant proteins against calcium precipitation by κ-carrageenan. Protein isolates—coconut △-△; glandless cottonseed ▲-▲; peanut ○-○; soy ●-●. (Protein conc—0.15%; $CaCl_2$—0.01M. Data represent average of three trials.)

the stability data obtained at a relatively low protein concentration (0.15%) may not be extrapolated to higher protein levels, such as 1–3%, since proportional increases in the carrageenan content result in a highly viscous product. The relationship between the protein stability and carrageenan ratio was neither linear nor identical for all the isolates. The reason for the observed differences in the solubility behavior of these complexes may be better understood after studying the calcium-dependent interaction properties of various fractions present in these heterogeneous protein preparations.

Table 1—Effect of sequence of ingredient incorporation on protein stability[a]

Sequence of ingredient incorporation	Stability of systems containing protein isolates			
	Coconut %	Glandless cottonseed %	Peanut %	Soy %
Ca^{++} added to protein-carrageenan mixture	100	86	100	70
Protein added to Ca^{++}-carrageenan mixture	99	85	100	72
Carrageenan added to Ca^{++}-protein mixture	12	25	15	35
Carrageenan not added to Ca^{++}-protein mixture	12	20	15	25

[a]Average of three trials

The formation of stable complexes with β-lipoproteins of blood (Bernfeld, 1965), the caseins of milk (Lin and Hansen, 1971) and the vegetable protein isolates in the present study suggest that the interaction of κ-carrageenan with calcium sensitive proteins at neutral pH ranges may be of a general polyelectrolyte type (Rees, 1969). The participation of ionic calcium in these interactions is apparent from the development of turbidity upon addition of calcium chloride to the protein-stabilizer mixtures. Earlier work on the morphology of the casein-carrageenan complexes suggested that ionic calcium was involved in the aggregation of protein molecules to compact bodies (Chakraborty, 1970), the interaction of double helices into junction zones of low protein reactivity and the complexing of protein bodies with the more reactive unordered areas of polysaccharide strands (Chakraborty and Hansen, 1971).

Due to the importance of ionic calcium in different phases of protein-stabilizer interaction, the sequence of intermixing protein, carrageenan and calcium solutions may be important to the final stability of the system. The effect of changing the normal sequence of adding calcium to the protein-carrageenan mixture on the resultant protein stability is reported in Table 1. Stability data indicates that carrageenan need not be premixed with the protein solutions prior to adding calcium, and that it could be incorporated simultaneously with ionic calcium without affecting the stability of the system. However, adding κ-carrageenan after mixing protein and calcium solutions lowered the stability values to the level obtained without any stabilizer. Thus, carrageenan appeared to be effective as long as the calcium mediated aggregation of proteins had not become too massive for colloidal stability.

Typically, all stable complexes consisted of an irregular fiber-like mass and many dark, globular particles (Fig. 2). On the basis of structural studies on polysaccharides (Rees, 1969), carrageenan would be expected to exhibit fiber-like character. Since $KMnO_4$ is generally recognized as a protein stain (Zobel and Beer, 1965), the dark globular particles were considered to be carrageenan-free protein bodies (F) and the electron dense regions of the fibrous mass were assumed to be the protein-rich areas (P) of the carrageenan-protein complex. The relatively electron-transparent areas of the fibrous mass were regarded as protein-free (PF) zones of the complex. Such interpretations have been used previously as a

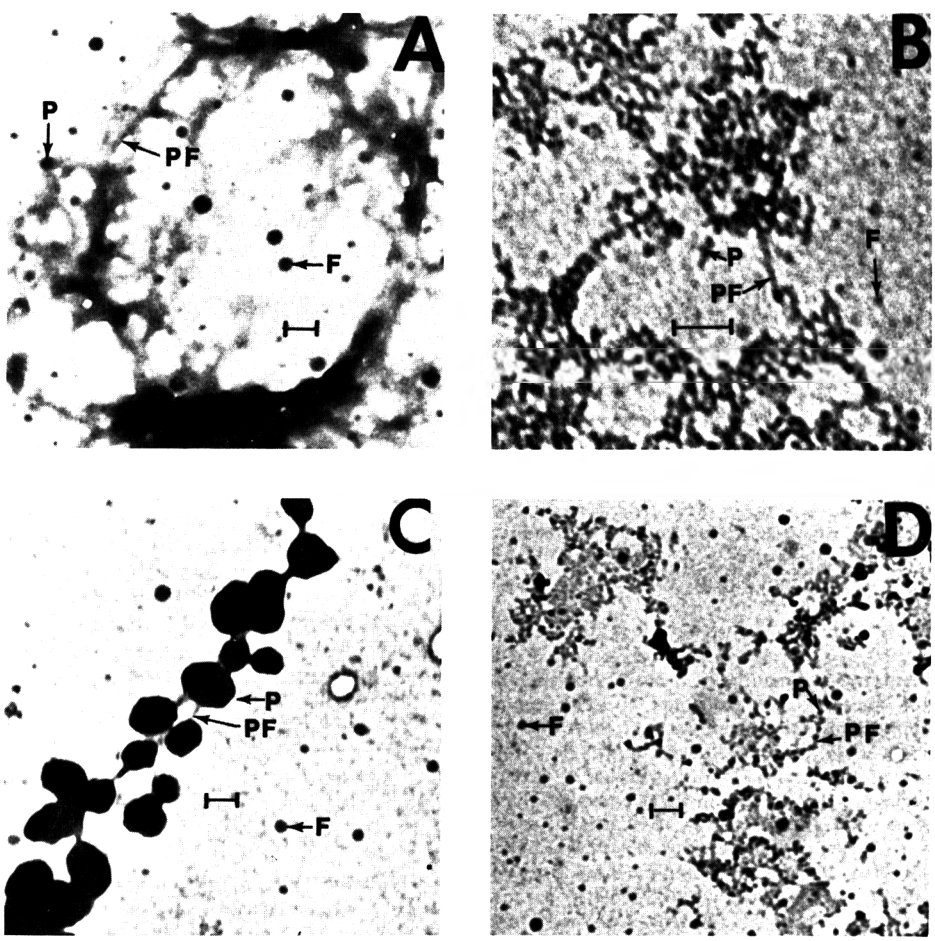

Fig. 2—Electron micrographs of κ-carrageenan stabilized complexes of vegetable protein isolates: (A) coconut; (B) cotton seed; (C) peanut; (D) soy. (F—free protein bodies; P—protein rich areas in the complex; PF—protein-free zones in the complex; — represents 1000 Å).

basis for analyzing electron photomicrographs of protein systems with and without κ-carrageenan (Chakraborty, 1970). Heterogeniety could be observed in the dimensional features of the free-protein bodies (F), the protein-rich (P), and the protein-free (PF) areas of the complexes. However, the quantification of such data was not attempted in this study.

Free-protein bodies were of approximately 100–200 Å diam in complexes containing glandless cottonseed and soy protein, and of about 200–500 Å diam in systems containing coconut and peanut proteins. The protein bodies may very well represent the globular fractions of heterogeneous protein isolates that were not involved in the calcium-mediated protein-hydrocolloid interaction.

The nature of protein aggregation on the carrageenan fibers was different for various isolates and was largely responsible for apparent morphological differences between the complexes. The protein aggregates ranged from diffused protein-rich areas about 500–1000 Å in diameter in coconut-protein complexes to compact protein particles of about 1000–2000 Å in diameter in peanut-proteins complexes. In contrast the protein bodies, appearing as arrays of small, compact globules in the glandless cottonseed and soy protein complexes were of about 100–200 Å diameter. The observed differences in the morphology of protein aggregates may possibly be related to the inherent structural features of the constituent proteins and their calcium-dependent interaction.

Although the various isolates aggregated differently, the interaction of carrageenan with these aggregates appeared to follow a basic pattern. In all cases, the protein aggregates interacted to a greater extent in some areas of polysaccharide fiber, and none in other areas, but in no case was the protein distribution uniform and continuous over the entire carrageenan structure. The localization of protein interaction in polysaccharide strands was also the characteristic feature of the carrageenan stabilized caseins (Chakraborty and Hansen, 1971). Relatively protein-free fiber regions of the polysaccharide very well may correspond to the ion-aggregated double helix regions of low protein reactivity and therefore, the morphology of the carrageenan stabilized plant proteins would also be consistent with the double helix junction zone model of Rees (1969). The calcium ion binding ability of κ-carrageenan was not determined in our study. However, Rees (1969) emphasized that the polyionic macromolecules interact largely by polyelectrolyte effect and not so much by salt bridges. For this reason, the chelation of calcium ions by κ-carrageenan and subsequently, higher protein solubility at lower calcium ion activity may be ruled out. The stabilization of these complexes is apparently achieved by entrapping small, calcium-aggregated, protein bodies in the carrageenan structure before such bodies could agglomerate further into large, colloidally unstable particles. The double helix junction zones may provide effective separation of these protein particles from each other and adequate solvation of the relatively protein-free hydrocolloid regions, thus imparting physical stability to the system.

The formation of stable complexes by the same hydrocolloid with different calcium-sensitive proteins suggests that the specificity of these reactions lies not so much in the interaction phenomenon involving the proteins and polysaccharide, but in the specific conformational features unique to κ-carrageenan. However, only a limited number of proteins were used in the present study. These proteins may not behave similarly in various calcium-mediated phenomena. Soy protein isolates and milk caseins are similar in their behavior in model systems containing κ-carrageenan and in milk dialysate (Chakraborty and Hansen, 1969). In these systems, both proteins are stabilized against precipitation by ionic calcium. However, the behavior of these proteins in systems containing carboxymethyl cellulose is quite different. Under similar conditions carboxymethyl cellulose precipitates casein (Hansen, 1968) and stabilizes soy protein (Chakraborty and Hansen, 1969). The manner in which the calcium sensitivity of proteins influences the calcium-mediated formation of double-helix junction zones is not known. Futhermore, no direct relationship between the protein solubility and double-helix content has been established. Before the role of double-helix junction zones in protein stabilization can be confirmed, further work will be necessary with homogenous preparations of other calcium sensitive proteins of known ion-binding characteristics.

REFERENCES

AOAC. 1970. "Official Methods of Analysis of Association of Official Analytical Chemists," 11th ed. p. 858. Association of Official Analytical Chemists, Washington, D.C.

Anderson, N.S., Campbell, J.W., Harding, M.M., Rees, D.A. and Samuel, J.W.B. 1969. X-ray diffraction studies of polysaccharide sulphates: Double helix models for κ- and ι-carrageenans. J. Mol. Biol. 45: 85.

Bernfeld, P. 1966. Interaction of polyanions with blood components. In "The Amino Sugars," ed. Balazs, E.A. and Jeanloz, R.W. Vol 2B, p. 251. Academic Press, New York and London.

Chakraborty, B.K. 1970. Electron microscopy of protein hydrocolloid interaction systems. Ph.D. dissertation, Ohio State University, Columbus, Ohio.

Chakraborty, B.K. and Hansen, P.M.T. 1969. Stabilization of soy protein isolates in milk-salt systems. J. Dairy Sci. 52: 886.

Chakraborty, B.K. and Hansen, P.M.T. 1971. Electron microscopy of protein hydrocolloid interaction systems. J. Dairy Sci. 54: 754.

Farkas, E. and Glicksman, M. 1967. Hydrocolloid rheology in the formulation of convenience foods. Food Technol. 21: 535.

Hansen, P.M.T. 1968. Stabilization of α_s-casein by carrageenan. J. Dairy Sci. 51: 192.

Lawhon, J.T. and Cater, C.M. 1971. Effect of processing method and pH of precipitation on the yields and functional properties of protein isolates from glandless cottonseed. J. Food Sci. 36: 372.

Lin, C.F. and Hansen, P.M.T. 1970. Stabilization of casein micelles by carrageenan. Macromolecules 3: 269.

Rees, D.A. 1969. Structure, conformation and mechanism in the formation of polysaccharide gels and networks. Adv. Carbohyd. Chem. 24: 267.

Samson, S.J., A.S., Khaund, R.N., Cater, C.M. and Mattil, K.F. 1971. Extractability of coconut proteins. J. Food Sci. 36: 725.

Zittle, C.A. 1965. Some properties of photoxidized κ-casein. J. Dairy Sci. 48: 1149.

Zobel, C.R. and Beer, M. 1965. The use of heavy metal salts as electron stains. Int. Rev. Cytol. 18: 363.

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Technical paper 9723 of the Texas Agricultural Experiment Station.

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FREE ARGININE CONTENT OF PEANUTS (Arachis hypogaea L.) AS A MEASURE OF SEED MATURITY

INTRODUCTION

SINCE PEANUTS are indeterminate in their growth habit (Sturkie and Williamson, 1951), those harvested at a given time always include a certain number of immature fruits. At present, methods to determine the degree of immaturity are subjective for the most part and are based on such factors as size, color of the testa, degree of darkening of the inside of the pod and seed characteristics (Barrs, 1962; Emery et al. 1966; Holley and Young, 1963; Mills, 1961; Teter, 1966; Toole et al., 1964).

A reliable objective procedure has been sought by growers, manufacturers and scientists for some time. Holley and Young (1963) reported that the amount of carotenoid pigments had some relationship to the degree of immaturity in peanuts, since the extract of immature peanuts absorbed light to a greater extent at 435 nm than mature peanuts. Because of interference of components unrelated to maturity, no quantitative interpretations could be developed. Emery et al. (1966) used a pigmentation method to differentiate maturity of farmers stock peanuts and found it to be relatively effective. However, the high concentration of pigments was associated with immaturity only when the peanuts were cured rapidly and not when they were cured by the traditional stockpile method. High absorption values provided a positive qualitative check for immaturity but low values did not.

Newell (1967) and Mason et al. (1969) reported a large decrease in arginine content of the Spanish peanut fruit as it matured. On the basis of their findings, an investigation was conducted to evaluate the usefulness of arginine content as a measure of immaturity in peanuts. This paper describes the development of a quantitative procedure applicable to the major peanut types.

EXPERIMENTAL

Arginine analysis

50g samples of freshly harvested or of dry-cured peanuts were homogenized in a Waring Blendor at high speed in 500 ml of 3N $HClO_4$ for 9 min. The flask was immersed in an ice-water bath to keep the extraction mixture cold. The suspension was filtered on a fast flowing fluted filter paper and the first 50 ml of filtrate was collected. After adjusting the pH of the filtrate to 8.0 with 2N KOH, the precipitate was removed by centrifugation. The supernatant was transferred to a 250 ml volumetric flask and diluted to volume with deionized, distilled water. Samples from this flask were diluted five-fold and the arginine was determined on 1 ml aliquots. The reaction was a colormetric one and based upon the procedure of Izumi (1965a, b).

Because of the nature of samples analyzed, the following precautions were observed: (1) the KOH solution was carefully protected against CO_2 absorption; (2) the acetic anhydride was kept free from water vapors and other contaminants that might alter its critical strength; (3) the KOBr solution was stored at 4°C in the dark and a new stock solution prepared each week.

Standard curves

Standard curves were prepared with ten serial dilutions containing from 0–30 μg of arginine. The developed color was found to be stable for 1 hr.

For recovery studies the procedure was exactly the same as for routine analyses except the filtration was completed and filtrate washed (washings added to filtrate) before a representative sample was taken. Also, the centrifugate was washed and the washings combined with the supernatant to insure quantitative transfer.

The precision and accuracy of the procedure was established with a flow procedure (Fig. 1) designed to allow differentiation of sampling error from inherent error of procedure manipulations. The flow diagram shows the sampling procedure starting with replicate 100g samples of the same variety of Spanish peanuts.

Calibration curves

Four major peanut types (Spanish, Runners, Virginias and Valencias) were analyzed including both freshly harvested and dry-cured peanuts segregated by subjective means (Pang, 1967) into four maturity classifications: mature, high intermediate, low intermediate and immature. Samples from the four maturity groups were combined as follows to obtain lots from 0–100% immaturity in increments of 10%: The arginine values for immature peanuts were taken to represent 100% immaturity while that of the mature peanuts was arbitrarily assigned 0% immaturity. 90% and 80% immature samples were formulated from calculated

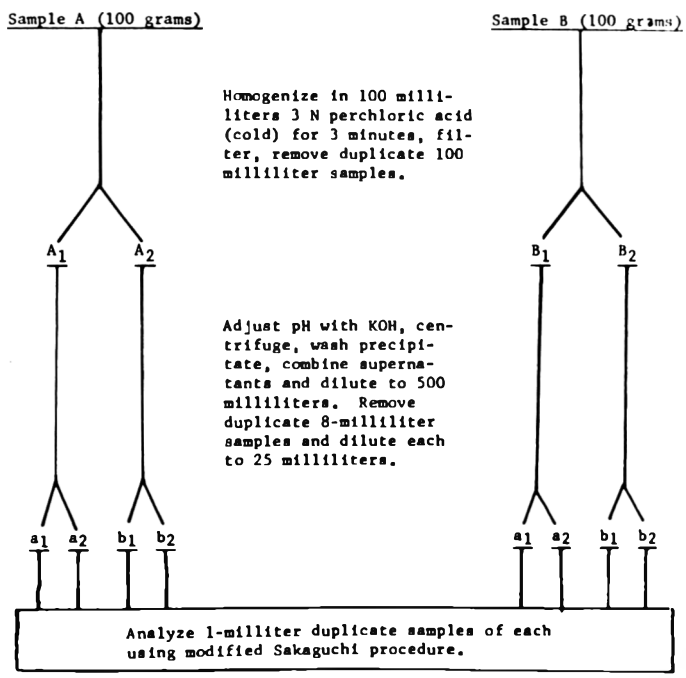

Fig. 1—Flow diagram of precision and accuracy experiment used in arginine assay system.

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amounts of each of the immature and low intermediate group. 70, 60 and 50% immature samples were formulated by combining calculated amounts of each of the high intermediate and mature groups. This procedure was necessary in order to have sufficient peanuts to supply 50g samples at all calibration levels, since most of the peanuts fell into the two more mature segregation categories (high intermediate and mature). The procedure provided calibration samples indicative of authentic samples of immature peanuts, since the lower immaturity calibration samples were made up of peanuts from the two segregations containing the lower immaturity (mature and high intermediate) while the higher immaturity calibration samples were made up of peanuts from the two higher immaturity segregations (low intermediate and immature).

Field analyses

To test the methodology under field conditions, peanuts of four varieties were grown at Perkins, Okla., in 1967, and harvested at weekly intervals during a 7-wk period. Care was taken to harvest all of the peanuts including those detached from the vine while removing the peanuts from the soil. Freshly harvested samples were shelled and segregated visually into four stages of maturity based on pericarp, seed coat color and thickness, and seed size. Each group was weighed and analyzed for arginine and moisture content.

RESULTS & DISCUSSION

THE SEARCH for a simple chemical procedure for determining arginine was quickly reduced to a recent modification of the Sakaguchi reaction (Izumi, 1965a) which embodies other modifications (Izumi, 1965b). A review of the literature revealed that it was a highly sensitive and accurate procedure for arginine in the presence of other amino acids because of minimal interference from other amino acids. To determine whether or not the procedure would measure free arginine in the protein-free extracts of peanuts, it was tested on samples to which measured amounts of arginine had been added to the cold perchloric acid used in extraction.

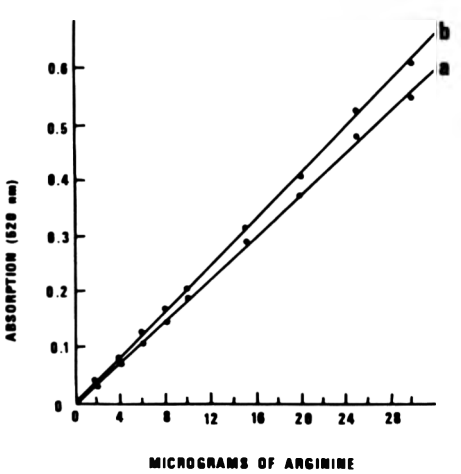

Fig. 2—Standard curves for the assay of free arginine using water (a) and peanut extract (b) as diluents.

Standard curves

Standard curves for arginine (Fig. 2) gave somewhat higher values when peanut extract was the diluent rather than water even though an allowance was made for the arginine content in the peanut extract. This was attributed to the presence of other amino acids such as glutamic acid, aspartic acid, and phenylalanine in perchlorate extracts of peanuts (Newell, 1967) that result in higher optical density readings (Izumi, 1965a). Ion exchange chromatography of several of these samples also supported these assumptions. In this procedure free amino acids other than arginine were present in the perchlorate extracts to the extent of about 20% of the arginine present.

The recovery values using the perchlorate extract as diluent on two separate recovery studies were near 100% which was within experimental error.

The summary of the results of the Sakaguchi analyses of the resulting samples from the precision and accuracy study are shown in Table 1. A brief inspection of the means and standard deviation values allowed conclusions to be drawn concerning sources of error. A standard deviation of not greater than ± 0.30 between duplicate samples showed that the precisions of manipulations involved in the Sakaguchi procedure were excellent with a coefficient of variation of 2.2%. This was indicated by submean I, since subsamples labeled 1 or 2 or a or b were all duplicates of the same subsample (see Fig. 1).

Errors that were present due to manipulation during preparation of the samples were apparent from examining values for submean II. Mean values for subsample A_1 and A_2 were considerably different whereas those for B_1 and B_2 were very close. Thus, degree of accuracy of the procedure was much less in subsampling within the A series even though the

Table 1—Precision and accuracy study for subsamples of replicate samples (A and B) of peanuts

Arginine values (mg/g peanuts) for duplicate Subsamples			Submeans + Standard Deviation I	II	III	Grand Mean
	$^{a}1,1$	12.9				
			13.0 ± 0.15			
	$^{a}1,2$	13.1				
A_1				13.10 ± 0.29		
	$^{b}1,1$	13.5				
			13.2 ± 0.43			
	$^{b}1,2$	12.9				
					12.57 ± 0.64(A)	
	$^{a}2,1$	11.6				
			11.9 ± 0.43			
	$^{a}2,2$	12.2				
A_2				12.05 ± 0.35		
	$^{b}2,1$	12.0				
			12.2 ± 0.29			
	$^{b}2,2$	12.4				
						13.73 ± 1.30
	$^{a}1,1$	15.2				
			15.2 ± 0.00			
	$^{a}1,2$	15.2				
B_1				14.90 ± 0.39		
	$^{b}1,1$	14.8				
			14.6 ± 0.29			
	$^{b}1,2$	14.4				
					14.89 ± 0.36(B)	
	$^{a}2,1$	14.8				
			15.1 ± 0.43			
	$^{a}2,2$	15.4				
B_2				14.88 ± 0.38		
	$^{b}2,1$	14.8				
			14.7 ± 0.22			
	$^{b}2,2$	14.5				
Pooled Std. Dev.			± 0.30	± 0.35	± 0.52	± 1.30
C.V.		1.7%	2.2%	2.6%	3.8%	9.5%

precision of the Sakaguchi procedure for arginine was excellent.

Apparently, from the comparison of the values for submean III, considerable error in accuracy was incurred between replicate samples A and B since the mean value for sample A was 2.32 μg/g peanuts lower than that for sample B. The amount of error involved was indicated by the standard deviation of 1.30 for the mean value of 13.73 for the replicates. After this precision study was completed, replicated sampling error was reduced considerably by blending the sample for a longer period of time. This change was incorporated in the procedure described herein.

Thus, precision and accuracy of the Sakaguchi procedure under these conditions was well within the sampling error for 100-g samples. This error amounted to 9.5% for a single 100-g sample or 3.8% for duplicate 100-g samples in this study. As a consequence of these results, differences between routine samples of less than 10% were not considered significant. Repetition of this procedure for 50-g samples revealed sampling error (8.7%) was about the same as that for 100-g samples.

Repeated analyses of several peanut samples revealed that excellent precision was obtained using the routine procedure even though no attempt was made for quantitative transfer of filtrate and centrifugate. This protocol was necessary to shorten the time for preparation of samples. The described technique was equally applicable to both wet and dried peanuts.

Calibration curves

The calibration samples were analyzed and the resulting curves for both raw and cured peanuts of the four major types are plotted in Figure 3. Thus, the degree of immaturity of an unknown sample of raw peanuts may be estimated from the corresponding calibration curve. Even though mature peanuts grown in one area have been consistent from year to year in free amino acid content, calibration curves should be prepared for each geographical area if the procedure is to be used routinely.

In practice, the important part of the calibration curves was that representing less than 50% immaturity since peanuts harvested under normal conditions would not possess immaturity higher than 50%. Also, the analyst should be reminded that this procedure measures mean percentage immaturity since some seeds would be very close to mature while others would be very immature.

Field test

Table 2 gives data on the amount of shelled peanuts within each maturity group for the four varieties. Results of the arginine analysis (Table 3) showed that the mature and high intermediate maturity groups usually gave similar values for arginine. The low intermediate

Table 2—Percentage of shelled peanuts in each maturity group of four varieties of freshly harvested peanuts grown at Perkins, Okla. in 1967

Variety	Maturity group	Percentage: Days from planting to harvest 123	130	137	144	151	158	172
Valencia	Mature	26	47	48	62	65	74	73
	High Int.	45	33	29	20	14	18	13
	Low Int.	19	16	20	17	18	8	8
	Immature	10	5	3	2	2	1	1
Argentine	Mature	42	58	67	77	78	81	82
	High Int.	32	24	19	9	11	13	11
	Low Int.	21	14	9	14	10	5	2
	Immature	4	4	5	1	1	1	0
Early Runner	Mature	–	5	11	8	34	40	49
	High Int.	52	53	53	49	38	34	27
	Low Int.	32	32	28	33	23	21	18
	Immature	16	11	8	9	5	5	4
NC-2	Mature	2	9	4	16	56	50	62
	High Int.	55	45	65	50	25	26	16
	Low Int.	29	36	22	26	15	20	12
	Immature	14	11	9	8	4	5	2

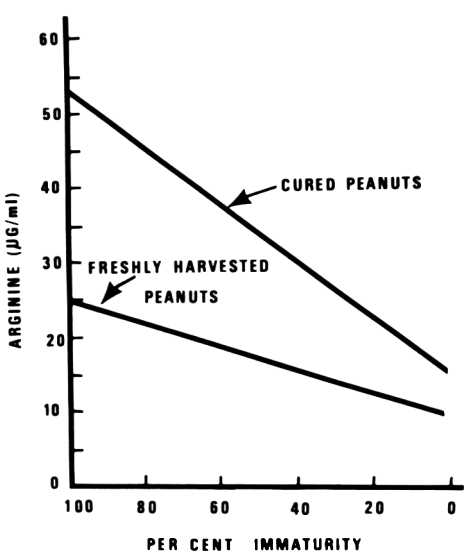

Fig. 3—Calibration curves for arginine in relation to the degree of immaturity.

Table 3—Arginine content in each maturity group of four varieties of uncured peanuts grown at Perkins, Okla. in 1967

Variety	Maturity group	Arginine content (μg/ml): Days from planting to harvest 123	130	137	144	151	158	172	Average
Valencia	Mature	6.4	5.5	7.9	8.6	11.8	10.2	9.2	8.5
	High Int.	8.6	7.1	11.0	8.6	9.4	9.9	9.8	9.2
	Low Int.	11.4	22.6	22.7	13.5	12.9	–	10.9	15.6
	Immature	23.2	–	–	–	–	–	–	23.2
Argentine	Mature	7.6	7.2	7.6	9.3	10.5	9.3	8.2	8.5
	High Int.	7.7	7.6	7.8	10.6	10.8	11.0	10.1	9.3
	Low Int.	13.9	14.3	15.5	14.0	13.4	–	–	14.2
	Immature	29.3	27.5	23.2	–	–	–	–	26.7
Early Runner	Mature	–	–	7.4	10.2	10.1	10.9	10.4	9.8
	High Int.	12.2	7.4	11.5	9.8	10.7	11.9	10.9	11.0
	Low Int.	19.6	16.4	19.8	14.0	17.1	16.7	14.9	16.9
	Immature	25.3	23.7	25.0	24.6	–	–	–	24.6
NC-2	Mature	–	10.5	12.8	10.4	10.6	11.5	10.9	11.1
	High Int.	14.1	8.6	9.9	9.7	10.7	11.0	11.2	10.7
	Low Int.	16.7	15.3	18.1	15.9	16.3	19.8	19.2	17.3
	Immature	23.9	25.0	24.9	–	–	–	–	24.6

group contained about 50% more arginine than the mature and high intermediate groups. Most of these peanut kernels were of the size for processing into peanut butter. Pang (1967) reported that peanut butter made with this group of peanuts was inferior in flavor to those made with mature peanuts. Argentine peanuts contained about 10% of the low intermediate group if harvested at about 140–150 days (Table 2). The immature peanuts which are usually quite small were extremely high in arginine, and Pang (1967) scored these lower in flavor than the low intermediate group. This supports the possible relationship of high arginine and poor flavor of peanut products and may be an additional advantage in the measurement of immaturity.

Based upon data in Tables 2 and 3, it was concluded that Argentine peanuts should be harvested at 158–172 days for highest quality peanuts. Organoleptic data on peanuts from the same location and same variety verified this postulate. If harvested at the recommended time of 140 days, a less desirable product would have resulted.

Thus, arginine content appears to be a sensitive, rapid means of determining the amount of immaturity in a sample of peanuts. The application of the procedure outlined should prove valuable in the measurement of maturity in both developing and harvested peanuts, and lead to a higher quality consumer product.

REFERENCES

Barrs, H.D. 1962. The relation between kernel development and time of harvesting of peanuts at Katherine, N.T. Aust. J. Expt. Agr. Anim. Husband. 2: 106.

Emery, D.A., Gupton, G.L. and Hexem, R.O. 1966. Indexing the maturation of varietal and segregating populations of virginia type peanuts. Proceedings of the Fourth National Peanut Research Conference, University of Georgia Experiment Station, Tifton, GA. July: 25.

Holley, K.T. and Young, C.T. 1963. The relation of peanut maturity and pigments. Proceedings of the Peanut Improvement Working Group, Oklahoma State University, Stillwater, Okla., p. 52.

Izumi, Y. 1965a. New Sakaguchi reaction. Anal. Biochem. 10: 218.

Izumi, Y. 1965b. New Sakaguchi reaction. 2. Anal. Biochem. 12: 1.

Mason, M.E., Newell, J.A., Johnson, B.R., Koehler, P.E. and Waller, G.R. 1969. Nonvolatile flavor components of peanuts. J. Agr. Food Chem. 17: 728.

Mills, W.T. 1961. Effective heat units as a system for predicting optimum time to harvest peanuts. Proceedings of the Amer. Soc. Agric. Engr., St. Joseph, Mich., p.1.

Newell, J.A. 1967. Precursors of typical and atypical roasted peanut flavor. Ph.D. dissertation, Oklahoma State University, Stillwater, Okla.

Pang, Li-Sung. 1967. The influence of maturity and time of harvesting Spanish peanuts on peanut butter quality. M.S. thesis, Oklahoma State University, Stillwater, Okla.

Sturkie, D.G. and Williamson, J.T. 1951. "The Peanuts, The Unpredictable Legume," p. 173. The National Fertilizer Association, Washington, D.C.

Teter, N.C. 1957. Physical and pathological factors affecting quality of peanuts as influenced by curing. Proc. Peanut Research Conference, p. 54.

Toole, V.K., Baily, W.K. and Toole, E.H. 1964. Factors influencing dormancy of peanut seeds. Plant Physiol. 39: 822.

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THE GRADING OF LOW-ESTER PECTIN FOR USE IN DESSERT GELS

INTRODUCTION

WITH THE DEVELOPMENT of improved low-methoxyl pectins (Anon., 1970a) and their increased use in a variety of gelled products (Egger, 1970), the need for a standardized method for the evaluation of low-methoxyl pectins has become increasingly important.

A number of objective and subjective tests of a destructive or nondestructive nature have been used with both model gels and fruit juice gels with varying degrees of success (Anon., 1947; Hills et al., 1942; Lange et al., 1961; Lopez and Li, 1968; McCready et al., 1944; Owens et al., 1947, 1949) but the literature contains no standard, or generally accepted procedure for the evaluation of low-ester pectin gels, or for the establishment of the grades of the pectins used in these gels.

The characteristics of a low-methoxyl pectin gel are influenced by the nature of the pectin and by the conditions of its use. Kertesz (1951) and Doesburg (1965) reviewed the literature and discussed the effects of such factors as degree of esterification, distribution of the methoxyl groups on the pectinic acid chain and molecular weight. Conditions employed during gel making such as calcium levels and pH of the gel were also included in their discussions.

Although there are considerable data in the literature concerning the effects of various factors upon low-methoxyl pectin gels, much of the information is of little value. The pectin samples are often poorly defined and gel-making procedures are not always adequately described. The use of so many instrumental devices in the gel evaluations is confusing when comparing data from various sources. It was thought that a study of the conditions of use of a low-methoxyl pectin gel using a well defined pectin sample in a carefully developed procedure would be useful. Because low-methoxyl pectin gels are often expected to have characteristics similar to those of high-solids jellies, testing procedures should preferably be analogous to those used in the case of high-solids jellies. Low-methoxyl pectins are frequently used in gelled dessert products and it seemed therefore appropriate to use these testing procedures on gels having a firmness and strength close to that of dessert gels.

This paper deals with some of the more important factors influencing the firmness and breaking strength of dessert type gels made with low-methoxyl pectin. Further, a possible grading procedure is described.

EXPERIMENTAL

Gel evaluation

In the evaluation of high-ester pectins, jellies made for testing are much firmer than those which would be made for actual use (IFT Committee, 1959). The textural characteristics sought in dessert gels are such that more information could be obtained about the gel if it were evaluated under conditions of use. This involved working with a relatively weak gel at reduced temperatures. Such gels are difficult to remove from standard ridgelimeter glasses and they do not stand up when finally turned out. The standard jelly glasses therefore had to be replaced by a more suitable container. Test gels, after cooking to the desired weight, were poured into Plexiglas® cylinders (50 mm long and 44 mm ID) which had masking tape collars wrapped around one end of each cylinder to extend about ¼ in. above the edge. The other end of each cylinder was attached to a sheet of plastic wrap with a small amount of silicon grease to prevent leakage. After pouring, the gels were covered with plastic wrap and stored at 5–7°C for 20–22 hr. Immediately after storage the covering and tape were removed and a wire cheese cutter was used to cut away the excess gel, leaving one of a standard height. The bottom wrap was then removed and the gel turned out onto a Plexiglas® plate using a small spatula to loosen the sides. The gel was allowed to sit for 1 min and was then measured for sag using a Sunkist Exchange Ridgelimeter (Sunkist Growers, Inc., Ontario, Calif.). Because the Plexiglas® cylinder was much shorter than the standard jelly glasses supplied with the ridgelimeter a supporting plate of 25 mm was used. The readings on the ridgelimeter scale were converted to actual gel sag measurements by using Figure 1.

Readings were taken on duplicate gels from the same batch, and where readings differed by more than 0.3 mm, the gel was remade.

Immediately after the sag readings were taken, the gel was cut crosswise into slices of 10 mm thickness and the bottom one was discarded. A Marine Colloids Gel Tester (Marine Colloids, Inc., Springfield, N.J.), fitted with a 10.9 mm diam plunger, was used to measure the breaking pressure of the 4 gel slices. Any reading differing by more than 10g with the next highest or lowest reading was discarded and average breaking strength values were calculated for each duplicate.

Gel preparation

A commercially available lemon gelatin dessert mix was prepared according to package instructions to use as a model. This gel had an average sag of 12.5 mm and the average breaking pressure was 95g at 11°C. A sample of Hi-Poly® Pectin, No. 3475 (Hi-Poly® is a registered trademark of Sunkist Growers, Inc., Sherman Oaks, Calif.) was then used to prepare a gel with similar sag and breaking pressure measurements by modifying the recipe for a low solids dessert gel supplied by Sunkist Growers, Inc. (Anon, 1970b). However, a sag of 12.5 mm and a breaking pressure of 95g were respectively at the upper and lower limits measurable with the equipment in use. For this reason a somewhat firmer gel having a sag of 10.5 mm at 11°C and a breaking pressure of 126g at 11°C was adopted as standard. Although somewhat

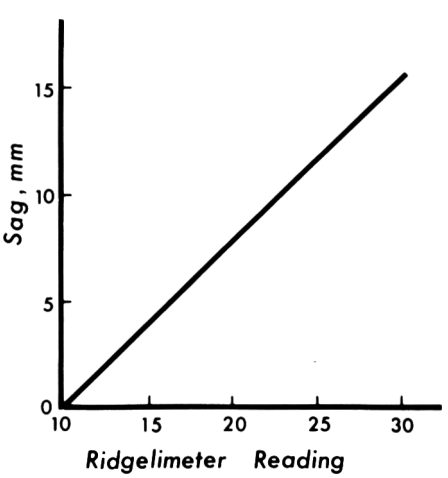

Fig. 1—Relationship between sag and ridgelimeter reading.

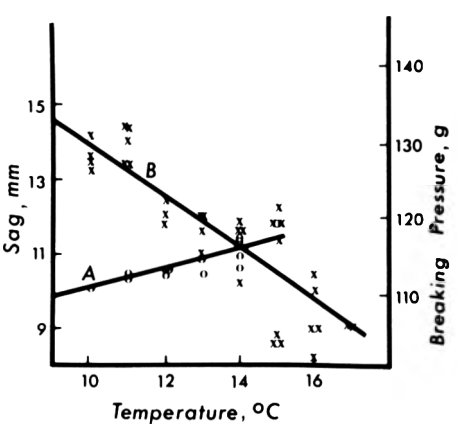

Fig. 2—The influence of temperature on sag (A) and breaking pressure.

firmer, this gel had a mouth feel very similar to a standard gelatin dessert. The procedure for making this gel was the following: To 350 ml water were added 40g sucrose, 5 ml of a solution containing 1.95g citric acid hydrate and 5 ml of a solution containing 1.8g sodium citrate dihydrate. The mixture was brought to a boil in a tared 3 qt stainless steel saucepan on a Corning hotplate. A 4g sample of Hi-Poly® Pectin, previously mixed with 35g sucrose, was added and the mixture was stirred for 140 sec. Then 20 ml calcium chloride solution containing 30 mg Ca^{++}/g Hi-Poly® Pectin were stirred in vigorously and the boiling continued until a final weight of 364g was reached. Total boiling time was kept as close as possible to 6 min. The mixture was then quickly skimmed to remove foam and poured into the prepared containers This standard gel had a pH of 3.8.

Determination of factors influencing gel character

The standard gel making procedure, with necessary modifications, was used to determine the effect of temperature, pectin concentration, cooking time, pH and calcium level on sag and breaking pressure values.

Analytical procedures

Moisture, ash, methoxyl content, galacturonic acid level and molecular weight were determined as described by Smit and Bryant (1967). For the determination of amide levels acid-washed and dried samples of ca. 1g were suspended in about 250 ml H_2O. This was mixed with 20 ml 10% NaOH and about 100 ml were then distilled over into a beaker containing 20 ml 0.1N HCl. Excess acid was titrated with 0.1N NaOH to pH 8. From this the number of amide groups present in the pectin was calculated.

RESULTS & DISCUSSION

THE Hi-Poly® Pectin used had a methoxyl content of 5.5%, a galacturonic acid level of 96.1% and a molecular weight of 115,000, all on an acid-washed and dried bases. The moisture, ash and standardizing sugar totalled 19.6% and the pectin contained 13 amide groups/100 galacturonide units.

Effect of temperature

Standard gels were allowed to remain at room temperature for various lengths of time after removal from storage before sag and breaking pressure measurements were taken. Temperature of the gels was measured immediately after each set of readings by inserting a thermometer into the center of a crushed portion of the gel.

It is well known that low-ester pectin gels soften with increasing temperatures and this is illustrated in Figure 2.

The equations for the straight lines best fitting the points on the graphs were: $y = 7.8 + 0.24x$ for the relationship between sag and temperature and $y = 165.11 - 3.5x$ for the relationship between breaking pressure and temperature. From these straight lines a correction of ± 0.25 was calculated for each degree above or below 11°C for sag values obtained in the range of 9–15°C. For the breaking pressure readings, a correction ± 3.3 was calculated for each degree above or below 11°C for a temperature range of 9–17°C. These corrections were used when necessary in the work reported below.

Effect of pectin concentration

The data presented in Figure 3 were obtained with standard gels in which only the amount of pectin and sucrose were varied in order to keep a constant soluble solids level.

At a constant calcium level, increase in pectin concentration results in a firmer, stronger gel since there are more free carboxyl groups present. Owens et al. (1949) found that increasing the pectin concentration increased the strength as a logarithmic function where strength was measured as the shear modulus by the rigidometer. However, in their work, the calcium:pectin ratio was kept constant as the pectin concentration changed. It is important to stress that the data reported here were obtained with one pectin preparation. The calcium sensitivity of a low-ester pectin is dependent on a variety of factors such as the presence of amide groups in the pectin, methods used during pectin manufacture, the solids content of the gel, etc. As a result, somewhat different results may be expected with other pectin preparations.

Effect of cooking time

Data presented in Figure 4 were obtained when standard gels having a pH of 3.8 were cooked for 6, 11, 14 and 18 min. With extended cooking times, sag increased and breaking pressure decreased somewhat. Increasing the cooking time is thought to increase the extent of depolymerization of the pectin resulting in a weaker gel.

Effect of pH

By using quantities ranging from 2–35 ml citric acid solution containing 390g citric acid hydrate/liter and adjusting the amount of water needed in the standard formula, a series of gels having different pH values were prepared and tested. The pH of the gel was measured with a Corning pH meter after the gel was evaluated.

The effect of pH on the sag and breaking pressure of a Hi-Poly® Pectin gel with a fixed calcium to pectin ratio is given in Figure 5. Low-methoxyl pectins can be used in products varying in pH from 2.5–6.5 (Anon., 1947). However, a maximum breaking pressure reading was obtained at pH 3.8 and a minimum sag was obtained at pH 3.3–3.7

For a low-methoxyl pectin of 32% esterification, Doesburg (1965) showed an increase in gel strength, measured as a compression modulus of elasticity, from pH 2.4–3.1, followed by a gradual decrease. Owens et al. (1949) showed for a low-methoxyl pectin, used with a con-

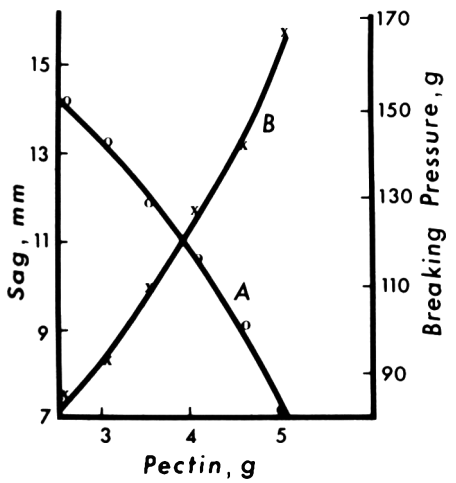

Fig. 3—The influence of pectin concentration on sag (A) and breaking pressure (B).

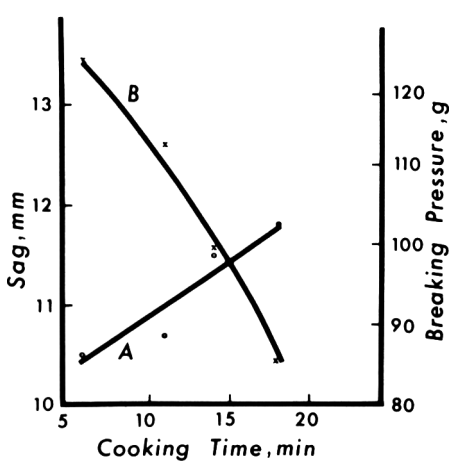

Fig. 4—The influence of cooking time on sag (A) and breaking pressure (B).

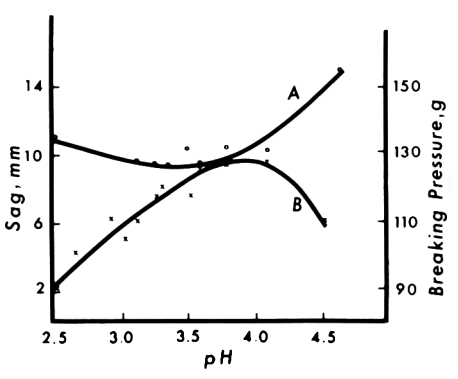

Fig. 5—The influence of pH on sag (A) and breaking pressure (B).

Table 1—Calculated factors used with different weights of pectin

Wt pectin (g)	Assumed F or S	Factor
2.5	140.0	0.625
3.0	116.7	0.750
3.5	100.0	0.875
4.0	87.5	1.000
4.5	77.8	1.125
5.0	70.0	1.250

stant calcium:pectin ratio of 40 mg/g, a maximum gel strength at pH 2.7, with values decreasing on either side of this pH. Gel strength was measured as the shear modulus.

Although the observation of an optimum pH for maximum gel strength for a given pectin under given conditions is found frequently in the literature, no explanation for the observation has been given. The calcium sensitivity of the pectin, as well as the chelating power of citrate will change with increasing pH and this may be responsible for the optima observed.

Effect of calcium concentration

From 15–40 ml of a calcium chloride solution was used in the standard jelly formula to give Ca^{++} levels of from 22.5–60 mg Ca^{++}/g of Hi-Poly® Pectin. Once again the water level was adjusted to keep the volume of added liquid constant.

The data obtained are presented in Figure 6. There is much evidence in the literature (Pilnik and Voragen, 1970) that gel formation is not merely due to "bonding" through primary valences but that secondary valences between calcium and hydroxyl groups also play an important role.

At a level of about 20 mg Ca^{++}/g of pectin, gels had high sag and low breaking pressures showing a lack of sufficient calcium. Increasing the calcium to 40 mg/g raised the breaking pressure to a maximum; beyond this point, more calcium brought a decrease in breaking pressure. Hills et al. (1942) also obtained maximum strength values, decreasing on either side of a certain optimum calcium level, when calcium level was plotted against jelly strength as measured by a Delaware jelly tester. Low strengths at high calcium levels appear to result from difficulties in obtaining a uniform calcium-pectin dispersion (Owens et al., 1949. In this case, pregelation often occurs resulting in an unevenly formed and generally weak gel.

Increasing the calcium level to about 50 mg/g resulted in a sharp decrease in sag. Sag still decreased slightly beyond this point and a minimum was obtained at around 60 mg/g in spite of the fact that slightly less than 45 mg Ca^{++}/g of pectin would have been sufficient to "bind" all the available carboxyl groups.

The slight increase in sag at very high calcium levels is probably due to the extreme pregelation which occurs under those conditions.

Pectin grading

A grading method was developed by following some of the principles used in the grading of high solids jellies (IFT Committee, 1959). The low-ester firmness (F) was defined as being the weight of the gel batch in g, divided by the weight of the pectin sample required to yield a gel of standard firmness having a sag of 10.5 mm at 11°C. The low-ester strength (S) was defined as being the weight of the gel batch in g, divided by the weight of pectin sample required to yield a gel having a standard breaking pressure of 126g at 11°C. These sag and breaking pressure values were obtained with a standard gel containing 4g Hi-Poly® Pectin. This pectin therefore had an F of 87.5 and an S of 87.5. By using more or less pectin, different F and S values would be assumed. For example, if 3.5g had been used, the assumed F and S values would have been 100; however, the true F and S values for this sample were 87.5. A factor was thus calculated to convert assumed F and S values to true F and S values, and these are given in Table 1.

Data showing the relationship of the amount of pectin to gel sag and breaking pressure were then used to plot calculated factors against sag and breaking pressure and these are given in Figure 7.

A relatively straight line relationship existed in the range of about 9.5–13.5 mm sag and about 90–135g breaking pressure. For gels falling outside of these limits it would probably be best to remake the gel using a larger or smaller amount of pectin. The use of these data to determine the true F or S value of an unknown low-ester pectin sample may be illustrated by using the following example: The standard procedure for gel making was followed using 4g of the sample. The assumed F and S values were therefore 87.5. With this particular sample the sag of the gel was 12 mm at 11°C. From Figure 7 the corresponding factor was 0.852. Therefore, the true F value was 87.5 × 0.852 or 74.2. For the same gel, the breaking pressure was 114g, giving a factor of 0.910 and a true S value of 78.7.

As shown earlier, sag and breaking pressure values should be corrected by ± 0.25 or ± 3.3 respectively for each degree above or below 11°C and these corrections would have been taken into account if readings were not made at 11°C.

Under the conditions used the pH of the standard gel was 3.8. However, it is quite possible that other low-ester pectin samples may give different final pH values when used in the standard gel formula. Different amounts of added citric acid may therefore be required than the quantity specified in order to have a final pH close to 3.8.

Although cumbersome, it is suggested that both sag and breaking pressure measurements be incorporated in the suggested grading procedure. It is possible to prepare low-ester pectins which may give gels having breaking pressure values above or considerably below 126g at a standard sag of 10.5 mm. Such gels will have a texture quite different from the texture associated with dessert gels. Manufacturers of low-ester pectin or of dessert gels containing low-ester pectin may therefore prefer to standardize low-ester pectins to a particular F value and also specify a desirable ratio of S/F.

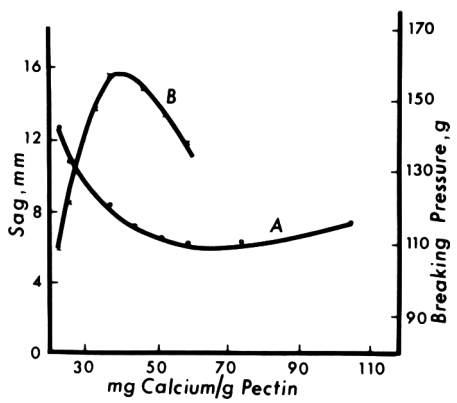

Fig. 6—The influence of calcium content on sag (A) and breaking pressure (B).

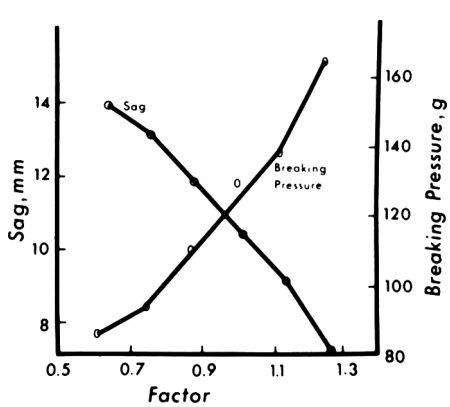

Fig. 7—Relation between calculated factor and sag or breaking pressure.

REFERENCES

Anon. 1947. Exchange Pectin L M. California Fruit Growers Exchange, Ontario, Calif.

Anon. 1970a. Citrus pectin forms reversible gels. Food Processing 31(4): 38.

Anon. 1970b. Gelled and dry gel mix products

with Hi-Poly® Pectin, ref. 521. Sunkist Growers, Inc., Ontario, Calif.
Doesburg, J.J. 1965. "Pectic Substances in Fresh and Preserved Fruits and Vegetables," p. 41. Institute for Research on Storage & Processing of Horticultural Produce, Wageningen, The Netherlands.
Egger, D.L. 1970. Filling, capping, collating system requires only one operator. Food Processing 31(4): 18.
Hills, C.H., White, W.W. and Baker, G.L. 1942. Low-sugar jellying pectinates. Proc. Inst. Food Technol., p. 47.
IFT Committee. 1959. Pectin standardization: Final report of the IFT Committee. Food Technol. 13: 496.
Kertesz, Z.I. 1951. "The Pectic Substances," p. 130. Interscience Publishers Inc., New York.
Lange, D., Bock, W. and TäuFel, K. 1961. Evaluation of strongly- and poorly-esterified apple pectin by the Tarr-Baker method. Ernährungsforschung 6: 65. [Chem. Abstracts 59: 22641e]
Lopez, A. and Li, L. 1968. Low-methoxyl pectin apple gels. Food Technol. 22: 1023.
McCready, R.M., Owens, H.S. and Maclay, W.D. 1944. Alkali-hydrolyzed pectins are potential industrial products. Food Ind. 16: 794.
Owens, H.S., McCready, R.M. and Maclay, W.D. 1949. Gelation characteristics of acid-precipitated pectinates. Food Technol. 3(3): 77.
Owens, H.S., Porter, O. and Maclay, W.D. 1947. New device for grading pectins. Food Ind. 19: 606.
Pilnik, W. and Voragen, A.G.J. 1970. Pectic substances and other uronides. In "The Biochemistry of Fruits and Their Products," ed. Hulme, A.C., vol. 1, p. 64. Academic Press, New York.
Smit, C.J.B. and Bryant, E.F. 1967. Properties of pectin fractions separated on diethylaminoethyl-cellulose columns. J. Food Sci. 32: 197.

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THE EFFECT OF DEMETHYLATION PROCEDURES ON THE QUALITY OF LOW-ESTER PECTINS USED IN DESSERT GELS

INTRODUCTION

FOUR TYPES of demethylating agents used to produce low-methoxyl pectins are listed by Joseph et al. (1949) as: (1) acid; (2) alkali; (3) enzyme; and (4) ammonia in alcohol or concentrated aqueous ammonia.

Low-methoxyl pectins produced by enzyme demethylation have been found to be inferior to those produced by other methods because of the nonrandom distribution of methyl ester groups among molecules of the pectin (Speiser and Eddy, 1946; Hills et al., 1949). This is in agreement with Kertesz (1951) that the enzyme removes the ester groups one by one along the chain while alkali and acid are assumed to remove these at random. The inferior strength of the enzyme demethylated pectins could also be related to the presence of more "ballast" materials, chiefly araban and galactan, since enzyme demethylation causes the removal of very little ballast. Acid demethylation removes ballast at approximately the same rate as it removes methyl ester groups, and thus an acid demethylated pectin would have a higher percentage of polygalacturonic acid (Hills et al., 1949).

Kertesz (1951) stated that the main disadvantage to acid de-esterification is its slowness. It may be speeded up by using higher temperatures, but this results in increased depolymerization of the pectin chains with an adverse effect on gel strength (Doesburg, 1965). Acid demethylation may be carried out in suspension in an organic solvent-acid mixture as recommended by Joseph (Kertesz, 1951), in a water-acid solution (Hills et al., 1942; Speiser et al., 1945) or in combination with the pectin extraction procedure (Woodmansee and Baker, 1949). Joseph's method is apt to give a heterogenous pectin preparation with respect to ester content since the outer surfaces of the pectin particles would be demethylated more than the inner areas (Kertesz, 1951).

Alkaline demethylation is rapid but the removal of methyl ester groups is accompanied by depolymerization of the pectin chain (Kertesz, 1951) and the rate of depolymerization increases faster than the rate of demethylation as temperature increases (Slavickova, 1961). Neukom and Deuel (1958) suggested that in the degradation of the pectin chain with alkali, the activated hydrogen at C(5) is removed and the glycosidic linkage is cleaved with the formation of a double bond between C(4) and C(5). However, careful control of temperature and alkali concentration can result in the preparation of useful low-methoxyl pectins (McCready et al., 1944; Joseph et al., 1949).

The use of ammonia in alcoholic systems or in concentrated aqueous ammonia systems results in a low-methoxyl pectin that differs in that it contains amide groups. The role of these amide groups during gel formation with ammonia demethylated pectins is not clear but at least two commercially produced low-ester pectins presently contain such groups (Bryant, 1959; Wiles and Smit, 1971).

This paper deals with low-ester pectins produced by acid and alkali demethylation, the characteristics of these pectins and the properties of gels prepared from them.

EXPERIMENTAL

Sample preparation

Demethylated pectin samples were prepared from a high molecular weight pectin supplied by Sunkist Growers Inc., Ontario, Calif. The pectin had been extracted from lemon peel with sulfurous acid solution, filtered, precipitated with aluminum chloride, pressed, shredded and dried.

Acid demethylation

Prior to demethylation 220g of the Al-coagulum were rehydrated in distilled water overnight. After excess water was removed by pressing, the pectin was suspended in 1350 ml 60% isopropanol (v/v) containing 150 ml conc HCl, stirred for 30 min, filtered and washed with 60% isopropanol (v/v). For demethylation, the pectin was stirred continuously with a mixture of 1680 ml isopropanol, 920 ml H_2O and 400 ml conc HCl in a 4000 ml beaker on a magnetic stirrer at around 25°C. Small samples, taken at intervals were washed with 60% isopropanol (v/v) until free of chlorides. After a final rinse with isopropanol and air drying, per cent esterification was estimated by titration. Larger samples taken during demethylation were treated in a similar fashion and buffered by suspension in a 60% isopropanol (v/v) solution containing sufficient $Na_2CO_3 \cdot H_2O$ to neutralize half of the free carboxyl groups as determined by titration with NaOH. These suspensions were shaken for 1 hr, left overnight, filtered, washed with isopropanol and air dried before grinding in a Wiley mill through a 60 mesh screen. This resulted in samples A-1 through A-5.

Table 1—Specific reaction-rate constants

Demethylation procedure	$k \times 10^{-3}$ min^{-1}
1.4N HCl at 25°C	0.1
NH_4OH at pH 11 and 5–7°C	25.1
NaOH at pH 8 and 5–7°C	61.6

Preparation of material for alkali demethylation

A 260g sample of starting material was acid washed using a mixture of 1020 ml isopropanol, 510 ml H_2O and 170 ml conc HCl, and then rinsed with 60% isopropanol (v/v) to remove aluminum. Stock pectin solutions of 3000 ml were made by dissolving 85g of wet washed pectin in H_2O. This weight of wet pectin was equivalent to 65g of the original dry pectin before acid washing.

Demethylation with NH_4OH

A stock pectin solution was demethylated at 5–7°C by adjustment to pH 11 using 350 ml conc analytical grade NH_4OH. After different time intervals the reaction was stopped by adding 400 ml conc HCl followed by precipitation with 6 liters of isopropanol. Ammonium hydroxide was used to adjust the pH of the alco-

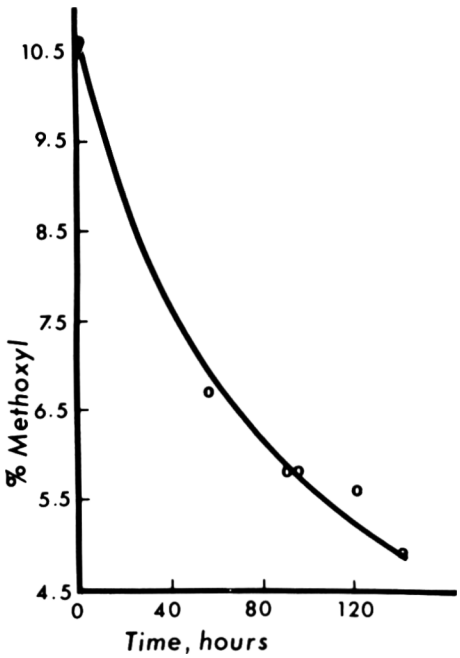

Fig. 1—Change in methoxyl content during demethylation with HCl at 25°C.

Table 2—Comparison of acid and alkali demethylated low-methoxyl pectin samples

Sample	% OCH_3[a]	% Gal. acid[a]	M.W. 1000	Moist. + ash (%)	% Groups per 100 gal. units –COOH	–NH_2	pH (1% sol)	F	S	Gal. Characteristics
A-1	6.7	91.4	175	16.7	57	0	3.7	70	106	Smooth
A-2	5.8	91.5	152	18.8	62	0	3.8	187	118	Brittle
A-3	5.6	95.8	130	14.5	64	0	3.8	199	121	Brittle
A-4	4.9	92.8	128	20.2	67	0	3.9	229	140	Brittle
A-5	4.0	91.0	124	18.5	71	0	4.0	235	108	Brittle
Na-3	5.3	95.4	121	18.8	65	0	3.5	83	low	Brittle
Na-1	4.6	90.7	118	14.9	68	0	3.6	109	low	Brittle
Na-2	4.1	92.0	124	17.4	73	0	3.5	244	101	Brittle
Am-1	5.0	94.2	120	17.5	45	21	4.2	low	57	Smooth
Am-2	4.6	95.0	124	14.3	47	22	4.3	51	77	Smooth
Am-3	4.3	97.7	117	14.8	46	26	3.8	206	126	Brittle

[a]Determined after washing with HCl and drying

hol slurry to between 4.0 and 4.5. The precipitated pectin was squeezed out in a cotton cloth and then washed in a Buchner funnel with 60% isopropanol until free of chlorides. After a final rinse with isopropanol and air drying, the pectin was ground in a Wiley mill through a 60 mesh screen. This resulted in samples Am-1 through Am-3.

Demethylation with NaOH

To prepare samples Na-1 and Na-2, 3000 ml portions of the sol were demethylated at 5–7°C by adjustment to pH 8 with 200 ml 1N NaOH. To stop the reaction, 120 ml conc HCl were added after 13 and 15 min respectively. Precipitation, washing and drying were the same as with the NH_4OH demethylated samples except that the pH of the alcohol slurry was adjusted to between 3.5 and 4.0 with 1N NaOH.

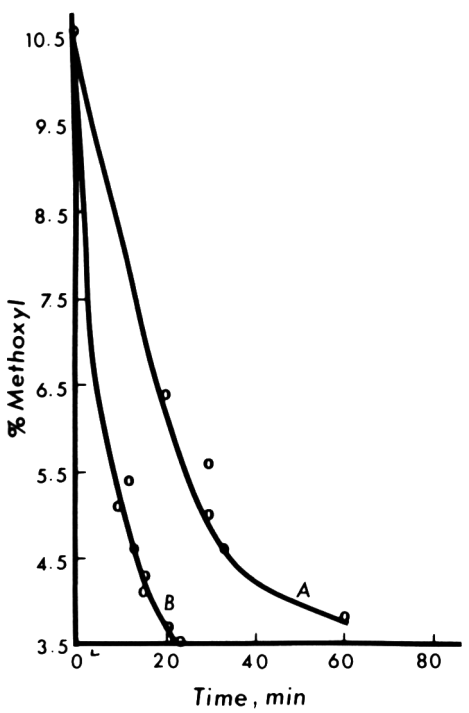

Fig. 2—Decrease in methoxyl content curing treatment with alkali. (A) NH_4OH at pH 11 and 5–7°C; (B) 1N NaOH at pH 8 and 5–7°C.

Sample Na-3 was prepared using 180 ml 1N NaOH and 3000 ml sol, followed by acidification with 100 ml conc HCl after 15 min of demethylation. Further treatment of the sample was the same as for samples Na-1 and Na-2.

Analytical

Methoxyl content, galacturonic acid content, moisture, ash, amide level and molecular weight were determined as described by Smit and Bryant (1967). Standard gels having a fixed Ca:pectin ratio of 30 mg Ca^{++}/g of pectin, a pH of 3.8 and a soluble solids level of around 22% were prepared and firmness (F) and strength (S) were determined as outlined by Black and Smit (1972). The amount of sag as measured with an Exchange Ridgelimeter was used as an indication of F while breaking pressure, as measured with a Marine colloids Gel Tester, was used as an indication of S.

RESULTS & DISCUSSION

Preparation of samples

The decrease in methoxyl content during acid demethylation is shown in Figure 1.

In the alkaline demethylations described in this work, ammonium hydroxide was used at pH 11 and a temperature of 5–7°C (Fig. 2). At this pH, the sodium hydroxide reaction was too difficult to control, hence a pH of 8 was used (Fig. 2). Even at this lower pH, demethylation proceeded so quickly that it was difficult to estimate the exact time required to produce a sample at a specific methoxyl level.

Plots of the logarithm of the methoxyl content against time gave a straight line relationship at high and intermediate methoxyl levels indicating a first order reaction with respect to methoxyl content with all three types of demethylation used.

Specific rate constants were calculated from the graphs of methoxyl level versus time using the formula:

$$k = \frac{2.303}{t} \log \frac{C_o}{C}$$

where C_o is the initial methoxyl level and C is the methoxyl level at time t. Results are shown in Table 1.

McCready et al. (1944) report a reaction-rate constant for de-esterification with HCl of 0.7×10^{-3} min^{-1} at pH 3 and 50°C. A k value of 0.1896×10^{-3} min^{-1} at 30°C with 0.87N hydrochloric acid was given by Speiser et al. (1945). These values agree favorably with those obtained in this work in which different conditions of temperature and acid concentration were used.

McCready et al. (1944) reported k values of 5×10^{-3} min^{-1} for demethylation with ammonium hydroxide and 9×10^{-3} min^{-1} for demethylation with sodium hydroxide at pH 11 and 15°C. These values are different from those reported in Table 1 but it should be recognized that differences in starting material, experimental conditions and the presence of cations will all have an effect on the rate of demethylation (Merrill and Weeks, 1946; Slavickova, 1961).

Comparison of samples

A high acid concentration was used to increase the extent of depolymerization during acid demethylation. In spite of this, the molecular weights of samples A-1 to A-4 are higher than that of the alkali demethylated samples (Table 2). Comparisons between acid and alkali demethylated samples are therefore difficult to make. However, the molecular weights of both A-5 and Na-2 are 124,000 and the samples have a similar number of free carboxyl groups/100 galacturonide units. This indicates that these samples may be compared as to gelling characteristics. Gels made from A-5 and Na-2 had firmness values (F) of 235 and 244 and strength values (S) of 108 and 101, respectively (Black and Smit, 1972). A subjective evaluation of the gels shows that they were quite brittle and coarse. Low methoxyl pectins de-esterified with HCl or NaOH therefore appeared to produce similar low solids gels, particu-

larly since the distribution of methoxyl groups in the two types of pectins is similar (Heri, 1962).

With the acid demethylated samples, an increase in F occurred as the methoxyl level decreased from 6.7 to 4.0. The F appeared to be approaching a maximum at 4.0% methoxyl. With these samples, the maximum S was reached around 4.9% methoxyl, decreasing on either side. In the presence of more free carboxyl groups firmer and stronger gels are normally obtained. However, as the number of free carboxyl groups increase, pregelation and decreased solubility become more evident resulting finally in weak, poor-textured gels. Gels made from all of the samples exhibited these characteristics at lower methoxyl levels.

The samples prepared by alkali demethylation had less range in methoxyl level than those obtained with acid demethylation but it is likely that the F and S values for these samples would also reach maxima at certain low methoxyl levels.

In general it would appear that the acid samples produced firmer, stronger gels at relatively high methoxyl levels. However, the high molecular weights of the acid demethylated samples contributed to this.

It could be reasoned that the amide groups play no active role in gel formation, serving only to tie up carboxyl groups. If this were true, low methoxyl pectins containing the same number of free carboxyl groups would be expected to exhibit similar gel characteristics, regardless of whether the remaining carboxyls were tied up as methyl esters or amides. However, a comparison of the acid and ammonia demethylated samples show that this is not so. From the data it appears that acid demethylated pectins with a carboxyl level of 46 groups/100 galacturonide units would have F and S values close to zero. In contrast, with 46 carboxyl groups/100 units sample Am-3 had a F of 206 and a S of 126 (Table 2).

The number of carboxyl groups/100 galacturonide units did not change significantly in the ammonia demethylated samples as the methoxyl level decreased from 5.0 to 4.3. However, the S and F values increased sharply. The increases could not be attributed to more available carboxyls but rather to the increased number of amide groups which may be involved in hydrogen bonding. As well, an increased number of carboxyl groups cannot be used to explain the change from a smooth to a brittle texture with these samples.

REFERENCES

Black, S.A. and Smit, C.J.B. 1972. The grading of low-ester pectin for use in dessert gels. J. Food Sci. In press.

Bryant, E.F. (ass. California Fruit Growers Exchange). 1959. Acid amides of pectinic acid and process for their preparation. U.S. Patent 2,480,710.

Doesburg, J.J. 1965. Some important characteristics of pectic substances. In "Pectic Substances in Fresh and Preserved Fruits and Vegetables," p. 21. Institute for Research on Storage and Processing of Horticultural Produce, Wageningen, The Netherlands.

Heri, W. 1962. Chromatographische Fraktionierung von Pektinstoffen an Diaethylaminoaethyl-Cellulose. Ph.D. thesis, Eidgenoessischen Technischen Hochschule, Zurich.

Hills, C.H., Mottern, H.H., Nutting, G.C. and Speiser, R. 1949. Enzyme-demethylated pectinates and their gelation. Food Technol. 3: 90.

Hills, C.H., White, W.W. and Baker, G.L. 1942. Low-sugar jellying pectinates. Proc. Inst. Food Technol., p. 47.

Joseph, G.H., Kieser, A.H. and Bryant, E.F. 1949. High-polymer, ammonia-demethylated pectinates and their gelation. Food Technol. 3: 85.

Kertesz, Z.I. 1951. Preparation and purification of pectic substances in the laboratory. In "The Pectic Substances," p. 94. Interscience Publishers, Inc., New York, N.Y.

McCready, R.M., Owens, H.S. and Maclay, W.D. 1944. Alkali-hydrolyzed pectins are potential industrial products. Food Ind. 16: 794.

Merrill, R.C. and Weeks, M. 1946. Kinetics of the de-esterification of pectin. J. Phys. Chem. 50: 75.

Neukom, H. and Deuel, H. 1958. Alkaline degradation of pectin. Chem. & Ind., p. 683.

Slavickova, A. 1961. The reaction of pectin with ammonia in the presence of calcium salts. The saponification, amidation and degradation. SBORNIK. Scientific papers from Inst. of Chem. Technol., Prague. Food Technol. 5(1): 73.

Smit, C.J.B. and Bryant, E.F. 1967. Properties of pectin fractions separated on diethylaminoethyl-cellulose columns. J. Food Sci. 32: 197.

Speiser, R. and Eddy, C.R. 1946. Effect of molecular weight and method of de-esterification on the gelling behavior of pectin. J. Am. Chem. Soc. 68: 287.

Speiser, R., Eddy, C.R. and Hills, C.H. 1945. Kinetics of de-esterification of pectin. J. Phys. Chem. 49: 563.

Wiles, R.R. and Smit, C.J.B. (ass. Sunkist Growers, Inc.). 1971. Method for producing pectins having high resistance to breakage and high capacity for gelling in the presence of calcium. U.S. Patent 3,622,559.

Woodmansee, C.W. and Baker, G.L. 1949. High-polymer, acid-demethylated pectinates and their gelation. Food Technol. 3: 82.

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INTERRELATIONSHIP BETWEEN CERTAIN PHYSICOCHEMICAL PROPERTIES OF RICE

INTRODUCTION

A SERIES OF studies on the interrelationship between various physicochemical properties of rice varieties was initiated in this laboratory in 1968. Results of water-uptake behavior (Bhattacharya and Sowbhagya, 1971), and improvement in amylose (Sowbhagya and Bhattacharya, 1971) and alkali reaction (Bhattacharya and Sowbhagya, 1972) tests of rice have been reported earlier. Results of the starch-iodine blue value test and moisture uptake at room temperature along with other parameters of these rice varieties and their interrelations are reported here.

MATERIALS & METHODS

Rice

The samples and their milling have been described (Bhattacharya and Sowbhagya, 1971). In all, 45 samples comprising 42 varieties were used in two series: Series I consisted of *indica*, dwarf *indica*, *japonica* (ponlai) varieties and their hybrids; Series II consisted mostly of newly developed *indica* × dwarf *indica* hybrid lines. All varieties were nonwaxy.

Methods

Apparent water uptake (W') at 80° and at 96°C (Bhattacharya and Sowbhagya, 1971), equilibrium moisture content on soaking in water at room temperature (EMC-S) (Indudhara Swamy et al., 1971), alkali score in 1.4% KOH (Bhattacharya and Sowbhagya, 1972), and amylose content (in defatted rice powder) (Sowbhagya and Bhattacharya, 1971) were determined as described. The alkali degradation types (Bhattacharya and Sowbhagya, 1972) were determined with 1% KOH for varieties giving too high scores and with 1.7% KOH for those giving too low scores in 1.4% KOH. Protein (N × 5.95) in brown rice was determined by Kjeldahl digestion. Viscography of rice flours (about 80 mesh, ground in a high-speed Raymond hammer mill) was carried out with a Brabender viscograph (Halick and Kelly, 1959).

Starch-iodine blue value (BV)

Following the procedure of Hall and Johnson (1966), extraction was carried out at boiling temperature (96°C) instead of at 77°C (Halick and Keneaster, 1956) to eliminate any possible influence of differences in the gelatinization temperature. However, 45-mesh powder was used instead of 80 mesh recommended, as it was equally satisfactory (although giving 1–3% lower reading). Also, the reading increased slightly up to 15–20 min extraction (both in 45- and 80-mesh powder); hence a 20-min extraction period was adopted instead of the 5 min recommended. Readings were taken in a Klett-Summerson photoelectric colorimeter with filter 60. A standard amylose (Nutritional Biochemicals Corp.) solution was included in all experiments; all results were expressed as apparent amylose contents of the rice instead of as the color readings as such.

BV with lower-temperature extracts (60°, 65°, 70°, 75°C) were determined similarly, using 500 mg powder and 50 ml water for the extraction in an appropriate constant-temperature water bath; an appropriate aliquot was used for color development. Iodine concentration was always maintained at 2 ml of 0.2% iodine (Halick and Keneaster, 1956) per 100 ml of final colored solution.

BV of the rice gruel (excess water remaining after cooking rice at 96°C) was also similarly determined. Rice (2.00g) was cooked in 20 ml water (Bhattacharya and Sowbhagya, 1971), and color was developed as above with 1 ml of the strained gruel (after standing in a test tube undisturbed for about 2 hr at room temperature) and made up to 50 ml. These Klett readings were not further converted into apparent amylose values.

Two to three replicates of all tests were run and the mean values taken.

RESULTS

THE VARIOUS RESULTS are shown in Table 1. Entries in the Table are divided into two parts, Series I and II, since the two series of samples were procured and tested separately.

Water uptake at 80°C

As discussed earlier (Bhattacharya and Sowbhagya, 1971), water uptake values were expressed as percentages of the water uptake at 96°C, whereby the effects of differences in surface area and various other interfering factors among the varieties were eliminated. From Table 1 it is clear that the values are, with some divergence, well related to the alkali scores ($r = 0.924^{***}$ for $n = 40$), and hence inversely with the gelatinization temperatures (Juliano et al., 1964a, b). Similar tests were also conducted at 70° and 75°C, but the best differentiation between varieties was given at 80°C.

Equilibrium moisture content on soaking in water at RT (EMC-S)

It was earlier shown in preliminary work from this laboratory (Idudhara Swamy et al., 1971) that the EMC-S differed slightly among different subspecies of rice. This was well borne out by the present data (Table 1). The value was around 28–29% moisture (wet basis) in the local *indica* varieties, and 30–31% moisture in dwarf *indicas* and a majority of their hybrids as well as in the *japonicas*. Separate tests with four waxy rice varieties (not otherwise included in these studies) showed their EMC-S to be 32–34% moisture.

Starch-iodine blue value (BV)

This test has been extensively employed in rice quality studies as an indirect estimate of the amylose content. But Juliano et al. (1968) showed recently that amylose content and BV were not proportional throughout: viz. the BV actually fell progressively once the amylose content exceeded 30%. They tried to explain this in terms of easier in situ retrogradation of amylose when it was present in high amounts.

Inasmuch as the BV represented nothing but the water-soluble amylose content of rice (under the set of conditions employed), the BV data were expressed here as apparent (i.e., water-soluble) amylose contents and also as water-soluble amylose as a percentage of the total amylose (Table 1). The latter data immediately revealed that while the BV and amylose content were indeed not constantly correlated, the departures were related not to high amylose contents but mainly to sub-species differences. Thus the 96°C water-soluble amylose was 40% or more (usually around 50%) of the total amylose for *indica* and *japonica* varieties, but below 40% (usually around 35%) for dwarf *indicas* and most of their hybrids. This test, nevertheless, gave very useful information.

BV at 65°C expressed as % of that at 96°C gave, with some exceptions, good correlation with the alkali scores ($r = 0.915^{***}$ for $n = 40$) and the water uptake values ($r = 0.912^{***}$ for $n = 40$) (Table 1), and hence inversely with the gelatinization temperature. This correlation is quite logical. Tests at 60°, 70° and 75°C gave less sharp correlation (i.e., for 45-mesh powder; for 85-mesh powder, best correlation was at 60°C).

BV of gruel

The BV of the excess water remaining after cooking of rice has often been determined, and many consider it gives a better correlation with rice quality than the classical BV data (Chikubu, 1967; Juliano et al., 1968). This aspect was investigated.

It was found that the gruel-BV data, expressed either as such or per unit surface area [cf. water uptake per unit surface area (Bhattacharya and Sowbhagya, 1971)] did not give any clear correlation with any other parameter (hence not reported). Cooking 13 diverse varieties (Series I) for 5, 10, 20 and 30 min each at 96°C showed that the logarithm of the gruel BV was directly proportional to the water uptake, the curves being all parallel to each other (a few selected curves are shown in Fig. 1). Since water uptake of rice at any given time of cooking (e.g., 20 min) itself differed appreciably from variety to variety due to differences in surface area and other physical factors (Bhattacharya and Sowbhagya, 1971), the corresponding gruel BV values varied still more due to the exponential relationship above. Thus the greater part of the difference between the gruel BV values of two varieties after cooking for a fixed time (e.g., 20 min) arose from factors (kernel shape, size, cracks, etc.) that were quite unrelated to their intrinsic quality. Hence this param-

Table 1—Some physicochemical properties of rice

Sl. No.	Variety[a,b]	Protein content[c] (brown rice) %	Amylose content[c] %	Alkali score[d]	$W'_{80°}$ / $W'_{96°}$ %	$BV_{65°}$ / $BV_{96°}$ %	EMC-S %	Soluble amylose %	Soluble amylose % of total amylose
				SERIES I					
1.	C.P.-231 (*i* × *j*)	7.1	17.2	0.0 (C)	24	1.6	30.4	7.3	42
2.	Rexoro (*i*)	8.2	25.4	0.5 (C)	26	1.3	28.5	13.4	53
3.	ADT-8 (*i*)	7.6	29.2	1.0 (A)	35	1.6	27.6	15.1	52
4.	Halubbulu (*i*)	7.1	29.8	1.4 (A)	35	1.6	27.0	15.2	51
5.	Khar (*i*)	7.7	29.2	1.5 (A)	41	2.6	28.6	15.4	53
6.	Vankasannam (*i*)	9.2	30.0	1.6 (A)	24	1.3	27.0	15.7	52
7.	S-749 (*i*)	8.4	28.8	2.4 (A)	38	1.7	28.7	12.5	44
8.	S-199 (*i*)	9.3	28.4	2.5 (A)	33	1.6	28.6	12.6	44
9.	S-661 (*i*)	8.4	29.1	2.5 (A)	38	1.5	28.3	14.0	47
10.	Kalma (*i*)	6.0	28.5	2.9 (A)	—	2.6	28.2	15.5	55
11.	S-139 (*i*)	7.5	28.5	3.1 (A)	36	2.3	27.6	15.1	53
12.	ADT-27 (*i* × *j*)	11.3	27.5	3.5 (B)	47	2.0	28.7	11.5	42
13.	S-317 (*i*)	9.0	29.5	3.5 (A)	—	2.7	28.1	15.2	52
14.	S-705 (*i*)	9.6	28.7	3.8 (A)	—	2.6	28.0	14.2	50
15.	S-718 (*i*)	8.7	28.8	3.9 (A)	42	2.2	28.6	11.9	41
16.	S-1092 (*i*)	6.5	29.6	4.0 (A)	48	2.0	28.8	13.2	45
17.	S-701 (*i*)	7.0	29.3	4.0 (A)	48	1.9	28.3	13.0	44
18.	SR-26B (*i*)	7.1	29.9	4.0 (A)	48	2.7	28.2	15.2	51
19.	Ch-2 (*i*)	11.5	27.5	4.4 (B)	44	3.0	29.0	10.5	42
20.	Taichung 65 (*j*)	7.0	21.7	6.0 (C)	74	8.1	31.4	11.7	54
21.	Tainan 3 (*j*)	7.8	21.1	6.5 (C)	80	6.4	31.4	10.6	52
22.	Taichung (N) 1 (di)	10.3	28.4	7.4 (B)	67	7.5	29.7	9.2	32
23.	IR-8 (*i* × di)	9.0	27.6	7.4 (B)	64	7.0	31.1	8.5	31
24.	Co-25 (*i*)	8.0	26.8	8.0 (A)	72	7.4	30.9	13.5	50
				SERIES II					
25.	AC-9800 (*i*)	9.4	24.5	0.6 (C)	33	2.0	28.3	13.9	57
26.	NSJ-198 (*i*)	8.2	28.2	1.0 (A)	34	1.4	28.5	15.9	56
27.	CR-10-5437 (*i* × di)	8.2	27.7	1.0 (B)	44	1.7	28.6	12.6	46
28.	IET-355 (*i* × di)	8.0	29.2	2.5[e](A)	43	3.2	29.3	12.4	42
29.	IET-528 (*i* × di)	7.7	29.3	3.5[e]	56	4.9	29.2	12.6	43
30.	IET-434 (*i* × di)	9.2	28.8	4.0[e]	46	3.2	29.7	11.9	41
31.	CR-1-6 (*i* × di)	7.5	27.0	6.0[e](C?)	55	4.8	29.5	10.4	38
32.	C-20 (*i* × di)	9.0	28.8	7.0[e]	71	7.1	30.0	10.0	35
33.	T(N) 2 (*i* × di)	7.1	29.2	7.0 (C?)	65	7.6	30.3	10.1	35
34.	IET-400 (*i* × di)	8.3	29.2	7.2 (B)	69	7.9	29.8	10.6	36
35.	UVT II-6664 (*i* × di)	8.6	27.4	7.3 (C?)	63	7.3	30.1	9.5	35
36.	UVT II-6725 (*i* × di)	7.8	28.6	7.3 (C?)	63	7.9	30.3	9.9	35
37.	UVT-6729 (*i* × di)	9.4	28.0	7.3	62	6.8	30.4	9.5	34
38.	C-3383 (*i* × di)	8.6	28.4	7.3	68	9.7	29.9	11.3	40
39.	CR-28-25 (*i* × di)	8.6	28.7	7.3	64	—	30.1	10.4	36
40.	CR-28-117 (*i* × di)	9.1	28.1	7.3	67	8.4	30.2	10.3	37
41.	IET-440 (*i* × di)	9.0	28.6	7.3	74	6.8	29.7	12.0	42
42.	T(N) 1 (di)	9.8	28.2	7.5	60	7.8	30.4	9.3	33
43.	IR-8 (*i* × di)	7.2	28.2	8.0	70	8.4	30.0	9.7	34

[a]Arranged in order of increasing alkali score (column 5)
[b]Letters in parentheses indicate sub-species: *i* = *indica*; di = dwarf *indica*; *j* = *japonica*.
[c]On dry basis
[d]The letters in parentheses indicate the alkali reaction type (Bhattacharya and Sowbhagya, 1972).
[e]These samples (No. 28–32) gave wide kernel-to-kernel variation in alkali score, showing segregation of the hybrids.

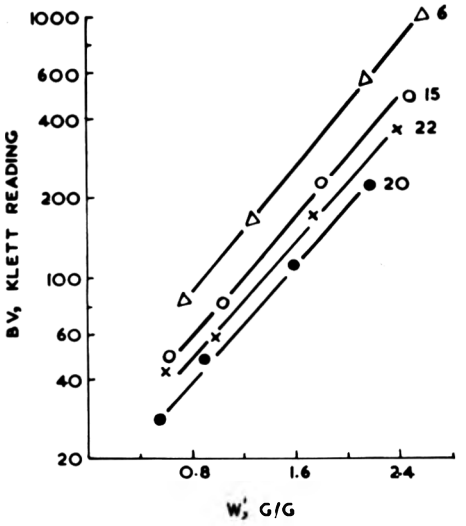

Fig. 1—Relationship between apparent water uptake (W') and the blue value of excess cooking water (BV) during cooking of rice (96° C). The numbers identify the samples (as in Table 1).

eter gave no useful information whatsoever about the intrinsic property of rice unless either (a) the rice was cooked for different periods and the interpolated value of BV at unit water uptake was taken; or (b) the BV was determined after cooking each variety for its optimal cooking period, at which time the water uptake for all varieties was more or less constant at 2.5 (Bhattacharya and Sowbhagya, 1971). However, as much information is probably given by the usual rice-powder BV test above, which is also simpler. The rice swelling coefficient test of Hampel (1958) also gives essentially the same information, but is much more complicated.

Pasting characteristics

Viscograms were obtained for most of the Series I samples. Unfortunately, the samples had become appreciably aged (over 2 yr) by the time these tests were run, and hence the curves were atypical (hence data not reported). However, in relative terms, the two dwarf *indica* varieties (Taichung Native 1 and IR 8) showed an appreciably higher setback value than their amylose contents warranted.

DISCUSSION

IT IS WELL-KNOWN that the alkali degradation score of rice bears an excellent inverse relationship to its gelatinization temperature (GT) (Juliano et al., 1964a, b). The present work shows that water uptake at 80°C and BV at 65°C (both expressed as percent of the respective parameter at boiling temperature) also give almost equally valid inverse estimates of the GT. However, there were some differences in the patterns of the three parameters, the reasons of which are not yet known, but which must have some important implications in terms of rice property. Hence it should be useful to determine all three parameters in studies of rice quality.

The simple property of equilibrium moisture content attained by rice on soaking in water at room temperature (EMC-S) can differentiate between rice types and thus promises to be a useful test. Its value in studies of parboiled rice has been demonstrated (Indudhara Swamy et al., 1971). The higher moisture affinity of waxy and *japonica* varieties is evidently due to their greater content of amylopectin. But why the dwarf *indicas*, with their high amylose contents, should also absorb a relatively high amount of moisture is not yet clear. Moreover, whether this property is related to the dwarf *indica* gene or only to their low GT is also not clear. Thus Co-25 (No. 24, Table 1), an *indica* with low GT, had high EMC-S, while those *indica* × dwarf *indica* hybrids which had a high GT simulated true *indicas* in having low EMC-S (see Series II, Table 1). On the other hand, the value of CP-231 (No. 1) shows that a low amylose content always led to a high EMC-S even if the variety had a very high GT.

The starch-iodine blue value (BV), expressed as 96°C water-soluble amylose as a percentage of the total amylose, also gives a sharp differentiation of the dwarf *indica* type. But here again it is not clear whether this is a reflection of the dwarf *indica* gene or that of a high amylose-low GT combination. Co-25 (No. 24), the only such variety among the *indicas*, behaved true to other *indica* types. But among the *indica* × dwarf *indica* hybrids (Series II), varieties with low GT behaved like true dwarf *indicas* but those with high GT like true *indicas*.

The reason for the lower amylose solubility in dwarf *indica* varieties could be due to a greater ease of its in situ retrogradation as suggested by Juliano et al. (1968) or to a somewhat higher molecular weight. It could also be due to a greater entanglement of the amylose fraction in dwarf *indica* with the micellar structure of the starch granule; Leach et al. (1959) speculated as such with reference to the lower amylose solubility in potato (20%) as compared to corn (50–60%) starch. These same authors also pointed out that potato starch has a greater equilibrium moisture content than cereal starches; so also is the case in dwarf *indica* rice.

The fact that the two dwarf *indicas* whose viscograms were tested gave higher setback values than their amylose contents warranted, may also have some relation to the low amylose solubility, the high EMC-S and the high amylose-low GT combination of these samples.

The implication of the alkali degradation type (Bhattacharya and Sowbhagya, 1972) of the varieties is not clear at this time, except that varieties with 25% amylose or less seem definitely to give the C type reaction.

The protein content seems to be unrelated to any other property tested.

It may be speculated that the soluble amylose content of rice (% by weight, next to last column of Table 1), although not an index of the total amylose content as earlier believed, may have some relationship to the eating quality of the variety. Samples with low BV can be expected to have a relatively low amylose:amylopectin ratio in their adhering cooking water, which might result in cohesiveness of such cooked rice, and vice versa. This may be why Halick and Keneaster (1956) found excellent correlation between the BV and the cooking quality of rice in 200 samples. However, simultaneous taste-panel tests are necessary to verify this hypothesis.

Further studies of the above parameters, including taste-panel and viscogram tests, in varieties with diverse and extreme characteristics may help in obtaining a clearer understanding of the physicochemical basis of rice quality.

REFERENCES

Bhattacharya, K.R. and Sowbhagya, C.M. 1971. Water uptake by rice during cooking. Cereal Sci. Today 16: 420.

Bhattacharya, K.R. and Sowbhagya, C.M. 1972. An improved alkali reaction test for rice quality. J. Food Tech. 7(3).

Chikubu, S. 1967. Chemical composition and quality of rice in Japan. Internat. Rice Comm. Newslet. Special issue: 146.

Halick, J.V. and Keneaster, K.K. 1956. The use of a starch-iodine blue test as a quality indicator of white milled rice. Cereal Chem. 33: 315.

Halick, J.V. and Kelly, V.J. 1959. Gelatinization and pasting characteristics of rice varieties as related to cooking behavior. Cereal Chem. 36: 91.

Hall, V.L. and Johnson, J.R. 1966. A revised starch-iodine blue test for raw milled rice. Cereal Chem. 43: 297.

Hampel, G. 1958. Determination of rice swelling coefficient. Getreide u. Mehl 8(6): 45.

Indudhara Swamy, Y.M., Ali, S.Z. and Bhattacharya, K.R. 1971. Hydration of raw and parboiled rice and paddy at room temperature. J. Food Sci. Technol. 8: 20.

Juliano, B.O., Bautista, G.M., Lugay, J.C. and Reyes, A.C. 1964a. Studies on the physicochemical properties of rice. J. Agr. Food Chem. 12: 131.

Juliano, B.O., Cagampang, G.B., Cruz, L.J. and Santiago, R.G. 1964b. Some physicochemical properties of rice in Southeast Asia. Cereal Chem. 41: 275.

Juliano, B.O., Cartaño, A.V. and Vidal, A.J. 1968. Note on a limitation of the starch-iodine blue test for milled rice amylose. Cereal Chem. 45: 63.

Leach, H.W., McCowen, L.D. and Schoch, T.J. 1959. Structure of the starch granule. 1. Swelling and solubility patterns of various starches. Cereal Chem. 36: 534.

Sowbhagya, C.M. and Bhattacharya, K.R. 1971. A simplified colorimetric method for determination of amylose content in rice. Stärke 23: 53.

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JFS APPLIED SCIENCE and ENGINEERING

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THE INFLUENCE OF AGING BEEF IN VACUUM

INTRODUCTION

NUMEROUS STUDIES on aging beef have been conducted. Most of the research has been concerned with the effect of time, temperature, or both on the eating characteristics of beef. Results have generally shown that aged beef is more tender than unaged beef (Hoagland et al., 1917; Moran and Smith, 1929; Tressler and Murray, 1932; Brady, 1937; Griswold and Wharton, 1941; Deatherage and Harsham, 1947; Harrison et al., 1949; Ramsbottom and Strandine, 1949; Paul and Bratzler, 1955; Wilson, 1957; Doty and Pierce, 1961; Busch et al., 1967; Webb et al., 1967; Larmond et al., 1969). Some research, however, has shown that the aging process has little or no effect on beef tenderness (McIntosh et al., 1942; Parrish et al., 1969).

Conventional aging of beef is accomplished by holding the meat between 30° and 40°F for various periods of time ranging from 3 days up to 3 or 4 wk. In most instances the amount of aging most beef undergoes is somewhat dependent upon the distribution system from the point of slaughter to the ultimate consumer.

In recent years, there has been a trend toward central breaking and vacuum packaging of primal and sub-primal cuts of beef. While improved sanitation and reduction of shrink have been the main reasons for vacuum packaging of beef, it has only been assumed that vacuum packaging has no effect on the natural aging processes (Wilson, 1957).

The purpose of this investigation was to study the effect of aging beef loins and ribs with and without vacuum packaging at two temperatures for 7 and 15 days on sensory characteristics, microbial counts and weight loss.

EXPERIMENTAL

12 PAIRED U.S. Choice grade beef short loins and ribs were obtained from 600–650 lb beef carcasses of "A" maturity with a "small" amount of marbling. Selection was done in the carcass on the day following slaughter at a commercial meat packing company.

The meat was held at 36–38°F upon arrival at the University Meats Lab. On the third day postmortem the short loins and ribs were cut in half giving a total of four portions from each side of the carcass. A 1-in. control steak was cut from the anterior end of each short loin for initial sensory evaluation.

Eight treatments were assigned to the portions of short loins and ribs in such a way that each location received each treatment at least once.

The treatments were:

1. Packaged in vacuum and aged 7 days at 32°F.
2. Packaged in vacuum and aged 15 days at 32°F.
3. Packaged in vacuum and aged 7 days at 40°F.
4. Packaged in vacuum and aged 15 days at 40°F.
5. No packaging, aged 7 days at 32°F.
6. No packaging, aged 15 days at 32°F.
7. No packaging, aged 7 days at 40°F.
8. No packaging, aged 15 days at 40°F.

The time periods of 7 or 15 days refer to aging time only, since all treatments began on the third day postmortem. In this discussion, only aging times will be used.

Initial microbial counts for each sample were determined by taking 1-sq. in. swab samples on the inside (rib cage) and outside (subcutaneous fat) surfaces of the paired ribs and short loins. Serial dilutions were made and plating was done on total place count agar. After 7 and 15 days aging, similar samples were taken from each treated portion.

All portions to receive a vacuum packaging treatment were partially wrapped in bone cloth to prevent bone puncture prior to packaging in a Cryovac S (2 mils) bag. A vacuum packaging machine was used to draw a vacuum of approximately 20 in. Hg and seal the packages.

Samples were weighed before and at the end of each aging period to determine weight loss. Shear values were determined from ½-in. cores obtained from 1-in. steaks, using a Warner-

Table 1—Effect of packaging treatment during aging on eating characteristics, microbial counts and weight loss of beef loins and ribs

Item	Treatments	
	Vacuum packaged	Not packaged
W-B shear	6.05	6.26
Taste panel tenderness	4.91	4.73
Taste panel juiciness	5.06	5.00
Taste panel flavor	5.04	4.94
Log bacteria counts	7.4149[a]	8.9508[b]
Weight loss, %	0.59[a]	4.37[b]

[a,b]Means bearing different superscripts are significantly different ($P < 0.05$).

Table 2—Effect of temperature and packaging treatment during aging on tenderness and weight loss of beef loins and ribs

Item	Treatment			
	Vacuum packaged		Not packaged	
	32°F	40°F	32°F	40°F
Tenderness panel scores	4.87[a]	4.96[a]	4.42[b]	5.04[a]
Weight loss, %	0.62[a]	0.57[a]	4.90[b]	3.85[c]

[a,b,c]Means bearing different superscripts are significantly different ($P < 0.05$).

[1] Present address: Central Foods, Inc., Cape Girardeau, Mo.

Bratzler shear device. The sample steaks were broiled at approximately 350°F to an internal temperature of approximately 155°F and then tempered in a 200°F oven for 5 min prior to sampling. Cores were taken from the lateral, central and medial positions of the longissimus muscle. Each core was sheared twice.

A six member trained panel evaluated steaks (same as used in shear tests) for tenderness, juiciness and flavor. A six point scale was used for tenderness as follows: 6 very tender; 5 tender; 4 slightly tender; 3 slightly tough; 2 tough; and 1 very undesirable.

RESULTS & DISCUSSION

Effect of packaging treatment

Although steaks from the portions aged in vacuum had slightly more desirable palatability characteristics than those aged with no packaging, Warner-Bratzler shear and taste panel scores were not significantly ($P > 0.05$) different between treatments (Table 1). However, vacuum packaged cuts had lower bacterial counts ($P < 0.05$). Portions aged without packaging had bacterial counts approximately 34 times greater than portions aged in vacuum. Although no shelf life studies were done on steaks fabricated from the aged portions, it is possible that steaks from portions aged in vacuum would have a longer retail shelf life due to the lower bacterial counts.

Percent weight loss during aging was also highly significantly ($P < 0.01$) affected by packaging. Vacuum packaged portions lost only 0.59% during aging while the unpackaged portions lost 4.37%. The reduction in shrinkage during aging appears to be a primary advantage of vacuum packaging.

Tenderness was significantly affected by a temperature × packaging treatment interaction as shown in Table 2. It appears that from a tenderness standpoint, if beef is to be aged at 32°F it is desirable to use vacuum packaging. Aging beef at 40°F in vacuum produced steaks which were only slightly less tender than those aged at 40°F with no packaging. The data in Table 2 also suggest that aging beef in vacuum minimizes the effect of temperature on tenderness since beef aged at 32°F in vacuum packages was nearly equal in tenderness to beef aged at 40°F in vacuum.

A temperature × package interaction affecting weight loss during storage was also observed as shown in Table 2. Significant difference in weight losses was observed between samples aged at 40°F with no packaging and 32°F with no packaging. Both of these treatments produced significantly higher weight losses than the treatments with vacuum packaging.

The effects of aging time and packaging treatment interaction on weight loss are given in Table 3. The differences in weight loss for beef aged 7 days in vacuum packaging and that aged 15 days in vacuum packaging are insignificant. Weight losses for portions aged with no packaging were substantially higher than portions aged in vacuum for both the 7- and 15-day aging periods. During 7-day aging, beef loin and rib portions aged with no packaging lost 2.44% more weight than those aged in vacuum packages while for the 15 days storage period the difference was 5.12%. Portions aged 15 days compared to 7 days not packaged had 2.80% more weight loss ($P < 0.05$).

Effect of temperature

The effects of temperature during aging on eating characteristics, microbial counts and weight loss are given in Table 4. A significant difference ($P < 0.01$) in Warner-Bratzler shear values was observed between steaks from portions aged at the two temperature treatments. Even though aging at 40°F is statistically superior to 32°F, the actual difference of 0.4 lb per 0.5-in. diameter core is not great. Taste panel tenderness evaluation also showed that steaks from portions aged at 40°F were more tender ($P < 0.01$) than those aged at 32°F. Aging beef at either temperature gave improved tenderness scores over the initial readings.

These results are in agreement with earlier reports (Griswold and Wharton, 1971; Deatherage and Reiman, 1946; Wilson, 1957; Sleeth, 1958; Busch, 1967) that increased aging temperatures increase the speed of tenderization in beef. The findings of the study disagree with the report of Moran and Smith (1929) who reported no observable difference in tenderness due to temperature between beef aged at 32–40°F and that aged at 41°F for periods up to 17 days. McIntosh et al. (1942) observed that aging beef loins at 34° and 50°F for 7 days with and without ultraviolet light and at 40°F for 14 days with ultraviolet light had no significant effect upon texture and tenderness. In a study of various time and temperature treatments during aging, Parrish et al. (1969) reported that Warner-Bratzler shear values were essentially unchanged for all treatments.

Sensory evaluation scores for both juiciness and flavor indicated no differences between beef aged at 32°F and that aged at 40°F. A "desirable" level of juiciness and flavor was maintained with each aging temperature. The data are similar to the findings of Sotola et al. (1943) and Sleeth et al. (1958) who reported that aging temperatures had no

Table 4—Effect of temperature on eating characteristics, microbial counts and weight loss of beef loins and ribs

Item	Initial 0 day	32°F	40°F
W-B shear, lbs	7.09[a]	6.36[b]	5.96[c]
Taste panel tenderness	3.86[a]	4.64[b]	5.00[c]
Taste panel juiciness	4.77	4.99	5.05
Taste panel flavor	4.77	4.99	5.00
Log bacteria counts	4.9314[a]	8.1643[b]	8.8909[c]
Weight loss, %	0.00	2.76[a]	2.21[b]

[a,b,c]Means bearing different superscripts are significantly different ($P < 0.05$)

Table 3—Effect of aging time and packaging treatment on weight loss of beef loins and ribs

Treatment	Mean percent weight loss
7-day aging in vacuum package	0.54[a]
15-day aging in vacuum	0.64[a]
7-day aging not packaged	2.98[b]
15-day aging not packaged	5.76[c]

[a,b,c]Means bearing different superscripts are significantly different ($P < 0.05$).

Table 5—Effect of aging time on eating characteristics, microbial counts and weight loss of beef loins and ribs

Item	0 day	7 days	15 days
W-B shear, lbs	7.09[a]	6.65[b]	5.66[c]
Taste panel tenderness	3.86[a]	4.56[b]	5.08[c]
Taste panel juiciness	4.77	4.96	5.09
Taste panel flavor	4.77	5.00	4.99
Log bacteria counts	4.9314[a]	7.7299[b]	8.9395[c]
Weight loss, %	0.00	1.76[a]	3.20[b]

[a,b,c]Means bearing different superscripts are significantly different ($P < 0.01$).

significant effect on flavor or juiciness.

Aging temperature had a highly significant ($P < 0.01$) effect on microbial counts of the aged portions. The increased bacterial counts on portions aged at 40°F were approximately 5.34 times greater than the increase for portions aged at 32°F. These results are in general agreement with the findings of Naumann et al. (1969) who reported that bacteria on fresh meat grew three times faster at 40°F than at 32°F.

Surprisingly, the mean weight loss of all portions held at 40°F was significantly ($P < 0.01$) lower than for those treated portions held at 32°F. No satisfactory explanation can be given as to why the higher temperature treatment would have a lower percent weight loss, since it is assumed that evaporation would proceed at a faster rate at higher temperatures. The percent relative humidity of the two aging rooms was 84% in the 40°F room and 81% in the 32°F room which does not seem different enough to affect weight loss.

Effect of aging time

Data presented in Table 5 show the effects of aging time of 7 and 15 days on shear and sensory characteristics. Warner-Bratzler shear values decreased significantly ($P < 0.01$) with increased aging time. Increases in taste panel tenderness scores with increased aging time were also significant ($P < 0.01$). However, the taste panel scores indicate that a slightly greater tenderization occurred during the first 7 days of aging rather than during the 7–15 days aging period as was indicated by the Warner-Bratzler shear values.

The results are in general agreement with numerous other researchers who observed increases in tenderness associated with increased time of aging (Hoagland et al., 1917; Moran and Smith, 1929; Tressler and Murray, 1932; Griswold and Wharton, 1941). Ramsbottom and Strandine (1949) reported beef to be more tender 2 hr postmortem than at any time thereafter for the next 6 days. At 12 days postmortem, the meat was considerably more tender than at 2 hr postmortem. During aging at 35°F, Harrison et al. (1949) found the greatest increases in tenderness to occur during the first 10 days. Husaini et al. (1950) aged carcasses for 15 days at 38°F and found increases in tenderness in all cases over the 3-day tenderness level. Doty and Pierce (1961) and Busch et al. (1967) also observed increases in tenderness due to increased time of aging. Larmond et al. (1969) reported a 25% increase in tenderness between 2 and 9 days of aging at 34°F. McIntosh et al. (1942) reported that aging loins at 34°F and 40°F for 0, 7 and 14 days had no significant effect on tenderness. The work of Parrish et al. (1969) revealed a decrease in tenderness from 4–7 days of aging.

Juiciness and flavor were not significantly affected by aging time. Nearly equal increases in juiciness and flavor scores were noted during the first 7 days of aging. Juiciness scores improved slightly from samples aged to 15 days while flavor scores remained unchanged as compared to the 7-day aging period.

Highly significant ($P < 0.01$) increases in surface bacterial counts were observed after 7 and 15 days of storage. The 7-day counts were over 600 times as great as the 0-day or initial bacterial counts. At the end of 15 days aging, the bacterial counts were 160 times greater than the 7-day counts.

Aging time had a highly significant ($P < 0.01$) effect on weight loss during aging. Samples aged 15 days had nearly twice as much weight loss than the samples aged for 7 days. However, the averaged weight loss per day was slightly lower for the 15-day period than for the 7-day period (0.212% vs. 0.251%). These weight losses expressed on a per day basis are lower than those reported by Sleeth et al. (1957).

REFERENCES

Brady, D.E. 1937. Study of the factors influencing tenderness and texture of beef. American Society of Animal Production Record. 136–38: 246.

Busch, W.A., Parrish, F.C. Jr. and Goll, D.E. 1967. Molecular properties of post-mortem muscle. 4. Effect of temperature on adenosine triphosphate degradation, isometric tension parameters and shear resistance of bovine muscles. J. Food Sci. 32: 390.

Deatherage, F.E. and Reiman, W. 1946. Measurement of beef tenderness and tenderization of beef by Tenderay process. Food Res. 11: 524.

Deatherage, F.E. and Harsham, A. 1947. Relation of tenderness of beef to aging time at 33–35° F. Food Res. 12: 164.

Doty, D.M. and Pierce, J.C. 1961. Beef muscle characteristics as related to carcass grade, carcass weight and degree of aging. Technical Bull. No. 1231, Agricultural Marketing Service, USDA.

Griswold, R.M. and Wharton, M.A. 1941. Effect of storage conditions on palatability of beef. Food Res. 6: 517.

Harrison, D.L., Lowe, B., McClurg, B.R. and Shearer, P.S. 1949. Physical, organoleptic, and histological changes in three grades of beef during aging. Food Technol. 3: 284.

Hoagland, R., McBryde, C.N. and Powick, W.C. 1917. Changes in fresh beef during cold storage. USDA Bull. 433.

Husaini, S.A., Deatherage, F.E. and Kunkle, L.E. 1950. Studies on meat. 2. Observations on relation of biochemical factors to changes in tenderness. Food Technol. 4: 366.

Larmond, E., Petrasovits, A. and Hill, P. 1969. Application of multiple paired comparisons in studying the effect of aging and finish on beef tenderness. Canadian J. An. Sci. 49: 51.

McIntosh, J.A., Sotola, J., Boggs, M., Roberts, J., Prouty, C.C. and Ensminger, M.E. 1942. The relation of ultraviolet light and temperature during aging to the quality of beef. Part 1. Washington State College Agric. Exp. Sta. Bull. 422.

Moran, T. and Smith, E.C. 1929. Post-mortem changes in animal tissues—the conditioning or ripening of beef. Food Investigations Special Report 36, Great Britain Dept. of Sci. & Ind. Res.

Naumann, H.D., Stringer, W.C. and Gould, P.F. 1969. Guidelines for handling prepackaged meat in retail stores. Manual 64, Reprint 11/69/5M, University of Missouri Ext. Div., Columbia, Mo.

Parrish, F.C. Jr., Rust, R.E., Popenhagen, G.R. and Miner, B.E. 1969. Effect of post-mortem aging time and temperature on beef muscle attributes. J. An. Sci. 29: 398.

Paul, P.E. and Bratzler, L.J. 1955. Studies of tenderness of beef. 2. Varying storage times and conditions. Food Res. 20: 626.

Ramsbottom, J.M. and Strandine, E.J. 1949. Initial physical and chemical changes in beef as related to tenderness. J. An. Sci. 8: 398.

Sotola, J., McIntosh, J.A., Prouty, C.C., Dobie, J.B., Ensminger, M.E. and McGregor, M.A. 1943. The relation to ultraviolet light and temperature during aging to quality of beef. Part 2. Washington State College Agri. Exp. Sta. Bull. 431.

Sleeth, R.B., Kelley, G.G. and Brady, D.E. 1958. Shrinkage and organoleptic characteristics of beef aged in controlled environments. Food Technol. 12: 80.

Tressler, D.K. and Murray, W.T. 1932. Tenderness of meat. 2. Determination of period of aging grade A beef required to produce a tender quick frozen product. Ind. Engr. Chem. 24: 890.

Webb, N.B., Kahlenberg, O.J., Naumann, H.D. and Hedrick, H.B. 1967. Biochemical factors affecting beef tenderness. J. Food Sci. 32: 1.

Wilson, J.D. 1957. Aging at high temperature. Proc. of Annual Recip. Meats Conf. 10: 45.

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PROTECTIVE PACKAGING MATERIALS FOR FRESH BEEF SHIPMENTS

INTRODUCTION

FRESH BEEF distribution presently involves shipments from packers directly to retail stores (store door delivery) or shipments from packers to central distribution warehouses and subsequent transport to retail stores. Between production at the packing plant and selection at the retail store, beef spends an average of 4 days of transportation time being routed through the distribution system and is subjected to 0.5% shrinkage per day (Weatherly et al., 1968). The present distribution system could therefore create an approximate loss of 440 million pounds of beef annually due to shrinkage alone. In addition, millions of pounds of beef are condemned annually as a result of being tainted, sour, putrid or contaminated. Currently, a major portion of the fresh beef is distributed as hanging quarters and primal cuts in refrigerated rail cars or in refrigerated trailer trucks. It has been suggested (USDA, 1966) that an inexpensive packaging material should be developed to protect beef carcasses and cuts from the contamination and shrinkage that occurs during distribution through the marketing channels. This study investigated the effects of protective packaging materials on shrinkage rates, bacterial counts and appearance characteristics of beef quarters and primal cuts.

EXPERIMENTAL

FIVE TEST SHIPMENTS were conducted in cooperation with industry to study the effects of protective packaging materials on beef quarters and primal cuts (Table 1). The quarters and primal cuts were either not wrapped (unprotected) or they were protected by paper bags, polyethylene bags or polyvinyl chloride film. As each test shipment was assembled, individual weights were obtained for quarters and/or primal cuts immediately prior to packaging. In test shipments 2, 3 and 5 the exposed lean surfaces of representative cuts were scored at points of origin and destination by use of a 9-point scale in which 9 = very light cherry red and 1 = black. Quarters and primal cuts in test shipments 2, 3, 4 and 5 were scored before and after shipment for appearance of the subcutaneous fat cover using a 6-point scale in which 6 = very fresh appearance and 1 = severe or extensive discoloration or desiccation. The trailers were loaded with quarters and primal cuts in the manner customarily used for beef shipments. As the shipments were being assembled, loading patterns and product density were noted. Temperature fluctuations during loading, transit and unloading were monitored by use of Ryan (Models D and F) recording thermometers which were positioned at various locations in each trailer. Each recording thermometer was checked for temperature accuracy before and after each shipment. At the point of load destination, each cut was weighed after removal of the protective wrap. Primal cuts and quarters were sampled for bacterial determinations by swabbing the areas of the fat surfaces corresponding to the round, rump, sirloin, shortloin, rib or chuck. A 1.0 sq cm sterile aluminum template was used and the samples were incubated on tryptone glucose yeast agar at 5°C for 7 days.

RESULTS & DISCUSSION

TEST SHIPMENT 1 consisted of 63 ribs (31 unprotected and 32 placed in polyethylene bags). The ribs were a part of a mixed beef load composed primarily of beef quarters. Data from recording thermometers used during loading and shipment revealed that the temperature inside the trailer was 22°C at the time of loading and that the average temperature during shipment was 4°C. The lowest temperatures attained at specific locations within the load during transit ranged from −1 to 8°C. Upon arrival at the load destination, noticeable amounts of moisture had accumulated on the subcutaneous fat surface of ribs wrapped in polyethylene bags. Bacterial counts were higher ($P < .01$) for the ribs packaged in polyethylene bags ($\log_{10} = 5.20$) than for ribs which were unprotected during shipment ($\log_{10} = 4.05$). Polyethylene bags reduced surface evaporation thereby maintaining a more favorable water activity (Aw) for bacterial growth. Stokes (1960) and Volz and Marsden (1963) reported similar relationships between temperature and/or

Table 1—Protective wrapping treatments for beef quarters and primal cuts in each test shipment

Test shipment	Distance traveled (miles)	Time intransit (hr)	Item	Protective wrapping material: Unprotected (n)	Paper bag (n)	Polyethylene bag (n)	Polyvinyl chloride film (n)
1	560	26	Ribs[a]	31		32	
2	270	19	Forequarters	8	8		6
			Hindquarters	8	8		6
			Chucks	7	6	6	6
			Rounds	12	13	13	12
3	1200	120	Forequarters	6	6	14	6
			Hindquarters	11	11	19	11
			Rounds	12	12	12	14
			Loins	3	4	4	4
4	1000	35	Forequarters	22	21	21	21
			Hindquarters	22	21	21	21
			Chucks	7	7	8	8
			Ribs	3	4	4	4
			Rounds	11	13	12	14
5	1350	85	Forequarters	24	22	22	22
			Hindquarters	24	22	22	22
			Chucks	16	18	18	18
			Rounds	3	4	4	4

[a]Ribs were placed in cardboard cartons either unprotected or after the protective wrap was applied.

[1]Present address: Armour & Co. Research Lab., Oak Brook, Ill.

water activity and microbial growth and Volz and Marsden (1963) suggested that refrigerated truck temperatures should be maintained at −2 to 0°C. Percent shrinkage during transit (Table 2) did not differ significantly between beef ribs that were unprotected (0.61%) and those which were packaged in polyethylene bags (0.57%). The lack of significant differences in shrinkage may have resulted from the protective nature of the cardboard cartons. Similar results were reported previously by Schneidau (1964).

Test shipment 2 consisted of 22 forequarters, 22 hindquarters, 25 chucks and 50 rounds. The quarters and primal cuts were loaded into a trailer that had an average temperature of 19°C during the 6-hr loading period. The lowest temperatures achieved at specific locations in the trailer during transit ranged from 3–11°C and the time required to achieve the lowest temperature at given locations in the load varied from 4.6–13.0 hr. The mechanical refrigeration unit was controlled by a thermostat setting of 2°C during the shipment. Temperatures at the rear of the trailer were the highest recorded, agreeing with the findings of Johnson et al. (1959). Palletized boxes that occupied a part of the trailer and a waxed paper partition used to separate fresh beef from the palletized boxes influenced air movement and ultimate temperatures within the trailer. In addition, primal cuts were loaded directly adjacent to the refrigeration unit. Loading in this manner prevented the full capacity of forced air flow from circulating, since the trailer was not equipped with ducts to convey air flow toward the rear of the trailer. Loading patterns, the presence of palletized boxes and paper partitions influenced normal air flow and prevented maintenance of desired temperatures during transit.

Table 2–Mean values for intransit shrinkage[a] in test shipments 1, 2, 3, 4 and 5

Test shipment	Item	Treatment			
		Unprotected	Paper bag	Polyethylene bag	Polyvinyl chloride film
1	Ribs	0.61[b]	–	0.57[b]	–
2	Chucks	0.71[b]	0.32[b]	0.22[b]	0.40[b]
2	Rounds	0.72[b]	0.35[b]	0.74[b]	0.31[b]
2	Forequarters	0.53[b]	0.38[b]	–	0.40[b]
2	Hindquarters	1.00[b]	0.57[bc]	–	0.26[c]
3	Loins	2.43[b]	1.63[b]	1.78[b]	0.93[b]
3	Rounds	2.87[b]	2.22[c]	1.51[d]	1.36[d]
3	Forequarters	1.24[b]	0.78[c]	0.52[cd]	0.29[d]
3	Hindquarters	1.34[b]	1.09[bc]	0.66[c]	0.82[c]
4	Sides	0.45[bc]	0.63[b]	0.22[d]	0.28[cd]
4	Chucks	0.30[b]	0.35[b]	0.03[b]	0.03[b]
4	Rounds	0.26[b]	0.08[b]	0.13[b]	0.07[b]
4	Ribs	1.25[b]	0.00[b]	0.55[b]	0.14[b]
5	Sides	1.93[b]	0.17[c]	0.05[c]	0.08[c]
5	Chucks	1.91[b]	0.93[c]	0.25[cd]	0.13[d]
5	Rounds	2.69[b]	1.46[c]	0.84[cd]	0.43[d]

[a]Computed as the percent weight loss during shipment
[bcd]Means on the same horizontal line bearing different superscripts differ significantly ($P < .05$).

Table 3–Mean values for muscle color score[a] in test shipments 2, 3, 4 and 5

Test shipment	Item	Muscle	Treatment			
			Unprotected	Paper bag	Polyethylene bag	Polyvinyl chloride film
2	Chucks	Longissimus	4.9[b]	5.3[b]	5.3[b]	5.3[b]
2	Chucks	Triceps	3.0[b]	3.2[b]	4.0[b]	4.0[b]
2	Rounds	Gracilis	1.2[b]	1.4[b]	1.7[b]	2.2[b]
2	Rounds	Quadriceps	2.5[b]	4.3[b]	3.8[b]	4.5[b]
3	Loins	Longissimus	1.7[b]	3.0[bc]	5.3[d]	3.5[c]
5	Chucks	Longissimus	1.3[b]	1.7[b]	2.4[c]	2.5[c]

[a]Based on a 9-point scale in which 9 = very light cherry red and 1 = black
[bcd]Means on the same horizontal line bearing different superscripts differ significantly ($P < .05$).

Weight losses for chucks, rounds and forequarters during transit did not differ significantly among packaging treatments (Table 2), but hindquarters that were wrapped in polyvinyl chloride film shrank less ($P < .05$) than those which were unprotected. Packaging materials were removed from quarters and cuts immediately after arrival at the warehouse and weights were subsequently obtained after 8 hr (chucks and rounds) and 27 hr (chucks) of storage in a 2°C cooler. Since there was no evidence of compensatory weight loss following bag or film removal, reduced shrinkage during transit results in economies which can be realized at the retail level. After 27 hr of 2°C storage at the warehouse, the chucks that were unprotected during transit (1.56%) had sustained greater ($P < .05$) shrinkage than those packaged in polyethylene bags (0.68%). No differences in bacterial numbers were observed between treatments (data are not presented in tabular form) and all counts were less than 4.00 ($\log_{10}$).

The 84 beef quarters and 65 primal cuts included in test shipment 3 were placed in a trailer that had an average temperature of 18°C during loading. The thermostat was set at 0.5°C during shipment and canvas sleeves located along the top center line distributed refrigerated air to the rear portion of the trailer. The average temperature along the top center line was −2°C and along the bottom center line was 5°C. Air circulation within the trailer was impaired by primal cuts which were stacked near the rear doors, by palletized cartons that were positioned below the refrigeration unit and/or by the compactness of the load. Differences in intransit shrinkage of loins between treatment groups (Table 2) were not consistent enough for statistical significance but the 2.43% shrinkage for unprotected loins as compared to the 0.93% shrinkage for loins in polyvinyl chloride film would be of economical importance. Rounds that were transported in polyethylene bags or in polyvinyl chloride film incurred less ($P < .05$) shrinkage than rounds which were unprotected or in paper bags while those in paper bags shrank less ($P < .05$) than the unprotected cuts. Both polyethylene bags and polyvinyl chloride film reduced ($P < .05$) shrinkage during the shipment of forequarters and hindquarters (Table 2). Bacterial counts did not differ significantly among treatment groups, but quarters and primal cuts which were shipped in polyethylene bags and polyvinyl chloride film evidenced accumulated moisture on the surfaces during shipment. Nevertheless, the number of microbes ($\log_{10}$ = less than 4.00) was well below the 1.0×10^7 count required for the formation of surface slime (Warnecke et al., 1966).

Test shipment 4 consisted of 85 fore-

quarters, 85 hindquarters, 30 chucks, 15 ribs and 50 rounds. The average temperature inside the trailer during loading was 19°C. The mechanical refrigeration unit was controlled by a thermostat setting of −0.5°C during the shipment. Intransit temperatures were collected in the middle and along both side walls at locations near the ceiling, through the center of the load and near the floor of the trailer by use of 24 recording thermometers. The temperatures averaged −3°C (−5 to 2°C) at the ceiling level, 0°C (−3 to 5°C) at midpoint level and 2°C (−1 to 5°C) at the floor level. While the front portions of the trailer received refrigeration at all levels, circulation and return air flow were restricted by the loading pattern such that temperatures above 2°C (2–5°C) prevailed at floor level in the rear portion of the trailer at all recording positions. Higher temperatures in the trailer resulted in higher internal temperatures of quarters and primal cuts in the trailer measured immediately after unloading. Ribs placed on the floor of the trailer near the rear doors had internal temperatures of 7–8°C, hindquarters hanging on the rail near the rear doors were 6–7°C and forequarters near the front of the trailer had internal temperatures of −1 to 2°C upon removal from the truck at the destination.

Intransit shrinkage data for sides and primal cuts in test shipment 4 are presented in Table 2. Beef sides that were wrapped in polyvinyl chloride film or polyethylene bags shrank less ($P < .05$) than sides that were wrapped in paper bags and those sides in polyethylene bags shrank less ($P < .05$) than sides that were unprotected during transit. Differences in shrinkage between treatments for chucks, ribs and rounds were not consistent enough for statistical significance. The low percentages of shrinkage which were incurred by the quarters and primal cuts during this shipment may have resulted from the short period of time intransit and restriction of air circulation within the trailer. As air circulation increases and the corresponding decrease in temperature occurs, there is an increase in moisture evaporation from meat surfaces and therefore more shrinkage (Gerrard, 1969; Ewell, 1935).

Intransit shrinkage data for sides, chucks and rounds in test shipment 5 are presented in Table 2. Sides that were protected by paper bags, polyethylene bags or polyvinyl chloride film incurred less ($P < .05$) shrinkage than the unprotected sides during transit. Chucks and rounds which were unprotected during transit sustained greater ($P < .05$) shrinkage than did those in paper bags, polyethylene bags or polyvinyl chloride film. Bacterial counts for cuts in polyethylene bags ($\log_{10} = 4.07$), polyvinyl chloride film ($\log_{10} = 3.71$), paper bags ($\log_{10} = 3.71$) and those from unprotected cuts ($\log_{10} = 3.52$) did not differ significantly. The slightly higher bacterial counts on cuts in polyethylene bags probably resulted from moisture retention on the meat surfaces, thereby maintaining an Aw level complementary to bacterial growth.

Primal cuts in test shipments 2, 3 and 5 were assigned desirability scores for muscle color at the point of origin and again upon arrival at the destination. Numerical values for cuts wrapped in paper, polyethylene or polyvinyl chloride were higher in every case than scores for unprotected cuts. However, statistical differences were noted only for loins and chucks in shipments 3 and 5, respectively. In these instances, cuts protected with polyethylene bags or polyvinyl chloride film were significantly ($P < .05$) brighter in lean color than those which were unprotected during shipment (Table 3).

Sides, chucks, rounds, ribs and loins in test shipments 2, 3, 4 and 5 were assigned desirability scores for appearance of the subcutaneous fat. Unprotected cuts or sides differed significantly from those protected by paper bags in 2 of 10 comparisons, from those in polyethylene bags in 9 of 10 comparisons and from beef wrapped in polyvinyl chloride film in 7 of 10 comparisons (Table 4). When the polyethylene bags and polyvinyl chloride film were removed upon arrival at the destination, the surfaces of cuts were more moist than corresponding surfaces of cuts that were transported unprotected or in paper bags. These cuts evidenced greater freshness and less discoloration and desiccation of the subcutaneous fat when compared to those that were unprotected or in paper bags during shipment. During subsequent storage, the surface moisture that had accumulated on cuts that were wrapped in polyethylene bags and polyvinyl chloride film evaporated, leaving the surface firm, dry and white. Meat cuts with firm, white fat surfaces and no discoloration of exposed lean enjoyed price negotiation advantages when quarters or cuts were presented to prospective buyers in a packer branch house receiving the cuts from test shipment 3. Of the 20 cuts selected as most desirable in appearance by a buyer, 10 had been wrapped in polyvinyl chloride film and 9 had been wrapped in polyethylene bags. These findings suggest that protection during shipments of 5 days or less with either polyethylene bags or polyvinyl chloride film would result in maximum product freshness and acceptability on arrival at the destination and that overall appearance is a price determining factor in determining the ultimate selling price of beef quarters and cuts. There was some evidence that polyethylene bags and polyvinyl chloride film should not be allowed to remain on quarters or primal cuts longer than 7 days following application due to the possibility of bacterial increases that may occur in the moist environment created by these protective packaging systems.

In data not presented in tabular form, time requirements for application and removal of packaging materials from 790 primal cuts or quarters were obtained. Man-seconds for application and removal of paper bags, polyethylene bags and polyvinyl chloride film from primal cuts were 27.6, 10.8; 27.1, 9.7 and 23.6, 14.7, respectively. Corresponding requirements for application and removal of paper bags, polyethylene bags and polyvinyl chloride film from beef quarters were 46.0, 17.5; 56.0, 10.6 and 38.7, 13.9 seconds, respectively. Packaging material costs for paper bags, polyethylene bags and polyvinyl chloride film were 16, 22 and 36 cents, respectively, for primal cuts and 34, 22 and 34 cents, respectively, for

Table 4—Mean values for appearance[a] of subcutaneous fat in test shipments 2, 3, 4 and 5

Test shipment	Item	Treatment			
		Unprotected	Paper bag	Polyethylene bag	Polyvinyl chloride film
2	Chucks	2.6[b]	2.7[b]	3.7[c]	3.0[bc]
2	Rounds	3.5[b]	3.8[b]	4.0[b]	3.5[b]
3	Loins	3.0[b]	3.7[bc]	4.0[c]	4.0[c]
3	Rounds	2.1[b]	3.4[c]	4.9[c]	5.2[d]
4	Sides	4.8[b]	5.1[b]	5.8[c]	5.5[c]
4	Chucks	3.6[b]	3.6[b]	4.4[c]	4.7[c]
4	Rounds	4.4[b]	4.7[b]	5.3[c]	5.6[c]
4	Ribs	4.7[b]	4.5[b]	6.0[c]	6.0[c]
5	Sides	1.7[b]	2.1[bc]	2.5[c]	2.2[bc]
5	Chucks	4.1[b]	4.4[c]	4.5[c]	4.6[c]

[a]Based on a 6-point scale in which 6 = very fresh appearance and 1 = severe or extensive discoloration or desiccation

[bcd]Means on the same horizontal line bearing different superscripts differ significantly ($P < .05$).

beef quarters. Using shrinkage data obtained from test shipment 5 and current product values for beef, net savings of $1.88, $2.15 and $2.14 per side; $0.28, $0.58 and $0.69 per round; and $0.27, $0.48 and $0.48 per chuck were realized by the use of paper bags, polyethylene bags or polyvinyl chloride film, respectively, during transit. These values include labor costs for application and removal of packaging materials, material costs and beef product values.

On the basis of these studies, it is concluded that:

(1) Temperature conditions within the five trailers used for these test shipments were generally inadequate for maintenance of quality in beef quarters and primal cuts. Temperatures within specific loads were affected by loading patterns, product densities, positions of palletized product or paper partitions, thermostat settings and the presence or absence of air conveyance ducts.

(2) The use of polyethylene bags or polyvinyl chloride film significantly ($P < .05$) reduced intransit shrinkage as compared to unprotected cuts or quarters in 7 of 16 comparisons. It was difficult to establish the effectiveness of paper bags in reducing shrinkage intransit because of excessive tearing during loading, shipment and unloading, but significant ($P < .05$) reductions in weight loss were noted in 5 of 16 comparisons. Economic feasibility comparisons revealed that protective packaging resulted in net savings of from $0.27 to $0.69 per wholesale cut and from $1.88 to $2.15 per side in reduced shrinkage loss.

(3) Lean color from cuts protected by polyethylene or polyvinyl chloride was significantly ($P < .05$) superior in brightness to that of unprotected cuts in 2 of 6 comparisons.

(4) Mean values for appearance of subcutaneous fat revealed that cuts protected by polyethylene bags were more desirable than unprotected cuts in 9 of 10 comparisons. Cuts wrapped in polyvinyl chloride film were considered superior ($P < .05$) to unprotected cuts in 7 of 10 comparisons.

(5) When polyethylene bags and polyvinyl chloride film were removed upon arrival at the destination, the surfaces of cuts were more moist than corresponding surfaces of cuts that were transported unprotected or in paper bags. Since an increase in Aw could increase bacterial growth, prolonged storage in plastic wrapping materials is not feasible. Microbial counts from cuts or quarters in these test shipments were significantly different only in comparison 1, but trended toward significance in shipment 5. Until further evidence is available polyethylene bags and polyvinyl chloride film should not be allowed to remain on quarters or primal cuts longer than 7 days following application.

REFERENCES

Ewell, A.W. 1935. The cooler storage of beef. Refrig. Engr. 29: 16.

Gerrard, F.R. 1969. High pressure spray washing. Bull. 65, p. 16. The Institute of Meat, London.

Johnson, H.D., Penny, R.W. and Guilfay, R.F. 1959. Rail car and "piggyback" transportation of freshly killed beef. USDA Mkt. Res. Rpt. 339.

Schneidau, R.E. 1964. Cooler shrink of pork carcasses and wholesale pork cuts. Res. Bull. 788. Ind. Agr. Exp. Sta.

Stokes, J.L. 1960. Growth of microorganisms at low temperatures. Proc. of the Twelfth Research Conf., p. 9. American Meat Institute Foundation, Chicago.

USDA. 1966. Report of task force for research on centralized meat packaging. Nutrition, Consumer and Industrial Use Research.

Volz, M.D. and Marsden, J.A. 1963. Centralized processing of fresh meat for retail stores. USDA Mktg. Res. Rpt. 628.

Warnecke, M.O., Ockerman, H.W., Weiser, H.H. and Cahill, V.R. 1966. Quality of processed comminuted meat as affected by microbial flora of the raw constituents. Food Technol. 20: 687.

Weatherly, E., Earle, W. and Brown, E. 1968. A cost comparison of four different methods of meat distribution. Research Highlights in Food Distribution. Dept. Agr. Eco. Rpt. 1. Cornell University, Ithaca.

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DEVELOPMENT OF A PROCESS FOR PREPARING A FISH PROTEIN CONCENTRATE WITH REHYDRATION AND EMULSIFYING CAPACITIES

INTRODUCTION

THE PRODUCTION of low fat content, deodorized fish flour (FPC) is a possible means of utilization of vast quantities of low value fish for direct human consumption. Many processes for making FPC have been advocated (Finch, 1970; Meade, 1971). Although some processes employing hot-solvent extraction to make FPC for animal feed have been commercially successful, production of FPC for human consumption has not fulfilled its promise (Holden, 1971).

Extensive reviews by Finch (1970) and Meade (1971) indicate that commercial efforts to make FPC for human consumption have concentrated on solvent extraction processes which employ isopropanol (IPA) ethylene dichloride, and to a lesser extent, ethanol and hexane as extracting solvents. In general, these processes result in a protein with good nutritional properties, little or no offensive odor and taste, and no functional properties (water regain, emulsification). Although much effort has been expended to make food products from FPC, particularly that made by IPA extraction, it appears that FPC without functional properties is mainly relegated to the role of food supplement (Holden, 1971). FPC might be more valuable if it could be eaten as a food on its own merits or used as a meat substitute. If the protein could be made to regain water to the original content in the fish (approximately 80%), a meat substitute might be made for a reasonable price from a comparatively high priced powder. Retention of the binding power of the original fish muscle would further increase the value of the protein (Devanney and Mahnken, 1970).

An ideal FPC process in addition to being economical would produce a product with the following attributes: (1) nutritive properties similar to the original fish muscle; (2) water regain to the original content of the fish muscle; (3) bacteriologically sterile; (4) odorless and tasteless or else possessing a pleasing odor and taste; (5) stable to atmospheric conditions; (6) no toxic residues. In addition, it would be highly desirable if the process resulted in the recovery of a high grade oil. The purpose of this investigation was to develop a procedure for making an FPC with all of these properties.

A preliminary report of this process was made at the Joint Texas Shrimping Association–Gulf States Marine Fisheries Commission Meeting (Cobb and Hyder, 1971).

Theoretical

Ample evidence exists that in the normal or undenatured state, a protein molecule is arranged in such a manner that hydrophilic or ionic groups are oriented toward the aqueous environment while hydrophobic groups are oriented toward the interior of the molecule (Butler, 1971). During the various hot solvent extraction processes which have been proposed for the preparation of FPC the protein molecule is exposed to a more hydrophobic solvent medium (ethanol, isopropanol, ethylene dichloride, hexane) than its normal aqueous environment. Such solvents lower the free energy of unfolding of the protein molecule (Tanford, 1962). Also, the heat energy is sufficient to break hydrogen and hydro-

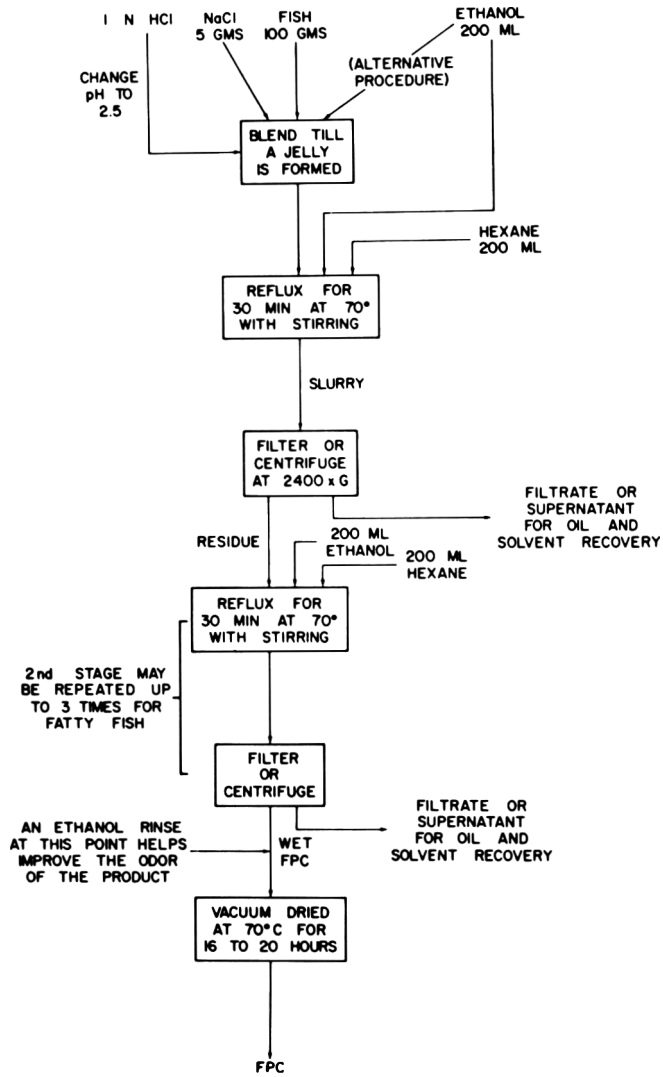

Fig. 1—Flow diagram for the preparation of TAMU-FPC. Decantation of hexane which would come prior to filtration or centrifugation is not shown as a separate step.

phobic bonds which help maintain the structure of the molecule. When possessing both positive and negative charges, the denatured molecules interact to form micelles with some of the hydrophobic side chains oriented toward the exterior of the micelle. The reorientation of the hydrophobic groups causes a corresponding reorientation of the hydrophilic groups toward the interior of the micelle, forming relatively high energy ionic bonds. The resulting micelle has a hydrophobic surface which blocks penetration of water to the ionic groups. The result is a very stable protein micelle which actually repels water.

In order for the protein molecule to be soluble or to retain rehydration capabilities, protein-protein interaction must be prevented. The simplest approach to prevent protein-protein interaction is by pH adjustment. If protein molecules have only positively or negatively charged groups, electrostatic repulsion will diminish protein-protein interaction and, hence, the formation of micelles. If micelle formation does occur, charged groups should be orientated toward the surface. The result should be a molecule or micelle which can interact with an aqueous medium, thereby retaining some rehydration properties.

At acid pH below 3.5 only C-terminal carboxyl groups should be ionized. The remainder of the charges on the molecule will be positive, i.e., NH_3^+ type. A similar situation exists at basic pH above 13.5 (pK_3 of arginine) except the molecule is negatively charged. Because of the distinct possibility of destruction of cysteine and racemization of some amino acids at high pH (Hill, 1965; Levene and Bass, 1928), an acid pH appears to be best for prevention of ionic interaction. However, conditions must be sufficiently mild to prevent destruction of tryptophan so that the protein retains its nutritive value.

EXPERIMENTAL

Materials

Atlantic croaker (*Micropogon undulatus*) 10–30 cm long were collected from boats during shrimp trawling operations in Galveston Bay and the Gulf of Mexico. Fish, not utilized immediately, were treated as follows: (1) scales, viscera and heads were removed from the fish, the carcases were then covered with distilled water in sealed plastic bags and stored at −20°C until used. Ethanol (95%) was obtained from several commerical sources. Hexane was obtained from two different sources: n-hexane, ACS reagent grade from J.T. Baker, and was used without further purification; hexanes, ACS reagent grade obtained from Fisher Scientific, were redistilled to reduce off-flavored impurities. Sodium chloride, food grade, was obtained from Morton Salt Co., Chicago, Ill.

Preparation of FPC (TAMU-process)

Fillets were removed from larger fish (above 20 cm long) and ground or minced into small pieces. Sodium chloride (5% by weight) was added and the material was blended in a Hoover blender until a smooth jelly was obtained. The pH of the jelly was adjusted to 2.5 with 1N HCl and 2 ml of ethanol per gram of fish were added. In an alternate procedure the ethanol was added and the pH of the resulting mixture was then adjusted with HCl until it stabilized at 2.5. The mixture was transferred to a round bottom extraction flask equipped with an efficient reflux condenser. Hexane equal to the volume of the ethanol was added. The mixture was rapidly stirred and heated in a water bath until the hexane began refluxing (approximately 70°C). After 30 min at reflux temperature the hexane was decanted and the ethanol was removed by filtration or centrifugation. The protein was returned to the extraction flask and amounts of ethanol and hexane equal to the original volumes were added. The procedure was repeated from two to four times depending upon the fat content of the fish. Figure 1 presents a flow diagram of the process. Because the hexane was decanted through the same filter employed to remove the ethanol, decantation is not illustrated as a separate step in Figure 1. A final ethanol rinse was necessary to remove odoriferous residues remaining from the hexane. The FPC was air-dried or vacuum-dried at 70°C in a desiccator connected to a water aspirator.

Effects of salt content

The effect of salt content on fish protein in acidified ethyl alcohol were measured on three different batches of fish. Fillets were removed from each batch and comminuted with a sausage grinder. The comminuted material was carefully mixed and two 100g portions for each salt level were removed. Each portion was blended in a Waring Blendor with an appropriate amount of salt. Then 200 ml of ethanol were added and the pH adjusted to 2.5 with N HCl. The material was rinsed with 300 ml of ethanol into extraction flasks fitted with condensors and heated in a water bath to 70°C for 30 min with slow stirring. The material was rinsed into 500 ml Nagalene bottles with an additional 100 ml of ethanol. The bottles were sealed, and the mixture was then centrifuged for 30 min. The total volume was measured. The supernatant was carefully withdrawn, measured and checked for protein content.

Methods of evaluation

Analyses of fish muscles and FPC were conducted in duplicate on samples randomly drawn from the homogenous mass of each batch or several batches pooled together. Fish muscles requiring dehydration prior to analyses were freeze-dried at 5°C for 55 hr in an Industrial Dynamics Pilot Plant Freeze Dryer Model CPF20.

Chemical analyses. Protein determinations were based on Kjeldahl nitrogen content x 6.25. Initial determinations were made by the AOAC (1965) method. However, sulfuric acid digestion with a mixture of selenium oxide and potassium sulfate as catalysts (Willard et al. 1956) was found to give higher and more reproducible values than the AOAC procedure and was subsequently used. Soluble protein in supernatants was estimated by the biuret procedure of Snow (1950).

Moisture and/or volatile substances were determined by heating to a constant weight in a drying oven at 100°C (AOAC, 1965).

Fat content was determined by extraction with diethyl ether in a Soxhlet extractor for 8 hr (AOAC, 1965).

Ash content was determined by ashing in a muffle furnace at 550°C (AOAC, 1965).

Amino acids were analyzed in a fully automated Beckman 120 Model C Amino Acid Analyzer. Samples were saturated with nitrogen and then hydrolyzed with 6N HCl under vacuum at 110°C (Stein and Moore, 1954). Tryptophan was determined by the alkaline hydrolysis procedure of Kohler and Palter (1967). Cysteine was analyzed by the performic acid oxidation method as described by Hirs (1967).

Microbiological examination. The detection of salmonella, staphylococci, clostridia and the enumeration of coliform, aerobic and anaerobic viable organisms were according to the *Recommended Methods for the Microbiological Examination of Foods* (APHA, 1967). Presence of putrefactive anaerobes and *Clostridium botulinum* was further determined on the basis of the recommendations of the Center for Disease Control, Atlanta, Ga. (USPHS, 1968).

Determination of protein yield. The yield of FPC was determined as percent protein recovered. Kjeldahl nitrogen values were used and periodically checked with true protein values calculated from amino acid analyses. The loss of protein during processing was computed by difference or by determining the amount of protein lost in the solvents.

Titration curve. A titration curve was developed by adding 0.0197N NaOH solution to the FPC solution (2g/100 ml) and measuring the corresponding change in pH.

Rehydration capacity of FPC. Estimates of the water holding capacity of FPC were made by rehydrating it in water at different pH and ionic strengths. Duplicate samples of FPC, 0.2g

Table 1–Effect of NaCl on ethanol recovery from the extraction at 70°C for 30 min of 100g of fish muscle with 600 ml ethanol at pH 2.5

NaCl (g)	Nature of protein	Volume of supernatant (ml)	Appearance of supernatant[a]
0	Gel	68–488	Turbid
1	Gel	333–516	Turbid
2	Gel and granular	448–502	Turbid
3	Gel and granular	514–647	Turbid
4	Granular	492–510	Turbid
5	Granular	503–526	Clear

[a]Total protein lost in supernatant ranged from 0.3–2.7 mg/100g based on Kjeldahl nitrogen determination

each, were rehydrated with 10 ml of distilled water in a 50 ml centrifuge tube for at least 1 hr at room temperature (25°C). The pH was adjusted with 1M NaOH or 1M HCl. The ionic strength was adjusted to the same value in each tube by adding 1M NaCl and distilled water until the final volume of the liquid was 15 ml. The rehydrated FPC was then centrifuged at 2400 x G for 30 min. The supernatant was removed very carefully by capillary pipette and its volume was measured. The amount of water retained by the FPC was calculated by subtracting the volume of supernatant from the total volume of the rehydration medium (15 ml). Correction for the evaporation loss during centrifugation was made by using water blanks.

The rehydration capacities at pH 2 and 5 were estimated in a similar way at the ionic strengths of 0.13, 0.3, 0.6, 0.9 and 1.2. The pH was adjusted with 1M NaOH. The solution was adjusted to desired ionic strength with NaCl.

For routine checks of rehydration capacity of FPC, approximately 0.5g samples were placed in 12 ml graduated conical test tubes. Water was added until swelling ceased. The tube was allowed to set for several minutes. The volume of the protein provided a good estimate of rehydration capacity.

Emulsifying capacity estimation. The emulsifying experiments were conducted according to the scheme of Carpenter and Saffle (1964). Concentrations of FPC ranging from 1–2.5g/100 ml of water were emulsified in a blender by adding corn oil continuously at 12 ml/min. The results were plotted as concentration of protein in water against ml of oil in the emulsion at the inversion point.

For routine checks of the emulsifying properties of the FPC 1g samples were rehydrated in 100 ml of water, 10 ml oil were added, then the resulting mixture was blended until smooth.

RESULTS

Process development

Table 1 presents the data on the extraction of protein from fish muscle at pH 2.5 with ethanol and different concentrations of NaCl. Gel formation was observed when the NaCl concentration was less than 3% of the weight of fish muscle. The protein residue or pellet obtained after centrifugation tended to be more granular as the NaCl concentration increased. At the 5% level of NaCl, separation of solvent by filtration was the easiest as the protein residue was granular and the supernatant was clear. Turbidity in the supernatants persisted from 0–4 percent NaCl. The material responsible for the turbidity appeared to clog the filter, thus making solvent removal difficult. The volume of recovered supernatant, which varied considerably with the lower salt concentrations, had an inverse relationship to the volume of the gel. The reason for the variation in gel formation has not been established. However, there was some evidence that gel formation may have depended upon the amount of water in the starting material. When water was added to the starting material gel formation was more evident.

Protein recoveries at pH 2, 2.5, 3, 4 and 7 are presented in Table 2. When ethanol and 5% NaCl was used as the extracting medium, the highest protein recovery was 61% at pH 2.5 and the lowest was 48% at pH 7. Recoveries from five samples of fatty fish yielding 8–10 ml of oil per 100g were even lower, ranging from 23–41%. This low recovery appeared to be caused by (1) a possible detergent effect of the lipid and (2) the need for additional extraction steps for lipid removal. Therefore, use of a secondary solvent was indicated. Hexane, an immiscible organic solvent which has been considered as a suitable solvent for the preparation of FPC by several workers (Pariser and Odland, 1963; Yanez et al. 1967) was used to increase the rate of extraction of neutral lipids. The addition of hexane to the system increased protein recovery, for this batch of fish, from 61–86 ± 1% (Table 2). Recovery values for some batches of fish appeared to be different. For some unfrozen fish 88–95% protein yields were obtained and for several batches of frozen fish yields as low as 76% were obtained.

When NaCl (5g/100g) was blended with comminuted fish prior to pH adjustment, the muscle was converted to a jelly (or paste). The jelly had been separated from bones and scales by a filter press. This required coarse screening, then fine screening with a 60-mesh screen. Rinsing of the residue with a small volume of 5% NaCl recovered additional protein. The addition of a small volume of 5% NaCl to the jellied fish did not appear to affect subsequent protein yields.

The fish had to be comminuted into very small particles prior to pH adjustment. If particles size was more than 2 or 3 mm, fishy odors were not removed by the extraction process. In addition, these particles became very hard upon drying and did not grind easily. The conversion of the muscle into a jelly reduced this problem. Inadequate stirring during extraction also resulted in large particle formation and incomplete removal of lipid. Sieving of the final product to remove larger particles usually removed any residual fishy odors.

If the protein was acidified prior to the addition of ethanol, the pH could be adjusted rapidly. However, if the ethanol was added prior to pH adjustment, the pH continued to change for about 30 min. In order to obtain proper pH adjustment, the protein had to be stirred vigorously. Approximately 19–20 meq of HCl were required to adjust the pH of

Table 2–Effects of pH and hexane on protein recovery from 100g of fish muscle blended with 5g of NaCl and extracted for 30 min at 70°C 3x with 200 ml of ethanol or 200 ml of ethanol plus hexane.

Hexane (ml)	pH	Protein recovery[a,b] (%)
–	2.0	54
–	2.5	61[c]
–	3.0	50
–	4.0	49
–	7.0	48
200	2.5	86±1

[a]Based on percent nitrogen recovery from fish muscle

[b]Recovery values are presented for the same batch of fish. Recovery values for the ethanol extracts are for two determinations with < 5% variation. Ranges for recovery values of five preparations for the ethanol-hexane extracts are presented.

[c]Recovery values from fish yielding 8–10 ml of oil per 100g of fillet ranged from 23–41% (5 samples).

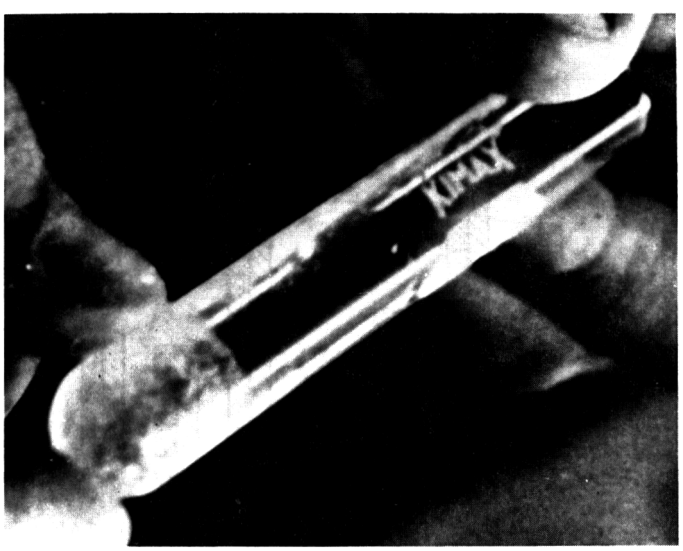

Fig. 2–Rehydrated TAMU-FPC gel, pH 2.5, 1g (0.87g protein)/15 ml.

100g of fish muscle to 2.5.

During the extraction process, the hexane remained as a distinct layer on top of the ethanol-water (from the fish) layer. The hexane layer appeared to contain most of the lipid while the protein remained in the ethanol-water layer. In preliminary experiments using 100g of fillets, 8–10 ml of oil were recovered from the combined hexane extracts. When fish did not show signs of oxidation (i.e., rancid odor), the oil was straw-colored and transparent with a mild fishy odor. The oil from rancid fish ranged in color from orange to red. Distillation of the ethanol (combined extracts) left a creamy residue of 6–7g. Adjustment of the pH of the ethanol to 9.0 with $Ca(OH)_2$ prior to distillation produced a precipitate (2.3g) which had a strong fishy odor and orange color, even when the fresh fish were used. Preliminary analysis indicated that the precipitate contained 38 – 40% protein (N x 6.25), with the remainder presumably Ca, moisture and lipids. Following recovery of the precipitate the residue remaining after distillation of the ethanol and water appeared to be largely crystalline, probably NaCl. In the later experiments where more accurate recovery of lipid was attempted, the neutral lipid level of the fish was so low (less than 0.5%) that accurate recovery of the oil was not possible. Distillation of the hexane extracts of fillets (100g) from these lean fish left straw-colored oil residues estimated at less than 0.5g. Distillation of the ethanol produced approximately the same amount of residue as from fatty fish.

Table 3–Proximate analysis of fish muscle and fish protein concentrate

Analysis[a]	Fish muscle			Fish protein concentrate		
	Batch I	Batch II	Batch III	Batch I[b]	Batch II	Batch III
Protein	18.80	18.77	17.88	86.18	87.74	87.08
Fat[c]	0.45	.59	0.42	0.36	0.31	0.35
Volatile	80.02	79.82	81.11	10.36	9.20	9.06
Ash	0.73	0.82	0.59	3.10	2.75	3.51

[a]Expressed as percentage
[b]Batch number of FPC corresponds to batch number of fish muscle from which the FPC was made.
[c]Diethyl ether-extracted

Properties of FPC

The FPC (TAMU-FPC) prepared by the acid-ethanol-hexane extraction procedure (TAMU-process) from *M. undulatus* was off-white in color, had no detectable odor and an almost undetectable sour taste prior to pH adjustment. The material is hydrophilic, i.e., readily takes up water and forms a gel (Fig. 2), but not hygroscopic. Samples have been left open up to 1 yr without odor reversion. The material was usually odorless but three minor problems with odor in the final product were encountered: (1) a petroleum odor from fish which had been contaminated with petroleum prior to arrival at the laboratory; (2) a petroleum odor from some lots of low grade hexane; and (3) fishy odors from samples which were not adequately extracted or else had large particles. Once the protein had been contaminated with the petroleum odors, they were difficult to remove.

The proximate analysis of the boneless fish muscle and the TAMU-FPC made from the muscle are presented in Table 3. The fish used for these studies were taken during the post-spawning period when they had very low fat contents. Preliminary data indicated that protein, moisture and fat content of FPC produced from fish with up to 10% fat were very similar (86–88%, 9–10%, < 0.5%, respectively) provided that two additional extractions were used to remove the excess lipid. The

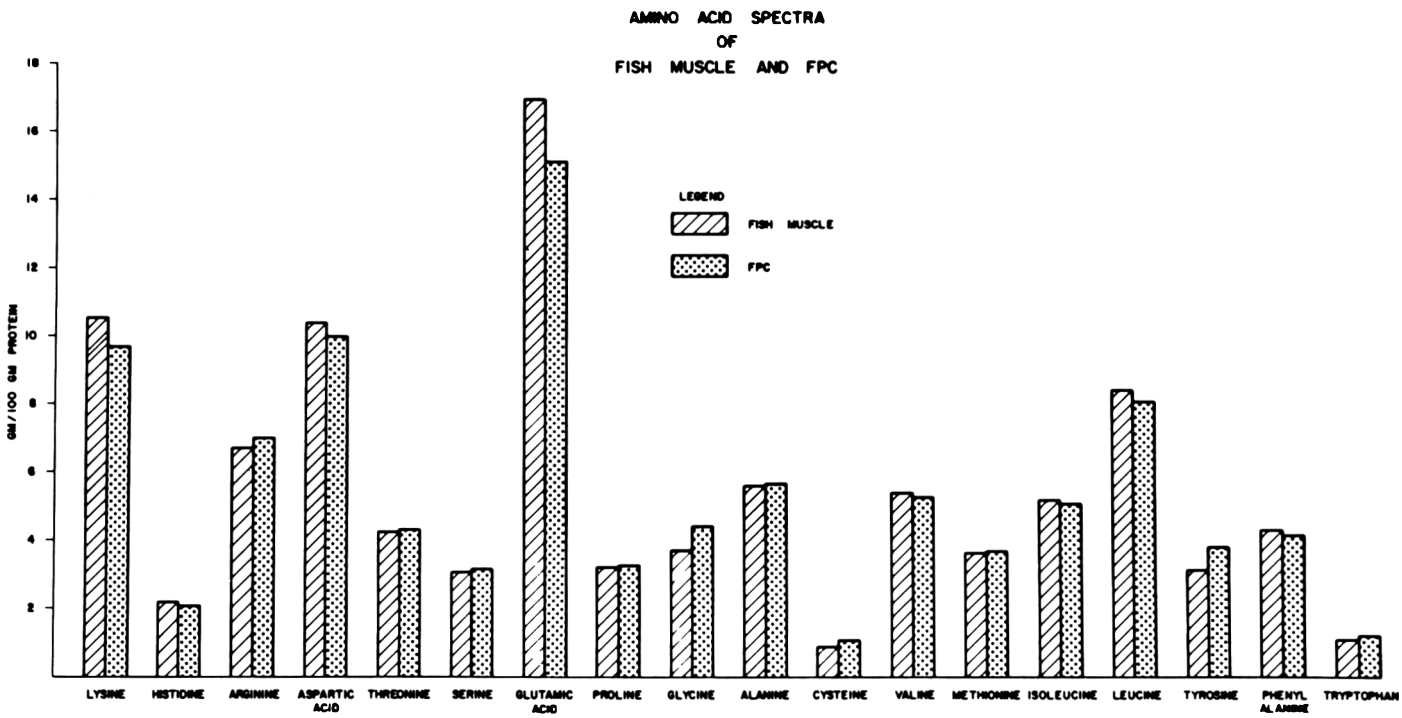

Fig. 3–Comparative histogram of amino acid content of fish muscle and TAMU-FPC. For more accurate comparison amino acid content was calculated on the basis of residue weights (mol wt–18).

volatile contents of these samples were high. From the odors evolved when the FPC was heated to 105°C some of the volatile residue appears to be ethanol. The protein content ranged between 86 and 88%. Ash content for samples prepared from boneless muscle ranged from 2.75–3.51%. When the samples were dried under the same conditions to a constant weight, the yield of the dry FPC provided a good estimate of protein recovery.

Amino acid analyses

The essential amino acid spectra of fish muscle and FPC made from the muscle were similar (Fig. 3). Of the essential amino acids, lysine was the only one with a lower level in the FPC than in the fish muscle. This suggested that a protein or peptide rich in lysine was lost during processing. Tryptophan, which might be destroyed by the acid conditions, was preserved.

Bacteriological examination of fish muscle and FPC

Duplicate samples of the three batches of fish muscle were examined for microbial flora prior to processing into FPC. Immediately after preparation of FPC, duplicate samples from each batch were removed aseptically and examined for the change in the bacterial flora. The results are presented in Table 4. No growth in the lowest dilution is reported as < 5 per gram. Anaerobic agar plate counts per gram of fish muscle were higher than the aerobic plate counts in Batch I and III and in Batch II they were almost equal. This suggested that there were very few strict anaerobes which was confirmed by further incubation of the anaerobes under aerobic conditions. Processing of the muscle into FPC destroyed most of the microorganisms. No viable organism was observed in any of the different batches except in one sample of Batch I. This probably was due to contamination during analysis since the duplicate sample did not produce growth, and no growth was evident in enrichment media (trypticase-soy agar). In general, the process appeared to yield sterile FPC. This was not unexpected since solvent and pH conditions were not conducive to bacterial survival.

Table 4—Bacteriological examination of fish muscle and fish protein concentrate

Sample	Batch[a] No.	Agar plate count/g Aerobic	Agar plate count/g Anaerobic	Coliform MPN/01.g
Fish muscle	I	2.2×10^4	7.1×10^4	3.6
	I	7.0×10^4	8.6×10^4	<1.8
	II	1.1×10^5	8.9×10^4	<1.8
	II	1.0×10^5	9.8×10^4	<1.8
	III	5.6×10^4	6.0×10^4	<1.8
	III	5.4×10^4	1.0×10^5	3.6
FPC	I	<5	<5	<1.8
	I	<5	25	<1.8
	II	<5	<5	<1.8
	II	<5	<5	<1.8
	III	<5	<5	<1.8
	III	<5	<5	<1.8

[a]Batch numbers of fish protein concentrate (FPC) correspond to batch numbers of fish muscle from which the FPC was made.

Functional properties of TAMU-FPC

Rehydration capacity. The rehydration properties of TAMU-FPC depend upon pH and ionic strength. Figure 4 shows the water holding capacity of three pooled samples of FPC at low ionic strength (0.13) under different conditions of pH. The lowest water holding capacity was in the region of pH 5–8. The water holding capacity decreased from 16 to 7 ml per gram as the pH increased from 2 to 7. As the pH was increased from 7 to 10 the water holding capacity increased from 7 to 20 ml per gram. In the pH zone of 5–8, which covers the pH of most sausage emulsions, the water holding capacity varied from 10 to 7 ml per gram. This was approximately twice the amount of the original water in the fish muscle (70–80% depending upon the fat content). Rehydration of FPC to the approximate original water content of fish muscle (20% FPC, 80% water) between pH 5 and 8 yielded gels having the consistency of a thick paste. The water holding capacity was markedly increased when the ionic strength was increased from 0.13 to 0.4 at pH 5 and then there was a gradual decrease with further increase of the ionic strength to 1.2 (Fig. 5). At pH 2 there was no change in the water holding capacity with the change of the ionic strength from 0.13 to 0.4. Then the water holding capacity decreased as the ionic strength increased to 0.9, after which it

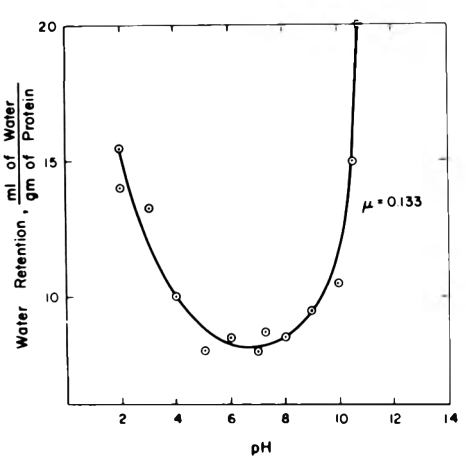

Fig. 4—Water holding capacity of TAMU-FPC at different pH. Each point represents the average of duplicate analyses with < 5% variation.

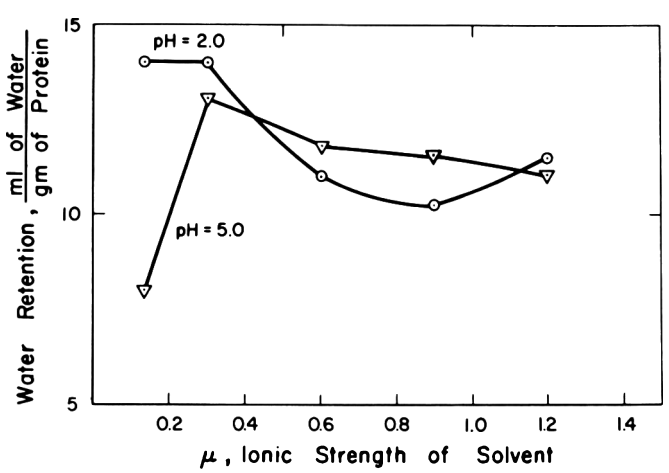

Fig. 5—Water holding capacity of TAMU-FPC at different ionic strength. Each point represents the average of duplicate analyses with < 5% variation.

appeared to increase. The water holding capacity may be affected by heat, as some rehydrated samples appeared to lose a small amount of water upon heating to 100°C in a boiling water bath. This was evident with only a few samples and did not occur if the samples had been emulsified with oil prior to heating.

Change of pH and salt content had a marked effect on the FPC which had been fully rehydrated at acid pH. Expulsion of water occurred when the pH was changed from 2 to 5. An increase in the water holding capacity at pH 5 was evident when a small amount of salt was added to the rehydrated FPC. The rehydrated FPC changed from a translucent gel to a pasty, cream-colored material as the pH was increased from 3–5.

The pH of FPC prior to drying appears to be critical for its rehydration capacity. Samples of FPC which were adjusted to pH 5–7 prior to drying did not swell when placed in water, suggesting that much of the rehydration capacity was lost. For many uses adjustment of pH of the FPC may be necessary. This has been accomplished by adding N NaOH to the rehydrated material or a calculated amount of Na_2CO_3, $NaHCO_3$, or $CaHPO_4$ to the FPC prior to rehydration. In experiments to make sausage emulsions with the FPC, which will be presented in detail in subsequent communications, a mixture of 6g of beef and 4g of rehydrated FPC (1 part dry FPC to 4 parts water) had a pH of 5. A titration curve of TAMU-FPC is presented in Figure 6.

Emulsifying capacity. The emulsifying capacity of TAMU-FPC was studied by rehydrating FPC in water and blending it with Wesson oil to the inversion point at which the emulsion could no longer hold any more oil. The inversion point was difficult to observe when low concentrations of FPC (below 0.75g of FPC on 100 ml of water) were used. Figure 7 shows the variation in the percent of oil with different concentrations of FPC in emulsions at the inversion point. At pH 3 the maximum oil content in the emulsion was 150 ml when the FPC concentration of 1g/100 ml was used. The addition of more FPC reduced rather than increased the oil uptake. The emulsions were thick, creamy and smooth (Fig. 8).

A study of the emulsifying properties at different pH was conducted with a concentration of FPC of 1g/100 ml of water. The amount of oil in the emulsion varied from a maximum of 155 ml at pH 2 to a minimum of 60 ml at pH 5 (Fig. 9). When the pH of the saturated emulsions made at pH 2 or 3 was changed to a more basic pH, a loss of oil and water was evident. If the emulsion was not fully saturated with oil, milky suspensions were often formed when the pH was adjusted to the region of 7. Temperatures up to 100°C did not appear to affect the emulsions.

Protein solubility. Protein solubility was too low for measurement in the pH range of 2–9.

DISCUSSION

FINCH (1970) states that proteins may be used in foods for their functional properties rather than their nutritive properties. He adds that FPC may be made by processes, which in order to secure desired physico-chemical properties, reduce protein quality. One such commercial process is the Wiking Eiweiss process in Germany (Shenstone, 1952) which produces an egg white substitute from fish protein. The product has good whipping properties but the manufacturing conditions of solubilization with hot alkali suggest that the protein is degraded. In contrast, the process developed in this study provides not only a protein with rehydration and emulsifying properties but it also preserves most of the essential amino acids originally present in the fish muscle. Preliminary feeding studies (Hyder, 1972) indicate that TAMU-FPC has a PER of better than 3.0. The slight loss of lysine that occurs during processing suggests that lysine rich proteins, possibly histones, or peptides are lost.

An extensive treatment of the theoretical approach which was used in developing this process is not possible at the present time. However, some evidence is available to support the concepts advocated. The sand-like character and resistance to rehydration of isopropanol-extracted FPC would be consistent with the theory advanced. As predicted by the theory, a hydrophobic "membrane" with perhaps some exposed polar groups and peptide bonds would be expected to be developed on the surface of the molecule. Such a surface membrane or shell has been proposed by Ackman et al. (1967). Initially, a condensed protein shell was proposed, but was later modified to a hydrated membrane (Ackman and Odense, 1968) on the basis of atmospheric water adsorption by the FPC and the failure to demonstrate a condensed zone of protein by eosin staining. A predominately hydrophobic membrane with some exposed polar groups and peptide bonds which can hydrogen bond with water (Tanford, 1962) would be expected to behave in the same manner.

Whether or not this process will be economical will depend upon a number of factors. It would be desirable to eliminate salt from the pretreatment phase. Preliminary results suggest that this step may be eliminated if filtration is

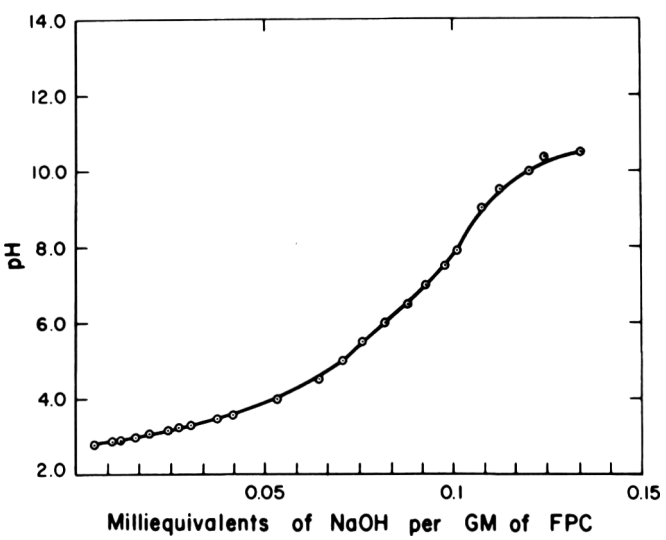

Fig. 6–Titration curve for TAMU-FPC.

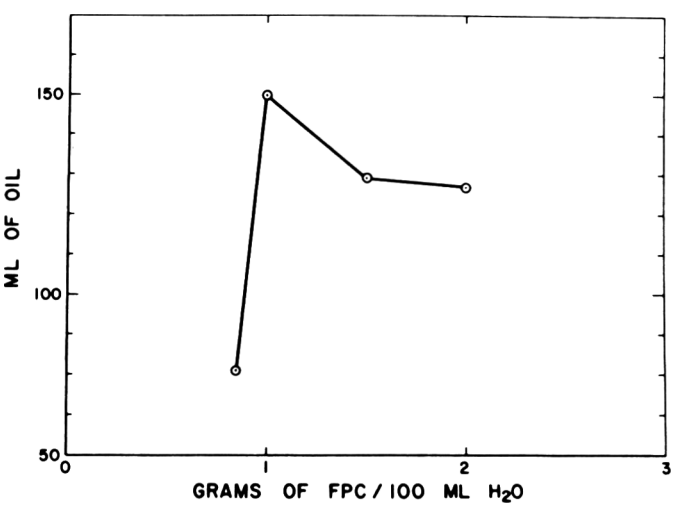

Fig. 7–Emulsifying capacity of different concentrations of TAMU-FPC (1g FPC = 0.87g protein) at pH 3.0. Each point represents duplicate analyses with < 5% variation.

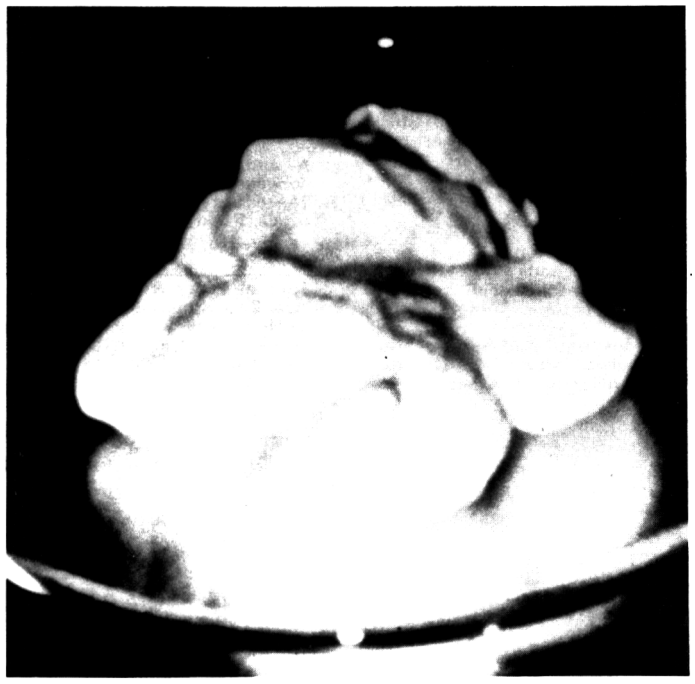

Fig. 8—TAMU-FPC corn oil emulsion.

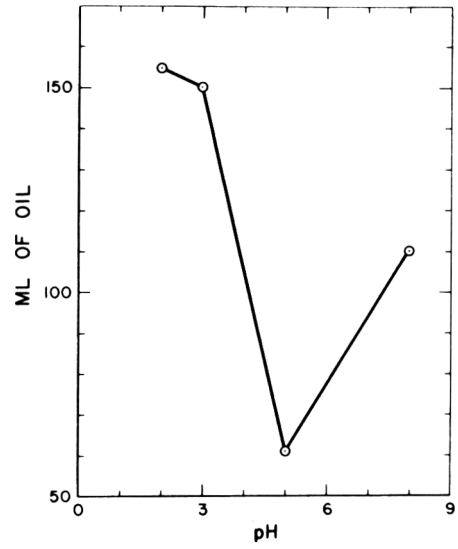

Fig. 9—Emulsifying capacity of TAMU-FPC (1g FPC = 0.87g protein) at different pH. Each point represents duplicate analyses with < 5% variation.

avoided but longer extraction time and a greater solvent-to-protein ratio may be needed. Simplification of the solvent system in order to use isopropanol rather than ethanol would probably be necessary in the United States and other countries which tax ethanol. The use of isopropanol, however, will also depend upon the degree of reaction with acid in the solvent medium. The presence of heavy metals such as zinc would be expected to increase the rate of reaction of alcohol and acid (Noller, 1958).

The use of hexane in the process has both advantages and disadvantages. Lovern (1966) has emphasized the importance of oil recovery by pointing out the fact that "it is the oily pelagic fish that can be most cheaply caught in greatest quantities." The economics of FPC manufactured from oily fish makes oil recovery a very important factor. Hexane promotes the removal of neutral lipids but apparently does not readily extract polar lipids from fish (Finch, 1970; Pariser and Odland, 1968). The processing of fish oils for edible products usually consists of degumming, refining, bleaching, hydrogenation and deodorization (Chang, 1967). The very low level of residue observed in the hexane extracts from the lean fish contrasted to that from the fat fish also suggested that hexane was extracting largely neutral lipids and, hence, a largely degummed and partially refined oil. Weighed against these advantages are the disadvantages of flammability, possible off-flavor residues, and the difficulty of using a dual solvent system. It should be pointed out that the Quintero process uses an ethanol-hexane solvent system for the manufacture of FPC from fish meal (Yanez et al. 1967).

In order to develop this process for commercial exploitation much more study is needed. The major advantage of this procedure lies in pretreatment to give functional properties to the final product. Considerable study needs to be devoted to drying the product. Although lower volatile levels have been achieved in some samples the usual level (approximately 10%) is not low enough. Preliminary experiments indicate that humidification and redrying, as has been used to reduce solvent residues in isopropanol-extracted FPC (Finch, 1970), may possibly allow replacement of organic solvent with water. Further drying of TAMU-FPC may be complicated by the probable availability of polar residues which can form hydrogen bonds with water (Tanford, 1962). A comprehensive study of solvent and by-product recovery is required to determine: (1) optimum solvent ratios; (2) amount of solvents lost per unit of FPC produced; (3) removal of undesirable odors from solvents during recovery; (4) if substitution of isopropanol for ethanol is possible; and (5) whether hexane would be used during the entire process or initial stages.

Mattil (1971), discussing the functionality of proteins, has emphasized that a protein should be soluble under the conditions of pH, ionic strength, etc., at which the protein will be used. At first glance this study appears to contradict these conclusions. TAMU-FPC, although not soluble, emulsifies oils. However, it should be pointed out that the gel-nature of the rehydrated protein indicates that its state may be very similar to that of a protein in solution. The anomalous behavior of TAMU-FPC in not increasing oil uptake as the protein level increased is more difficult to explain. Perhaps, 1g of FPC per 100 ml of water represents the optimum concentration for this protein. The emulsions at this concentration were quite thick.

REFERENCES

Ackman, R.G., Hingley, H.J. and Power, H.E. 1967. Determination of isopropyl alcohol in fish protein concentrate by solvent extraction and gas-liquid chromatography. J. Fish. Res. Bd. Canada 24(7): 1521.

Ackman, R.G. and Odense, P.H. 1968. Reinterpretation of isopropyl alcohol retention in fish protein concentrate. J. Fish. Res. Bd. Canada 25(4): 805.

AOAC. 1965. "Official Methods of Analysis." Association of Official Agricultural Chemists, Washington, D.C.

APHA. 1967. "Recommended Methods for the Microbiological Examination of Foods," 2nd ed. American Public Health Association, N.Y.

Butler, L. 1971. Protein structure and properties. J. Amer. Oil Chem. Soc. 48: 101.

Carpenter, J.A. and Saffle, R.L. 1964. A simple method of estimating the emulsifying capacity of various sausage meats. J. Food Sci. 29: 774.

Chang, S.S. 1967. Processing of fish oils. In "Fish Oils," ed. Stansby, M.E., p. 206. Avi Publishing Co., Westport, Conn.

Cobb, B.F. III and Hyder, K. 1971. The utilization of trash fish. Proc. Gulf States Marine Fish. Comm. In press.

Devanney, J.W. III and Mahnken, G. 1970. The economics of fish protein concentrate. Rep. MITSG71-3, USDC PB197157. Clearinghouse Fed. Sci. Tech. Inf., Springfield, Va.

Finch, R. 1970. Fish protein for human foods, In "CRC Critical Reviews in Food Technology" 1(4): 519.

Hill, R.L. 1965. Hydrolysis of proteins. Advan. Protein Chem. 20: 37.

Hirs, C.H.W. 1967. Determination of cystine as cysteic acid. Methods in Enzymology 11: 59.

Holden, C. 1971. Fish flour: protein supplement has yet to fulfill expectations. Science 173: 410.

Hyder, K. 1972. The development of a process for preparing a fish protein concentrate with functional properties. Ph.D. thesis, Texas A&M University, College Station, Texas.

Kohler, G.O. and Palter, R. 1967. Studies on methods for amino acid analysis of wheat products. Cereal Chem. 44: 512.

Levene, P.A. and Bass, L.W. 1928. Action of alkali on casein. J. Biol. Chem. 78: 145.

Lovern, J.A. 1966. General outlook for edible protein concentrate. In "World Protein Resources. Advances in Chemistry," Series 57, p. 37. Amer. Chem. Soc., Washington, D.C.

Mattil, K.F. 1971. The functional requirements of protein foods. J. Amer. Oil Chem. Soc. 48: 477.

Meade, T.L. 1971. Recent advances in the fish by-products industry. In "CRC Critical Reviews in Food Technology" 2(1): 1.

Noller, C.R. 1958. "Textbook of Organic Chemistry," 2nd ed. W.B. Saunders Co., Philadelphia, Pa.

Pariser, E.R. and Odland, E. 1963. MIT-UNICEF studies on the production of fish protein concentrate for human consumption. Comm. Fish. Review 25: 6.

Shenstone, F.S. 1952. Egg white and yolk substitutes: Wiking Eiweiss. Food Pres. Quart. 13: 45.

Snow, J.M. 1950. Proteins in fish muscle. 2. Colorimetric estimation of fish muscle protein. J. Fish. Res. Bd. Canada 7: 594.

Stein, W.H. and Moore, S. 1954. The free amino acids of human plasma. J. Biol. Chem. 211: 915.

Tanford, C. 1962. Contribution of hydrophobic interactions to the stability of the globular conformation of proteins. J. Amer. Chem. Soc. 84: 4240.

USPHS. 1968. "Laboratory Methods in Anaerobic Bacteriology." NCDC Laboratory Manual, Public Health Service Publication No. 1803, Washington, D.C.

Willard, H.H., Furman, N.H. and Bricker, C.E. 1956. "Elements of Quantitative Analysis," 4th ed., p. 161. D. Van Nostrand Co., Princeton, N.J.

Yanez, E., Barja, I., Monckeberg, F., Maccioni, A. and Donoso, G. 1967. The fish protein concentrate story. 6. Quintero fish protein concentrate: Protein quality and use in foods. Food Technol. 21: 1604.

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TEXTURE MEASUREMENT OF INDIVIDUAL COOKED DRY BEANS BY THE PUNCTURE TEST

INTRODUCTION

THE MAJORITY of objective texture-measuring devices and methods are designed to obtain some average measurement of the textural property of the food. In the case of foods that occur in small size units, a large number of units are usually used in a single test in order to obtain this averaging effect. An example is the well-known Tenderometer that is widely used for grading the textural quality of fresh, green peas. The advantages of this type of test are that it is much quicker to perform one test, for example, on 200 peas at one time than 200 tests on 200 peas and also that there is less calculation required to analyze the results. As a recent example, Voisey and Larmond (1971) studied the texture of canned baked beans using the Tenderometer, Shear Press and several extrusion cells. There are times, however, when it becomes desirable to measure the texture of each individual piece of food in order to determine how widely single units vary from the average value. When outstandingly hard units are present, an instrument such as the Tenderometer fails to detect them, but their presence can be painfully evident when chewed.

The puncture test is a simple test that can be performed quickly, and it is a good index of textural quality for a number of commodities. Bourne (1966), reviewed puncture testing techniques and their application in measuring texture of many different foods. In studies on the cookability of dry pinto, lima, and white beans, Morris (1963) and Burr et al. (1968) mounted 100 metal punches 1/16-in. diam, each weighing 90g, over 100 dry beans that were held in small metal cups. The apparatus was immersed in boiling water in order to cook the beans. When the beans reached a certain degree of softness, the punch penetrated the beans. The number of beans that had been punctured was recorded at regular intervals. A graph of the percentage of cooked beans (i.e., soft enough to be punctured under a standard force of 90g) vs. the cooking time was sigmoid in shape. The time required for 50% of the beans to be punctured was taken as the cooking time. This apparatus was used to study the effect of various storage conditions on the rate of cooking of dry beans (Morris, 1964) but no attempt was made to evaluate the texture of that small proportion of beans that cooked slowly.

The Pabst Texture Tester (R.E. Pabst Co., 5115 Westheimer, Houston, TX 77027) is specifically designed for repetitive measurements on a large number of "bite-size" units of food. In this machine a set of small shearing blades or a circular punch is caused to shear or puncture a small unit of food and the force required to puncture each unit is recorded on a small strip-chart recorder. The machine is designed for automatically feeding units that are approximately spherical in shape, such as peas and peanuts. It can be supplied with an automatic digital readout which counts the number of units in each of five force ranges. Proctor et al. (1956) measured the maximum force to

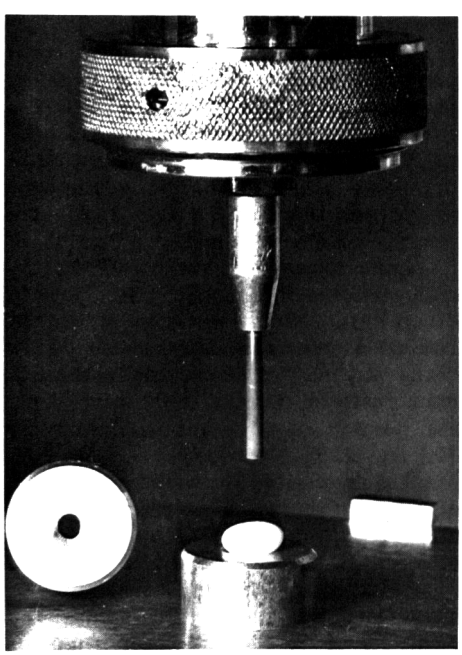

Fig. 1—Photograph of cooked bean set in aluminum block in readiness for puncture test. Spare aluminum block at side shows countersink hole that centers the bean.

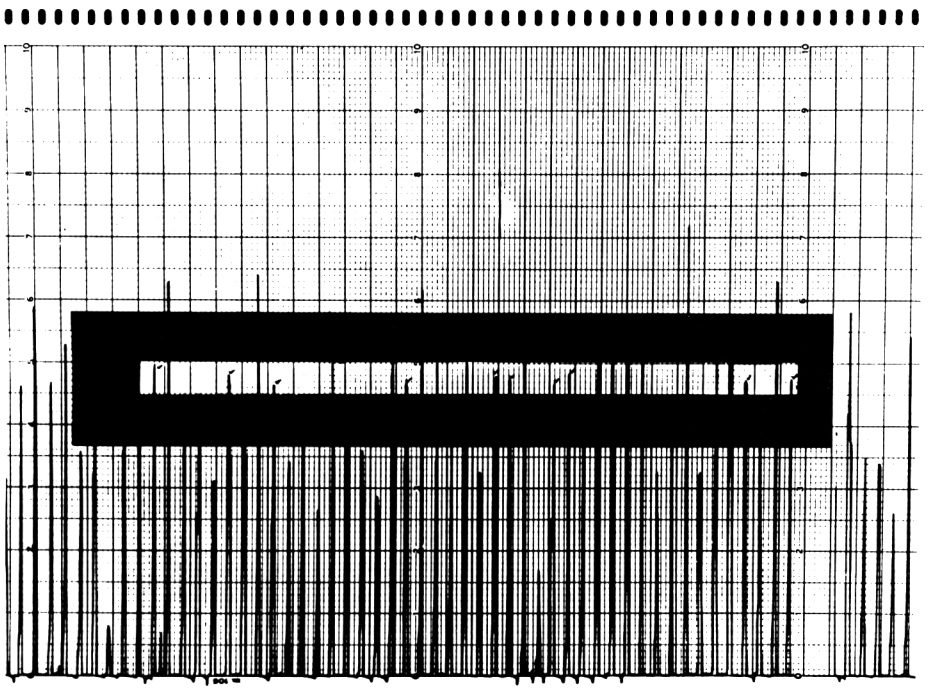

Fig. 2—Photograph of portion of Instron chart showing force peaks from puncture tests on single beans. The mask focuses attention on a 50-g range of force. Note the check marks on the force peaks inside of masked area. Full scale load = 1,000g.

crush single peas in the M.I.T. Denture Tenderometer and found that approximately 1 hr was required to test 72 peas.

The Instron Universal Testing Machine has been adapted for use in measuring various textural aspects of foods (Bourne et al. 1966). This paper describes how it was used for simple, rapid routine puncture measurements on cooked dry beans. The procedure is not as automatic as the Pabst machine, because it requires the full-time attention of one operator. On the other hand, the 10-in. wide chart of the Instron enables data to be recorded more accurately than the 2-in. wide Pabst chart.

EXPERIMENTAL

A CIRCULAR, flat faced steel punch 1/8 in. diam is attached to the inverted load cell of the Instron. Centered directly beneath it is a small aluminum block that has a 7/32 in. diam hole drilled through it. A countersink on the upper side helps to center a cooked bean and hold it in place (see Fig. 1). The B load cell set to 1000g full scale load is used. Crosshead speed is 30 cm/min and chart speed is 5 cm/min. In this test the only parameter needed is peak force. Running the chart at a slower speed than the crosshead speed reduces the quantity of chart paper used and enables the data to be picked off more quickly because the peak heights are closer together. The extension cycle controls on the Instron are adjusted until the punch moves between a lower point just below the countersink and an upper point about 1–2 cm above the upper surface of the aluminum block. The lower point is set by manually operating the Instron until the punch face is in the desired lower position and adjusting the lower extension cycling control to zero. The desired upper position is then manually set and the upper extension cycling control is adjusted to zero. The machine is then switched to EXTENSION CYCLE. When the Instron is started the punch automatically moves in a reciprocating stroke between the two pre-set distance limits and the operator is not required to touch any of the controls until he wishes to stop the machine.

When the machine is running, the operator simply places one bean in the countersink hole. When it has been punched, he removes this bean and replaces it with another bean before the punch descends again. A small brush or a piece of wire with a small hook in the end often facilitates removal of the punctured bean. Once the machine has been set up, one operator can puncture 10 beans per minute. If the operator finds he has insufficient time to remove the punctured bean and position the next bean in place before the punch descends again he has three ways in which to give himself more time: (a) lengthen the stroke by adjusting the upper distance setting on the Instron cycle control; (b) run the crosshead at a slower speed; or (c) puncture a bean on every second stroke.

Pea beans, marrow beans and soybeans were size-graded on a set of bean sieves in manner described previously (Bourne, 1967) and one sieve size only was used for each test. The beans were soaked overnight in tap water at ambient temperature, then cooked in live steam for 30 min at 250°F in a pressure retort. When cool, 500 intact beans from each sample were punctured by the technique described above.

In the study on the rate of softening of pea beans, size-graded beans were soaked overnight, then cooked for various times by immersion in boiling water, followed by immediate cooling in cold water. 120 intact beans from each cooking time were punctured.

RESULTS & DISCUSSION

FIGURE 2 is a photograph of a portion of the chart obtained in one of these tests. The usual method to obtain the required data from the chart is to read off the peak heights from each curve in units of force. However, since the purpose of this type of experiment is usually to find the number of peaks in each force range, there is a more rapid way of extracting the data from the chart with very little loss in accuracy. The force scale of 100 units is divided into 20 sections, each 5 force units wide. (The number of divisions can be more or less if desired but we find 20 sections gives sufficient accuracy and high read-off speed.) Two long rulers are laid along the chart to expose only the first section of 5 force units. The number of peaks in this section is counted off without recording the actual force of each curve. The two rulers are then moved up to expose only the second section of 5 force units and the number of peaks in this section is counted. This process is repeated until the entire force range has been covered. A small checkmark is made near each peak as it is counted. This prevents counting a peak twice and also helps to locate any peaks that have been missed. The technique is simple, and it allows the data from many beans to be read off quickly.

Figure 3 shows the number of beans in each puncture force range. Every peak in excess of 1,000g has been shown as 1000g on this graph. The number of pea beans and marrow beans reaches a maximum between 300 and 400g force, while the number of soybeans peak between 200 and 250g force. The curve for soybeans approximates the shape of a normal distribution curve. The pea beans and marrow beans also approximate a normal distribution curve, except that there is a small long tailing off of a small number of beans into the higher force range. The presence of this small proportion of harder beans would not be detected in any test such as the Tenderometer, Shear Press, or extrusion cells which measures the average textural quality of a large

Table 1–Puncture test on individual cooked pea beans

Cook time (min)	Puncture force Highest (g)	Lowest (g)	Mean (g)	Ratio: highest-lowest / mean
30	1525	280	523	2.38
60	503	68	201	2.16
90	372	70	165	1.84
120	262	39	139	1.61
150	236	32	123	1.66
180	301	52	129	1.94
240	200	35	96	1.71
300	207	30	89	1.86

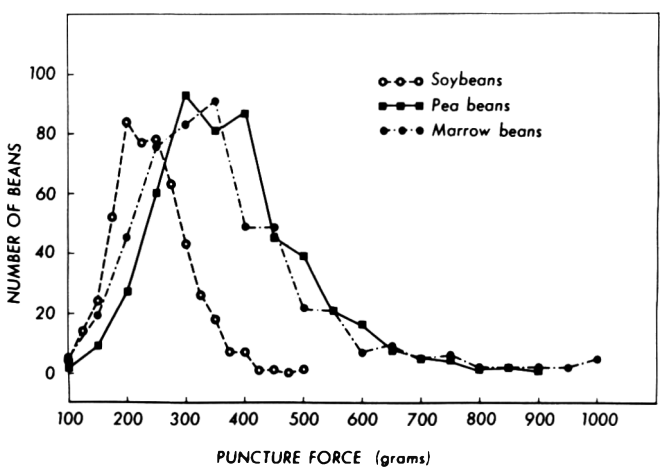

Fig. 3–Number of beans in each puncture force range using 1/8 in. diam punch: 50g force range increments for pea beans and marrow beans; 25g force range increments for soybeans.

number of beans in a single test; however, these few hard beans are easily detected in the mouth because only 2 or 3 beans are chewed between the teeth at any one instant.

Table 1 shows the range of puncture force and the calculated mean puncture force for pea beans cooked in boiling water for various times. The mean value is calculated as a weighted average from the number of beans and mid-point force in each force range. The mean force values show the average softening of the beans as cooking time is prolonged. The force range shows that, on an absolute basis, the difference in puncture force between the firmest and softest beans narrows as cooking continues. After 30 min of cooking there is a difference of 1245g of force between the softest and firmest bean; after 60 min the span is reduced to 435g force; and after 240 min it is 165g. However, on a proportional basis there is little narrowing of the range between the firmest and softest bean after 90 min of cooking time. This is shown by the ratio of range to mean force which is fairly constant after 90 min cooking time (Table 1).

We have used the same basic procedure for rapid puncture testing of a number of commodities including strawberries (Ourecky et al., 1968), raspberries, grapes, green peas, roasted dry soybeans, cherries, peanuts and sliced vegetables. Foods that have a larger diameter than pea beans require a longer stroke length which results in a testing rate lower than ten units per minute. When nonspherical products are tested (e.g., sliced beets and carrots) it is preferable to use a flat plate without a countersink to support the unit of food. This technique is very useful in any studies in which the piece-to-piece variation of texture in a food is wanted without expending an excessive amount of time to obtain the data.

REFERENCES

Bourne, M.C. 1966. Measurement of shear and compression components of puncture tests. J. Food Sci. 31: 282.

Bourne, M.C., Moyer, J.C. and Hand, D.B. 1966. Measurement of food texture by a Universal Testing Machine. Food Technol. 20: 170.

Bourne, M.C. 1967. Size, density and hardshell in dry beans. Food Technol. 21: 335.

Burr, H.K., Kon, S. and Morris, H.J. 1968. Cooking rates of dry beans as influenced by moisture content and temperature and time of storage. Food Technol. 22: 336.

Morris, H.J. 1963. Cooking quality of dry beans. Proc. 6th Annual Dry Bean Conf., p. 11. WURRD, USDA Albany, Calif.

Morris, H.J. 1964. Changes in cooking qualities of raw beans as influenced by moisture content and storage time. Proc. 7th Annual Dry Bean Conf., p. 37. WURRD, USDA Albany, Calif.

Ourecky, D.K. and Bourne, M.C. 1968. Measurement of strawberry texture with an Instron machine. Proc. Am. Soc. Hort. Sci. 93: 317.

Proctor, B.E., Davison, S. and Brody, A.L. 1956. A recording strain gage denture tenderometer for food. 3. Correlation with subjective tests and the Canco Tenderometer. Food Technol. 10: 344.

Voisey, P.W. and Larmond, E. 1971. Texture of baked beans—A comparison of several methods of measurement. J. Texture Studies 2: 96.

Ms received 4/8/72; revised 6/3/72; accepted 6/5/72.

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RATE LIMITING MECHANISMS IN CAUSTIC POTATO PEELING

INTRODUCTION

CAUSTIC PEELING of fruits and vegetables has been employed in commercial food processing for many years. However, like many industrial processes, relatively little is known concerning the fundamental mechanisms which govern the rate at which peeling takes place. While it is always better to be aware of fundamentals, it is not always necessary in order to develop a commercially feasible process. Such is the case with caustic peeling operations.

Added incentive for fundamental research into this area has been recently provided by the necessity of solving water pollution problems (Mercer et al., 1971). This has been a particularly vexing problem for the potato processing industry in Idaho where a significantly large portion of the pollution load can be attributed directly to the peeling operation. Forced to reevaluate the caustic peeling process, Graham et al. (1969) reported on the development of the so-called "dry peel" process which has resulted in significant reductions in the pollution load. Nevertheless there still does not seem to be any insight into the mechanisms which govern the peeling rates. Thus this work was initiated with two end goals in mind: (1) to determine the rate limiting mechanisms in caustic potato peeling; and (2) to demonstrate the feasibility of utilizing standard mathematical modeling techniques to commercial food processing operations. It was hoped that the achievement of the first goal might suggest changes in the "wet" peeling process which would be of value to those processors who cannot presently afford to install the dry peel process.

As might be expected, most of the research connected with caustic potato peeling was conducted prior to 1960 and is summarized by Talburt and Smith (1962). Basically the wet process consists in immersing the potatoes in a hot (180–220°F) concentrated (15–25 wt %) solution of sodium hydroxide (NaOH). The peel is apparently loosened as a result of starch gelatinization with the depth of the loosened peel determined by the residence time of the potatoes within the caustic bath. Typical residence times range from 3–8 min (Harrington et al., 1956). Early workers such as Olsen (1941) and Lankler and Morgan (1944) claimed that wetting agents improved the peeling rate but to our knowledge they are not used in any commercial process. Most of the research results are reported in the form of "peel loss" as a function of caustic concentration, temperature and time. Peel loss is of course the most important variable for a processor but its subjective nature is less than desirable from a research point of view since it does not provide information on the mechanisms involved.

The success of the dry peel process does provide some clues to the rate governing mechanisms however. In this process the potatoes are dipped in a less concentrated solution for a short time, held at room temperature for 3–5 min and then heated with infrared radiation for 2–3 min at 1600°F. The result is that the peeling is confined to the outer regions of the potato, resulting in less peel loss and in a peel which is much easier to remove mechanically. The sensitivity of the process to temperature suggests a rate due to chemical reaction while the necessity for "holding" the potato for 3–5 min prior to heating suggests a penetration, or mass transfer, problem. The development of the equations of the next section are based on a model which considers simultaneous mass transfer and chemical reaction. The model is then tested by experiments to determine which rate process is governing.

Analysis

We restrict our analysis to a single potato immersed in a caustic solution at constant temperature. Since typical peel times are usually less than 8 min only the outer region of the potato is affected and the geometry can be taken, to a first approximation, as a slab. We shall also assume that the process can be described by a shrinking core model (Denbigh and Turner, 1971) as shown in Figure 1. In this model the chemical reaction is assumed to take place *only* at the outer edge of an unreacted core and the rate is determined by the rate of the reaction itself and/or the rate at which caustic can be delivered to the unreacted core by diffusional mechanisms. At this point we should point out that we are assuming that gelatinization is a chemical reaction between the caustic (hydroxyl ion) and the starch. The correct mechanism of the gelatinization is probably a combination of physicochemical phenomena (Whistler and Paschall, 1965) but such microscopic details are not necessary for our analysis.

It is well known that the hydroxyl ion promotes gelatinization (Whistler and Paschall, 1965) and thus we would expect caustic to be consumed as the process continues. While this is no doubt true, experiments conducted by McFarland (1971) failed to show a measureable change in caustic concentration even under the most severe conditions. As a result, experimental determination of peeling rates could not be based on changes in caustic concentration since it was imperceptible. However, it is also well known that during caustic potato peeling the solution (and the potato) turns a yellow color. Although Talburt and Smith (1962) claim that this is probably due to the reaction between the caustic and flavinoids, we are of the opinion that it is actually due to a reaction between the caustic and chlorogenic acid. We base this conclusion on the fact that when caustic and pure chlorogenic acid are brought

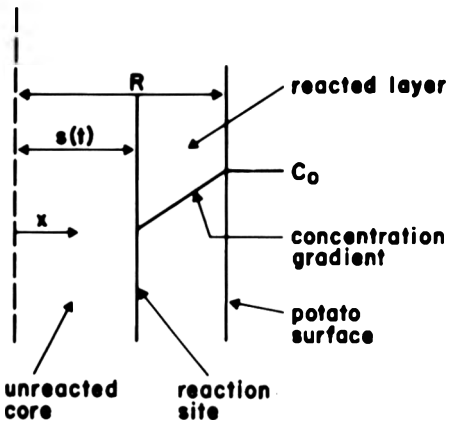

Fig. 1—Sketch of shrinking core model.

together in the same approximate concentrations as exist in the peeling process, the solution acquires a yellow color. In addition, it is known that chlorogenic acid is more heavily concentrated in the outer skin (Talburt and Smith, 1962) which is consistent with our own experiments which will be discussed later in the paper.

In view of this discussion the pertinent reactions can be written in simplistic form as

$$\text{[CAUSTIC]} + \text{[STARCH]} \rightarrow \text{[PRODUCTS (GELATINIZATION)]}$$

$$C + a\,St \rightarrow bP \qquad (1)$$

$$\text{[CAUSTIC]} + \begin{bmatrix}\text{CHLOROGENIC} \\ \text{ACID}\end{bmatrix} \rightarrow \text{[SALT (yellow)]} + [H_2O]$$

$$C + m\,A \rightarrow n\,Y + \ldots \qquad (2)$$

Since we were unable to monitor concentration changes in reaction (1), we based our experimental work on reaction (2) where the progress of the yellow product could be followed by colorimetry. We assume that the rate of reaction (2) is first order with respect to caustic and that it is given by

$$r = k'' C_A^m C_c \qquad (3)$$

where k'' is a reaction rate constant based on surface area (Bird et al., 1960).

With the kinetics in mind we are now in a position to analyze the rate processes in the context of the shrinking core model. As is typical in shrinking core analyses we shall assume quasi-steady state conditions which merely states that there is no accumulation of mass in the reacted layer of Figure 1. A differential mass balance on the caustic gives,

$$\frac{\partial}{\partial x}\left(D\frac{\partial C_c}{\partial x}\right) = 0 \qquad (4)$$

where D is an effective diffusivity of the reacted potato. The boundary conditions applying to (4) are the chemical reaction rate at x = s and constant caustic concentration at x = R. That is

$$\text{at } x = s : D\frac{\partial C_c}{\partial x} = k'' C_A^m C_c \qquad (5)$$

$$\text{at } x = R : C_c = C_o \qquad (6)$$

The solution for the flux of caustic through the reacted layer can be obtained from equations (4)–(6) as

$$N_c = \frac{C_o}{\frac{(R-s)}{D} - \frac{1}{k'' C_A^m}} \qquad (7)$$

For experimental purposes we are interested in the concentration of the yellow product of reaction (2) in the bath surrounding the potato. From the assumed stoichiometry the flux of the yellow product leaving the potato is

$$N_Y = -n N_c \qquad (8)$$

and the time rate of change of the concentration of the product in the *bath* is given by

$$V\frac{dC_Y^B}{dt} = N_Y A \qquad (9)$$

with V the volume of the bath, and A the surface area of the potato. Substituting (7) and (8) into (9) and integrating

$$C_Y^B = \frac{n C_o k'' C_A^m A}{V}\int_0^t \frac{dt}{1 - \beta(s - R)} \qquad (10)$$

In equation (10) s is a function of time and $\beta = k'' C_A^m/D$.

To complete the integration of (10) we must have an expression for s as a function of time. Assuming that the chlorogenic acid is evenly distributed throughout the outer portion of the potato, a mass balance gives

$$C_A\frac{ds}{dt} = -\frac{m}{n}N_Y = \frac{m C_o k'' C_A^m}{1 - \beta(s - R)} \qquad (11)$$

which, upon integration, yields

$$1 - \beta(s - R) = (1 + \alpha t)^{1/2} \qquad (12)$$

where $\alpha = m(k'')^2 C_A^{2m-1} C_o/D$.

Substituting (12) into (10) and completing the integration we arrive at an expression for the concentration of the yellow salt product in the bath as a function of time.

$$C_Y^B = \frac{2 n C_o k'' C_A^m}{\alpha}\frac{A}{V}\left[(1 + \alpha t)^{1/2} - 1\right] \qquad (13)$$

Equivalent reaction and mass transfer rates. The result in equation (13) was arrived at by giving equal weight to chemical reaction and mass transfer rates. Thus if C_Y^B V/A C_o were plotted versus $(1 + \alpha t)^{1/2}$ the result should be a straight line of slope 2 n k'' C_A^m/α if both rate processes were equally important. However since we have no way of predetermining α, this is not possible. Instead, if (13) is differentiated with respect to time and evaluated at time zero we obtain

$$\frac{V}{AC_o}\left(\frac{dC_Y^B}{dt}\right)_{t=0} = n k'' C_A^m \qquad (14)$$

Thus the initial slope should be a constant at a given temperature and would probably be expected to vary exponentially with temperature since it is proportional to the reaction rate constant (Denbigh and Turner, 1972).

Mass transfer limited. If we assume that the reaction rate is much larger than the mass transfer (diffusion) rate, then in the limit $k'' \rightarrow \infty$ and the concentration of caustic at the reaction site, s, would be zero. Returning to (13) we see that α is proportional to k''^2, or

$$\alpha = \phi(k'')^2 \qquad (15)$$

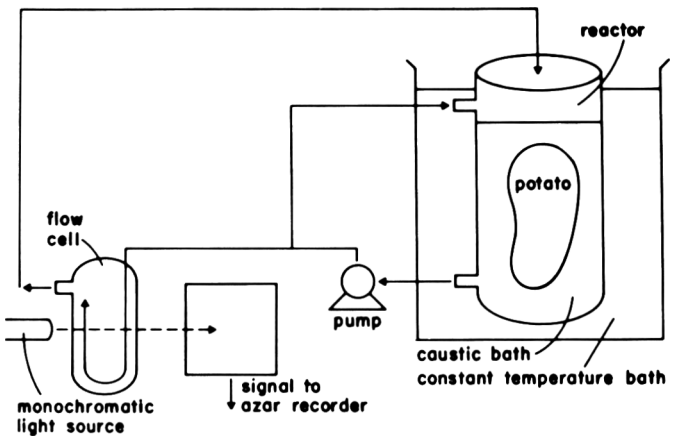

Fig. 2–Schematic of experimental apparatus.

and (13) can be rewritten

$$\frac{V}{AC_o} C_Y^B = \frac{2 n C_A^m}{\phi} \left[\left(\frac{1}{k''^2} + \phi t \right)^{1/2} - \frac{1}{k''} \right] \quad (16)$$

As $k'' \rightarrow \infty$ then, equation (16) reduces to

$$\frac{VC_Y^B}{AC_o^{1/2}} = \left(\frac{4 n^2 D C_A}{m} \right)^{1/2} t^{1/2} \quad (17)$$

Consequently if the process is mass transfer limited, a plot of the left hand side of (17) versus $t^{1/2}$ should yield a straight line. Note also that the slope of this line should only be *weakly* dependent on temperature (i.e., only to the extent that D depends on temperature).

Reaction rate limited. If only the chemical reaction rate is important, then the shrinking core model is not necessary. In this case we can consider the reaction to take place on the outer surface of the potato and the time rate of change of C_Y^B is given by

$$V \frac{dC_Y^B}{dt} = (n k'' C_o C_A^m) A$$

Integration then yields

$$\frac{VC_Y^B}{AC_o} = n k'' C_A^m t \quad (18)$$

Equation (18) predicts that, for a reaction rate limited process, a plot of VC_Y^B/AC_o versus time should yield a straight line of slope $n k'' C_A^m$.

EXPERIMENTAL

AS ALREADY MENTIONED, our preliminary experiments were directed to the determination of changes in the caustic concentration as a function of time (by titration). However it was found that there were only minute changes in caustic concentration (McFarland, 1971) and thus we were forced to look for some other result of the process which could be measured more readily. The approach we finally chose was to monitor the color change of the bath as a function of time. Since the yellow product is probably not a direct product of the gelatinization process [see equations (1) and (2)], we are implicitly assuming that reaction (2) will have the same *time varying* characteristics as reaction (1).

The experimental equipment is shown schematically in Figure 2. The reactors were pyrex glass and dimensioned to minimize the volume of caustic required to totally immerse the potato. The reactor was maintained at constant temperature by a fluidized sand bed heater and the caustic was continually circulated through the reactor by a peristaltic pump. A tee connection in the pump line provided a continual flow of fresh solution to the spectrophotometer flow cell. The cell volume was approximately 10 cc and the estimated lag time of the colorimeter measurements was 5 sec. Colorimeter measurements were made by a Beckman Spectronic 20 at a wavelength of 350μ and recorded as a continuous function of time on an Azar recorder. Assuming a Beer's Law dependency (Marron and Prutton, 1958) of light transmission on concentration, the concentration of 'Y' in the bath can be calculated from

$$C_Y^B = -K \ln(I/I_o) \quad (19)$$

where K is the absorbancy index multiplied by the path length (a constant for a given temperature, species and path length).

The potatoes used in the experiments were Idaho Russetts supplied by the Aberdeen Experimental Station. A wide variety of sizes and shapes were studied with post harvest times ranging from 1–6 months. Prior to peeling they were stored at 45°F. Although NaOH is used commercially, all our experiments were conducted with reagent grade potassium hydroxide (KOH). KOH was chosen in order to complement another project being conducted in the Food Science Dept. at the University of Idaho (Shen, 1972). A few of the experimental runs were duplicated using NaOH and it was concluded that the results were essentially equivalent to those obtained with KOH. This is also consistent with Shen's (1972) conclusions.

Experimental conditions ranged in temperature from 105–170°F and in caustic concentrations (by weight) from 5–25%. Measurements included the volume of caustic employed, the surface area of the potato and the weight of the potato before and after immersion. Surface areas were determined by a method developed by McFarland (1971).

RESULTS

IN VIEW OF the analytical and experimental approaches described above it is immediately apparent that a quantitative verification of equations (14), (17) or (18) would be near impossible. Considering equation (18) for example with C_Y^B given by equation (19), we do not have values for n, k'', C_A, m or K. Moreover some of these parameters will vary from potato to potato. The same is true of the parameters in (14) and (17). Consequently our analyses were restricted to determining how C_Y^B varied with time. That is, to see if the appropriate straight lines developed when either mass transfer or reaction rate limitations was assumed. The slopes of the resulting lines would then be lumped parameters which included all of the unknown values.

Figure 3 shows the results of assuming that the process is reaction rate limited. According to equation (18), the ordinate should vary linearly with time at a given temperature. As can be seen from the plot, a straight line does develop between times of 1 and 6 min. What is more, the slope of the line is steeper as temperature increases which would also be expected since reaction rate constants are strongly dependent upon temperature. At times greater than 6 min the caustic must penetrate deeper into the potato and diffusion becomes a problem, causing a departure from the linear lines of Figure 3.

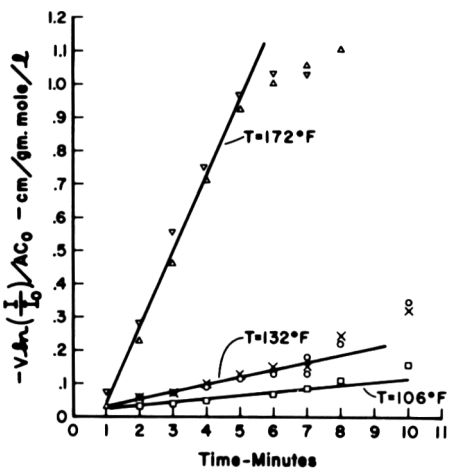

Fig. 3–Test of reaction rate controlled assumption.

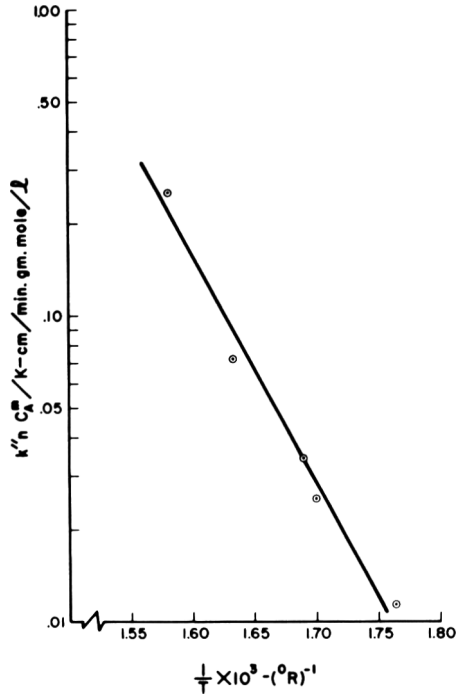

Fig. 4–Verification of Arrhenius' Reaction Rate Law.

Of more interest however is the fact that none of the straight lines go through the origin, indicating that something other than the chemical reaction is affecting the rate. Since during the first minute or two the caustic is primarily in contact with the outer skin of the potato, we can conclude that the composition of the outer skin presents a very different environment to the caustic than does the potato beneath the skin. This could possibly explain the previously reported success of Olsen (1941) and Lankler and Morgan (1944) in utilizing wetting agents to increase peeling rates.

Since it appears that the process is reaction rate controlled over the major portion of the processing time, the data were also tested to determine the dependence of the reaction rate constant on temperature. According to Arrhenius' Law (Denbigh and Turner, 1971) the rate constant should vary exponentially with temperature according to

$$k'' = k_o \exp - [E^*/RT] \qquad (20)$$

Referring to equations (18) and (19), the slopes of the straight lines in Figure 3 should equal n k'' C_A^m/K. Thus a plot of the slopes of Figure 3 versus 1/T on semi-log paper should yield a straight line of slope $-E^*/R$. Figure 4 shows the results of such an analysis and as can be seen an excellent straight line is obtained corresponding to an energy of activation, E*, equal to 34.6 Kcal/g mole.

The results of assuming the opposite extreme, mass transfer rate limited, are shown in Figures 5 and 6. All the data plotted in Figure 5 correspond to a temperature of approximately 150°F and reflect experiments conducted on potatoes from two different crops. According to equation (17) a straight line should develop when the ordinate is plotted versus $t^{1/2}$ if the process is mass transfer rate limited. Over the first 3 or 4 min this is certainly the case although the slopes are quite different for the two crops. From equation (17) the pertinent parameters which might be expected to vary from crop to crop are the concentration of chlorogenic acid and the diffusivity (the latter because it is an "effective" value which depends on the composition and porosity of the outer potato). However the results of Figure 5 would require the product of these two parameters to differ by a factor of ten between the two crops. Since little quantitative information is known concerning the concentration of chlorogenic acid in potatoes, it is difficult to judge whether this might explain the discrepancy. It should also be kept in mind that the concentration of acid in the potato is not uniform as we have assumed in the model and perhaps a different distribution within the potato also contributes to the problem.

One additional characteristic which should separate mass transfer problems from chemical reaction problems is their dependence on temperature. As we have already seen the temperature dependence of chemical reaction rates is exponential whereas it is only a weak influence on mass transfer rates. Figure 6 provides a dramatic illustration of these effects. All the data, corresponding to temperatures between 128–167°F, fall on the same straight line for the first 3 min of the process, indicating mass transfer limitations. After 3 min, however, the run with the highest temperature shows a rapidly increasing

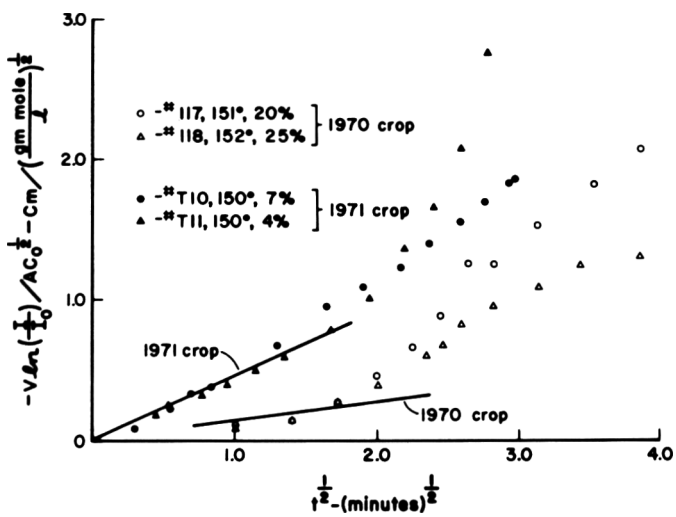

Fig. 5–Test of shrinking core–diffusion controlled assumption (T = 150° F).

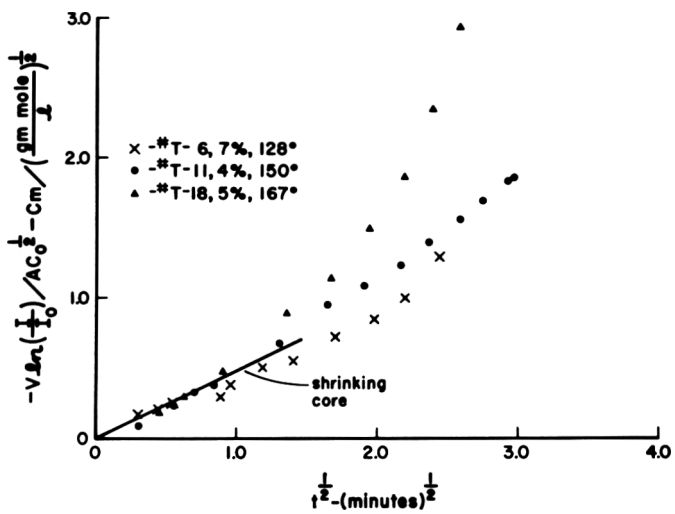

Fig. 6–Effect of temperature on the shrinking core–diffusion controlled assumption.

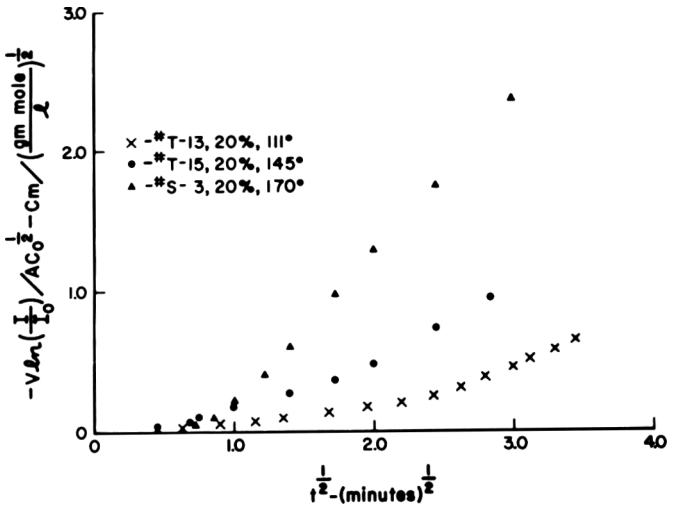

Fig. 7—Effect of temperature on the shrinking core—diffusion controlled assumption (pre-peeled potatoes).

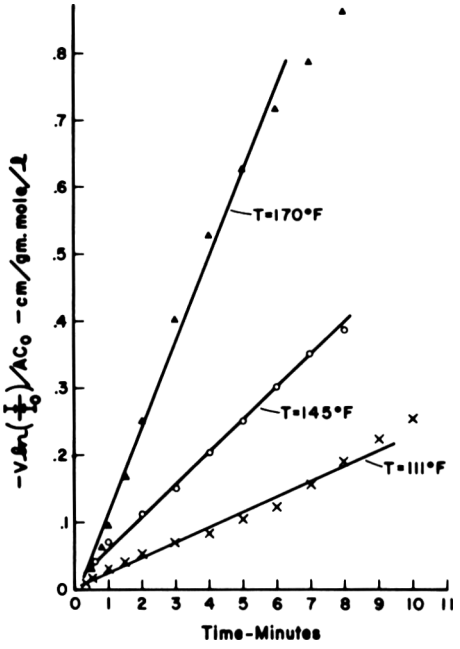

Fig. 8—Effect of temperature on the reaction rate controlled assumption (pre-peeled potatoes).

slope indicating a large rate of production of the product, Y. This is clearly representative of a chemical reaction rate.

At this point the results all tend to point to the same conclusion. The process is mass transfer limited over the first few minutes of contact with the caustic and subsequently becomes reaction rate controlled. Since the caustic is primarily in contact with the outer skin during this initial time period we decided to subject "prepeeled" potatoes to this same analysis. The experiments were run in the same manner as those described above except that prior to immersion, the outer skin was carefully scraped off. The results of these experiments are shown in Figures 7 and 8. The data are plotted according to the shrinking core mass transfer model in Figure 7 but there is no one straight line which would correlate the data over the first 2 or 3 minutes. However, when chemical reaction rate limitations are assumed, as in Figure 8, the data plot on almost perfect straight lines. Not only are the slopes dependent on temperature but they all come very close to going through the origin. Contrasting these results with Figure 3 where the slopes did not go through the origin, we can conclude that mass tranfer limitations are eliminated by prepeeling the potato.

DISCUSSION

IT IS WORTHWHILE at this point to consider the implications of these results and to reconsider the various assumptions and limitations that were built into the analysis. Dealing with the latter first, the key to our approach is the assumption that the reaction between caustic and chlorogenic acid is in some way representative of the gelatinization which loosens the potato peel. There is no way to prove this *à priori* and we know of no work where the subject has been considered. This is not surprising when one realizes that there are still a number of controversies concerned with the chemical and physical nature of starch granules. Whether chlorogenic acid is an active part of chemically induced gelatinization or merely a competitor in the various caustic reactions, cannot be answered at this time. Assuming the worse, it is still an excellent indicator of the ability of the caustic to penetrate the potato. This alone validates our approach and is probably very representative of the mass transfer problem if not qualitatively representative of the chemical reactions.

An additional question which always arises when dealing with a biological system is the repeatability of the data. A good example of this is the variations noted between the two crops in connection with the plots in Figure 5. Our approach circumvents many of these problems by combining as many variables as possible and looking at qualitative *changes* with time (i.e., linear or nonlinear). Variations from potato to potato within the same crop were accounted for by careful measurements of surface area—the most sensitive potato variable in our analysis. Many experiments were run for repeatability checks and most data were within a 10% spread which is about as good as can be expected.

The implications of these results may have far reaching consequences. We are of the opinion that the success of the "dry peel" process can be explained by our findings. Namely, caustic peeling is a mass transfer limited process until the outer peel has been penetrated. At this point it becomes a chemical reaction rate controlled process. Thus, in the dry peel process the original "dip" and holding time correspond to the mass transfer process. Once the outer skin is penetrated, the process becomes very sensitive to temperature and thus infrared heating is of great benefit at this stage of the process. Of additional significance is the fact that the diffusion of the caustic into the potato is fairly insensitive to temperature and thus one wonders about the advantage of heating the caustic solution. One possibility is that there is a wetting problem which is aided by higher temperatures. If so, it might be advantageous to use wetting agents in combination with low temperature caustic baths prior to the infrared heating operation. These and other questions which have been raised by this work should be tested by some careful pilot plant projects to determine whether additional savings can be garnered from the dry peel process or whether there is not a more economical way to wet peel.

NOTATION

A	surface area of potato cm^2
C_A	concentration of chlorogenic acid, g mole/l
C_c	concentration of caustic, g mole/l
C_o	concentration of caustic in bath, g mole/l

C_Y^B	concentration of yellow salt in bath, g mole/l
D	effective diffusivity of caustic in potato, cm^2/min
I/I_o	fraction of light transmitted
K	absorbancy index, l/g mole – cm
k''	surface reaction rate constant, $(g - mole)^{-m}/cm^2 - min$
m	stoichiometric coefficient, equation (2)
N_c	molar flux of caustic, g mole/cm^2 – min
N_Y	molar flux of yellow salt, g mole/cm^2 – min
n	stoichiometric coefficient, equation (2)
R	outer radius of potato, cm
r	reaction rate, g mole/cm^2 – min
s	radius of unreacted core, cm
T	temperature, °R
t	time, minutes
V	volume of caustic solution, cc
x	spatial coordinate, Figure 1
α	$m\,k''^2\,C_A^{2m-1}\,C_o/D$, $(g\ moles)^2/cm^4$
β	$k''\,C_A^m/D$, $1/cm^4$
ϕ	parameter defined by equation (15)

REFERENCES

Bird, R.B., Stewart, W.E. and Lightfoot, E.N. 1960. "Transport Phenomena." John Wiley & Sons, New York, N.Y.

Denbigh, K.G. and Turner, J.C.R. 1971. "Chemical Reactor Theory," 2nd ed. Cambridge University Press, London.

Graham, R.P., Huxsoll, C.C., Hart, M.R., Weaver, M.L. and Morgan, A.I. 1969. "Dry" caustic peeling of potatoes. Food Technol. 23: 61.

Harrington, W.O., Mayer, P.C., Olson, R.L., Mullins, W.R. and Potter, A.L. 1956. Observations on pre-peeling of potatoes. Food Technol. 10: 347.

Lankler, J.G. and Morgan, O.M. 1944. How wetting agents improve the chemical peeling process. Food Ind. 16: 888.

Marron, S.H. and Prutton, C.F. 1958. "Principles of Physical Chemistry," 3rd ed. MacMillan Co., New York, N.Y.

McFarland, D.M. 1971. Reaction rate studies of caustic potato peeling. Presented at the Northwest Regional Meeting of the student chapters of the A.I.Ch.E., Corvallis, Oregon.

Mercer, W.A., Ralls, J.W. and Rose, W.W. 1971. Food process modification to abate pollution. Presented at the 64th annual A.I.Ch.E. meeting, San Francisco, Calif.

Olsen, I.T. 1941. Wetting agents speed chemical peeling. Food Ind. 13(4): 51.

Shen, W.W. 1972. Substitution of potassium hydroxide for sodium hydroxide in lye potato peeling. M.S. thesis, University of Idaho, Moscow, Id.

Talburt, W.F. and Smith, O. 1962. "Potato Processing." Avi Publishing Co., Westport Conn.

Whistler, R.L. and Paschall, E.F. 1965. Fundamental Aspects. In "Starch: Chemistry and Technology," Vol 1. Academic Press, New York, N.Y.

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SURVEY OF FOOD INGREDIENT–DDT REACTIONS UNDER THERMAL PROCESSING CONDITIONS

INTRODUCTION

THE PERSISTENCE of DDT [1,1,1-trichloro-2,2-bis-(p-chlorophenyl)ethane] and its transformation products in the environment mean that much of the material applied for crop protection and the control of disease-transmitting insects still contaminates soil, air and water. Even with the restricted use of DDT and TDE [1,1-dichloro-2,2-bis-(p-chlorophenyl)ethane] (DDD) programmed for the future, all types of food and feed will contain measurable amounts of these compounds for many years to come. DDT was the first organic compound to be used on a large scale for insect control. In the twenty-seven years of its use, the total amount of DDT introduced into the environment has been estimated at 3.05 billion pounds (Woodwell et al., 1971).

The subject of the effect of processing on pesticide residues in foods has been reviewed by Liska and Stadelman (1969). The loss of DDT during thermal processing of food was first demonstrated by Tressler (1947). He reported essentially complete recovery of DDT in water or pH buffer on heating at 100°C for 4 hr in sealed glass tubes; the same conditions in tin cans gave a 20% reduction in DDT levels. When DDT was heated with tomato juice at 100°C for 20 min in tin cans, the DDT content was reduced 69%. A 25 min heating of DDT with peaches, applesauce and green beans gave reductions of 49, 58 and 25%, respectively. The most definitive study of the effect of thermal processing on DDT residues in food was that reported by Farrow and co-workers (1966). These workers established that TDE (DDD) was the major transformation product from DDT during thermal processing of spinach. A study of the relation of the material of construction of cooking vessels to the changes in DDT during heating has been reported by DeLoach and Hemphill (1971).

It is desirable to obtain more information in order to define which food components are responsible for the transformations of DDT to other compounds during thermal processing. This report describes the results of a preliminary investigation of the changes in aqueous solutions or suspensions of DDT during heating with vitamins, amino acids, peptides, casein, yeast nucleic acid and diphosphopyridine nucleotide.

EXPERIMENTAL

THE DDT STANDARD used was prepared from a sample of carbon-14 ring-labeled material purchased in 1963 (6 yr of storage in a crystalline state before use in the experiments summarized in this report). The purity of the radioactive DDT was found to be 95.3% p,p'-isomer by thin-layer chromatography. A mixture of 78.8 mg of the radioactive DDT (specific activity 8.475 μc/mg) with 620.8 mg of pure p,p'-DDT was dissolved in acetone and made up to a volume of 100.0 ml. The calculated radioactivity from the label statement on the storage vial was 6.67 μc/ml of solution; the measured radioactivity of 50 μliter portions of the standard solution was 6.46 μc/ml.

A 5-liter round bottom Pyrex flask, heated by a Glascol mantle, was used for the thermal reactions of this survey. The flask was partially filled with 2 liter of water or phosphate buffer and the food ingredient(s). Four porous stone boiling chips (Hengar granules) were used to promote smooth boiling. 2 ml of the acetone solution of radioactive DDT was added to the solution (or suspension) from a pipette in such a way that a finely divided suspension was formed. The weight of DDT added was 13.98 mg which corresponded to a concentration of 7.0 ppm. The suspension was heated in a uniform way for each experiment by setting a voltage regulator at 115/140 volts. The vapor from the boiling suspension was passed through a Kjeldahl trap to keep particulate material out of the distillate. The distillate was cooled in a long water-cooled condenser and the condensate collected in a graduated cylinder. The heating period was 2.0 hr during which time 1000 ± 50 ml of distillate was collected. The trap and condenser were rinsed with petroleum ether and the distillate extracted with four 150 ml portions of petroleum ether. The combined rinsings and extracts were dried over anhydrous sodium sulfate and concentrated to a volume of 10–20 ml by distillation through a fractionating column. Liquid scintillation counting of 1-ml portions of the distillate from the final stages of the concentration demonstrated that no radioactivity had been carried over to the distillate. The pot residue liquid was cooled, the pH taken, and extracted with three 150 ml portions of chloroform. The chloroform extract was dried and concentrated in the same way as described for the petroleum ether extracts.

Separations of the components in the DDT standard and in the extracts of the distillate and pot liquid were made using thin-layer chromatographv. A slurry was prepared by mixing 60g of aluminum oxide G, type E (E. Merck AG) with 95 ml of distilled water and 3g of lead-manganese activated calcium silicate phosphor (Sylvania Electric Products, Inc., Towanda, Pa.). The mixture was shaken manually in a 250 ml Erlenmeyer flask for 1.5 min. A Kensco applicator (Kensington Scientific Corp., Oakland, Calif.) with a 250μ gate was used to apply the slurry to six 8 × 8 × 0.25 in. glass plates. After air drying for approximately 4 hr, the alumina was activated by heating 16 hr at 130°C in an oven.

The following standards were applied in petroleum ether solution to the origin line of the thin-layer plates using glass capillary tubes: 1,1-dichloro-2,2-bis-(p-chlorophenyl)ethene (DDE) was synthesized from p,p'-DDT by the method of Grummett et al., 1945; the purity was found to be 99% by gas chromatography. 1-Chloro-2,2-bis-(p-chlorophenyl)ethene (DDDE) was synthesized from TDE under the same conditions used for the preparation of DDE and its purity was found to be 97% by gas chromatography. DDT was a ESA Pesticide Reference Standard purchased from City Chemical Corp., New York, N.Y., Code WB 22; no impurity could be detected in the DDT standard by gas chromatography or thin-layer chromatography. TDE was purchased from Aldrich Chemical Co., Milwaukee, Wis. (Catalog No. B 3953-3); it was 99% pure by gas chromatog-

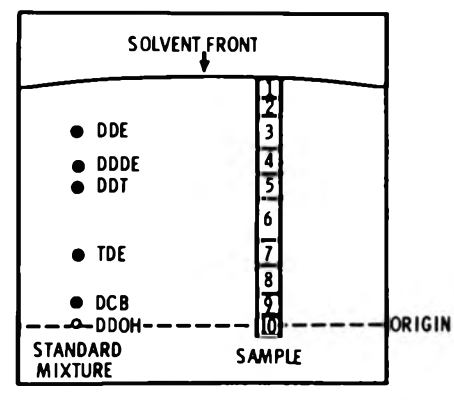

Fig. 1–Separation of mixture of standards on thin-layer chromatogram and plate sections used for counting of radioactivity

Table 1–R_f values for separation of DDT and related compounds by thin-layer chromatography

Compound symbol	R_f
DDE	.77
DDDE	.62
DDT	.54
TDE	.28
DCB	.10
DDOH	0.01

raphy. p,p'- Dichlorobenzophenone (DCB) was synthesized from DDE by the method of Grummett et al., 1952. After crystallization from ethanol, the DCB melted at 145–146°C and was 98% pure by thin-layer chromatography. 1,1,1-Trichloro-2,2-bis-(p-chlorophenyl)ethanol (DDOH) was a purified sample (WSZ 25:38 C) supplied by Rohm and Haas, Philadelphia, Pa. and the material was 99% pure by gas chromatography. The amount of standard applied was 5–25 μg depending on the intensity of quenching found for each standard compound. The concentrate of the extracts of the heated solutions (or suspensions) were applied with a 50 μl syringe in small increments so that the spot size did not exceed 5 mm in diameter. The plates were developed by immersing the bottom 0.5 in. of the plate nearest the origin line in n-heptane contained in a covered rectangular glass jar. After air drying, the plates were examined under ultraviolet light of 253 nm wavelength. The pesticides appeared as blue spots on a pink background. All of the compounds used as standards, except DDOH, showed strong quenching. A schematic representation of a typical thin-layer plate separation is shown in Figure 1. The R_f values measured for the separation of the compounds used as standards in this survey are tabulated in Table 1.

The region of the plate where the radioactive standard or the extracts of heated mixtures showed quenching were ruled off into 10 sections. A division of the region was made according to the position of standard compounds on each developed plate. In general, the plate sections corresponded to known compounds as follows: 3, DDE; 4, DDDE; 5, DDT; 7, TDE; 9, DCB; and 10, DDOH. The alumina represented by each plate fraction was picked up and transferred to a 20 ml counting vial by a vacuum device. The adsorbent mixture was suspended in 10 ml of toluene and 10 ml of a solution of 7.5g of 2,5-diphenyloxazole (PPO) and 0.15g of 1,4-bis-2-(5-phenyloxazoyl) benzene (POPOP) in 1 liter of toluene was added. The samples were counted in a Packard Tri-Carb Liquid Scintillation Spectrometer Model 3202 for 10 min. A channel ratio quench correction curve was used to determine counting efficiencies and the DPM were calculated and recorded.

RESULTS & DISCUSSION

THE RESULTS of analysis of the relative percentages of radioactivity in five thin-layer plate fractions from chromatography of the standard solution are tabulated in Table 2.

The precision for five sets of analyses of the radioactive DDT standard was good as shown by the data in Table 2. These results established a confidence level for the results of the analyses of extracts obtained from the single experiments of heating DDT with food component(s). The total radioactivity was not always accounted for in the regions corresponding to the five thin-layer chromatographic plate areas assigned to the DDT transformation products. Radioactivity found in other plate areas than those used for estimation of the five transformation products was due to traces of unidentified compounds. The composition of the

Table 2–Composition of standard DDT solution at two stages in project period

Period of analysis	DPM applied	Percent of radioactivity in region of				
		DDT	DDE	TDE	DCB	DDOH
Start of project	143,476	94.7	1.28	0.33	0.85	1.64
Start of project	81,124	95.5	1.28	0.36	1.13	1.50
Start of project	143,892	95.7	1.22	0.31	1.08	1.47
Finish of project	14,611	93.0	1.49	0.90	1.21	1.95
Finish of project	14,018	93.3	1.63	1.00	0.96	2.04
Average		94.4	1.38	0.58	1.05	1.72
Std Dev		0.62	0.086	0.18	0.071	0.14

Table 3–Thermal reactions of DDT at various pH values for 2 hr at 100°C

Food ingredient	pH		Concentration	Extract	Percentage of radioactivity in region of					
	Start	Finish			DDE	DDDE	DDT	TDE	DCB	DDOH
None	3.30	3.03	0.10 molar phosphate	Pet. ether	1.8	0.0	95.0	.62	1.6	.74
None	3.30	3.03	0.10 molar phosphate	$CHCl_3$	0.52	0.0	92.6	0.97	0.49	4.9
None	6.86	6.91	0.10 molar phosphate	Pet. ether	2.0	0.0	94.7	0.63	1.2	0.89
None	6.86	6.91	0.10 molar phosphate	$CHCl_3$	1.3	0.0	89.4	1.8	1.0	4.1
None	8.00	8.10	0.10 molar phosphate	Pet. ether	4.9	1.3	89.1	.35	2.6	0.70
None	8.00	8.10	0.10 molar phosphate	$CHCl_3$	3.1	0.90	93.2	0.66	0.40	0.77
None	8.00	8.10	0.10 molar phosphate	Pet. ether	4.8	1.3	89.3	0.35	2.5	0.60
None	7.02	7.52	Distilled H_2O	Pet. ether	2.2	0.0	93.4	1.8	1.2	1.0

Table 4–Thermal reactions of DDT with various food constituents for 2 hr at 100°C

Food ingredient	pH		Concentration ppm	Extract	Percentage of radioactivity in region of					
	Start	Finish			DDE	DDDE	DDT	TDE	DCB	DDOH
Yeast nucleic acid	7.10	7.12	50	Pet. ether	2.0	1.2	91.0	3.2	1.2	0.3
Yeast nucleic acid			50	$CHCl_3$	1.5	1.3	90.8	2.3	0.0	2.6
Diphospho-pyridine nucleotide	7.10	7.15	125	Pet. ether	2.9	2.0	89.9	0.95	2.6	1.2
Diphospho-pyridine nucleotide			125	$CHCl_3$	1.1	1.3	91.4	1.6	1.0	2.3
Casein	6.85	6.88	2500 (suspension)	Pet. ether	2.0	0.0	91.0	3.4	1.6	0.83

radioactive DDT standard solution changed with time during the 10 wk active experimental part of the survey. Presumably, this change was due to the effect of light, beta emission from the carbon-14 labeled compounds and wall catalysis during storage of the acetone solution in a glass volumetric flask exposed to interior room light. The loss of DDT in the standard solution during the storage period was 2.3%.

The results of the heating of radioactive DDT in distilled water or phosphate buffer solutions of various pH are tabulated in Table 3.

The experiments using buffer solutions or food ingredients were usually single determinations and, therefore, no statistical analysis of results is possible. The values reported are for extracts of the collected distillate or the pot residue. An independent check of the results of the distillate extract analysis was frequently carried out by analyzing the chloroform extract of the pot residue. Due to differences in steam volatility among the various DDT analogs and transformation products, the results of the analysis of the distillate extract and the pot residue extract were different. The pattern of results for the petroleum ether and the chloroform extracts were quite consistent. In the two cases where the thermal reaction part of the experiment was repeated (Table 3, pH 8 lines; Table 5, arginine lines) the agreement of results was good for the four most abundant components of the mixture.

It should be stressed that the identity of the compound in each thin-layer plate section was assigned by R_f values alone. The assignment of DDE, DDT and TDE was made with considerable confidence as these compounds are ubiquitous to most environmental samples of DDT and have been detected and confirmed by gas chromatography in our Laboratory many times. The other regions of the thin-layer plates contain compounds which can only be assigned a description of "chromatographic polarity similar to DCB and DDOH, respectively." The very small quantities of material represented in the plate regions for compounds other than DDT made it impractical to obtain more positive identification by infrared or mass spectrometry.

From the data in Table 3, it can be concluded that in the pH range of 3–8 (in which almost all foods fall), pH alone is not a major factor in DDT transformations during thermal conversions.

The effect of heating DDT with three types of food components is summarized in Table 4.

The results of the survey of the reactions of DDT with four amino acids and two peptides are tabulated in Table 5. The most remarkable result of this survey is the higher level of conversion of DDT to TDE shown by cysteine and glutathione compared with the other food ingredients examined. It appears that the sulfhydryl hydrogens can participate in the hydrogenolysis reaction involved in

Table 5–Thermal reactions of DDT with amino acids and peptides for 2 hr at 100°C

Food ingredient	pH		Concentration		Percentage of radioactivity in region of					
	Start	Finish	ppm	Extract	DDE	DDDE	DDT	TDE	DCB	DDOH
Arginine	7.10	NR[a]	100	Pet. ether	3.1	0.5	93.8	0.70	1.3	0.40
Arginine	7.10	NR	100	$CHCl_3$	2.9	0.8	93.5	0.98	0.67	2.5
Arginine	7.10	NR	100	Pet. ether	2.7	0.0	94.6	0.95	0.0	0.73
Arginine	7.10	NR	100	$CHCl_3$	2.6	0.50	93.1	0.92	1.3	1.2
Lysine	7.10	NR	100	Pet. ether	2.2	0.52	92.1	1.1	0.85	2.7
Lysine	7.10	NR	100	$CHCl_3$	2.1	0.53	92.6	1.1	0.84	2.7
Histidine	7.10	7.12	100	Pet. ether	1.5	0.0	91.5	3.9	0.86	1.1
Cysteine HCl	7.10	6.42	100	Pet. ether	1.7	0.0	84.6	9.6	1.3	0.84
Glutathione	7.10	6.88	100	Pet. ether	1.3	0.52	88.6	2.7	3.1	2.4
Aspartylphenyl-alanine methyl ester	7.10	6.84	100	Pet. ether	2.0	1.1	92.8	0.79	0.84	1.8

[a]Not recorded

Table 6–Thermal reactions of DDT with several water soluble vitamins for 2 hr at 100°C

Food ingredient	pH		Concentration		Percentage of radioactivity in region of					
	Start	Finish	ppm	Extract	DDE	DDDE	DDT	TDE	DCB	DDOH
Thiamine HCl	7.10	6.85	5	Pet. ether	2.1	1.3	92.6	0.90	1.7	0.0
Thiamine HCl	7.10	6.85	5	$CHCl_3$	1.3	1.5	90.4	1.5	0.91	2.0
Folic acid	7.10	6.93	25	Pet. ether	2.1	0.9	91.2	1.5	2.0	0.30
Folic acid	7.10	6.93	25	$CHCl_3$	2.2	2.1	92.9	1.1	0.0	1.9
Riboflavin	7.10	7.15	10	Pet. ether	3.4	1.0	89.2	1.7	1.9	0.0
Riboflavin	7.10	7.15	10	$CHCl_3$	2.3	0.0	81.0	2.0	3.6	5.9
Choline chloride	7.10	7.13	150	Pet. ether	1.8	1.4	90.4	1.1	0.86	3.5
Niacin	7.10	7.11	30	Pet. ether	1.5	0.90	91.3	1.7	1.7	2.0
Niacin	7.10	7.11	30	$CHCl_3$	0.76	0.0	91.9	2.0	0.69	3.4
Ascorbic acid	6.87	6.83	200	Pet. ether	2.1	0.36	92.5	0.88	1.3	1.3
Ascorbic acid			200							
Ferrous sulfate	6.45	6.88	40	Pet. ether	1.6	0.73	93.3	0.32	0.62	0.63
DiNa EDTA			20							
Hydrogen peroxide			10g/liter							
Same 4 components mixture as above			Same as above	$CHCl_3$	1.3	0.0	92.4	2.4	1.7	2.2

Table 7–Thermal reaction of DDT with a nine component mixture for 2 hr at 100°C

Food ingredient	pH		Concentration		Percentage of radioactivity in region of					
	Start	Finish	ppm	Extract	DDE	DDDE	DDT	TDE	DCB	DDOH
Riboflavin			10							
Thiamine HCl			5							
Folic acid			25							
Yeast nucleic acid			50	Pet. ether	1.8	1.3	89.1	3.6	1.0	1.8
Cysteine HCl	6.43	6.34	100	$CHCl_3$	1.3	1.0	76.0	5.9	1.4	5.3
Niacin			30							
Choline chloride			150							
Ascorbic acid			200							
Glutathione			100							

the conversion of DDT to TDE. Histidine also appeared to have some activity in this conversion. A combination of these individual reactions of amino acids residues may be the basis for a possible role for proteins in DDT transformations during thermal processing of foods.

The changes in DDT by heating with water soluble vitamins as summarized in Table 6 were small with the possible exception of riboflavin. It is interesting that the ene-diol hydrogens of ascorbic acid do not appear to be very effective in the DDT to TDE tranformation when compared with the results found with cysteine.

The results of the reaction of a mixture of nine components with the radioactive DDT at 100°C for 2 hr are tabulated in Table 7. The heating of DDT with nine components did not result in a completely additive decrease in DDT content. The DDT level was reduced by 20% and not by 40% as might have been predicted by a summation of the conversions caused by the individual compounds. This result is due to two factors: first, all nine compounds are competing for the same quantity of DDT and, second, possible interaction between various pairs of the nine components may have changed their state of reactivity toward DDT.

In a commercial situation when a canned food is heated at 120°C (248°F) the rate of reaction for each individual ingredient-DDT reaction would be increased approximately 4 times over the rate at 100°C used in this survey (Getman and Daniels, 1945). In a complex food mixture, there would be many individual ingredient-DDT reactions taking place simultaneously. Even if each individual reaction reduced the DDT content by only 1–5%, the cumulative result would be almost complete conversion of DDT to other compounds.

Further work to provide a better understanding of the significance of the conversion of DDT to other compounds during food processing should be devoted to a more precise identification of each compound formed and to determining the toxicology of the transformation products.

REFERENCES

DeLoach, H.K. and Hemphill, D.D. 1971. Effect of cooking utensil composition and contents on the reductive dechlorination of DDT to DDD. J. Assoc. Off. Agric. Chem. 54: 1352.

Farrow, R.P., Elkins, E.R. and Cook, R.W. 1966. Conversion of DDT to TDE in canned spinach. J. Agric. Food Chem. 14: 430.

Getman, F.H. and Daniels, F. 1945. "Outlines of Physical Chemistry," p. 363. John Wiley and Sons, Inc., New York, N.Y.

Grummett, O., Buck, A.C. and Jenkins, A. 1945. 1,1-Di(p-chlorophenyl)-1,2,2,2-tetrachloroethane. J. Amer. Chem. Soc. 67: 155.

Grummett, O., Marsh, D. and Stearns, J.A. 1952. Analysis and properties of bis-(p-chlorophenyl)-methylcarbinol. Anal. Chem. 24: 702.

Liska, B.J. and Stadelman, W.J. 1969. Effects of processing on pesticides in foods. Residue Rev. 29: 61.

Tressler, C.J. Jr. 1947. The recovery of DDT from canned foods and its stability during processing. J. Assoc. Off. Agric. Chem. 30: 140.

Woodwell, G.M., Craig, P.P. and Johnson, H.A. 1971. DDT in the biosphere: Where does it go. Science 174: 1101.

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EVALUATION OF TRITICALE FOR THE MANUFACTURE OF NOODLES

INTRODUCTION

PASTA PRODUCTS such as macaroni, spaghetti and noodles are very popular in Europe and the Western Hemisphere. In the Eastern world they are relatively rare, but again can be found in various forms of noodles in Southeast Asia, China and Japan (Irvine, 1971).

Durum wheat is the preferred raw material since the wheat is very hard and can be processed into semolina. It also requires little water compared with other wheats to form a dough. The lower the absorption the less water needs to be removed from the pasta products during the drying operation (Irvine, 1971).

Wheats other than durum are used in many parts of the world to produce pasta products. In Asia and the Far East, noodles are produced from wheat flour rather than semolina. Japan uses domestic soft wheat varieties. In Italy, everything from the best durum to the softest common wheat has been used.

Besides semolina or flour, a number of other food ingredients are sometimes added to pasta products (Irvine, 1971; Paulsen, 1961). The most common is egg. Eggs are used in the manufacture of noodles in the German-speaking countries of Europe and also in the U.S. and Canada. In some countries, such as India, high protein additives have been used with wheat flours (Paulsen, 1961; Irvine, 1971).

With the rapid increase in population, especially in developing countries, there is a need for cereal grains with a higher yield and also a higher protein content compared to wheat (Knipfel, 1969). Triticale, a polyploid hybrid cereal produced by cross-breeding wheat and rye was found to have such qualifications (Unrau and Jenkins, 1964; Villegas et al., 1968). The amino acid composition of triticale and the results of animal feeding experiments indicated that this cereal has a higher protein value than wheat. The lysine and sulfur amino acid content of triticale has been found to be higher than that of wheat (Kies and Fox, 1970a, b). It has recently been shown that this new cereal grain can be used for the production of breads (Lorenz et al., 1972). Another important question is whether it can be used for the production of pasta products.

Evaluation techniques for pasta products have been of primary concern to those in industrial and quality laboratories. The quality of cooked pasta products depends upon several factors—how the product holds up to cooking, how much water is absorbed, the loss of solids in the cooking water, the color of the products and their firmness (Walsh et al., 1969).

Several spaghetti-quality factors have been shown to be related to protein composition (Walsh and Gilles, 1971). Matsuo and Irvine (1970) demonstrated the influence of gluten strength on spaghetti cooking quality. Gluten quality was shown to be the major factor determining cooking quality.

Color is one of the most important considerations in assessing durum wheat quality. Small differences in pasta product color are most readily detected by visual observation, but optical reflectance data are preferred (Irvine, 1971; Corneluissen et al., 1962; Matsuo and Irvine, 1967). Statistical analyses showed a high correlation between visual and optical reflectance measurements of color (Walsh et al., 1969).

This paper evaluates the possibility of using triticale in the manufacture of noodles through a comparison with semolina and durum flour which are used commercially in pasta products and all-purpose flour which is normally used in home-made noodles.

MATERIALS & METHODS

Preparation of noodles

Noodles were prepared with an Imperia noodle machine (Tipo-Lusso, Italy) using all-purpose flour, durum flour, triticale flour as well as semolina. The flours and the semolina which was used as the control, were analyzed by AACC procedures (1962) for moisture, protein and ash. The dough absorption was 40% for all flours on a 14% moisture basis. Regular noodles were made from flour or semolina and water only. Egg noodles were prepared from flour or semolina, whole eggs and salt; the whole eggs contributing all the necessary moisture. The doughs were mixed in a Hobart N-50

Table 1—Key for panel evaluation of firmness and flavor of noodles

Firmness		Flavor	
Mushy	1	Like	1
Soft	2–3	Like slightly	2
Slightly soft	4	Undecided	3
Preferred firmness	5	Dislike slightly	4
Slightly firm	6–7	Dislike	5
Too firm	8–9		
Tough	10		

Table 2—Flour composition and noodle characteristics

	Flour composition			Uncooked noodle characteristics: Color		Texture	
	% Moisture	% Protein*	% Ash**	Reg Noodles	Egg Noodles	Reg Noodles	Egg Noodles
All-purpose flour	9.0	11.9	0.51	off-white	grayish yellow	brittle	hard
Semolina	12.3	12.2	0.62	yellow	deep yellow	hard	hard
Durum flour	11.5	12.1	0.58	grayish yellow	yellow	hard	hard
Triticale flour	9.3	13.3	0.53	off-white	grayish yellow	brittle	hard

*N × 5.7
**on 14% m.b.

mixer with bowl and paddle for 30 sec at speed 1 followed by 1 min at speed 2. The dough was sheeted with the noodle machine to a thickness of 2 mm and allowed to rest for 15 min before being processed into individual noodles, 4 mm wide and 2 mm thick. The noodles were dried overnight at room temperature and placed into plastic bags till needed.

Fig. 1–Comparison of regular noodles and egg noodles made with the experimental flours: Column 1 = regular noodles; Column 2 = egg noodles.

Quality tests

The firmness and flavor of the regular noodles and egg noodles were evaluated by panels. The panel for evaluating the regular noodles consisted of 10 untrained college students and the panel for evaluating the egg noodles consisted of 15 untrained college students. The samples were randomized, coded and cooked for 10, 15, 25 and 30 min, respectively, on different days, just before consideration by the panels. Codes for each sample were visable to each panel member by marking the plates on which the samples were presented. The panel members were asked to determine firmness by cutting the noodles with a fork and also to taste the noodles to arrive at a flavor judgement. The panel was instructed to disregard color in the evaluation of firmness and flavor. The firmness and the flavor of the noodles were evaluated using the key given in Table 1. Two replications of each test were done on different days.

Cooked weight of noodles was determined by cooking 40g of noodles in 600 ml of tap water for 10, 15, 25 and 30 min, respectively draining the noodles for 2 min and recording the weight of the drained noodles.

Cooking loss was measured by centrifuging the water, recovered during the cooked weight determination, at 2000 rpm for 20 min using an IEC International Centrifuge, pouring off the supernatant and weighing the residue.

Cooking loss and cooked weight of both regular and egg noodles were determined every time noodles were prepared for a panel evaluation.

For the determination of percent transmittance of the cooking water 20g of noodles were cooked in 300 ml of water in a porcelain-lined Flex-Seal Pressure Cooker sauce pan at atmospheric pressure for 10, 15, 25 and 30 min, respectively. The water was poured into the sauce pan and brought to boil after which the noodles were added. After cooking, the noodles were drained for 2 min and the liquid was centrifuged in an IEC International Centrifuge at 2000 rpm for 15 min. The percent transmittance of the liquid was read with a Bausch and Lomb spectrophotometer at 400 mμ.

The data of this study were analyzed by two-way analyses of variance and have been combined in Table 4.

RESULTS & DISCUSSION

Flour composition and noodle characteristics

The analyses of the flours and the semolina as well as the characteristics of the uncooked noodles are given in Table 2. The triticale flour was higher in protein than the other samples. The ash contents were similar.

Regular noodles prepared with the all-purpose flour and the triticale flour showed an off-white color in contrast to the yellow color of the semolina and durum noodles. The addition of eggs to the noodle recipe narrowed this color difference as seen in Table 2 and in Figure 1.

The all-purpose flour and the triticale flour produced regular noodles with a brittle texture compared with the hard texture of the semolina and durum noodles. The addition of eggs eliminated noticeable texture differences among the noodles prepared from the different flours.

Sensory evaluation

The change in firmness of regular noodles with cooking times is illustrated in Figure 2. Generally, the noodles became softer as cooking times increased. After 10 min of cooking, the triticale noodles were considerably softer than the semolina noodles indicating a shorter cooking time for the triticale noodles. The firmness of the triticale noodles changed less with longer cooking than that of the noodles produced with the other flours. This would indicate a greater cooking tolerance for triticale noodles,

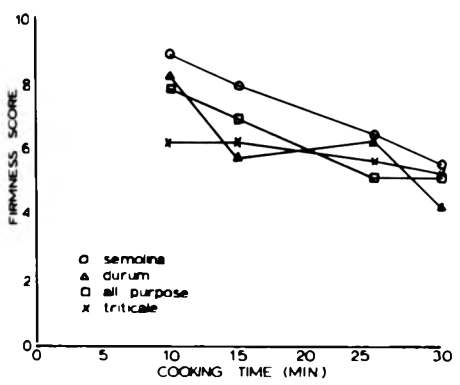

Fig. 2–The effect of cooking time on the firmness of regular noodles.

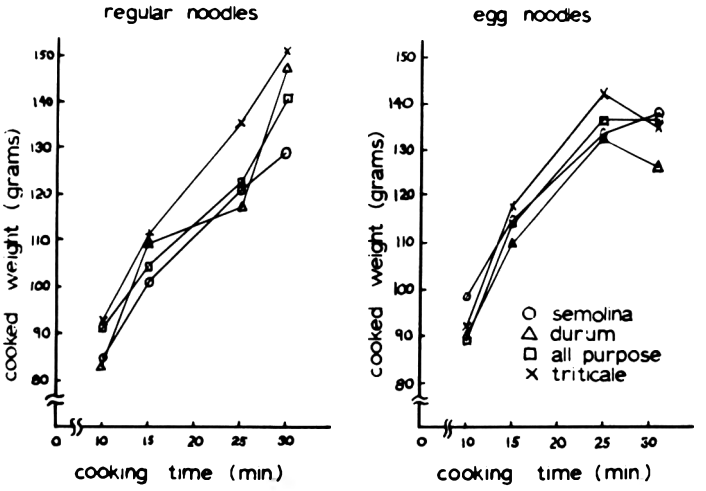

Fig. 3–The effect of cooking time on the cooked weight of noodles (40g of noodles cooked in 600 ml of water).

which would be an advantage in large-scale food preparation. The firmness score of semolina noodles changed approximately 40% extending the cooking time from 10 min to 30 min. For the triticale noodles this change in firmness amounted to only 15%.

Average firmness scores of both the regular- and the egg noodles are given in Table 3. The regular noodles were significantly different ($\alpha = 0.01$) in firmness due to the flours used and also due to increased cooking times. Triticale had the shortest cooking time, while semolina had the longest. The firmness of the egg noodles was not significantly different among the four flours. Differences in cooking time, however, produced a significant difference ($\alpha = 0.01$) in firmness.

A statistical analysis of the flavor scores of the regular noodles indicated no significant differences ($\alpha = 0.05$) among flours. Cooking times also had no effect on flavor scores. However, the data shown in Table 3 demonstrated a trend towards a better flavor with longer cooking times except for the triticale noodles which showed an opposite trend. The egg noodles showed no significant differences ($\alpha = 0.05$) in flavor due to the choice of flour in preparing these noodles. Cooking times, however, produced a significantly different ($\alpha = 0.05$) flavor score. The flavor of all egg noodles was best after only 10 min of cooking, generally, and then became slightly less acceptable with longer cooking times.

Cooked weight

The effect of cooking time on the cooked weight of regular and egg noodles is illustrated in Figure 3. The longer the cooking time the higher the cooked weight. Regular noodles made from triticale flour had the highest cooked weight after 10, 15, 25 and 30 min of cooking, respectively, while the semolina noodles, which were regarded as the control in this study, showed lower cooked weights comparing corresponding cooking times. The cooked weight of the regular noodles

Table 3—Sensory evaluation scores, percent weight gain, cooking loss and transmittance of noodles

		Sensory evaluation scores				Percent weight gain, cooking loss and transmittance of noodles					
		Firmness scores		Flavor scores		Cooked weight (% wt gain)		Cooking loss (% of cooked wt)		% Transmittance of cooking water	
	Cooking time	Regular noodles*	Egg noodles*	Regular noodles**	Egg noodles**	Regular noodles	Egg noodles	Regular noodles	Egg noodles	Regular noodles	Egg noodles
	10	7.8	8.1	3.3	2.4	127	122	11.6	6.8	85.4	70.6
All-	15	6.9	6.8	2.9	2.8	161	183	10.7	5.4	84.0	70.2
purpose	25	5.1	3.9	2.5	3.1	206	242	9.2	5.0	74.5	68.2
flour	30	5.1	4.3	2.7	3.0	251	242	8.5	4.8	72.4	60.2
	10	8.9	7.4	3.0	2.9	113	145	10.4	8.8	66.2	65.5
	15	7.9	6.5	3.0	2.8	154	187	10.4	7.0	59.6	63.7
Semolina	25	6.4	5.0	2.8	3.1	202	230	8.4	6.3	38.3	32.1
	30	5.5	4.0	2.6	3.5	224	224	7.9	6.1	36.1	30.0
	10	8.2	8.0	3.0	2.5	109	123	9.5	6.2	55.5	65.6
Durum	15	5.7	7.0	2.4	2.3	174	175	8.5	5.2	38.5	59.1
flour	25	6.2	4.8	2.3	2.8	193	234	9.4	4.7	34.5	45.5
	30	4.2	5.3	3.2	3.0	271	215	8.1	4.8	33.7	44.2
	10	6.2	7.7	2.9	2.8	133	131	14.8	9.0	77.1	67.5
Triticale	15	6.2	6.1	3.3	3.0	181	194	13.9	6.5	73.3	59.3
flour	25	5.6	4.3	3.3	3.1	236	258	12.2	6.2	67.6	58.3
	30	5.2	4.6	3.4	2.8	277	238	11.4	7.3	47.0	54.6

*The higher the score the firmer the noodles
**The lower the score the better the flavor

Table 4—Statistical data (AOV)—regular- and egg noodles

Source of variation	Firmness			Flavor			Cooked weight			Cooking loss		
	df	MS	F	df	MS	F	df	MS	F	df	MS	F
Regular noodles												
Flour	3	28.4	11.2*	3	3.5	3.1 n.s.	3	4284.3	134.1*	3	9.7	4.1*
Cooking time	3	124.6	49.1*	3	1.8	1.6 n.s.	3	239.3	7.5*	3	59.3	25.2*
Flour × cooking time	9	9.5	3.8*	9	2.0	1.8 n.s.	9	46.7	1.5 n.s.	9	1.0	0.4 n.s.
Error	304	2.5	–	304	1.1	–	16	31.9	–	16	2.4	–
Egg noodles												
Flour	3	7.6	2.7 n.s.	3	2.9	1.9 n.s.	3	3400.7	22.1*	3	1.7	1.5 n.s.
Cooking time	3	284.4	101.4*	3	6.7	4.4*	3	80.6	0.5 n.s.	3	13.7	12.5*
Flour × cooking time	9	5.3	1.9 n.s.	9	1.3	0.8 n.s.	9	26.4	0.2 n.s.	9	0.4	0.4 n.s.
Error	432	2.8	–	432	1.5	–	16	153.5	–	16	1.1	–

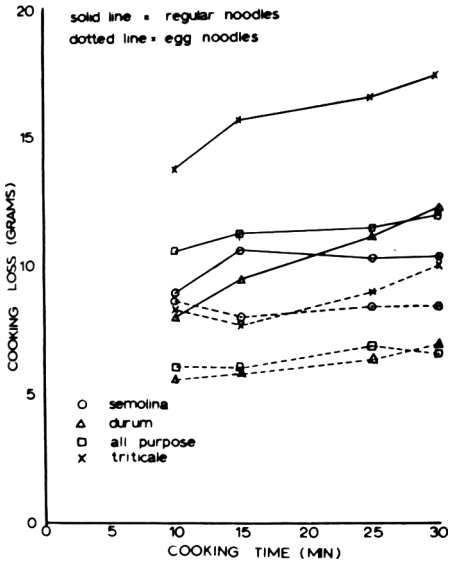

Fig. 4—The effect of cooking time on cooking loss of regular- and egg noodles.

and egg noodles expressed as percent weight gain is presented in Table 3. There is a significant difference ($\alpha = 0.05$) in cooked weight among flours with both the regular- and the egg noodles. The triticale noodles showed a considerably higher cooked weight than the other samples. Cooking the regular noodles for longer times produced significant increases in cooked weight ($\alpha = 0.05$) in each sample. Cooking times did not affect cooked weight of egg noodles.

Cooking loss

The effect of cooking time on cooking loss of regular- and egg noodles is illustrated in Figure 4. Cooking losses increased as cooking time increased. Of the regular noodles, those made from triticale showed considerably higher cooking losses than any of the others. Producing egg noodles, however, greatly reduced these cooking losses when using triticale due to the binding effect of the eggs. Egg noodles made from semolina and triticale flour had approximately the same cooking losses for up to 20 min of cooking. After 30 min of cooking, the cooking losses of the triticale noodles were slightly higher. Egg noodles made from durum- and all-purpose flour had the lowest cooking losses. The statistical analysis of the cooking loss data revealed that the choice of the flour and the cooking time produced significant differences ($\alpha = 0.05$) in cooking loss. The egg noodle data showed no differences in cooking loss among the flours. Cooking times, however, caused significant differences ($\alpha = 0.05$) in cooking loss.

The cooking losses expressed as percent of cooked weight are given in Table 3. As can be seen, the percent cooking losses actually decreased with longer cooking times since cooked weight increased more, percentage-wise, than cooking loss with longer cooking times. The regular triticale noodles had the highest percentage loss while the durum noodles had the lowest. This cooking loss, however, was considerably reduced by adding eggs to the noodle formulation.

Among the regular noodles those made from the all-purpose and the triticale flour showed the highest percent transmittance of the cooking waters. The average percent transmittance of eight determinations per noodle sample are given in Table 3. The yellow color of the regular semolina- and durum flour noodles produced darker cooking waters. The percent transmittance decreased with longer cooking times. While the color of the uncooked regular noodles varied considerably depending on the flour used (Table 2) this difference was only slight after 30 min of cooking for all but the all-purpose flour noodles. This was due to the loss of color pigments on cooking as indicated by a lower percent transmittance. The addition of eggs to the formula showed a slight decrease in the percent transmittance of the cooking water from noodles made with the all-purpose flour, semolina, and triticale flour but stabilized the color of the durum flour noodles.

CONCLUSIONS

A COMPARISON of triticale flour with durum flour, semolina and all-purpose flour for the manufacture of noodles indicated that regular triticale noodles had the shortest cooking time and the greatest cooking tolerance as determined through firmness evaluations by a panel. There were no flavor differences among the different regular- and egg noodles due to the choice of flour.

Both regular- and egg triticale noodles showed considerably higher cooked weights than the other samples. The regular triticale noodles, however, also showed a higher cooking loss than other noodles. Producing egg noodles greatly reduced these cooking losses when using triticale.

While the color of the uncooked regular noodles varied considerably—the all-purpose- and triticale noodles having the lightest color—this difference was only slight after 30 min of cooking due to the loss of color pigment from the durum- and semolina noodles. The addition of eggs to the noodle recipe narrowed this color difference in the uncooked noodles. It was concluded that triticale can be used for the manufacture of noodles.

REFERENCES

AACC. 1962. "Cereal Laboratory Methods," 7th ed. American Association of Cereal Chemists, St. Paul, Minn.

Corneluissen, P.E., Sibbitt, L.D. and Gilles, K.A. 1962. Color prediction of macaroni products. Ann. Proc. N.D. Acad. Sci. 16: 6.

Irvine, G.N. 1971. Durum wheat and paste products. In "Wheat Chemistry and Technology," 2nd ed., p. 777. AACC Monograph Series. American Association of Cereal Chemists, St. Paul, Minn.

Kies, C. and Fox, M.H. 1970a. Determination of the first-limiting amino acid of wheat and triticale grain for humans. Cereal Chem. 47: 615.

Kies, C. and Fox, M.H. 1970b. Protein nutritive value of wheat and triticale grain for humans, studied at two levels of protein intake. Cereal Chem. 47: 671.

Knipfel, J.E. 1969. Comparative protein quality of triticale, wheat and rye. Cereal Chem. 46: 313.

Lorenz, K., Welsh, J., Normann, R. and Maga, J. 1972. Comparative mixing and baking properties of wheat- and triticale flours. Cereal Chem. 49: 187.

Matsuo, R.R. and Irvine, G.N. 1967. Macaroni browness. Cereal Chem. 44: 78.

Matsuo, R.R. and Irvine, G.N. 1970. Effect of gluten on cooking quality of spaghetti. Cereal Chem. 47: 173.

Paulsen, T.M. 1961. A study of macaroni products containing soy flour. Food Technol. 15(3): 118.

Unrau, A.M. and Jenkins, B.D. 1964. Investigations on synthetic cereal species. Milling, baking and some compositional characteristics of some triticale and parental species. Cereal Chem. 41: 365.

Villegas, E., McDonald, C.E. and Gilles, K.A. 1968. Variability in the lysine content of wheat, rye and triticale proteins. Res. Bull. No. 10. Int. Maize and Wheat Improvement Center, Mexico D.F., Mexico.

Walsh, D.E., Gilles, K.A. and Shuey, W.C. 1969. Color determination of spaghetti by the tristimulus method. Cereal Chem. 47: 7.

Walsh, D.E. and Gilles, K.A. 1971. The influence of protein composition on spaghetti quality. Cereal Chem. 48: 544.

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COLORIMETRIC FURFURAL MEASUREMENT AS AN INDEX OF DETERIORATION IN STORED CITRUS JUICES

INTRODUCTION

THE FRESH taste of orange juice is a difficult property to preserve over long periods of storage. Unless juice is kept refrigerated at temperatures close to freezing, a disagreeable odor and off-flavor develop. Many chemical changes in citrus products occurring during storage have been identified in work covering two decades. From the early work of Huskins and Swift (1953), Blair et al. (1952) and Kirchner and Miller (1957) to recent studies by Rymal et al. (1968) and Nagy and Nordby (1970), a considerable range of chemical, chromatographic and spectroscopic methods have been successfully applied to terpene and lipid constituents. Nevertheless, there has remained a need for an analysis which would serve as a useful index of overall deterioration, in particular by showing some correlation with other evidence of deterioration, such as off-flavor, and by having the sensitivity to detect its onset at a very early stage.

Of the various known chemicals formed when juice deteriorates—peroxides, aldehydes, oxygenated terpenes and furfural—the last of these has perhaps the most satisfactory features for the desired analysis. The furfural content of fresh juice is virtually zero, whereas large amounts have been recognized in temperature aged juice by Rymal et al. (1968) and by Dinsmore and Nagy (1971). The volatility of furfural is sufficiently high to permit its rapid removal from juice by distillation or stripping procedures, moreover by these methods the low levels in juice can be considerably concentrated. Finally, high analytical sensitivity is available by means of direct ultraviolet spectrophotometry or by colorimetric determination. In recognition of these factors Blair (1965) made an exploratory study of stored canned orange juice, using colorimetric furfural determination as an index of deterioration. That study dealt with relatively long storage periods and high furfural levels, and although the results did not clearly show furfural detection at the onset of off-flavor, they indicated that with some further refinement this should be possible.

The present work incorporates those refinements needed for a sensitive and reliable analysis. The more sensitive color reaction of furfural with aniline has replaced the reaction with benzidine used by Blair, and the rapid distillation procedure of Scott and Veldhuis (1966) has been exhaustively tested for recovery efficiency on samples of juice reinforced with known amounts of furfural. It was felt both these developments should increase applicability of the furfural reaction considerably. As an illustrative application we have made colorimetric furfural determinations on orange juice samples stored at five temperatures at approximately 2-wk intervals over a 12-wk period, and the results have been correlated with taste evaluations. Where degradation was negligible after 12 wk, additional furfural measurements were made on equivalent juices, not necessarily of the same batch, after 6 months and 15 months of storage.

MATERIALS & METHODS

Juice

Commercially-processed orange juice from the late-season orange crop was obtained from Citrus World, Inc., Lake Wales, Fla. The late-season orange crop used for processing was composed essentially of the Valencia variety. Chemical analysis showed this juice to have a °Brix/acid value of 14.2. The juice was pasteurized according to the following procedure. The juice was brought from ambient temperature to 80°C in 4 sec and kept at 80°C for 6 sec. It was then brought up to pasteurization temperature of 96–99°C and kept at this level for 2 sec. Following the short pasteurization treatment, the juice was brought to 5°C in 4–6 sec and finally filled into 1-qt glass containers. This glass-packed orange juice was then taken directly from the assembly line and placed in lockers at 5, 10, 16, 21 and 30°C.

Flavor evaluation

The juices were tasted when freshly opened, after having first been brought to room temperature. Four experienced tasters were instructed to rank the randomly arranged juices in sequence from best to worst.

Furfural standards

A 1.00×10^{-2} molar solution was prepared

Table 1—Recovery of furfural by distillation: percentage of total furfural found in consecutive 5-ml fractions of distillate from aqueous samples

Sample vol	Fraction	Recovery from 100 μg/liter samples (run 1)	(run 2)	(run 3)	(run 4)	Recovery from 1000 μg/liter samples (run 1)	(run 2)	(run 3)	(run 4)
100 ml	1	40%	41%	45%	47%	40%	40%	37%	35%
	2	20	25	25	25	27	25	23	23
	(1 + 2)	60	66	70	72	67	65	60	58
200 ml	1	27	21	24	24	22	22	23	24
	2	20	15	17	15	16	18	15	13
	3	12	13	14	13	12	11	12	11
	4	8	8	12	11	10	11	11	9
	(1 + 2)[a]	47	36	41	39	38	40	38	37
	(1 + 2 + 3 + 4)	67	57	67	63	60	62	61	57

[a]Note that the fraction 1 from 100 ml sample and the sum of fractions 1 and 2 from 200 ml sample amount to identical 5% by volume proportions of the original sample.

by dissolving 96 mg of freshly distilled furfural in 100 ml of distilled water and was kept refrigerated at 5°C. Calculated volumes added to water or to juice gave standards in the range 10^{-4} to 10^{-6} molar. For convenience, note that the following relationships are equivalent at low furfural levels: 10^{-5} molar = 1 mg/liter = 1 part per million.

Furfural determination

Four widely used methods were studied and were standardized as follows:

Ultraviolet absorption. Measure peak at 275 mμ against base line drawn approximately from 240–310 mμ. $\epsilon = 1.4 \times 10^4$ M^{-1} cm^{-1}.

10% Aniline color. Prepare reagent by diluting 5 ml of freshly distilled aniline to 50 ml by volume with glacial acetic acid. Combine 2 ml sample, 2 ml ethyl alcohol and 1 ml reagent at room temperature. Measure maximum color at 515 mμ after 15–20 min, after which the color fades. $\epsilon = 1.7 \times 10^4$ m^{-1} cm^{-1}.

2% Aniline color. Prepare reagent by 1:5 dilution of 10% reagent with acetic acid. Combine 1 ml sample, 2 ml ethyl alcohol and 2 ml reagent. Read color at 515 mμ after 1 hr at room temperature. Slow fading commences after 1–1½ hr. $\epsilon = 6.2 \times 10^3$ M^{-1} cm^{-1}.

2% Benzidine color. Weigh 1g of benzidine (free base) into 50 ml of acetic acid. Combine 2 ml sample, 1 ml ethyl alcohol and 2 ml reagent. Run blank on 2 ml water. Read color at 550 mμ after 1 hr at room temperature. $\epsilon = 1 \times 10^4$ M^{-1} cm^{-1}.

Basic directions for the above-named color reactions were adapted from prodecures of Snell and Snell (1953) and AOAC (1965).

Distillation

The apparatus is that described by Scott and Veldhuis (1966). The heater control was adjusted to obtain a distillation rate of about 3 ml/min. Cooled water (ca. 18°C) was circulated through the condenser, and previously chilled tubes, marked at 5 ml, were used for collecting the distillate. In routine determinations a 200-ml sample of juice, containing about 1 ml of Dow Corning Antifoam C, was distilled only long enough to collect two 5 ml fractions.

Stripping

The apparatus consisted of a 1-liter flask equipped with fritted tube for nitrogen bubbler, 30 cm Vigreux column for water condensation and a single trap containing about 4 ml ice water for capturing furfural.

RESULTS & DISCUSSION

Furfural determination

Colorimetric reaction with aniline proved to be the best analysis, combining high sensitivity with negligible "blank." Trace amounts of furfural, which were lost against the UV background absorption of other juice volatiles or against the appreciable benzidine color "blank," were easily recognized by the aniline reaction. The 10% aniline reagent gives more intense but less stable color than the 2% reagent and so is particularly useful for furfural levels below 10^{-6} molar, the usual range in our juice distillates.

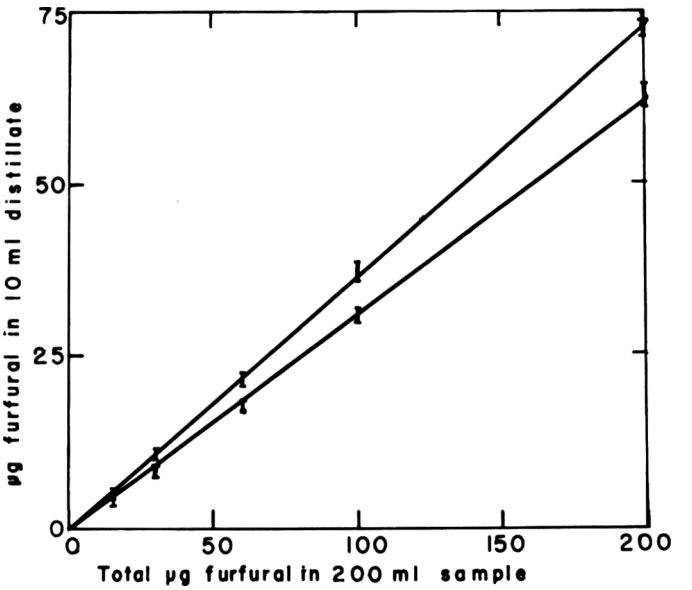

Fig. 1—Furfural recoveries by distillation from aqueous samples (upper curve) and from orange juice (lower curve). Range of four results is shown

Distillation efficiency

Close to 30% of the total furfural in a sample collects in the first 5% by volume of distillate. The concentration of furfural in this distillate is therefore enriched 6-fold relative to the original level, permitting an easy calculation of furfural in the sample. The consistency of the 30% recovery, is independent of sample size and holds without change over the practical concentration range of furfural in juice.

These results were established by two series of experiments which were carried out in quadruplicate: the first, with aqueous samples, to determine the best volumes of sample and distillate to take for analysis, and the second to establish a value for percentage recovery from juice. From the analysis on consecutive 5 ml fractions in Table 1 it is seen that close to 38% of an aqueous sample's total furfural collects in the first 5% by volume of distillate—that is, in the first 5 ml of distillate from a 100 ml sample and in the first 10 ml from a 200 ml sample. A 10-fold change in the amount of furfural does not change the average percentage recovery significantly. Out of all 16 results 13 are in the range 35–41% recovery. As would be expected, the reproducibility is poorer for the distillations involving smaller amounts. Seven out of eight of the distillations from 200 ml samples gave recoveries of 36–41%. Statistical analysis of these eight results shows that the 47% value can be rejected with better than 90% confidence and that the other seven have a median and 95% confidence interval of 38 ± 1.6. Although continuing the distillation increases furfural recovery, it does so at greater dilution and so works against obtaining a high enrichment factor.

Table 2—Comparison of furfural recoveries by distillation from water and from spiked orange juice

Furfural added to 200 ml sample	% Recovered in 10 ml of distillate[a]	
	from water	from juice
200 μg	35–36	30–32
100	36–38	30–32
60	36–37	27–30
30	34–36	26–31

[a]Range of four results

Table 3—Furfural levels in glass-packed orange juice: relation to storage time, temperature and off-flavor (furfural content in μg/liter)

Storage °C	2 Wk	4 Wk	7 Wk	9 Wk	12 Wk
10	None	None	None	None	Possible trace
16	–	None	–	30	35*
21	–	Trace	30*	50*	100*
30	40*	80*	160**	210**	400**

*Mild off-flavor
**Considerable off-flavor

Table 4—Stability of glass-packed orange juice on the basis of first discernible furfural

Storage °C	Onset of 30 μg/liter furfural
5	Well over 1½ yr
10	Probably 6–9 months
16	9 wk
21	6 wk
30	Under 2 wk

The results of the second study are shown in Table 2 and in Figure 1. Parallel distillations from water and from orange juice show consistently lower recoveries from juice, with no significant variation over a wide range of absolute furfural levels. The graphical slope of 0.30 establishes 30% as the best recovery value to use with juice, with a 95% confidence interval of less than ± 2.

Preliminary work with a nitrogen stripping procedure showed it to be less efficient. Recoveries amounted usually to only about 10% of the furfural in a 500 ml sample, held at 60°C, and stripped by nitrogen for 1–1½ hr at 40 ml per minute flow rate.

Tables 3 and 4 show the appearance and progressive increase of furfural in juice with severity of storage conditions. The parallel development of furfural and of off-flavor over a 12-wk period are documented in Table 3: higher storage temperatures lead to earlier recognition of off-flavor and to earlier colorimetric detection of furfural. The development of 30 μg/liter of furfural is used as the criterion of "first discernible furfural" in Table 4. The estimates of storage stability at 5°C and 10°C were obtained from the observation that there was still no detectable furfural after 15 months at 5°C, while an unmeasurable colorimetric trace was detected after 6 months at 10°C; the other results follow from Table 3. Since tasters could not always recognize off-flavor in samples which had developed furfural at this low a level, it seems certain that furfural measurement possesses more than sufficient sensitivity to reveal the onset of sufficient deterioration to cause off-flavor. Moreover the furfural values provide a useful index of the progress of deterioration beyond this point and enable various samples to be compared as to overall storage abuse.

The storage results just described are included here primarily to illustrate potential uses of furfural indexing and are in every respect a preliminary study. Storage studies with adequate replication and with far more elaborate organoleptic evaluation are now in progress on a variety of citrus juices.

Off-flavor

Despite the correlation which seems to exist between off-flavor and furfural, the latter must be regarded strictly as an index of those substances possessing the off-odor of temperature-aged juice. Furfural itself added to a control juice at the 200–2000 μg/liter level was not recognizable by the taste panel. If rancidity of meat, fish and dairy products, as reviewed by Schultz et al. (1962) is any guide, lipid degradation is often a chief source of malodorous products. The origin of furfural in citrus products is thought by Huelin (1955) to derive from decomposition of ascorbic acid. A more direct involvement of furfural in off-flavor is the proposal of Blair (1964) that furfural combines with hydrogen sulfide, also present in juice, to form highly odoriferous thiofurfural. Our investigation of the actual presence of thiofurfural has not progressed far enough to yield conclusive results. It is our opinion that a direct off-flavor connection with furfural is not a necessary postulate to accepting furfural in its own right as a useful index of juice deterioration and concomitant off-flavor development.

REFERENCES

AOAC. 1965. "Official Methods of Analysis," 10th ed, page 140. Assoc. Off. Agric. Chem., Washington, D.C.

Blair, J.S., Godar, E.M., Masters, J.E. and Riester, D.W. 1952. Exploratory experiments to identify chemical reactions causing flavor deterioration during storage of canned orange juice. Food Res. 17: 235.

Blair, J.S. 1964. Unpublished work at Florida Citrus Experiment Station.

Blair, J.S. 1965. Unpublished work at Florida Citrus Experiment Station.

Dinsmore, H.L. and Nagy, S. 1971. A rapid gas chromatographic method for studying volatile carbonyl compounds from orange juice and their changes during storage. J. Agr. Food Chem. 19: 517.

Huelin, F.E. 1953. Studies on the anaerobic decomposition of ascorbic acid. Food Res. 18: 633.

Huskins, C.W. and Swift, L.J. 1953. Storage changes in the phosphorus, nitrogen and fatty acid constituents of the lipid in canned Florida Valencia orange juice. Food Res. 18: 360.

Kirchner, J.G. and Miller, J.M. 1957. Volatile water-soluble and oil constituents of Valencia orange juice. J. Agr. Food Chem. 5: 283.

Nagy, S. and Nordby, H.E. 1970. The effects of storage conditions on the lipid composition of commercially prepared orange juice. J. Agr. Food Chem. 18: 593.

Rymal, K.S., Wolford, R.W., Ahmed, E.M. and Dennison, R.A. 1968. Changes in volatile flavor constituents of canned single-strength orange juice as influenced by storage temperature. Food Technol. 22: 1592.

Schultz, H.W., Day, E.A. and Sinnhuber, R.O. 1962. "Lipids and Their Oxidation." Avi Publishing Co., Inc., Westport, Conn.

Snell, F.D. and Snell, C.T. 1953. "Colorimetric Methods of Analysis," 3rd ed, Vol 3. Van Nostrand, New York.

Scott, W.C. and Veldhuis, M.K. 1966. Rapid estimation of recoverable oil in citrus juices by bromate titration. J. Assoc. Analy. Chem. 49: 628.

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Mention of brand names is for identification and does not imply recommendation by the U.S. Dept. of Agric.

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NITROGEN EXTRACTABILITY AND MOISTURE ADSORPTION CHARACTERISTICS OF SUNFLOWER SEED PRODUCTS

INTRODUCTION

SUNFLOWER MEAL and protein isolate offer considerable potential for use as protein supplements in human nutrition. Uses of these products include the addition of sunflower meal in bread making and tortilla chips and the preparation of textured vegetable protein from the protein isolate (McGregor, 1970; Talley et al., 1970).

Conventionally prepared sunflower meal develops a dark color during baking and a green color under alkaline conditions (Clandinin, 1958). The sunflower protein isolate obtained from this meal also has a dark green defect. Polyphenols such as chlorogenic acid have been implicated in the pigmentation of these products by Sechet-Sirat et al. (1959). A method using sodium sulfite and aqueous iso-proponol to remove polyphenols from sunflower meal has been reported by Gheyasuddin et al. (1970). More recently, Sosulski et al. (1972) demonstrated that these pigments could be diffused from intact kernels in an aqueous acid system.

To determine the potential of any protein concentrate or isolate a study of its functional properties is essential. In the present investigation the usefulness of sunflower kernels, treated and untreated sunflower meals and isolates has been evaluated through measurements of nitrogen extractability and moisture adsorption.

EXPERIMENTAL

DEHULLED SUNFLOWER kernels, obtained from a commercial supplier (Co-op Vegetable Oil Mills Ltd., Altona, Manitoba) were ground to an 80 mesh size, extracted with hexane and desolventized under vacuum at 45°C. Soybean meal was prepared by the same process from Altona soybeans. Another portion of the sunflower kernels was treated with 0.001N HCl at 80°C for 6 hr to diffuse out phenolic compounds from intact kernels according to a method described by Sosulski et al. (1972). The treated seeds were air dried in an oven at 40°C for 4 hr, ground and extracted with hexane. The resulting meal was called 80° diffusion extracted meal (DE meal-80). A second diffusion extraction procedure was developed in which the seeds were treated at 60°C for 12 hr and is referred to as 60° diffusion extracted meal (DE meal-60).

Protein was isolated from the meal and DE meal-60 by extraction with 0.02% NaOH followed by iso-electric precipitation as described by Cater et al. (1970). The protein isolate was washed twice with acidified water (pH 4.5) and then re-suspended by adjusting the pH to 7.0 with 0.02% NaOH prior to freeze drying. The protein isolate obtained from the meal was termed isolate and that prepared from DE meal-60 was designated diffusion extracted isolate (DE isolate).

The meals and isolates were analyzed for moisture, oil, protein (N × 6.25), ash and crude fiber according to AOAC (1970) methods.

The nitrogen extractability values were determined in duplicate over a pH range of 1–12 according to the method of Cater et al. (1970). The nitrogen content of the supernatant was measured by a semi micro-Kjeldahl method suggested by Bremner (1960). The results were expressed as percent of total nitrogen extracted from the sample and were plotted against pH to obtain the nitrogen extractability profiles. Soybean meal, prepared in a manner similar to sunflower meal, was used as a reference in this experiment.

Moisture adsorption of the meals and protein isolates was determined by holding these samples at temperatures of 5, 20 and 30°C. At each temperature, five different relative humidities ranging from 11–98% were produced by using concentrated salt solutions as recommended by Rockland (1960). 25 ml of a saturated solution were added to 12 oz battery jars. Aluminum weighing dishes containing 2g samples were set on top of inverted beakers previously placed in the jars. The containers were sealed with ground glass covers and kept at the specified temperatures. To determine the equilibrium moisture contents (EMC) the samples were weighed at 0, 24 and subsequently at 48 hr intervals until no further change in weight was observed. Equilibrium was usually established in about 168 hr except for the meal samples which required about 236 hr. The percent EMC was plotted against equilibrium relative humidity (ERH) to determine the moisture adsorption isotherms. A soy protein isolate (Supro-610 obtained from Ralston Purina Ltd.) was used for comparison purposes.

To study the adsorption of dehulled oilseeds (meats) about 40g each of sunflower, rapeseed and soybeans were soaked in excess water (ca. 500 ml) at 50°C in a beaker. This high temperature was maintained in an attempt to delay germination. Samples were removed at intervals of 15 min during the first hour and then at hourly intervals for a total period of 4 hr. At each sampling, about 4g of seeds were removed from the beaker and placed on a 100 mesh screen for 10 min to allow for drainage of free moisture. The samples were then weighed and the moisture content determined at 100°C for 16 hr. The percent moisture was plotted against time to determine the moisture uptake by the seeds.

RESULTS & DISCUSSION

THE PROXIMATE composition of sunflower kernels (meats) untreated and diffusion-extracted meals and protein isolates are reported in Table 1. While sunflower kernels contained 55% oil, the residual oil levels in the meals were less than 5% with the isolates being essentially free of oil. The crude protein contents of the meal and DE meal-80 were more than 60%. The slightly lower level of protein in the DE meal-60 sample could be due to nitrogen losses which occurred during the 12 hr diffusion process. The isolate and DE isolate contained over 90% protein. The diffusion extraction process resulted in lower amounts of ash and smaller levels of nitrogen-free extract in the two DE meals due to losses of minerals and soluble sugars during the treatment. Consequently, the percent of crude fiber in

Table 1—Proximate analysis of samples

Constituents	Sunflower kernel	Untreated		Treated (Diffusion-extracted)		
		Meal	Isolate	Meal-80	Meal-60	Isolate
		(%)				
Moisture	1.8	7.5	4.1	6.0	3.7	1.4
Oil	55.4	1.2	0.0	4.2	4.5	0.6
Protein (N × 6.25)	28.2	60.1	94.3	60.4	55.1	93.0
Ash	3.6	8.3	1.6	6.3	5.4	3.8
Crude fiber	2.4	5.2	0.0	9.3	14.8	0.9
Nitrogen-free extract	8.6	17.7	0.0	13.8	16.5	0.3

the treated meals was higher than that in the untreated meal.

Sunflower kernels were grey to off-white in appearance and the meal was grey colored. Sunflower isolate, prepared under alkaline conditions, developed a dark green color which is attributed to the oxidation of chlorogenic acid. The diffusion extraction process improved the color of the meal to an off-white shade, whereas the DE-isolate was tan colored.

The nitrogen extractability curve for soybean meal (Fig. 1) was similar to that obtained previously (Smith, 1958), with relatively high solubilities being observed below pH 3.0 and above pH 6.0. While a narrower curve would be desirable, the profile for sunflower meal does show good extractability at pH 8.0 and above, being over 90% soluble in this range as compared to 80% for soybean meal. The point of minimum solubility for sunflower meal appeared to be near pH 4.0 and at lower pH values was less soluble than soybean meal. This loss of solubility can possibly be due to the peptide-polyphenol interactions as reported by Loomis and Battaile (1966). The profile for sunflower meal is in close agreement with data obtained by Cater et al. (1970). The sunflower isolate was more soluble than the meal and showed a sharp minimum solubility point at pH 4.0. The maximum extractability for the isolate was observed at pH 6, while that for the meal occurred only at pH 8 and above.

In general, diffusion-extraction at elevated temperatures appeared to denature a portion of the meal proteins and thereby lowered their nitrogen extractability. The extractability curve for DE meal-60 (Fig. 2) was lower than that of the untreated meal (Fig. 1), with a broader range of insolubility between pH 3 and 7. The 80° diffusion process denatured the proteins to such an extent that solubility was less than 10% over the entire pH range of 1–11. The DE isolate prepared from DE meal-60 showed solubilities of near 100% at pH 7.0 and above but these proteins were also generally insoluble between pH values of 3 and 7.0. This was in marked contrast to the sharp minimum solubility point obtained for the isolate in Figure 1. The profile for DE meal-60 compares favorably with that for soybean meal. Protein isolates were not prepared from DE meal-80 as poor yields were obtained on a bench scale. The profile for DE-isolate (Fig. 2) is similar to that of the 50% aqueous iso-propanol isolate prepared by Cater et al. (1970). Thus the 60° diffusion-extraction procedure could be considered as an alternate method to remove undesirable color from sunflower products.

The moisture adsorption isotherms for the various samples at 5, 20 and 30°C are presented in Figure 3. Little difference was noted in the moisture contents of samples between relative humidities of 10 and 55%. However, marked changes were noted between 60 and 98% ERH, with the meal adsorbing the most moisture. Treatment received by DE meal-60 reduced its capacity to adsorb moisture when compared to the untreated meal sample. An opposite trend was observed for the isolates as the DE isolate showed greater moisture adsorption than the untreated isolate. Due to changes in vapor pressure at each relative humidity, moisture adsorption was inversely related to the sample temperature. All samples tested showed better moisture adsorption characteristics than those reported by Rasekh et al. (1971) for fish protein concentrate. The meal adsorbed more moisture than soy protein isolate (Supro-610) which could be due to the presence of the carbohydrate fraction in the meal. The increase in moisture content at higher relative humidities according to Caurie (1971) could be due to the product being in a zone of exclusively condensed water molecules. In the lower humidity zones of exclusively gaseous, or mixed gaseous and condensed water molecules, however, the differences in moisture uptake would be less.

The information from this study on moisture adsorption would be useful in evaluating the storage life of sunflower

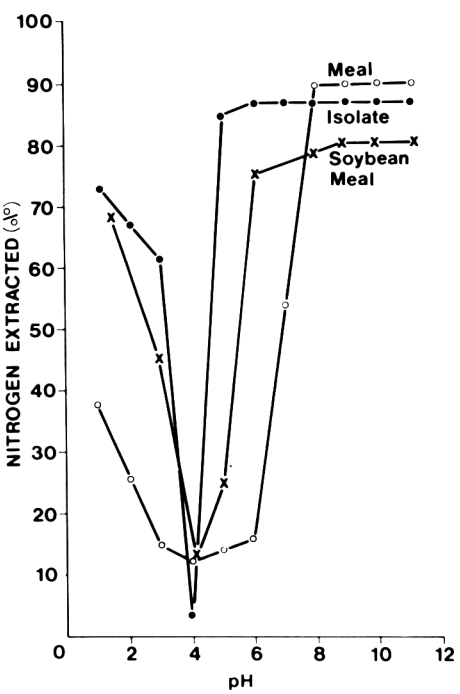

Fig. 1—Nitrogen extractability profiles for untreated sunflower meal, protein isolate and soybean meal.

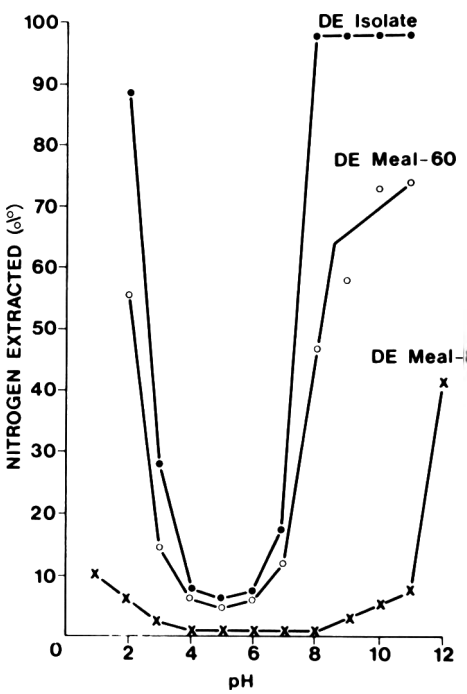

Fig. 2—Nitrogen extractability profiles for diffusion-extracted sunflower meal and protein isolate.

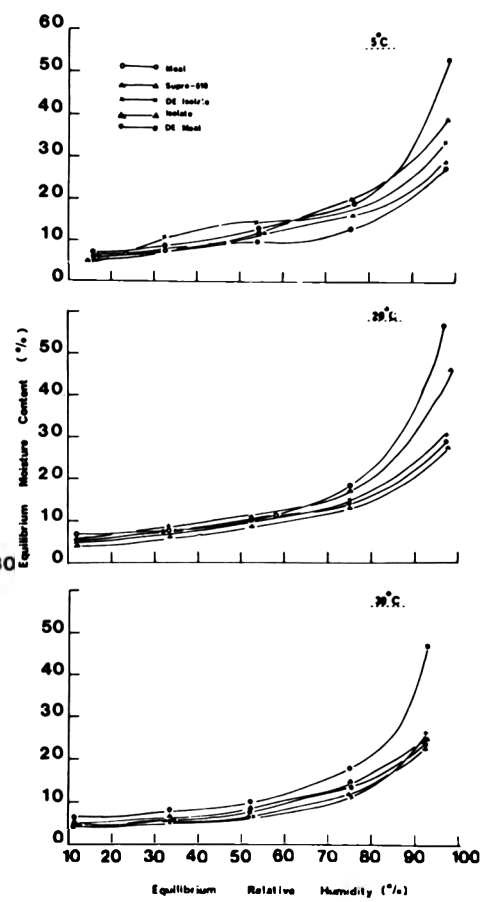

Fig. 3—Moisture adsorption isotherms for the meals and isolates at various temperatures and relative humidities.

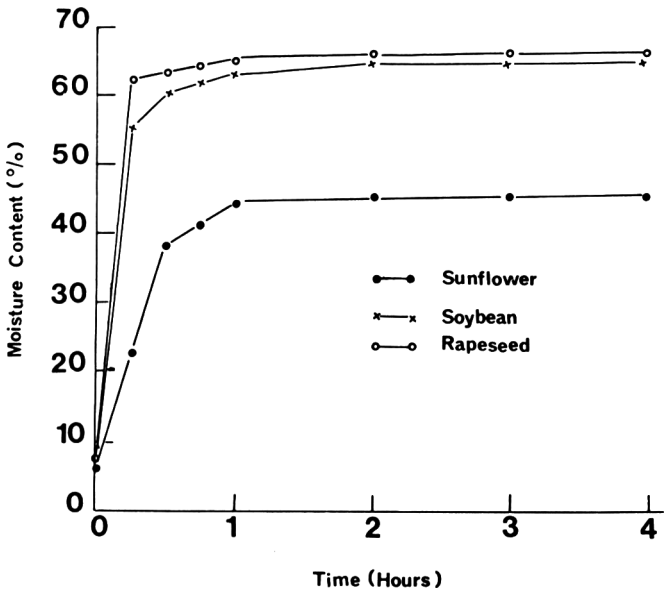

Fig. 4–Moisture uptake by dehulled oilseeds at 50 °C. (Results represent data obtained from three trials.)

meal and isolates. According to Jay (1970) a water activity of 0.6 or less is necessary for control of microbial growth. Safe moisture contents corresponding to a water activity of 0.6 or 60% ERH can be calculated from Figure 3. For example, at 5°C sunflower meal could be stored with 15% moisture without suffering microbial action, whereas at 30°C the safe moisture content for the meal would only be 11% or less.

Preliminary work was carried out to study the hydration properties of sunflower kernels in relation to those of rapeseed and soybean meats (Fig. 4). Oilseeds were observed to adsorb water rapidly during the first hour of soaking before reaching an equilibrium. This rapid hydration was indicative of moisture uptake by carbohydrate or protein fractions. Since seed proteins do not hydrate rapidly within 30 min (Wall, 1964) the increase in moisture content was due presumably to the carbohydrate fraction of the seed. Sunflower was unique in that water absorption was slower in the initial phase and much lower total water uptake was achieved after 4 hr. The sunflower sample absorbed 40% of its seed weight with water while rapeseed and soybean took up 60%. These variations may be due to differences in type and quantity of carbohydrate material.

The present data on nitrogen extractability for treated and untreated meals should be of assistance in devising technology for commercial preparation of sunflower flour, concentrate and protein isolate. Sunflower proteins have a desirable narrow minimum solubility point and relatively high nitrogen extractability above pH 8.0. Unfortunately, the specific treatments to remove pigments have adverse effects on protein solubility. While the 60° process resulted in improved color, the meal and isolate thus obtained showed evidence of protein denaturation. Further studies on processes which can be conducted at lower temperatures are continuing to produce sunflower products which could increase their use in formulated foods.

REFERENCES

AOAC. 1970. "Official Methods of Analysis." 11th ed. Association of Official Agricultural Chemists, Washington, D.C.

Bremner, J. 1960. Determination of nitrogen in soil by Kjeldahl method. J. Agric. Sci. 55: 11.

Cater, C.M., Gheyasuddin, S., Mattil, K.F. and Colvin, B.M. 1970. Recent developments in the production of sunflower protein isolates, p. 91. Proc. 4th Intl. Sunflower Conf., Memphis, Tenn.

Caurie, M. 1971. A single layer moisture adsorption theory as a basis for the stability and availability of moisture in dehydrated foods. J. Food Technol. 6: 133.

Clandinin, D.R. 1958. Sunflower seed oil meal. In "Processed Plant Protein Foodstuff," ed. Altschul, A.M. Academic Press Publ. Inc., New York.

Gheyasuddin, S., Cater, C.M. and Mattil, K.F. 1970. A method for the preparation of a colorless sunflower protein isolate. Food Technol. 24: 36.

Jay, J.M. 1970. "Modern Food Microbiology." Van Nostrand Reinhold Co., N.Y.

Loomis, W.D. and Battaile, J. 1966. Plant phenolic compounds and the isolation of plant enzymes. Phytochemistry 5: 423.

McGregor, D. 1970. Formulation of new sunflower seed products. Proc. 4th Intl. Sunflower Conf., Memphis, Tenn.

Rasekh, J.G., Stillings, B.R. and Dubrow, D.L. 1971. Moisture adsorption of fish protein concentrate at various relative humidities and temperature. J. Food Sci. 36: 705.

Rockland, B. 1960. Saturated salt solutions for static control of relative humidity between 5 and 40°C. J. Anal. Chem. Soc. 32: 1375.

Sechet-Sirat, J., Masqueitar, J. and Tayeay, F. 1959. Pigments in sunflower seeds. I. Chlorogenic Acid. Bull. Soc. Chim. Biol. 41: 1059.

Smith, A.K. 1958. Vegetable protein isolates. In "Processed Plant Protein Foodstuffs," ed. Altschul, A.M. Academic Press Publ. Inc. New York.

Sosulski, F.W., McCleary, C.W. and Soliman, F.S. 1972. Diffusion extraction of chlorogenic acid from sunflower seed. In press.

Talley, L.D., Brummett, B.J.C. and Burns, E.E. 1970. Utilization of sunflower in human food products. Proc. 4th Intl. Sunflower Conf., Memphis, Tenn.

Wall, J.S. 1964. Cereal Proteins. In "Symposium on Foods: Proteins and Their Reactions," ed. Schultz, H.W. and Anglemier, A.F. Avi Publ. Co. Inc., Westport, Conn.

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PALATABILITY OF PANCAKES AND COOKED CORN MEAL FORTIFIED WITH LEGUME FLOURS

INTRODUCTION

IN THE LAST few years, much attention has been focused on the nutritional adequacy of the American diet. Frequent references have been made to "empty" calories particularly with regard to snacks and breakfast foods, which are most frequently consumed by young people. When consumed with milk, however, the breakfast foods obviously provide useful nutrition. The reasons for the success of such foods may be many including convenience, ease of preparation, etc., but probably the principal reason is palatability—taste, aroma, texture, appearance, etc.

In general, people tend to select their food on the basis of what they enjoy eating rather than on its nutritional value. The most nutritious food in the world is ineffective unless it is accepted and consumed by the population for which it is intended. Therefore, it would appear that the most painless and effective way of improving the nutrition of groups who need it is by upgrading the nutritional value of foods they like. However, nutritional fortification must be accomplished without reducing acceptability of the products. This paper explores the possibility of blending cereal products such as cornmeal and pancake mixes with laboratory prepared navy and pinto bean powders to improve protein quality without significant reduction in palatability. Comparative studies with commercial soy flours were also made.

MATERIALS & METHODS

THE EFFECT OF nutritional fortification on sensory acceptability was conducted on pancakes and corn meal products. These foods were not selected for their significance in the diet but illustrate the principle of fortification without loss of acceptability.

Pancake mix

The basic dry mix consisted of the following:

All purpose white flour	100g
Sugar	12g
Nonfat-dry milk solids	10g
Baking powder	10g
Salt	3g

The batter was prepared by mixing the dry mix with one egg, one tablespoon of salad oil and 180 ml of cold water in a kitchen mixer operated at low speed until the mixture was thoroughly hydrated and smooth. This represents the standard or control pancake mix. When the mix was fortified with varying amounts of dry legume powders, the flour content was reduced by the amount of legume powder added.

The "cakes" were cooked in an oiled electric fry pan set at 420°F. Each cake consisted of 1 tablespoon of batter. The cooked cakes were served to the judges on oven-warmed plates coded with random numbers. Each individual booth where the testing was done contained a supply of maple syrup and butter to be used according to individual taste.

Cornmeal mix

50g of whole ground cornmeal was slowly added to 360 ml of boiling water, with constant stirring until the mixture reached a full boil. The mixture was then cooked for 30 min over boiling water in a double boiler. After cooking, 3 tablespoons of tomato sauce and 3 tablespoons of dry grated Parmesan cheese were uniformly stirred into the cornmeal. This represents the standard or control cornmeal mixture. The effect of fortification with dry legume powders was determined by replacing varying amounts of the dry cornmeal with an equal weight of legume powder.

Legume powders

Legume powders were chosen as the fortifying agents because of our interest in the increased utilization of beans and bean products, and because they are a reasonably good source of protein and other nutrients (Watt and Merrill, 1963). Replacement of 25–50% wheat flour or cornmeal with dry bean powder significantly increases the protein content of the food mixtures described above.

The bean powders were prepared from whole dry navy and pinto beans according to the process described by Morris (1961). Some of the powders prepared by the process described by Kon et al. (1970) were fortified with 0.6% methionine. Other powders were prepared by grinding dry whole peeled beans in a hammermill and sieving through a 100-mesh screen. The soybean flours (S_1-bakers defatted, S_2-defatted toasted, S_3-full fat, S_4-defatted) and pancake mixes were obtained from commercial sources.

Sensory evaluation

The unfortified products described above served as the standard or control for all levels of fortification with legume products.

Most of the sensory tests were conducted with a panel of 20 experienced judges selected on the basis of acuity and reliability. Some of the tests were conducted with larger groups of untrained laboratory personnel for the purpose of estimating relative acceptability of some of the fortified products.

The sensory tests used were paired comparison, duo-trio and hedonic scale (ASTM, 1968). Significance of the results obtained in the paired tests was determined by the sign test (Gacula et al., 1971).

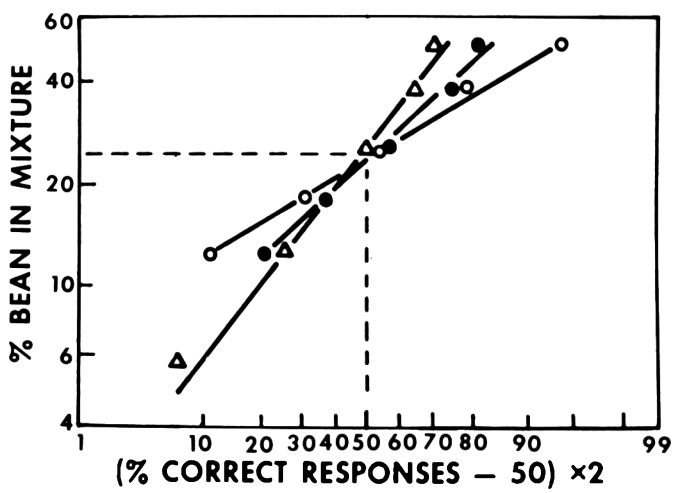

Fig. 1—Relation between percentage of legume in cornmeal mixture (dry weight basis) and detection of flavor difference between mixture and 100% cornmeal control (duo-trio test). △—defatted toasted soy (S_2); ●—ground, cooked white navy beans; ○—acid processed freeze-dried white navy bean powder fortified with methionine. Thresholds and 95% confidence limits: △—25%, 20–32%; ●—24%, 22–27%; ○—23%, 20–27%.

All sensory tests were conducted in a room containing individual booths and supplied with air passed through a bank of granular charcoal cannisters and maintained at a constant 74 ± 2°F and 50% R.H. The room was maintained under slight positive pressure to prevent outside unconditioned air from entering. Each judge was presented with one pair of samples per session with a score sheet giving appropriate information and instructions for each of the different tests. Pancakes were served hot from the griddle and the corn meal was held at 160°F until served. The tests were conducted under subdued lighting (7.5 watt green bulbs) to prevent possible color or appearance differences from influencing flavor judgments. Fortified and unfortified samples were presented first in each pair an equal number of times to prevent first sample bias. The judges were allowed to swallow the samples if they wished, and running water was available for rinsing between samples.

RESULTS & DISCUSSION

Corn meal mixtures

The relation between percentage of dry legume powder in corn meal and detection of flavor difference between 100% corn meal control and legume-corn meal mixture is shown in Figure 1. The duo-trio data were corrected for guessing the correct answer by subtracting 50 from the percentage of correct responses and multiplying by 2. When the product of this transformation was plotted against percentage of legume in mixture on log probability paper, reasonably good straight lines were obtained for the various legume products. In this form, the data appear to meet the requirements of the dose-response procedure described by Litchfield and Wilcoxon (1949). In this case, the LD_{50} or point where 50% of the responses actually detect the additive is considered the threshold or amount of legume that causes a noticeable difference in taste. This degree of discrimination corresponds to 75% correct responses in the uncorrected duo-trio data. Thresholds for the different legume products in corn meal varied from 23–25% of the final mixture with 95% confidence intervals ranging from 20–32%. Data obtained from the duo-trio test indicated that many of those who correctly matched duplicates considered the sample with added legume as having a better flavor than the unfortified control. To explore the possibility of improved flavor in the sample containing legume powder, the tests were repeated in the form of a paired comparison in which the subjects were asked to indicate which sample in the pair had the better flavor. Percentage of the subjects indicating better flavor in the sample containing legumes was plotted against percentage of legume in the mixture (Fig. 2).

It is interesting to note that a significant proportion of the subjects considered samples with 12.5–25% toasted defatted soy or ground cooked white navy beans as having a better flavor than the legume-free control. Even when the proportion of these two bean products was raised to 50% of the mixture, 60% of the subjects still considered the bean products as having a better flavor. Samples containing acid-processed legume powder gave nonsignificant results over the entire range of 12.5–50%. However, in the 12.5–25% range, preference was in favor of the bean sample while at the 50% level it was slightly in favor of the control samples. The lower flavor preference for products with the acid processed legume powder were probably due to the 0.6% added methionine in this powder. In order to obtain a general overall estimate of the relative acceptability of the control and 25% legume fortified corn mixtures, the products were evaluated on a 9 to 1 like-dislike hedonic scale. Figure 3 shows the cumulative percentage of responses

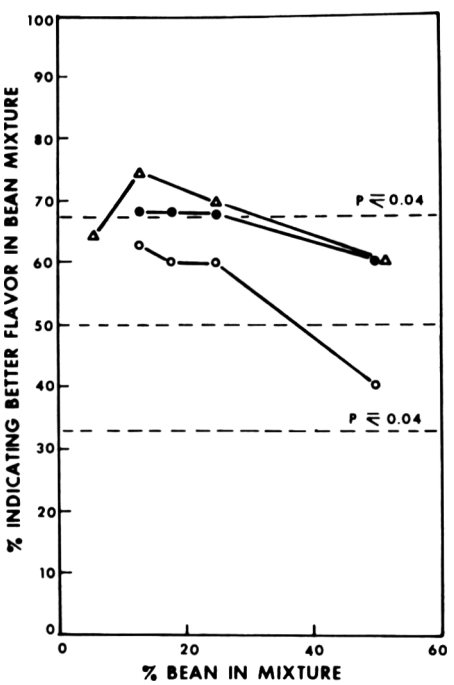

Fig. 2–Relation between percentage of legume in cornmeal mixture (dry weight basis) and percentage of responses indicating better flavor in mixture compared to 100% cornmeal control (paired comparison test, n = 40). ●–ground cooked white navy beans; ○–acid processed freeze-dried white navy bean fortified with methionine; △–defatted toasted soy (S_2).

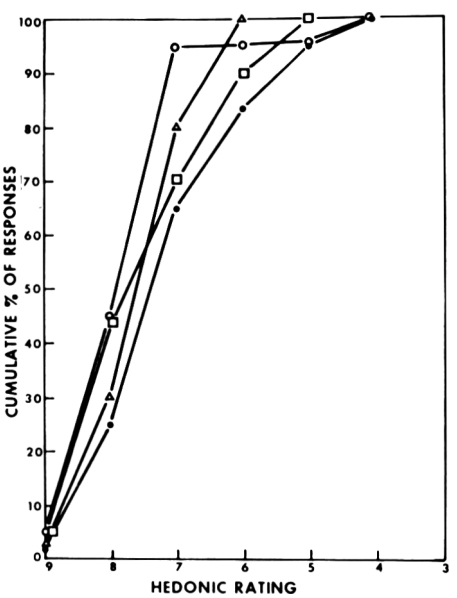

Fig. 3–Cumulative percentage of panel responses as a function of hedonic rating of legume-cornmeal mixtures. 9–like extremely, 8–like very much, 7–like moderately, 6–like slightly, 5–neither like nor dislike, 4–dislike slightly, 3–dislike moderately, 2–dislike very much, 1–dislike extremely. ●–control, all cornmeal; ○–25% defatted, toasted soy (S_2); △–25% acid processed freeze-dried white navy bean powder fortified with methionine; □–25% drum-dried pinto beans. Average hedonic rating: ●–6.7, ○–7.4, △–7.2, □–7.1; ● vs. ○ significant at P ≤ 0.003, others N.S.

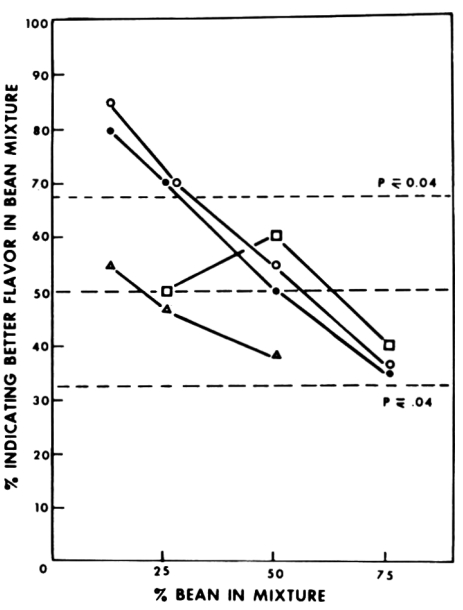

Fig. 4–Relationship between flavor responses from pancakes prepared from mixes containing various percentages of dry ground legumes or legume products (paired test, N = 40). ●–defatted toasted soy flour (S_2); ○–defatted bakers soy flour (S_1); △–acid processed freeze-dried white navy bean powder fortified with methionine. □–drum dried pinto beans.

Table 1—Nutrient changes in cooked pancakes and corn meal resulting from partial replacement of wheat flour and corn meal with navy bean and defatted soy flours

	Cooked pancakes			Cooked corn meal		
	Wheat flour fortified with:			Corn meal fortified with:		
Nutrients[a]	Unfortified control	30% navy bean flour	30% defatted soy flour	Unfortified control	30% navy bean flour	30% defatted soy flour
Protein	7.0	8.1	10.5	4.1	5.1	6.9
Calcium	57	69	82	62	72	81
Phosphorous	100	138	158	103	116	133
Potassium	111	199	283	78	146	192
Iron	0.7	1.3	1.7	0.6	1.0	1.2
Thiamin	0.05	0.13	0.15	0.10	0.12	0.15
Riboflavin	0.12	0.14	0.15	0.07	0.08	0.09
Niacin	0.34	0.48	0.52	0.51	0.54	0.56

[a]Protein—g/100g cooked product; all other nutrients—mg/100g cooked product

assigning values from 9 to 4 for like extremely to dislike slightly. The graph readily shows the percentage of responses which assigned a given hedonic rating or higher. Thus less than 10% of the panel assigned values of 9, but 65–95% of the panel assigned values of 7 or higher. The mean hedonic ratings varied from 6.7–7.4. The largest difference in acceptability of the samples was between control and 25% defatted toasted soy. This difference was significant at $P \leq 0.003$ in favor of the legume product and appears to confirm the results obtained in the paired tests. The other legume products were not significantly different from the control even though all were rated somewhat higher. All legume-containing products received ratings of like slightly (6) to like extremely (9) by 90–100% of the panel.

Results from three different types of sensory tests suggest that at least 25% legume can be added to this particular cornmeal product without adversely affecting the sensory characteristics of the mixture. Even more surprising is the fact that this particular panel appeared to prefer the samples with 25% defatted toasted soy over the all cornmeal control. The approximate nutritional improvement which could be achieved in such mixtures can be calculated from the tabular nutrient values listed for the individual components of the mixture (Watt and Merrill, 1963). Table 1 gives calculated values for a limited number of nutrients and mixtures. Considering protein, it is readily apparent that the greatest improvement is obtained by the use of defatted soy products since they contain over twice as much protein as ordinary dry beans.

Pancake mixes

In pancake mixes, as little as 10% legume addition to the basic mix was significantly detectable by the duo-trio

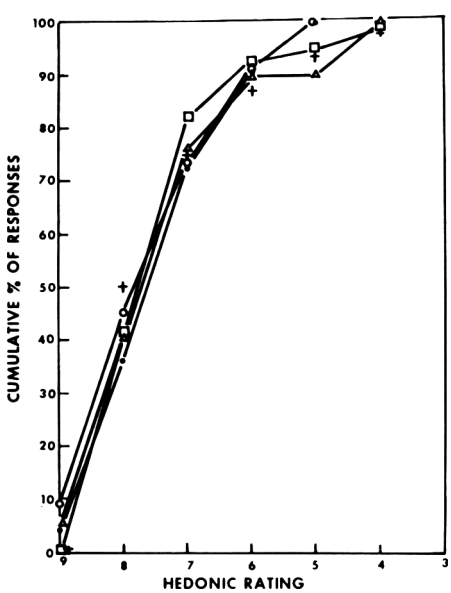

Fig. 5—Cumulative percentage of panel responses as a function of hedonic rating of pancakes prepared with various amounts of toasted soy (S_2). ●—unfortified mix; ○—12.5/2% (S_2) in mix; △—25% (S_2) in mix; □—50% (S_2) in mix. ● to □, n = 20–22. + [50% (S_2) in mix], n = 52. Average hedonic rating: ●—7.0, ○—7.2, △—7.0, □—7.1, + —7.0.

Table 2—Flavor comparisons of pancakes prepared from laboratory mix, commercial mixes and the same mixes fortified with soybean products

		No. of judgments indicating better flavor for:		
Comparison[a]	N	First sample	Second sample	Probability
LM vs 25% S_2 in LM	40	12	28	0.017
LM vs 50% S_2 in LM	52[b]	24	28	–
LM vs 25% S_3 in LM	40	9	31	0.0007
LM vs 50% S_3 in LM	40	12	28	0.017
25% S_2 in LM vs 25% S_3 in LM	40	16	24	0.268
50% S_2 in LM vs 50% S_3 in LM	40	10	30	0.002
LM vs 25% S_4 in LM	40	13	27	0.038
LM vs 50% S_4 in LM	40	10	30	0.002
25% S_2 in LM vs S_5	20	10	10	–
50% S_2 in LM vs S_5	20	10	10	–
25% S_1 in LM vs S_5	40	12	28	0.017
CM_1 vs 25% S_2 in CM_1	40	18	22	–
CM_1 vs 50% S_2 in CM_1	40	15	25	0.154
CM_2 vs 25% S_2 in CM_2	40	14	26	0.081
CM_2 vs 25% S_2 in LM	40	12	28	0.017
CM_2 vs 50% S_2 in LM	40	16	24	0.268
CM_3 vs 50% S_2 in LM	20	2	18	0.0004
CM_4 vs 50% S_2 in LM	40	12	28	0.017

[a]LM—laboratory mix; S_1—commercial bakers defatted soy; S_2—commercial defatted toasted soy; S_3—commercial full fat soy; S_4—commercial defatted soy; S_5—commercial pancake mix containing soy; CM_1 to CM_4—commercial pancake mixes

[b]Random sample of 52 laboratory employees not on the regular panel

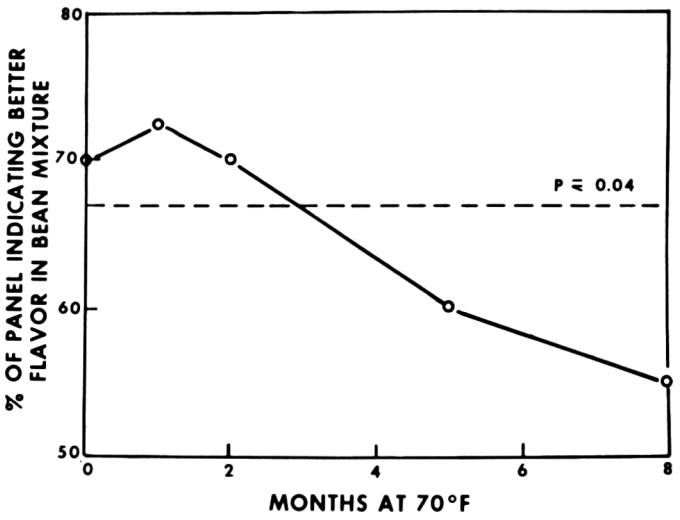

Fig. 6–Effect of storage at 70°F on flavor of pancakes prepared from mixes with and without 25% defatted toasted soy (S_2) (n = 40).

test. Since results from the cornmeal experiments indicated possible flavor improvements by certain levels of legume fortification, the sensory tests on pancakes were primarily by paired comparison and hedonic rating.

Results from the paired tests were plotted against percentage of legume in the mix (Fig. 4). Significant proportions of the panel indicated that 12.5–25% addition of both soy products to the mix gave the finished cake a better flavor.

As the percentage of soy in the mix was increased to 50 and 75%, the percentage of responses in favor of the additive dropped sharply in linear fashion. Even at 50% addition, however, the panel was about evenly divided in their judgment as to which sample had the better flavor. The mixes containing ordinary dry bean products were not significantly preferred at any of the levels tested, but neither was the control, even when the bean level was raised to 75%. Except for the acid-processed legume powder, at least 50% or more of this particular panel considered the cakes made from mixes containing up to 50% legume powder as having a better flavor than the control.

Further information on the relative acceptability of pancakes made from mixes containing 12.5–50% toasted soy flour is shown in Figure 5. It is clear that the average hedonic ratings of all samples including control were very similar, ranging from 7.0–7.2. Analysis of the data did not show any significant differences among samples. Less than 10% of the panel gave the samples a rating of 9, but 87–92% rated all products at 6 or higher. The data, obtained from 52 untrained laboratory persons, are of particular interest in that they are very similar to that obtained by the smaller regular panel, and indicate that 75% of this group assigned the 50% fortified cakes a rating of 7.0 or higher.

Table 2 shows results of paired comparisons between cakes prepared from laboratory and commercial mixes with and without soybean powder fortification. The S_1 and S_2 defatted soy flours are the same as those used in all previous fortification tests and the other products are as described in Table 2.

Slightly more than half of the 52 untrained laboratory subjects considered the 50% (S_2) fortified laboratory mix as having a better flavor than the unfortified control. This result is in agreement with the hedonic ratings obtained from a similar untrained panel and indicates no significant dislike or rejection of the fortified sample.

The full fat soy flour (S_3) at the 50% level appears to improve flavor of the laboratory mixes more than the defatted toasted sample (S_2). Defatted soy sample (S_4) appears to offer flavor improvement at both 25 and 50% levels over the unfortified laboratory mix. The laboratory mix fortified with 25 and 50% S_2 was not significantly different from a commercial pancake mix containing soy flour, but the commercial mix was significantly preferred over the laboratory mix containing 25% S_1. Addition of 25–50% S_2 to unfortified commercial pancake mixes did not appear to cause significant flavor preferences, but a majority of the responses indicated better flavor in the fortified samples. The laboratory mix fortified with 25 and 50% S_2 was significantly preferred over unfortified commercial mixes in 3 out of 4 tests. Thus, it appears that fortification with legume products to increase nutritional value of pancake mixes and cornmeal products would not be hindered on the basis of introduction of objectionable flavors. On the contrary, the evidence seems to indicate that flavor of the fortified products may be preferable to a substantial number of persons. While these results do not necessarily represent those which would be obtained by consumers in general, they do indicate the possibility of legume fortified products and illustrate the principle of fortification without significant loss of palatability. Somewhat similar results were obtained with soy fortified corn and wheat breads by Bookwalter et al. (1971 a, b). Such fortification would not only increase protein content of relatively low protein products, but would also raise the levels of other nutrients such as calcium, phosphorus, iron, potassium, thiamin and niacin. At the 50% level of fortification, the percentage of some of these nutrients would be more than doubled.

The sensory results up to this point were all obtained either immediately or within a few days of fortification. To determine the effect of possible ingredient interactions during storage at ordinary temperatures, laboratory mixes with and without 25% S_2 were packaged in polyethylene bags and stored at 70°F. After 1, 2, 5 and 8 months of storage, pancakes were prepared from fortified and unfortified mixes and evaluated for flavor. Figure 6 shows a significant preference for the fortified cakes at 0, 1 and 2 months of storage. Percentage of the panel considering the fortified sample as better in flavor declined to 60% after 5 months and 55% after 8 months. Thus the preference for the fortified sample becomes insignificant between 2 and 5 months at 70°F, but even after 8 months at this temperature, no adverse flavor effects attributable to fortification were noticeable.

REFERENCES

ASTM. 1968. "Manual on Sensory Testing Methods." ASTM Special Technical Publication No. 434.

Bookwalter, G.N., Kwolek, W.F., Black, L.T. and Griffin, E.L. Jr. 1971. Cornmeal/soy flour blends: Characteristics and food applications. J. Food Sci. 36: 1026.

Bookwalter, G.N., Mustakas, G.C., Kwolek, W.F., McGhee, J.E. and Albrecht, W.J. 1971. Full-fat soy flour extrusion cooked: properties and food uses. J. Food Sci. 36: 5.

Gacula, M.C. Jr., Moran, J.M. and Reaume, J.B. 1971. Use of the sign test in sensory testing. Food Product Development 5(6): 98.

Kon, S., Wagner, J.R., Guadagni, D.G. and Horvat, R.J. 1970. pH adjustment control of oxidative off-flavors during grinding of raw legume seeds. J. Food Sci. 35: 343.

Litchfield, J.T. Jr. and Wilcoxon, F. 1949. A simplified method of evaluating dose-effect experiments. J. Pharmacol. Exptl. Therap. 96: 99.

Morris, H.J. 1961. Instant bean powders. Proc. Fifth Ann. Dry Bean Research Conf., Denver, Colo., p. 4. USDA, ARS, WURDD, Albany, Calif.

Watt, B.K. and Merrill, A.L. 1963. "Composition of Foods." U.S. Dept. Agr. Handbook No. 8.

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EVALUATION OF A PROTEIN CONCENTRATE PRODUCED FROM GLANDLESS COTTONSEED FLOUR BY A WET-EXTRACTION PROCESS

INTRODUCTION

PROTEIN CONCENTRATES are currently finding utility as a protein ingredient in a wide variety of foods. The quantity of soy protein concentrate (by definition containing a minimum of 70% protein on a moisture-free basis) used for food in America in 1969 is reported to have been 33 million pounds (Hammonds and Call, 1970). These and other investigators predict an even brighter future for this type food ingredient because of its dual capability of providing important functional characteristics and good nutritional properties and its cost advantage over protein isolate. Finished products containing soy protein concentrates are employed principally in processed meat products, baked goods, breakfast cereals and dietary wafers (Meyer, 1971).

Protein concentrates from glandless cottonseed have better flavor than corresponding soy products and it is anticipated that a substantial market will exist for them since their cost of production should be comparable (Smith, 1971). Preparation of glandless cottonseed concentrates has been demonstrated to be feasible by either wet or dry processing procedures (Martinez et al., 1970). Each type process yields a concentrate that differs somewhat in composition and functionality.

A major deterrent to using a wet-extraction procedure has been the by-product liquid extract yielded. The utilization or disposal of this material has posed processing and/or pollution problems. Recently, such an extract from glandless cottonseed flour using the process under study was shown to contain a whippable substance with commercial potential as an egg white extender or substitute (Lawhon et al., 1972). This could greatly enhance the economic attractiveness of this protein concentrate process.

In the present study, wet-extracted concentrate spray dried at two pH levels was evaluated for use in protein-fortified bread and as a component in meat loaf to reduce juice and fat cook-out during baking and reduce meat requirements.

[1] Operated by the Texas Engineering Experiment Station for the Natural Fibers & Food Protein Committee of Texas.

EXPERIMENTAL

Wet extraction process

Protein concentrate was produced by extracting 10 lb of glandless cottonseed flour with tap water adjusted to pH 4, the point of minimum protein solubility, with either hydrochloric or phosphoric acid. The flour-water mixture was continuously stirred at 25°C for 40 min (6:1 solvent to solids ratio by weight). The pH was readjusted with 85% phosphoric acid until stabilized. The slurry was then separated with a continuous centrifuge producing 1800 gravities into a liquid extract containing a solubilized whipping substance and a solids residue. The solids residue was subsequently remixed with tap water at pH 4 (2:1 solvent to solids ratio based on original weight of flour) until uniformly dispersed and recentrifuged to remove liquid extract not separated initially.

The solids residue (protein concentrate) was divided into two portions for spray drying. One portion was spray dried "as is" (pH 4.5 ca) and another was adjusted to pH 6.8 and spray dried. 1N NaOH was used for pH adjustment.

The spray dryer used was a co-current, electrically-heated, cone-bottom type which utilized a two-fluid nozzle for feed atomization. Inlet air temperature was held at 300–310°F and outlet air temperature at 185–200°F during drying. Processing methods used to prepare the glandless cottonseed flour extracted are described in a subsequent section.

Nitrogen solubility profiles

Nitrogen solubility was determined at pH 3, 4, 5, 7 and 9 on the parent flour and on spray-dried and freeze-dried protein concentrates using the following modification of a method by Lyman et al. (1953):

(a) 1g of dry product was dispersed into 100 ml of water adjusted to pH of measurement.
(b) After stirring 5 min, the mixture was readjusted to the original pH. After an additional 5 min of stirring, the pH was rechecked and adjusted if necessary.
(c) The mixture was heated for 30 min in a 37.5°C water bath and then shaken for 30 min at 25°C.
(d) The mixture was then centrifuged at 2000 rpm for 20 min, filtered, and a 70-ml aliquot removed for determining soluble N by standard procedure (AOCS, 1971).

Analytical procedures

The amino acid analysis of protein concentrates and cottonseed flours used in the study (with the exception of tryptophan and cystine) was quantitatively determined by the procedure developed by Spackman et al. (1958). Tryptophan was determined by the method of Kohler and Palter (1967). Cystine was measured using a modification of the procedure by Schram et al. (1954).

Samples were hydrolyzed for determination of all amino acids except cystine and tryptophan in constant-boiling HCl for 24 hr under a nitrogen flush. Procedures for preparing protein hydrolysate for cystine and tryptophan are specified in the methods cited.

Moisture, oil, nitrogen, crude fiber, ash and free and total gossypol were determined according to standard AOCS methods (AOCS, 1971). Sugars were determined by a standard AOAC procedure (AOAC, 1965).

Baking protein-fortified breads

The utility of wet-processed protein concentrate for protein enrichment of wheat flour breads was tested in a wheat flour-concentrate blend proportioned to yield 17.5% protein on a 14% moisture basis. Based on previous measurements by Rooney et al. (1972), this level of protein in the blend increases the protein in the bread by more than 20%.

Experimental design and materials preparation. A randomized complete-block design was followed in conducting baking trials. Seven treatments consisting of six cottonseed materials plus an all-wheat flour control were included in the experimental design. One complete block of seven treatments (one loaf per treatment) was baked on a given day. Three blocks of treatments were baked during the experiment.

Processing methods used to prepare cottonseed flours and concentrates compared in the baking trials and the treatment number assigned each product were as follows:

Treatment 1 was LCP glanded cottonseed flour which was prepared at the USDA Southern Marketing & Nutrition Research Div., New Orleans, La. The process has previously been described in the literature (Gastrock, 1968; Vix et al., 1971).

Treatment 2 was the glandless cottonseed flour from which the wet-process protein concentrate was produced. This flour was prepared from Rogers GL-7 glandless cottonseed. Coarse kernels conditioned to 150°F and 7.25% moisture were flaked with flaking rolls to 0.010 in. thickness and extracted with commercial hexane in a scale-model Crown solvent extractor at the Crown Iron Works pilot plant, Minneapolis, Minn. Extracted flakes ("marc") were desolventized at 150–160°F by indirect steam heat without sparge steam added. Flakes were comminuted with a Fitzpatrick comminuting machine to pass an 80 mesh sieve.

Treatment 3 was wet-process protein concentrate spray dried at pH 4.5.

Treatment 4 was wet-process protein concentrate spray dried at pH 6.8.

Treatment 5 was protein concentrate prepared from glandless cottonseed flour by air classification. This concentrate was prepared at the Oilseed Products Div. (OPD) pilot plant using an Alpine Multi-plex Laboratory Zigzag Classifier 100 MZR. The classifier yielded 67% of its discharge as a high-protein fraction and 33% as a low-protein fraction.

Treatment 6 was the glandless cottonseed

Table 1–Data on cottonseed flours and protein concentrates compared in bread baking experiments

Sample analyzed	Moisture %	Oil %	Protein (Nx6.25) %	Protein sol. %	Gossypol free %	Gossypol total %	Crude fiber %	Ash %	Total sugars %	Color readings %
LCP glanded cs. flour	4.7	1.3	66.25 (69.52)[a]	98.2	0.023	0.023	2.0	8.8	9.8	84.2
Glandless cs. flour used in wet proc.	6.9	1.1	59.75 (64.18)	99.3	0.017	0.017	2.2	6.9	13.2	86.3
Wet proc. prot. conc. dried at pH 4.5	5.6	1.4	70.19 (74.31)	97.1	0.001	0.001	2.8	7.5	0	78.5
Wet proc. prot. conc. dried at pH 6.8	6.1	1.4	69.00 (73.51)	97.4	0.005	0.005	2.8	11.1	0	70.3
Air classified prot. conc.	10.8	1.4	61.00 (68.35)	99.9	0.030	0.030	1.8	7.7	10.5	86.1
Glandless cs. flour used in air classif.	11.6	1.0	56.00 (63.35)	97.6	0.043	0.043	2.1	7.1	12.4	84.1

[a]Values in parenthesis are on a moisture-free basis.

flour from which Treatment 5 was produced. This flour was prepared from Watson GL-16 glandless cottonseed at the OPD pilot plant. Flakes rolled to 0.010 in. thickness were extracted with commercial hexane without prior conditioning. Desolventization was with warm air at 90°F. Dry, defatted flakes were comminuted with an Alpine Kolloplex Laboratory Mill 160Z to pass an 80 mesh sieve.

Treatment 7 was a strong gluten, straight grade, commercial wheat flour which was bleached, brominated and malted. The flour was used as a control in the experimental bakings. It contained 14% protein and 0.4% ash (14% moisture basis).

Baking procedure. Pound loaves of bread were baked by a straight-dough procedure using a baking formula previously published by Rooney et al. (1972). Doughs were mixed with a Model 120-A Hobart mixer in a McDuffy bowl. 1% of sodium stearoyl-2 lactylate (SSL) was added to each of the doughs except the control. After fermentation doughs were scaled to 540g., rested 20 min, moulded, proofed to height and baked for 30 min at 430°F in an electric reel-type oven. Loaf volumes after baking were determined by rape seed displacement. The loaves were weighed, stored overnight and measurements of crumb reflectance recorded.

A Photovolt Model 610 reflectance meter was used to measure lightness of crumb color. The meter was equipped with a green filter and calibrated against a magnesium oxide plate. Five observations were read for each bread loaf.

Dough mixing properties. A Brabender farinograph equipped with a 50g stainless steel bowl was used to study the mixing properties of doughs. Tests were conducted according to a standard AACC procedure (1970). Water absorption was taken as the amount of water required to center the curve on the 500 Brabender unit line. No SSL was added to doughs studied with the farinograph.

Color measurements on cottonseed materials. Objective measurments of lightness of color in dry protein concentrates and flours were made using a Model AC-1 Gardner Automatic Color-Difference Meter. Readings were taken using the L scale of the meter after standardizing it with a ceramic tile having a calculated L value of 77.3. Two readings were obtained on each material.

Evaluation of protein concentrate for use in meat loaf

Five sets of two meat loaves each were baked to test the utility of wet-process protein

Table 2–Amino acid analysis of cottonseed flours and protein concentrates used in bread baking

Amino acids	L.C.P. flour g/16gN	Glandless cottonseed flour for wet proc. g/16gN	Wet process protein conc. dried at pH 4.5 g/16gN	Wet process protein conc. dried at pH 6.8 g/16gN	Air classif. protein conc. g/16gN	Glandless cottonseed flour for air classif. g/16gN
Lysine	4.2	4.0	3.6	4.0	4.0	4.2
Histidine	2.7	2.6	2.8	2.9	2.8	2.8
Ammonia	1.9	1.8	1.8	1.9	1.9	1.8
Arginine	12.1	11.7	10.8	11.3	11.3	11.4
Tryptophan	1.4	1.5	1.4	1.5	1.5	1.5
Cystine	2.2	2.4	1.9	2.1	2.5	2.5
Aspartic acid	9.1	9.1	9.2	8.9	8.8	8.7
Threonine	3.1	3.2	3.2	3.1	3.1	3.2
Serine	4.2	4.3	4.4	4.4	4.2	4.0
Glutamic acid	21.1	20.5	20.0	19.7	20.0	19.7
Proline	3.7	3.8	3.9	3.7	3.9	3.8
Glycine	4.0	4.2	3.9	3.9	4.0	4.0
Alanine	3.7	3.9	3.9	3.9	3.7	3.7
Valine	4.4	4.5	4.9	4.9	4.4	4.4
Methionine	1.5	1.4	1.8	1.5	1.6	1.6
Isoleucine	3.0	3.1	3.4	3.2	3.0	3.1
Leucine	5.7	6.0	6.4	6.2	5.7	5.7
Tyrosine	3.1	3.3	3.4	3.4	3.3	3.0
Phenylalanine	5.7	6.0	6.3	6.2	5.3	5.3
Totals	96.6	97.2	96.6	94.4	95.0	94.2
Available Lysine	3.6	3.8	3.1	3.1	3.6	3.5

concentrate as a meat loaf ingredient. A formula developed by Vegetable Protein Products Dept., Swift Chemical Co., Oak Brook, IL 60521 was used in the baking trials. In each trial, a meat loaf containing 2 lb of good quality ground chuck beef was baked as the control loaf. In a second loaf, 25% of the meat was replaced by concentrate which has been partially rehydrated. Two levels of concentrate were tested with the quantity of water used in rehydration held constant. In trials with a lower level, 41.3g of concentrate and 124 ml of water replaced 1/2 lb of meat. The higher level of protein concentrate was ultimately chosen to maintain the protein level in the cooked loaf at a level equal to or higher than the level in the all-meat control. Data presented in Table 4 are based on tests at the higher level. Ingredients in control and test loaves were similarly mixed and baked in the same oven at 350°F for 1-1/4 hr. After baking, the loaves were removed from pyrex baking dishes and allowed to drain for 10 min on a rack over a collecting pan. Cook-out drained from each loaf and cook-out from corresponding baking dishes was poured into 250 ml glass graduated cylinders for measurement of fat and water phases. The cook-out in each instance was strained through a 20 mesh strainer lined with three layers of cheesecloth to remove small meat fragments. Concentrate included in the test loaf was prepared by freeze drying at pH 6.8.

Fat phases were analyzed for fat and moisture and volatiles content. Water phases and samples of control and test meat loaves were analyzed for fat, nitrogen and moisture and volatiles content. Each type loaf was weighed before and after baking.

Formal organoleptic tests including statistical analysis of control and test loaf scores were not conducted. However, samples from two sets of loaves were submitted on three consecutive days to an untrained taste panel of 10 judges for their responses to the two meat loaf types.

RESULTS & DISCUSSION

Properties and yield of protein concentrate from the wet process

Table 1 contains analytical data on the wet-process concentrate and other cottonseed materials compared with it in bread baking experiments. By the commonly accepted definition of a protein concentrate (i.e., 70% protein on a moisture-free basis) the LCP glanded material could more correctly be referred to as a protein concentrate. However, since the originators of the process refer to this product as a flour in the literature (Gastrock, 1968; Vix et al., 1971) this designation has been used throughout. Conversely, the air-classified material as prepared for these experiments fell slightly below the 70% protein level. Nevertheless, it has been referred to as a protein concentrate since it is produced by further processing of a cottonseed flour. Products containing 70% or more protein have been produced by this technique from flours prepared from a different variety of glandless cottonseed.

The data in Table 1 reflect changes in cottonseed flour induced by the wet-extraction process. Sugars, for example, were reduced to an insignificant amount. Removal of these carbohydrates and other soluble matter increased the protein content by approximately 10% on a dry basis. Ash, crude fiber and oil contents were increased. The higher ash content of concentrate dried at pH 6.8 is attributable to the addition of NaOH for pH adjustment. The absence of sugars in wet-processed concentrate is a notable difference between it and products produced by nonaqueous processes.

Wet-process concentrate in dry form was not as light in color as the other cottonseed materials as shown in Table 1. The color of a commercial soy protein isolate (Promine D) was read for use as a standard of reference. The soy isolate gave a reading of 80.1. Concentrate dried at pH 4.5 yielded a lighter-colored dry product than pH 6.8 concentrate. However, bread baked with pH 6.8 concentrate gave a higher crumb reflectance reading than pH 4.5 bread. This is attributed to the poor loaf volume and coarse-grained texture of pH 4.5 bread.

Comparison of amino acid compositions are possible using data in Table 2. Available lysine (i.e., that lysine with epsilon amino groups unbound) in the wet-process concentrate was somewhat lower than in its parent flour.

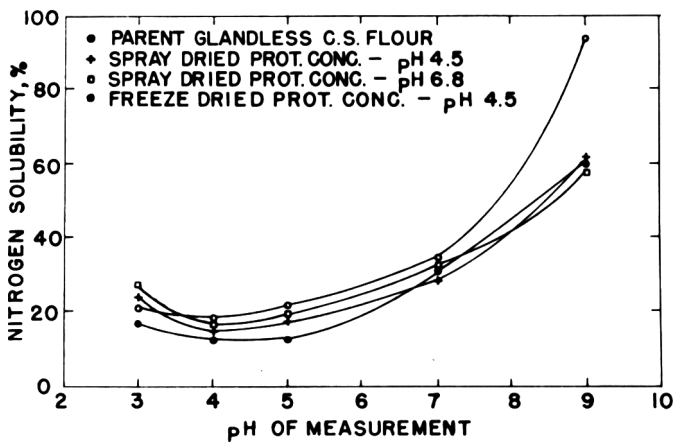

Fig. 1—Nitrogen solubility at different pH of measurement in wet-extracted protein concentrates and the parent glandless cottonseed flour.

Table 3—Properties of bread baked with wheat flour blended with cottonseed protein concentrate or cottonseed flour to 17.5% protein level[a]

Treatment no.	Materials	Material in blends[b] g	Loaf volume* cc	Specific loaf volume* cc/g	Crumb reflectance* %
1	LCP glanded cottonseed flour	7.6	2983.3[a]	6.80[a]	52.6[c]
2	GL-7 glandless cottonseed flour	8.5	2835.0[b]	6.37[b]	54.8[b]
3	Wet-proc. glandless protein conc. dried at pH 4.5	7.0	1900.0[c]	4.13[c]	50.1[d]
4	Wet-proc. glandless protein conc. dried at pH 6.8	7.1	2760.0[b]	6.11[b]	54.9[b]
5	Air classified glandless protein conc. from GL-16 glandless flour	7.8	3050.0[a]	6.89[a]	57.6[a]
6	GL-16 glandless cottonseed flour	8.7	2858.3[b]	6.27[b]	54.6[b]
7	Wheat flour control	0	3013.3[a]	6.79[a]	58.5[a]

[a]1% sodium stearoyl-2 lactylate added as dough conditioner

[b]Values are grams of cottonseed material required to make 100g of wheat flour-cottonseed material blend expressed on 14% moisture basis.

*Property values shown to be significantly different by range tests at the 5% level are indicated by different alphabetical superscripts.

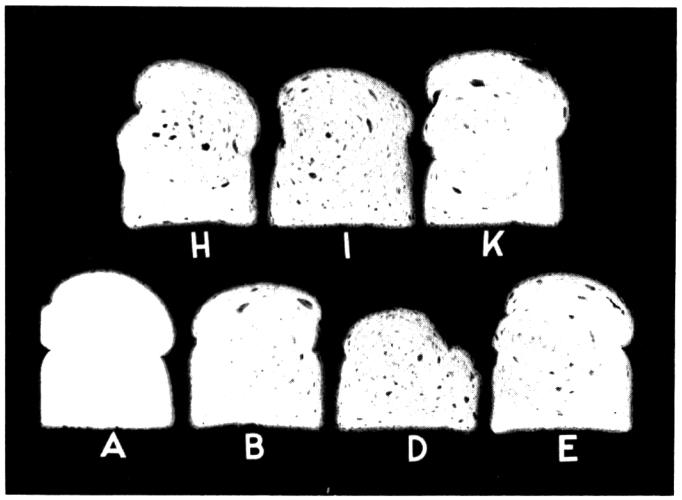

Fig. 2–Pound loaves of bread baked with blends of wheat flour and cottonseed protein concentrate or cottonseed flours at 17.5% protein level with 1% SSL added. Loaf A contains 100% wheat flour (Trt. 7); Loaf B contains GL-7 glandless cottonseed flour (Trt. 2); Loaf D contains wet-process protein concentrate dried at pH 4.5 (Trt. 3); Loaf E contains wet-process protein concentrate dried at pH 6.8 (Trt. 4); Loaf H contains LCP glanded cottonseed flour (Trt. 1); Loaf I contains GL-16 glandless cottonseed flour (Trt. 6); and Loaf K contains air-classified protein concentrate (Trt. 5).

Figure 1 shows the nitrogen solubility of the wet-process concentrate to have been changed substantially from that of the parent flour only at pH 9 or higher.

Yields of wet-process concentrate were calculated from multiple 400g bench-scale extractions and a single 10 lb laboratory extraction. The weight of concentrate produced equalled 58.5% of the weight of flour extracted. The concentrate contained 72.5% of the total nitrogen in the source flour.

Evaluation of breads enriched with protein concentrate

Table 3 shows properties of breads baked. Values given are treatment means from three replications. An analyses of variance (ANOVA) was performed on values obtained for each of the three properties shown.

Treatments were significantly different at the 1% level of significance for each of the three properties.

Duncan's new multiple range test was applied to compare among treatment means for each bread property. Property values shown to be significantly different by range tests at the 5% level are indicated in Table 3 by different alphabetical superscripts.

Loaves containing air-classified glandless concentrate, 100% wheat flour and LCP glanded flour were significantly larger in volume than loaves from other blends. There was no real difference among loaf volumes of breads containing these three treatments.

Perhaps, the most important observation from these data is the marked effect which pH of spray drying had on baking properties of the wet-process concentrate. The poor quality of bread baked with concentrate dried at pH 4.5 is quite apparent in Figure 2. Bread baked with the concentrate dried at pH 6.8 was equal in loaf volume to bread containing the glandless cottonseed flours. No attempt was made to determine the optimal pH of spray drying wet-processed concentrate to give the best loaf volume.

Range tests results on specific loaf volume means were identical with results from loaf volume means at the 5% level of significance. Test results of crumb reflectance, a measure of color lightness, showed the wheat control and air-classified protein concentrate to be significantly higher than a second group which again consisted of pH 6.8 wet concentrate, GL-7 flour and Gl-16 flour. LCP flour was darker in color than the second group but lighter than wet-process concentrate dried at the lower pH. The poorer color of LCP flour bread is attributed to the glanded cottonseed from which it was derived. The low reflectance of the pH 4.5 concentrate was caused by

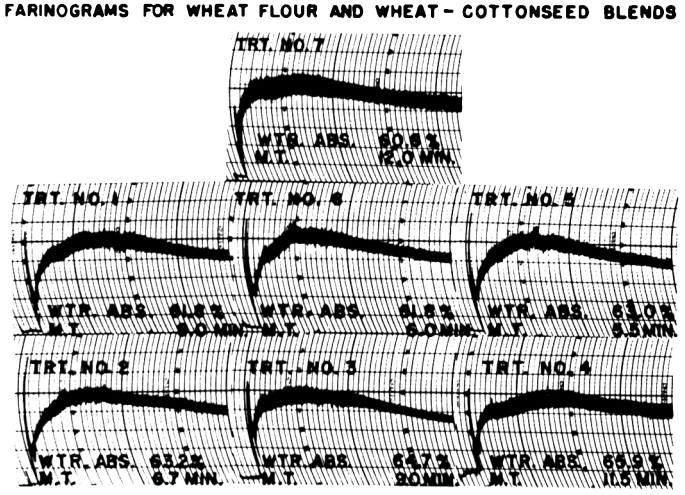

Fig. 3–Farinograph mixing curves of wheat flour-cottonseed protein concentrate and wheat flour-cottonseed flour blends at 17.5% protein level without SSL added. Water absorption and mixing tolerance times are shown. Control is 100% wheat flour.

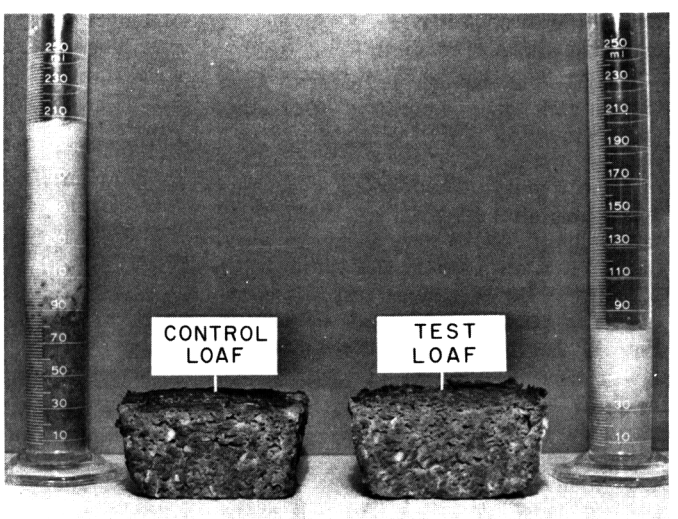

Fig. 4–Test and control meat loaves and the cook-out from each.

Table 4—Properties of meat loaves and liquids cooked out with and without wet-process protein concentrate in the loaves

Data description	Control loaf	Test loaf
Volumes and weights of cook-out:		
Fat phase volume, ml.	65.1	26.0
Fat phase weight, g.	53.8	20.3
Water phase volume, ml.	100.0	47.5
Water phase weight, g.	105.8	50.3
Total volume of cook-out, ml.	165.1	73.5
Total weight of cook-out, g.	159.6	70.6
Sample analyses:		
Fat phase		
moist. & volatiles, %	7.1	7.7
fat, %	92.9	92.2
Water phase		
moist. & volatiles, %	85.9	83.1
fat, %	2.6	2.4
protein (Nx6.25), %	5.1	7.1
Meat loaf		
moist. & volatiles, %	62.5	61.7
fat, %	12.6	12.4
protein (Nx6.25), %	19.7	19.6

its poor loaf volume and coarse-grained texture.

Mixing properties of doughs prepared from the wheat flour control and wheat-cottonseed blends without SSL added are reflected in the farinograms shown in Figure 3. The presence of cottonseed concentrates or flours in the doughs had the effect of increasing water absorption in all instances. The largest increases were in doughs containing wet-process concentrate and specifically the concentrate which was spray dried at pH 6.8.

Mixing tolerance or stability of the doughs as interpreted from each curve is also shown. Wet-process concentrate dried at pH 6.8 yielded the most stable dough of any of the blends. Dough containing this material was only slightly less stable than the control dough.

Evaluation of meat loaves containing protein concentrate

Figure 4 shows test and control meat loaves and their respective cook-outs. Data shown in Table 4 are from two sets of loaves baked from two batches of good quality ground chuck purchased approximately 1 wk apart at a commercial supermarket. The average weight of cook-out from control loaves was found to be 2.26 times the weight of test loaf cook-out. The cook-outs (as may be seen in Fig. 4) divided into a fat phase and a water phase. The fat phases from control loaf cook-outs averaged 2.67 times the weight of the fat phases from test loaf cook-outs. The control loaf water phases averaged 2.10 times the weight of test loaf water phases. It is apparent from the analytical data on the cook-out fractions in Table 4 that the test loaf water phase was higher in protein than the control loaf water phase. However, because of the lesser weight of the test fraction the overall loss of original protein in cook-out was reduced from 2.35% for the control loaf to 1.7% for the test loaf. The level of protein concentrate added to the test loaf was chosen to impart to it the protein level of the all-meat loaf. The moisture, fat, and protein contents of the two type loaves were essentially the same.

The consensus of the panel tasting sets of loaves on three consecutive days was that both loaves were quite acceptable with the test loaf being milder or more bland tasting than the all-meat loaf. The panel expressed a slight preference for the control loaf. It tasted more highly seasoned than the test loaf using the same formulation. The panel described the test loaf as being somewhat softer or smoother in texture.

Further investigations related to this work are contemplated in two specific areas:

(1) Preparation of wet-process concentrate spray dried at different pH to determine which pH gives optimal bread baking properties.
(2) Wet-extraction of different oilseed flours to produce protein concentrates which would then be blended to obtain a concentrate of enchanced quality. Oilseed flours extracted will be selected so the amino acid compositions of the concentrates produced will complement each other in a concentrate blend. The whippable extract is also enhanced in quality by synergistic effects of oilseed protein blends.

REFERENCES

AACC. 1970. "Approved Methods." American Association of Cereal Chemists, St. Paul, Minn.
AOAC. 1965. "Official Methods of Analysis," 10th ed. Association of Official Agricultural Chemists, Washington, D.C.
AOCS. 1971. "Official and Tentative Methods." 3rd ed. American Oil Chemists' Society, Chicago.
Gastrock, E.A. 1968. Edible flour from cottonseed by liquid classification using hexane. Presented at the Conference on Protein-Rich Food Products from Oilseeds, New Orleans, La. in May.
Hammonds, T.M. and Call, D. 1970. Publication A.E. Res. 320. Cornell University, Ithaca, N.Y.
Kohler, G.O. and Palter, R. 1967. Studies on methods for amino acid analysis of wheat products. Cereal Chem. 44: 512.
Lawhon, J.T., Cater, C.M. and Mattil, K.F. 1972. A whippable extract from glandless cottonseed flour. J. Food Sci. 37: 317.
Lyman, C.M., Chang, W.Y. and Couch, J.R. 1953. Evaluation of protein quality in cottonseed meals by chick growth and by a chemical index method. J. of Nutrition 49: 679.
Martinez, W.H., Berardi, L.C. and Goldblatt, L.A. 1970. Cottonseed protein products—Composition and functionality. Agr. & Food Chem. 18(6): 961.
Meyer, E.W. 1971. Oilseed protein concentrates and isolates. J. Am. Oil Chem. Soc. 48(9): 484.
Rooney, L.W., Gustafson, C.B., Clark, S.P. and Cater, C.M. 1972. Comparison of the baking properties of several oilseed flours. J. Food Sci. 37: 14.
Schram, E., Moore, S. and Bigwood, E.J. 1954. Chromatographic determination of cystine as cysteic acid. Biochem. J. 57: 33.
Smith, Allan K. 1971. Practical considerations in commercial utilization of oilseeds. J. Am. Oil Chem. Soc. 48(1): 38.
Spackman, D.H., Stein, W.H. and Moore, S. 1958. Automatic recording apparatus for use in the chromatography of amino acid. Anal. Chem. 30: 1190.
Vix, H.L.E., Eaves, P.H., Gardner, H.K. and Lambou, M.G. 1971. Degossypolized cottonseed flour—The liquid cyclone process. J. Am. Oil Chem. Soc. 48(10): 611.
Wilding, M.D. 1970. Oilseed proteins present utilization patterns. J. Am. Oil Chem. Soc. 57(10): 398.

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RESEARCH NOTES

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A Research Note

SPORE COUNTS OF THERMOPHILIC AEROBIC BACTERIA IN SOIL

INTRODUCTION

THE NUMBER OF spores of aerobic thermophilic spore-forming bacteria in soil is of interest to the canning industry. Spore counts were used by the National Canners Association as an index of the efficiency of washing asparagus (Mercer et al., 1960) and tomatoes (Mercer and Olson, 1969). Spores entering the finished product may come from various sources including soil, ingredients and processing plants. Only the latter two sources have received more attention than the first, which has not been investigated as much by the food microbiologist or by bacteriologists in general. The density of spores of thermophiles has not been studied in any detail nor have factors influencing sporulation in soil been investigated.

The data reported herein were obtained as part of our ecological studies with thermophilic aerobic spore-forming bacteria. Spore counts are correlated with organic matter and soil pH.

MATERIALS & METHODS

Soil collection

The upper 2–3 in. of soil were sieved (8 mesh stainless steel screen) and then placed in a plastic bag. These samples were air dried.

Soil was collected from the following locations: Hawaiian Islands (28 samples), Jamaica (29 samples), Majuro in the Marshall Islands, Moen and Fefan in Turk Lagoon (Eastern Caroline Islands) (17 samples), Muir Woods in California (11 samples), the Delta region in the Terminous-Lodi-Stockton, California area (9 samples) and in the Sonora Desert near Tucson, Arizona (19 samples). Yugoslavian soil samples (20 samples) were collected by Dr. George Smith of the University of Missouri, Columbia.

Agricultural as well as nonagricultural soils were collected in order to obtain a spectrum of fertility. The soils came from four large geographical areas based upon temperature: tropics, Micronesia; subtropics, Hawaii and Jamaica; desert, Arizona; and temperate, California and Yugoslavia.

Chemical analyses

The methods were those of Graham (1959) and Fisher (1968). Organic matter of California soils was determined in a muffle oven at 550°C.

Spore counts

Spore counts were made on the same samples as the chemical analyses. 10g of soil (in 100g of sterile water) were boiled for 30 min. The moisture lost during boiling was replaced with sterile distilled water. The samples were plated, (dextrose-tryptone agar) incubated (65°C for 24 hr) and counted.

Statistical analyses

The spore counts were converted into the log of the spore count + 1.0. This conversion permitted the inclusion of zero counts. Correlation coefficients were computed between spore counts, soil pH and % organic matter (Snedecor, 1956).

RESULTS & DISCUSSION

Temperate areas

Spore counts of soil collected in the Terminous-Lodi-Stockton area of California in which considerable asparagus is grown were not correlated with either soil pH or organic matter. The average spore count was 60,000 per g with a range of 2,000–212,000 per g. Even though the organic matter was high in these peat soils (18.2–69.0%), the number of spores was not as high as in Yugoslavian soils. The possible reason for this was the soil pH which ranged from 5.4–6.9 with a mean pH of 6.6.

In a forest environment (in Muir Woods National Monument), the soil from the forest floor had considerable organic matter; much of this was decomposing slowly because redwoods resist decay. The mean spore count of 85 per g (with a range of 0–400 per g) was considerably lower in this environment than the counts from asparagus fields in California. This low spore count was probably due to the low soil pH (range 5.3–6.1 with a mean of 5.8) and to the low temperature of the soil on the forest floor, which is shaded much of the day.

Organic matter and pH were positively correlated (r=0.45*) with spore counts of soil from Yugoslavia. The mean spore count was 373,000 per g with a range of 30–5,050,000 per g. Soil from Yugoslavia had the highest spore count of any soil tested. The mean pH of 7.2 of the soil was ideal for sporulation. Also, the pH range (6.3–8.2) spanned pH's which were good for sporulation to occur. The spore counts were positively correlated with soil pH (r=0.51*).

Subtropical areas

Soil pH was positively correlated with spore counts of Hawaiian soils (r=0.49**). The mean spore count for Hawaiian soils was 3,000 per g (with a range of 0–73,000 per g). There was a considerable range (4.2–8.4) in the pH of the soil. The more acidic soils were void of spores or were very low in numbers of spores.

The limited range of pH (6.3–8.1) with a mean of 7.5 in the Jamaican soils was also ideal for sporulation. However, neither pH nor organic matter (range of 1.0–7.5 with a mean of 4.1%) was correlated with spore counts (range of 82–27,000 with a mean of 2,000) in these soils.

Tropical areas

In order to obtain soil with a wide range of pH, alkaline samples from the Marshall Islands (pH 7.6–8.0) were compared statistically with samples from the Eastern Caroline Islands, which were more acid (pH 4.2–7.5). When this was done, soil pH was positively correlated with spore counts (r=0.92**). As with

the Hawaiian soils with acidic pH's, there were none or very few spores. The mean spore count for this area was 10,000 per g. However, if only the spore counts of the more acid soils from Eastern Caroline Islands are considered, the mean spore count was 250 per g.

Desert areas

Spore counts from the Sonora Desert in Arizona were positively correlated with pH (r=0.63*). The mean pH of 7.7 was good for sporulation to occur but the lower pH of 5.5 (range 5.5–8.3) was not. However, most pH's were greater than pH 7.0. The mean spore count was 1,000 spores per g with a range of 0–3,930.

All soils

When the data of all soil samples (133) were considered, pH was positively correlated with spore counts, (r=0.60**).

Organic matter is essential for growth of bacteria. According to Alexander (1961), *Bacillus* develop in large numbers only in the presence of suitable carbonaceous nutrients. If this were true for thermophiles, one would expect to find very high spore counts in the peat soil of California because of the very high organic matter (which furnishes both carbon and nitrogen). However, the soil pH ranged from 5.4–6.9. The more acidic soils tend to prevent the growth of such sporeformers as *B. stearothermophilus*. According to *Bergey's Manual* (Breed et al., 1957), only *B. stearothermophilus* grows at 65°C and at temperatures above. This species does not grow at pH 5.0. Wolf and Barker (1968), on the other hand, list type A of *B. coagulans* as being able to grow at 65°C at pH 6.2.

Ordal (1957) stated that pH for sporulation is similar to that for growth but the range is narrower. A strain of *B. coagulans* he studied produced more spores at pH 7.0 and produced very low spore yields at pH 6.0. Vinter (1969), too, stated that pH optimum for sporogenesis is close to pH 7.0.

Organic matter was positively correlated with spore counts in only the Yugoslavian soils. Even though the organic matter was high in many soils, the level of spores found was relatively low. From the data in this study, it appears that soil pH is much more important than the level of organic matter in determining if sporulation occurs or not.

REFERENCES

Alexander, M. 1961. "Introduction to Soil Microbiology." John Wiley and Sons, New York, N.Y.

Breed, R.S., Murray, E.G.D. and Smith, N.R. 1957. "Bergey's Manual of Determinative Bacteriology." The Williams & Wilkins Co., Baltimore, Md.

Fisher, T.R. 1968. "Soil Testing Procedures for Missouri County Soil Testing Laboratories." Dept. of Agronomy, University of Missouri, Columbia, Mo.

Graham, E.R. 1959. An Explanation of Theory and Methods of Soil Testing. University of Missouri Agricultural Experimental Station Bull. 734.

Mercer, W.A. and Olson, N.A. 1969. "Tomato Infield Washing Station Study." National Canners Assoc., Berkeley, Calif.

Mercer, W.A., Rose, W.W., Regier, L.W. and Chapman, J.E. 1960. "Better Washing of Asparagus to Improve Quality and Prevent Spoilage." National Canners Assoc., Berkeley, Calif.

Snedecor, G.W. 1956. "Statistical Methods." The Iowa State College Press, Ames, Iowa.

Ordal, J. 1957. The effect of nutritional and environmental conditions of sporulation. In "Spores," ed. Halvorson, H.O. Am. Inst. of Biol. Sci. Washington, D.C.

Vinter, V. 1969. Physiology and biochemistry of sporulation. In "The Bacteriol Spore," ed. Gould, G.W. and Hurst, A. Academic Press. New York.

Wolf, J. and Backer, A.N. 1968. The genus Bacillus: Aids to the identification of its species. In "Identification Methods for Microbiologists," ed. Gibbs, B.M. and Shapton, D.A. Academic Press. New York.

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A Research Note

NITROSAMINES AND THE INHIBITION OF Clostridia IN MEDIUM HEATED WITH SODIUM NITRITE

INTRODUCTION

PERIGO et al. (1967) and Perigo and Roberts (1968) described the formation of an inhibitor for the growth of several species of *Clostridium* during the sterilization of a culture medium in the presence of $NaNO_2$. They concluded that nitrite reacted with a component of the medium and the inhibitory activity differed from that of nitrite alone. The nature of the inhibitor is not known. It was further shown by Johnston et al. (1969) that in meat suspensions heated with $NaNO_2$ the inhibitory action was due to NO_2^-, although meat heated for 4 days at 80°C required less NO_2^- to inhibit *Cl. botulinum* than suspensions of meat not receiving the additional heat treatment. The extended heat treatment of the meat may have caused decomposition of the proteins with liberation of amino acids, peptides, and possibly, amines. Furthermore, the medium used by Perigo et al. contains yeast extract, beef extract and enzymic digests of meat thus making it rich in amines and amino acids. Nitrite reacts with amines and amino acids forming N-nitroso compounds, either N-nitrosamines or N-nitrosamides which are toxic and carcinogenic to animals and mutagenic to various species of microorganisms (Adelberg et al., 1965; Marquardt et al., 1963). We investigated the possibility that the "Perigo inhibitor" might be certain volatile N-nitroso compounds.

MATERIALS & METHODS

THE MEDIUM of Perigo et al. (1967) was used at pH 7.0 to reduce possibility of interference by HNO_2 at lower pH levels. Tryptone, peptone, yeast extract, and beef extract were obtained from Difco Labs. Sodium nitrite was added to tubes of medium so that, by doubled dilution, levels ranging from 640 to 1.25 ppm were obtained. In one set of conditions the NO_2^- was autoclaved in the medium at 15 psig for 15 min; in a second set of conditions the medium and NO_2^- solutions were autoclaved separately, then mixed aseptically. *Cl. botulinum B1218* (USDA culture) was grown at 35°C for 24 hr in thioglycollate medium (Difco), diluted 10-fold with water, and one drop added to each tube containing 4 ml of the Perigo medium. The medium was overlayered with Vaspar and gentle heating used to drive out excess air. The tubes were incubated at 35°C for 18 hr and the minimum inhibitory concentration (MIC) taken as the tube in which there was no visible growth or gas production.

To analyze for the presence of nitroso compounds a batch of medium was autoclaved at 15 psig for 20 min, NO_2^- added at 640 ppm and the whole re-autoclaved at 6 psig for 20 min. 200 ml of heated culture medium was extracted 2× with 200 ml each time of CH_2Cl_2 after saturation with NaCl. The combined extracts were treated with anhydrous Na_2SO_4 and concentrated to 5 ml in a Kuderna-Danish concentrator using a steam bath. The solution was washed 2× with 5 ml each 0.1M HCl to remove some interfering compounds, dried with anhydrous Na_2SO_4 and concentrated to 0.5 ml. The concentrate was analyzed for certain volatile nitrosamines by gas chromatography, using a Varian Model 1740 instrument equipped with two 9 ft × 1/8 in. OD stainless steel columns packed with 15% Carbowax 20M-TPA on 60–80 Gas-Chrom P. The standard flame ionization detector was modified for use as an alkali flame ionization detector as described by Howard et al. (1970). Flow conditions were: helium, 58; hydrogen, 45; and air, 188 ml/min. Injector port and detector temperatures were 190° and 250°C, respectively. Column temperature was programmed from 115–185°C at 4°/min.

Confirmation of nitrosamines was carried out with the same gas chromatograph connected to a DuPont Model 21-492 mass spectrometer. The GC column was maintained at 115° and injection port and detector temperatures at 200° and 230° respectively. The column effluent was split 1:1 and passed into the mass spectrometer via an inlet line heated to 200°C. The mass spectra were obtained at an ionizing voltage of 70 ev and an ion source temperature of 200°C.

RESULTS & DISCUSSION

THE RESULTS OBSERVED on growing the *Cl. botulinum* in the nitrite-treated media confirmed the observations of Perigo et al. (1967) and Perigo and Roberts (1968). The MIC of NO_2^- in the medium autoclaved with NO_2^- was 40 ppm; in the medium to which NO_2^- had been added aseptically, the MIC was 640 ppm. An inhibitor of the growth of clostridia was formed under the conditions described.

An extract of a batch of medium prepared with 640 ppm NO_2^- (subsequent testing with *Cl. botulinum* showed the MIC was 10 ppm) was examined for the presence of nitrosamines. The chromatogram in Figure 1 shows peaks with retention times similar to those for N-nitrosodimethylamine, N-nitrosomethylethylamine, and N-nitrosomethylpropylamine. However, structure confirmation with the mass spectrometer showed that none of these components contained a nitrosamine.

The inhibitory activity of a number of nitrosamines against *Cl. botulinum* was examined. The following nitroso compounds were placed into solution (200 mg in 10 ml water) and sterilized by Seitz filtration: N-nitrosodimethylamine (DMNA), N-nitrosodiethylamine, N-nitrosoproline, N-nitrosomorpholine, N-methyl-N-nitrosoethylamine, N-nitrosodiethanolamine, 1-nitrosopiperidine, N-nitrosodiphenylamine, N-methyl-N-nitrosoaniline and N-nitrosomethylurea. These nitroso compounds were prepared and purified in the laboratory. All preparations except the last three were added to the culture medium, at pH 7.0, to give solutions containing 10, 100 and 1000 ppm nitroso compound. The last three compounds were too insoluble so saturated solutions were prepared and two $\log_{10}$ dilutions made. Inoculation

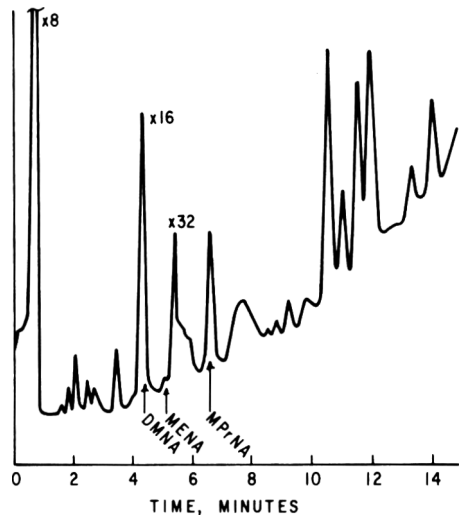

Fig. 1—Gas chromatogram of the extract of culture medium. Arrows indicate retention times for N-nitrosodimethylamine (DMNA), N-nitrosomethylethylamine (MENA), and N-nitrosomethylpropylamine (MPNA).

and incubation were as described. None of the nitroso compounds showed inhibitory action against *Cl. botulinum* even at the highest concentration tested.

The "Perigo inhibitor" does not appear to be a nitrosamine detectable under the experimental conditions used, nor do the several nitrosamines tested appear to be inhibitory to the growth of clostridia.

REFERENCES

Adelberg, E.A., Mandel, M. and Chen, G.C.C. 1965. Optimal conditions for mutagenesis by N-methyl-N′-nitro-N-nitrosoguanidine in Escherichia coli K12. Biochem. Biophys. Res. Commun. 18: 788.

Howard, J.W., Fazio, T. and Watts, J.O. 1970. Extraction and gas chromatographic determination of N-nitrosodimethylamine in smoked fish. Application of smoked nitrite-treated chub. J. Assoc. Offic. Anal. Chem. 53: 269.

Johnston, M.A., Pivnick, H. and Samson, J.M. 1969. Inhibition of Clostridium botulinum by sodium nitrite in a bacteriological medium and in meat. Can. Inst. Food Technol. J. 2: 52.

Marquardt, H., Zimmerman, F.K. and Schwaier, R. 1963. Nitrosamides as mutagenic agents. Naturwissen. 50: 625.

Perigo, J.A. and Roberts, T.A. 1968. Inhibition of clostridia by nitrite. J. Food Technol. 3: 91.

Perigo, J.A., Whiting, E. and Bashford, T.E. 1967. Observations on the inhibition of vegetative cells of Clostridium sporogenes by nitrite which has been autoclaved in a laboratory medium, discussed in the context of sub-lethally processed cured meats. J. Food Technol. 2: 377.

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Reference to brand or firm names does not constitute endorsement by the U.S. Dept. of Agric. over others of a similar nature not mentioned.

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A Research Note

SOME REACTION PRODUCTS FROM NONENZYMATIC BROWNING OF GLUCOSE AND METHIONINE

INTRODUCTION

ARROYO AND LILLARD (1970) recently reported the reaction products from nonenzymic browning of glucose and the sulfur-containing amino acids, methionine, cysteine and cystine. The cystine-glucose system yielded no significant odor, and the cysteine-glucose mixture gave an overcooked egg odor which was attributed to the presence of H_2S. The reaction mixture from glucose and methionine exhibited a characteristic boiled potato-like odor. On the basis of their data and observations, they attributed the odor to mercaptans. However, this conclusion contradicts previous literature, and results obtained in this laboratory. The heating of glucose with amino acids initiates browning reactions which yield a variety of intermediates including dicarbonyl compounds, such as pyruvaldehyde (Hodge, 1967). Dicarbonyls are then available to participate directly in Strecker degradations of amino acids. In the case of methionine the expected primary Strecker degradation product is methional (3-methylmercaptopropanal). The odor of this compound has been described as boiled potato-like. It also has been referred to as brothy, cheesey and cabbage-like, depending on available odor concentrations and chemical purity. Although Arroyo and Lillard (1970) observed a potato-like aroma from heated glucose-methionine mixtures, they did not isolate methional. In addition, these workers reported the identification of propanal in the methionine-glucose mixture, and stated that it was an expected Strecker degradation compound from methionine. This communication relates the results of a reexamination of the carbonyl products of the glucose-methionine heat induced browning reaction.

EXPERIMENTAL

DL-METHIONINE (Aldrich Chemical Co.) was twice recrystallized from a water-ethanol system to yield an odor-free dry crystalline material. Equimolar amounts (0.01M) of glucose (Allied Chemical) and methionine were dissolved in distilled water. Aliquots of the stock solution were heated in a boiling water bath for 2 hr, or were refluxed with continuous boiling for 2 hr. After cooling, 100-ml samples of the reaction mixtures were reacted for 12 hr with equal volumes of saturated 2,4-dinitrophenyl-hydrazine (2,4-DNP) solution in 5.0N HCl. The mixtures were exhaustively extracted with carbonyl-free hexane (Hornstein and Crowe, 1962) that subsequently was removed under vacuum after drying over anhydrous sodium sulfate. Magnesia adsorption columns (Schwartz et al., 1962) were employed to separate the monocarbonyl derivatives into characteristically colored bands which were collected, and each class was verified by measuring the wavelength of maximum absorption (Day, 1965). Class fractions were separated into individual components by the thin-layer chromatography (TLC) procedure of Badings and Wassink (1963). This technique employs a Keiscl-guhr G support coated with Carbowax 400, and the system is developed with light petroleum ether (bp 100–120°C). After development, plates were sprayed with 0.25M ethanolic NaOH to produce confirmatory spot colors.

Additional aliquots of the stock glucose-methionine reaction mixtures were employed for odor assessments by four experienced judges. Series of samples were prepared with reaction mixtures and authentic methional. Appropriate samples were combined with equal volumes of (a) 3% $HgCl_2$ to precipitate mercaptans and sulfides; (b) saturated 2,4-DNP in 5N HCl to remove carbonyls; and (c) distilled water to serve as a control. All samples were then shaken slowly for 1 hr prior to evaluation. The 2,4-DNP treated samples were neutralized with 5N NaOH to remove HCl before odor assessment. In the case of $HgCl_2$-treated samples, representative samples were acidified with equal volumes of 4N HCl, and shaken for an additional 30 min to regenerate mercaptides before final evaluations.

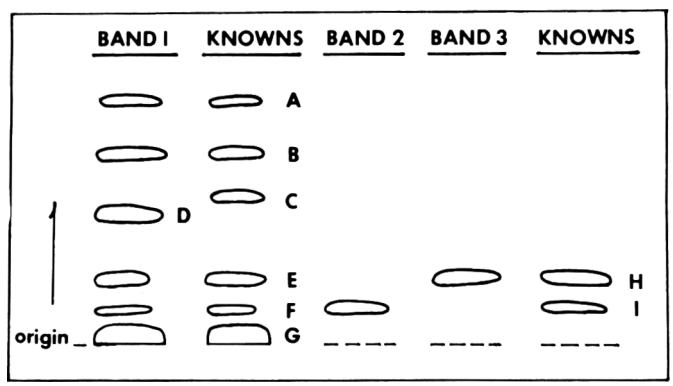

Fig. 1—TLC separation of reaction mixture 2,4-DNPHs from a magnesia adsorption column. Identification of spots: A, acetone; B, butanal; C, propanal; D, unknown; E, ethanal; F, methanal; G, methional; H, furfural; and I, 2-propenal.

Table 1—Characteristics of 2,4-DNPHs found in glucose-methionine reaction mixtures

2,4-DNPH	Figure ref.	λmax $CHCl_3$ (nm)	TLC spot color
Acetone	A	363	Tan
Butanal	B	358	Brown
Unknown	D	250[a]	Pastel blue
Ethanal	E	356	Brown
Methanal	F	346	Brown
Methional	G	355	Dark brown
Furfural	H	376	Maroon
2-Propenal	I	366	Rust red

[a]Also λ_{max} $CHCl_3$ at 770 nm

Table 2—Odor evaluations of methionine-glucose browning reaction mixtures[a]

Sample	Odor
Heated glucose-methionine	Cabbage-like, Boiled potato-like
Heated glucose-methionine + $HgCl_2$	Odor eliminated
Heated glucose-methionine + $HgCl_2$ treated with HCl	Cabbage-like, Boiled potato-like Odor regenerated
Heated glucose-methionine + 2,4-DNP	Cabbage-like, dimethyl disulfide-like

[a]Authentic 0.5 ppm methional samples behaved identically to reaction mixtures, except the cabbage-like odor was not present.

RESULTS & DISCUSSION

THE SIMMERED and refluxed reaction mixtures both exhibited a characteristic cabbagey, boiled potato-like aroma, but the development of brown pigment was apparent only in the refluxed sample. The refluxed samples had much more pronounced potato-like odors, and later were observed to contain larger amounts of 2,4-DNPHs. Only three bands representing alkanals, alk-2-enals and furfural 2,4-DNPHs, were obtained from the magnesia adsorption column. TLC separations (Fig. 1) showed that acetone and an unidentified component moved with the alkanal fraction. Acetone has been proposed as a product of nonenzymatic browning (Bavisotto and Roch, 1960). Methional was estimated to be the most abundant alkanal derivative present followed in decreasing order by ethanal, methanal, butanal and acetone. Band 2 from the magnesia column contained only 2-propenal (acrolein) and band 3 contained furfural. Table 1 presents confirmatory spectral and TLC spot color data for the carbonyls recovered from the reaction mixtures.

Contrary to the reports of El'odé et al. (1966) and Arroyo and Lillard (1970), propanal was not found in the reaction mixtures. Instead abundant quantities of 2-propenal were recovered in agreement with Ballance (1961) who reported 2-propenal as a methionine degradation product. The immediate precursor of 2-propenal is methional which is quite unstable. Upon rechromatographing TLC purified methional 2,4-DNPH, a second spot corresponding to the 2-propenal 2,4-DNPH was observed.

The results of odor evaluations are summarized in Table 2. The absence of a distinguishable odor after $HgCl_2$ additions demonstrated that sulfur compounds were more important than the nonsulfurous carbonyl compounds, and that these carbonyls contributed very little to the aroma. Arroyo and Lillard (1970) isolated and regenerated mercaptide derivatives, and with gas chromatography (GC) characterized methyl, ethyl and propyl mercaptan along with dimethyl sulfide and dimethyl disulfide. Methional, a sulfide, was not observed. Low GC operating temperatures, 48°C for a silicone rubber column and 58°C for a Carbowax column, allowed the detection of the more volatile mercaptans and sulfides, but prevented the elution and detection of methional. The elimination of the boiled potato-like odor with 2,4-DNP, while leaving a cabbage-like odor, indicates that mercaptans and sulfides could provide a modifying influence upon the overall flavor. However, the similar changes in odor characteristics experienced by authentic methional and reaction mixtures when treated with selective reagents, combined with the characterization of abundant methional 2,4-DNPH, provide strong evidence for the role of methional in the boiled potato-like aroma of methionine browning mixtures.

REFERENCES

Arroyo, P.T. and Lillard, D.A. 1970. Identification of carbonyl and sulfur compounds from nonenzymatic browning reactions of glucose and sulfur containing amino acids. J. Food Sci. 35: 769.

Badings, H.T. and Wassink, J.G. 1963. Separation and identification of aliphatic aldehydes and ketones by thin-layer chromatography of the 2,4-dinitrophenylhydrazones. Neth. Milk Dairy J. 17: 132.

Ballance, P.E. 1961. Production of the volatile compounds related to the flavor of foods from the Strecker degradation of DL-methionine. J. Sci. Food Agr. 12: 532.

Bavisotto, V.S. and Roch, L.A. 1960. Gas chromatography of volatiles in beer during its brewing, fermentation and storage. Proc. Am. Soc. Brewing Chem., p. 101.

Day, E.A. 1965. Some properties of carbonyl compounds encountered in the flavor isolates of dairy products. A review. Food Technol. 19: 1585.

El'odé, K.E., Dornseifer, T.P., Keith, E.S. and Powers, J.J. 1966. Effects of pH and temperature on the carbonyls and aromas produced in heated amino acid-sugar mixtures. J. Food Sci. 31: 351.

Hodge, J.E. 1967. Origin of flavor in foods. Nonenzymatic browning reactions. In "The Chemistry and Physiology of Flavors," p. 465. Avi Publishing Co., Westport, Conn.

Hornstein, I. and Crowe, P.E. 1962. Carbonyl removal from nonoxygenated solvents. Anal. Chem. 34: 1037.

Schwartz, D.P., Parks, O.W. and Keeney, M. 1962. Separation of 2,4-dinitrophenylhydrazone derivatives of aliphatic monocarbonyls into classes on magnesia. Anal. Chem. 34: 669.

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A Research Note

STABILITY OF INOSINIC ACID, INOSINE AND HYPOXANTHINE IN AQUEOUS SOLUTIONS

INTRODUCTION

MUCH ATTENTION has been devoted to the changes of 5′ribonucleotides especially in meat and vegetables during storage and different technological procedures (Davídek and Khan, 1968; Jones and Murray, 1962; Khan et al., 1968; Schormüller and Grosch, 1967). Davídek and Khan (1968) and Khan et al. (1968) found correlation between the amount of inosinic acid (IMP) and quality of poultry meat during frozen storage and storage at aseptic chilling conditions.

Postmortem 5′ribonucleotides are enzymatically degradated and the rate of this reaction is given by enzymatic activity of used muscle tissue. In frozen samples this activity increases with increasing damage of cells and subcellular membranes.

The changes of 5′ribonucleotides during thermal treatment of meat and meat products were studied by Macy et al. (1970a,b). During the heating of meat samples, concentration of adenylic acid (AMP) increased while that of IMP decreased. The increase in AMP is due most probably to the decomposition of residual amounts of adenosinetri-(ATP) and di-phosphate (ADP) present. The decrease of IMP concentration precipitates higher enzymatic activity during the beginning stage of heat treatment. Some experiments with the stability of purine compounds were done by Albert and Brown (1954).

Regarding the fact that the amount of IMP is in good correlation with the quality of meat, we were interested in the behavior of IMP and its degradation products in systems without enzymatic activity. For these studies we used model aqueous solutions of IMP, inosine and hypoxanthine.

EXPERIMENTAL

TESTS WERE MADE on solutions containing IMP (1 mg/ml) or inosine and hypoxanthine (2 mg/ml) in buffer solutions having pH values between 2–12 and ionic strength $I \leq 0.20$. All studies were made at 90°C. Estimation of IMP was carried out on Dowex 1 × 8 (formate, 200–400 mesh) column (1 × 4 cm) using 10 ml of test solution according to Davídek and Janícek (1971). The column was first eluted with water (50 ml) to separate the degradation product of IMP and then with HCl (4N, 15 ml) to separate IMP.

Inosine and hypoxanthine were separated on Dowex 50W × 4 (Na^+, 200–400 mesh) column (1 × 20 cm) using formic acid and sodium formate buffer [pH 3.0, $(Na^+) = 0.05$] as described by Davídek et al. (1971).

The amounts of IMP, inosine and hypoxanthine were determined by ultraviolet spectroscopy at 248 nm.

RESULTS & DISCUSSION

WE FOUND that IMP is degradated by a series of subsequent reactions of the first order, as illustrated in Figure 1.

In Figure 2 the dependence of logarithm of rate constants on pH is given. The fastest decomposition of all compounds studied was found in acidic medium. With increasing pH value the reaction rate of decomposition of IMP, inosine and hypoxanthine decreases (up to pH approximately 10) and then increases again. From results obtained in buffer solutions with different ratios of buffer components at constant pH value can be drawn the conclusion that these reactions are generally acid and base catalyzed. The catalytic effect decreases as follows:

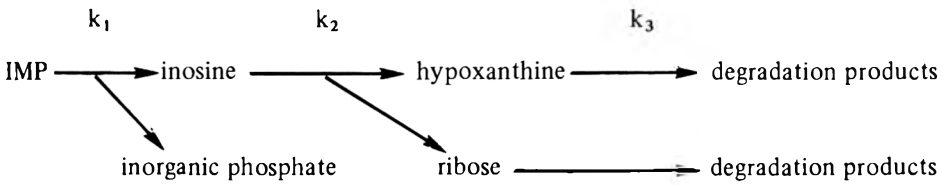

Fig. 1—Degradation of IMP by a series of reactions of the first order.

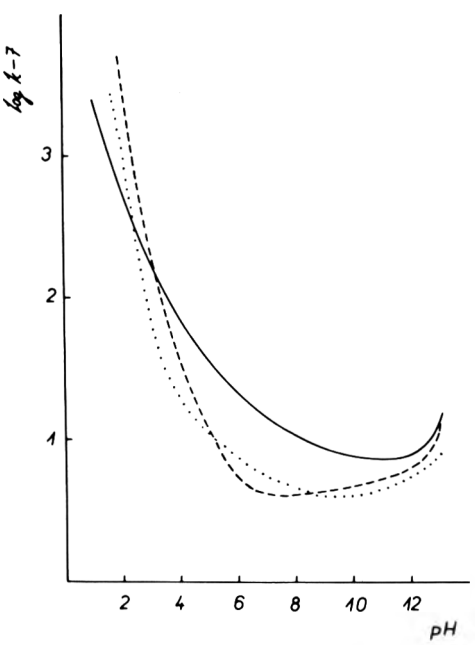

Fig. 2—Logarithm of rate constants plotted against pH: ——IMP; ···· inosine; ····· hypoxanthine.

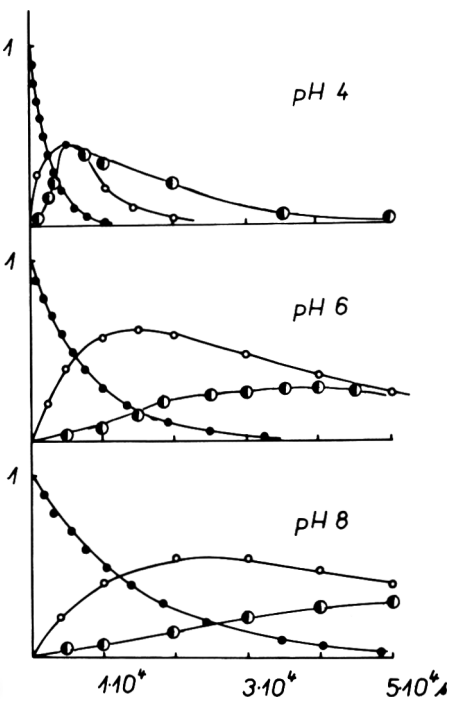

Fig. 3—Time dependence of degradation of IMP, inosine and hypoxanthine: • IMP; ○ inosine; ◉ hypoxanthine.

$H_3O^+ > OH^- > HA > A^- > H_2O$, where HA is a nondissociated weak acid and A^- its anion. The catalytic effect of H_3O^+ ions was approximately 100 times greater than OH^- ions.

No influence of ionic strength was found in acetate buffer solution (pH 4.0) up to 0.20.

The substituent in the 6 position of the pyrimidine cycle had no influence on reaction rate; the rate of decomposition of guanylic acid (GMP) was practically the same as that of IMP.

The influence of temperature on decomposition of IMP was studied in buffer solutions pH 3, 4 and 5 in the temperature region 60–98°C. The activation energy calculated was found to be 34.0, 30.4 and 28.1 kcal/mol, respectively.

The concentration of IMP, inosine and hypoxanthine are given by equations 1–3 (Zahradník, 1959).

$$[IMP] = [IMP]_0 e^{-k_1 \tau} \quad (1)$$

$$[INO] = [IMP]_0 \frac{k_1}{k_2 - k_1}(e^{-k_1 \tau} - e^{-k_2 \tau}) \quad (2)$$

$$[HX] = [IMP]_0 \cdot k_1 \cdot k_2 \left\{ \frac{e^{-k_1 \tau}}{(k_2 - k_1)(k_3 - k_1)} - \frac{e^{-k_2 \tau}}{(k_2 - k_1)(k_3 - k_2)} + \frac{e^{-k_3 \tau}}{(k_3 - k_2)(k_3 - k_1)} \right\} \quad (3)$$

where $[IMP]_0$ is concentration of IMP in time $\tau = 0$, [IMP], [INO] and [HX] are concentrations of IMP, inosine and hypoxanthine in time τ and k_1, k_2, k_3, are rate constants of decomposition of IMP, inosine and hypoxanthine, respectively.

The achieved results proved the high stability of IMP, inosine and hypoxanthine, especially in weakly acidic and neutral solutions (Fig. 3). From these results the conclusion can be drawn that after the inactivation of enzymatic systems the 5′ ribonucleotides, especially IMP are practically stable.

REFERENCES

Albert, A. and Brown, D.Y. 1954. Purine studies. Stability to acid and alkali, solubility, ionization, and comparison with pteridines. J. Chem. Soc. 2060.

Davídek, J. and Janíček, G. 1971. Bestimmung der Inosinsäure im Muskelgewebe. Z. Lebensm. Untersuch. u. -Forsch. 146: 1.

Davídek, J. and Khan, A.W. 1968. Estimation of inosinic acid in chicken muscle and its formation and degradation during postmortem aging. J. Food Sci. 32: 155.

Davídek, J., Velíšek, J., Grundová, K. and Janíček, G. 1971. Bestimmung von Nucleotiden, Nucleosiden und Basen der Nucleinsäuren in tierischen Geweben. Z. Lebensm. Untersuch. u. -Forsch. 146: 83.

Jones, M.R. and Murray, J. 1962. Degradation of adenine- and hypoxanthine-nucleotide in the muscle of chill-stored trawled cod. J. Sci. Food. Agr. 13: 475.

Khan, A.W., Davídek, J. and Lentz, C.P. 1968. Degradation of inosinic acid in chicken muscle during aseptic storage and its possible use as an index of quality. J. Food Sci. 33: 25.

Macy, R.L. Jr., Neumann, D.H. and Bailey, M.E. 1970a. Water-soluble flavor and odor precursors of meat. 3. Changes in nucleotides, total nucleosides and bases of beef, pork and lamb during heating. J. Food Sci. 35: 78.

Macy, R.L. Jr., Neumann, D.H. and Bailey, M.E. 1970b. Water-soluble flavor and odor precursors of meat. 4. Influence of cooking on nucleosides and bases of beef steaks and roasts and their relationship to flavor, aroma and juiciness. J. Food Sci. 35: 81.

Schein, A.H. and d'Angelo, Ch. 1969. Base analysis of RNA. Applications for RNA analysis of effects of heating purines and pyrimidine nucleotides with 1N HCl at 100°C for 1 hour. Anal. Biochem. 29: 323.

Schormüller, J. and Grosch, W. 1967. Nucleoside und heterocyclische Basen der Erbse (Pisum sativum). Mögliche Veränderungen während der enzymatischen Phase des Kochprozesses. Z. Lebensm. Untersuch. u. -Forsch. 134: 162.

Zahradník, R. 1959. Kinetické rovnice homogenních reakcí. Chem. listy 53: 56.

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A Research Note

DETERMINATION OF BROMINATED VEGETABLE OIL CONCENTRATIONS IN SOFT DRINKS USING A SPECIFIC ION ELECTRODE

INTRODUCTION

BROMINATED vegetable oils (BVO) have been widely used in the soft drink industry as "weighting agents" for flavoring oils. As a result of toxicity studies by the Canadian Food and Drug Directorate (1969), the Food and Drug Administration removed BVO from the list of Generally Recognized As Safe (GRAS) substances (Anonymous, 1970a) and established an interim usage level in soft drinks at 15 parts per million (ppm) (Anonymous, 1970b). At this time, it was considered desirable to develop a method for BVO estimation at low concentrations.

Previous work on BVO determination in soft drinks has employed gas-liquid chromatography and X-ray fluorescence spectroscopy (Conacher et al., 1970). For our purposes it was felt that a method determining total bromide would provide the best information. In addition, the method had to be suitable for multiple routine analyses. It was found that a debromination procedure (after Egli, 1969) used in conjunction with a bromide specific ion electrode gave acceptable quantitation. The results of this study are reported in this research note.

EXPERIMENTAL

Materials

All chemicals used were reagent grade. The titrations were carried out on a Corning model 12 pH meter (expanded millivolt scale) with a Beckman fiber junction calomel reference electrode and a Beckman bromide Selectlon^{TM} indicator electrode (solid state crystal type).

Procedure

Orange-flavored beverages containing 15 ppm BVO (brominated soybean oil, sp gr 1.3323, Br assay 39.5%, Swift and Co.) were prepared as test samples.

A 500-g sample of this beverage was extracted with 3 × 100-ml portions of diethyl ether, the extracts washed with water, and the solvent removed on a rotary evaporator. To the residue were successively added 10 ml dimethylformamide (DMF), 50 mg palladium on charcoal (Pd/C:10%) and 10 ml sodium borohydride solution (3g $NaBH_4$ in 100 ml 2N NaOH). The reaction mixture was refluxed 45 min and the volatile amine produced by the reaction was absorbed in aqueous HCl. The solution was cooled and the excess $NaBH_4$ destroyed with the addition of 20 ml acetone. Following this treatment, the solution was distilled at atmospheric pressure to remove 20 ml of distillate, which was discarded. Next, the cooled solution was filtered quantitatively (Whatman No. 1 paper) and diluted with 20 ml of 1N H_2SO_4 (total volume of filtrate about 100 ml, pH about 3.6).

Following cessation of effervescence, the sample was titrated with 0.0500N $AgNO_3$ and the titration monitored with a bromide Select-Ion^{TM} electrode. A salt bridge junction (4% agar in saturated KNO_3 soln) was employed to prevent the calomel reference electrode from being clogged with AgCl.

A "blank" sample was also treated according to the above method.

RESULTS & DISCUSSION

MILLIVOLTS graphed versus milliliters titrant gave a typical potentiometric titration curve for bromide ion. However, since the specific ion electrode potential proved unstable near the equivalence point and required 3–5 min to equilibrate, Gran's Plot Paper (Anonymous, 1970c) was employed for end point extrapolation (see Fig. 1). The use of this type of graph paper required very few data points, and these were taken before the equivalence point at 0.1–0.2 ml

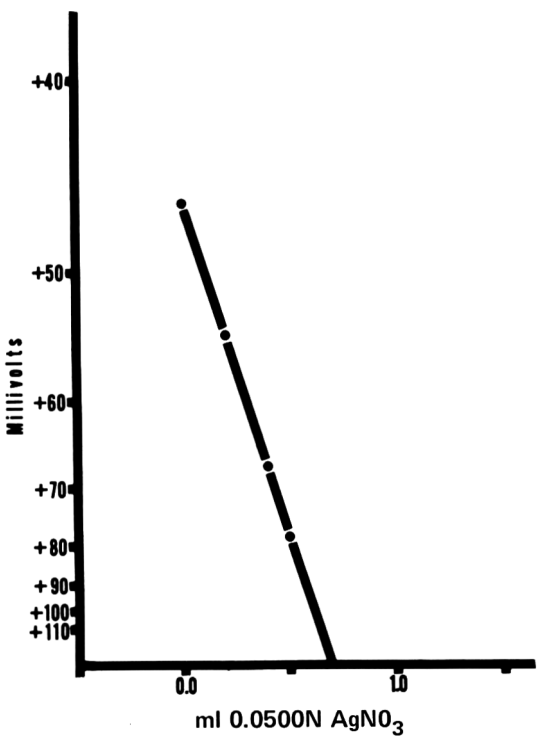

Fig. 1—Gran's Plot of the titration of a 15 ppm BVO beverage.

Table 1—Determination of BVO in a beverage

Sample no.	Grams beverage extracted	ppm BVO added	ppm BVO found	% BVO recovery
1	500	15	14	93
2	500	15	14	93
3	500	15	14	93

increments, thus eliminating the region of electrode instability.

Samples of the brominated soybean oil were treated according to the above procedure to determine the degree of reaction completion. The dehalogenation ranged from 95.1–96.1% (five samples), the average value being 95.6% ± 0.8.

At a level of 15 ppm BVO, the accuracy and precision of this method were $\pm < 1$ ppm and ± 1 ppm, respectively (see Table 1).

Information obtained from four BVO manufacturing companies indicated that the Br assay in various brominated oils (i.e., soybean, sesame, olive, etc.) of maximum specific gravity ranged from approximately 39.0–43.0%. Thus, BVO determined in commercial beverages by the above method should be assigned a range by applying this percent factor in the calculation:

$$\text{ppm} = \frac{(\text{mg } Br^- \text{ found})\,(1000)}{(\%\text{ dehalogenation})\,(\text{g beverage extracted})\,(\%\text{ Br assay})}$$

The BVO range of a 15 ppm beverage would be 14–16 ppm.

Since the initiation of this investigation on the quantitation of BVO in soft drinks, a recent article (Wendt, 1972) indicates that the present tolerance level of 15 ppm may be raised as the result of tests on BVO levels three times higher than the no-effect level of the Canadian Food and Drug Directorate. This author has tested BVO levels greater than 15 ppm, and they can also be determined by the procedure reported here. Levels less than 15 ppm were not tested.

Additional work still needs to be done on the beverages with BVO levels greater than 15 ppm and on brominated oils other than soybean.

REFERENCES

Anonymous. 1970a. Food additives. Brominated vegetable oils, removal from list of generally recognized as safe. Federal Register 35: 1049.

Anonymous. 1970b. Amending regulation—food additive. Federal Register 35: 12062.

Anonymous. 1970c. Specific ion electrode technology. Newsletter 11(11 and 12): 49. Orion Research Inc. Cambridge, Mass.

Canadian Food and Drug Directorate. 1969. Biochemical and pathological changes in animals fed brominated vegetable oil. Food Cosmet. Toxicol. 7: 25.

Conacher, H.B.S., Chadha, R.K. and Saharasbudhe, M.R. 1969. Determination of brominated vegetable oils in soft drinks by gas-liquid chromatography. J. Am. Oil Chem. Soc. 46: 558.

Conacher, H.B.S., Meranger, J.C. and Leroux, J. 1970. Levels of brominated vegetable oils in soft drinks by X-ray fluorescence and gas-liquid chromatography. J. Assoc. Off. Agric. Chem. 53(3): 571.

Egli, R.A. 1969. Neue Bestimmungsmethode für organisch gebundenes Jod, Brom und Chlor. Z. Anal. Chem. 247: 39.

Wendt, A.S. 1972. BVO. The Flavour Ind. January.

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A Research Note

EFFECT OF SONIC ENERGY ON THE AIR DRYING OF APPLE AND SWEET POTATO CUBES

INTRODUCTION

SONIC ENERGY has been shown to hasten the drying of particulate materials (Greguss, 1963; Purdy et al., 1971). Bartolome et al. (1969) found a three-fold sonic energy induced increase in the drying rate of white potato cylinders 3/8 in. in diameter and 1-1/4 in. long. Best results were obtained during the early drying stages using 120°F air, flowing at 9.1 fps (feet per second) with 8,100 Hertz sound at 118 dB, the maximum level for their equipment.

Sound energy was found to contribute little or no heat. These workers concluded that the increased drying rate possibly resulted from improved heat transfer and a pumping action by the sound waves in the food, causing water vapor to be brought to the surface more rapidly.

We attempted to confirm the catalytic effect of sound energy on increasing drying rates of piece-form foods and to develop a better understanding of the mechanism by which sound alters the rate of drying over a range of moisture contents.

EXPERIMENTAL

FIGURE 1 shows a diagram of the drying apparatus. Two types of pneumatic stem jet whistles (Branson Pneumatic Sound Generator, output 138 dB at 13,000 Hz; Astrobeam Model 461 Sonic Generator, output 140 dB at 9,800 Hz) and an electronically driven loud speaker (Model ID-60, University Loudspeakers operated at 135 dB at 8,100 Hz) were evaluated as sound sources. The pneumatic sound generators were isolated by a 0.0015 in. polyethylene membrane to prevent air from the whistle from passing over the samples.

A centrifugal fan provided electrically heated air at 5, 10 and 20 feet per second (fps) and 125°F, 150°F and 175°F in the 4 in. ID sample treatment section. This section was fitted with a pitot tube, microphone port and metal sample holder. The holder, which could be located 10-40 in. from the sound source, provided 5 stiff prongs on a horizontal line across the diameter of the tube. The center prong was a thermocouple to measure piece temperature. Cubes of food were held stationary on the prongs during drying. The prongs were stiff enough to prevent sample vibration by air movement.

Sound levels were measured and monitored during drying with a Dynasciences Model 614 Condenser microphone (Dynasciences Corp., Instrument Systems Div., Chatswourh, Calif.) and General Radio (General Radio Inc., Concord, Mass.) 1900A Wave Analyzer calibrated with a 1560-P Sound Level Meter. A microphone traverse along the test chamber showed the presence of standing waves. The sample holder was located at positions of maximum and minimum sound intensity in comparative runs.

Unblanched white potato, apple and yellow sweet potato were cut into 2 cc cubes using a jig. Drying rates were determined by weight loss versus time using air with an average moisture content of 0.0055 lb of water per lb of dry air. Replicate runs were made with and without sound energy with the sample holder located at the measured maximum intensity location. Three air velocities and three temperatures were used. Weight loss was measured using the average weight of 5 cubes for each point. New cubes were used for each drying interval.

RESULTS

SONIC ENERGY from each of the three sources used did not appear to affect the drying rate of unblanched white potato, apple, or yellow sweet potato cubes. Representative drying curves for apple and sweet potato for various treatment conditions are shown in Figures 2 and 3. White potato composition was found to vary too greatly from piece to piece and

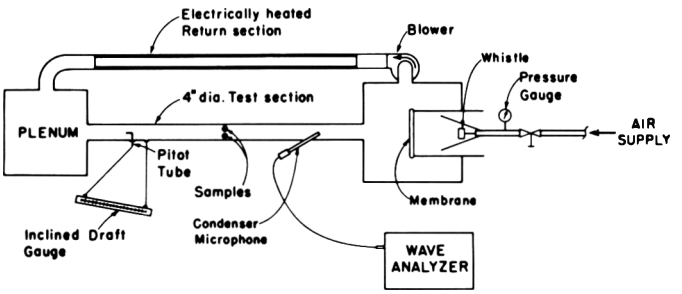

Fig. 1—Apparatus for sonic energy drying.

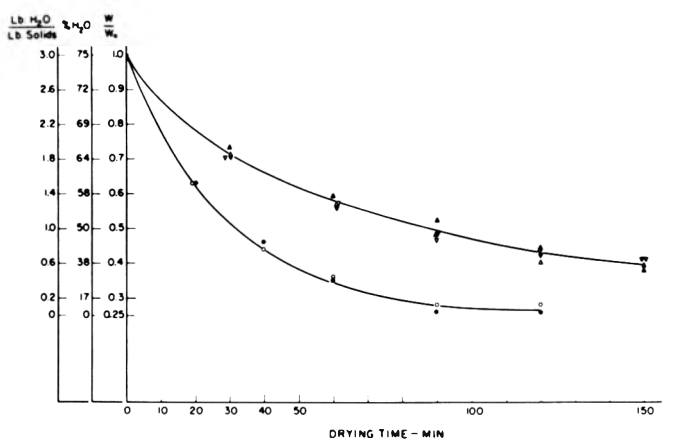

Fig. 2—Drying curves for unblanched 2 cc sweet potato cubes of 25% solids. Open symbols with sound (138 dB, 13,000 Hertz); solid symbols without sound: ●●● 175°F, 10 fps; ▲▲▲ 125°F, 5 fps; ▼▼▼ 125°F, 10 fps.

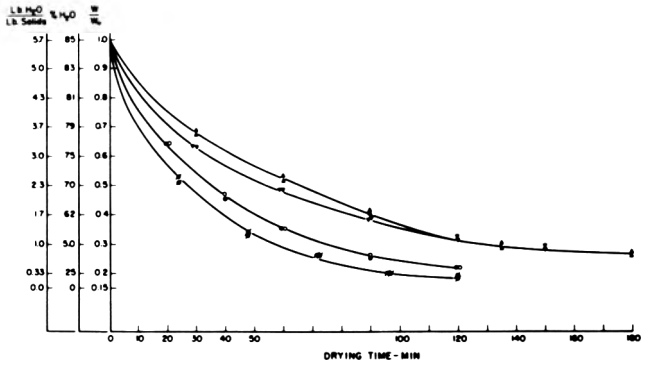

Fig. 3—Drying curves for unblanched 2 cc apple cubes of 15% solids. Open symbols with sound (138 dB, 13,000 Hertz); solid symbols without sound: ●●● 175°F, 5 fps; ◆◆◆ 175°F, 10 fps; ▲▲▲ 125°F, 5 fps; ▼▼▼ 125°F, 10 fps.

from tuber to tuber to allow use as a test material. The effects of air velocity and temperature on the drying rate of apple cubes are clearly illustrated in Figure 3. These curves indicate that the system had the precision to demonstrate small changes, if present, in drying rate due to sound. Increasing air velocity from 5 to 10 fps only increased the initial drying rate. Water vapor pressure at 125°F tends to make mass transfer the limiting step beyond 65% weight reduction. At 175°F an increase in air velocity affected drying rates down to 75% weight reduction. The higher vapor pressure of water required higher heat transfer rates to achieve a maximum drying rate. Sound frequency, intensity and source had no effect on these curves.

We concluded that sonic energy did not seem to affect drying rates in our experimental apparatus under drying conditions limited by heat or mass transfer in piece-form foods. Hoff (1970) explained that careful tuning of the sound source and sample were required. If this is true, then exposure of a sample at a measured high intensity sound level at a given point is not sufficient to obtain an increased drying rate. This would expain the difference in our results and those of Bartolome et al. (1969).

REFERENCES

Bartolome, L.G., Hoff, J.E. and Purdy, K.R. 1969. Effect of resonant acoustic vibrations on drying rates of potato cylinders. Food Technol. 23(3): 47.

Greguss, P. 1963. The mechanism and possible applications of drying by ultrasonic irradiation. Ultrasonics 1: 83.

Hoff, J.E. 1970. Personal communication and letter, 17 June 1970.

Purdy, K.R., Simmons, G.W., Hribar, A.E. and Griggs, E.I. 1971. Acoustically augmented convective drying. Chem. Eng. Progress Symposium Series on Sonochemical Engineering 67(109): 55.

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

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A Research Note

FILM OBSERVATIONS AT AN OIL-WATER INTERFACE

INTRODUCTION

CUMPER AND ALEXANDER (1950) proposed that film formation by proteins at air-water interfaces occurs in three stages: (1) diffusion to and adsorption at the interface; (2) surface denaturation by uncoiling of the protein molecule; and (3) protein coagulation. Formation of interfacial films by emulsifiers in oil-in-water emulsions may have a significant effect on the stability of the emulsion (Becher, 1965). Osipow et al. (1957) stated that interfacial films create an energy barrier against dispersed globule coalescence.

F. M. Ascherson in 1838 observed that on simple contact of oil with an aqueous protein sol, a protein skin formed at the interface (Bikkerman, 1958). Film formation at an oil-water interface has been reported to occur for a wide variety of substances: gum acacia (Shotton and White, 1963); tragacanth, saponin, sodium oleate and gelatin (Bikkerman, 1958); soy sodium proteinate, potassium caseinate and nonfat dry milk (Pearson et al., 1965), serum albumin (Cockbain and McRoberts, 1953) and salt-soluble proteins of muscle tissue (Swift et al., 1961; Helmer and Saffle, 1963; Meyer et al., 1964; Borchert et al., 1967).

This report describes an experimental technique which allows the researcher to substantiate and further investigate interfacial film formation at the oil-water boundary.

EXPERIMENTAL

VISUAL OBSERVATION and photographic records of the characteristics of interfacial films were conducted with a modification of a microscopic technique developed by Shotton and White (1963). A 60 mm diam petri dish was placed on the stage of an A.O. Spencer Model 635 Pathostar microscope equipped with a 35 mm camera. Aqueous solutions of substances tested for interfacial film formation were placed in the dish. A stationary 1 ml syringe containing corn oil and equipped with a flat-tip 24 gauge needle was used to introduce and age oil globules in the aqueous solution for 10 min. Following the procedure of Shotton and White (1963), the volume of oil was gradually decreased from the initial volume and evidence of positive film formation identified by a wrinkling and folding at the surface of the oil globule.

Proteinaceous substances tested were 1% salt-soluble protein of cow meat and beef hearts, 0.5% protein as sodium caseinate and 2.5% protein as soy sodium proteinate. Nonprotein substances examined were 1.0% propylene glycol alginate and 2.5% gum acacia.

RESULTS & DISCUSSION

INTERFACIAL FILM formation by salt-soluble protein of cow meat and beef hearts is shown in Figure 1a and 1b, respectively. Extensive folding of the film occurred at the interface when the oil globules were slowly reduced in volume. Several observers in our laboratory described the deformation as a "wrinkling" plastic bag effect. Similar results were obtained for sodium caseinate (Figure 1c) and gum acacia (Figure 1d). The film appearance of gum acacia is in agreement with that reported by Shotton and White (1963).

From photomicrographs of emulsions prepared using model systems, Swift et al. (1961) and Pearson et al. (1965) have shown that both meat salt-soluble protein and potassium caseinate encapsulate oil globules in the emulsion system. Both groups of workers attributed this formation to denaturation of protein at the oil-water interface. The presence of protein membranes in sausage emulsions have been recorded by Helmer and Saffle (1963), Meyer et al. (1964) and Borchert et al. (1967). Heat processing the emulsions resulted in rupture of the fat-encapsulating protein matrix (Borchert et al., 1967).

Soy sodium proteinate and propylene glycol alginate showed no evidence of film formation (Figure 1e and 1f, respectively). When oil droplets were slowly withdrawn from solutions of these substances, no folding or striations developed. Pearson et al. (1965) reported that in oil-in-water emulsions prepared with soy sodium proteinate, protein layers formed around oil globules when the solution was at pH 10.7 but not at pH 5.6. The pH of the soy sodium proteinate solution in this study was 7.6.

The ability to form interfacial films

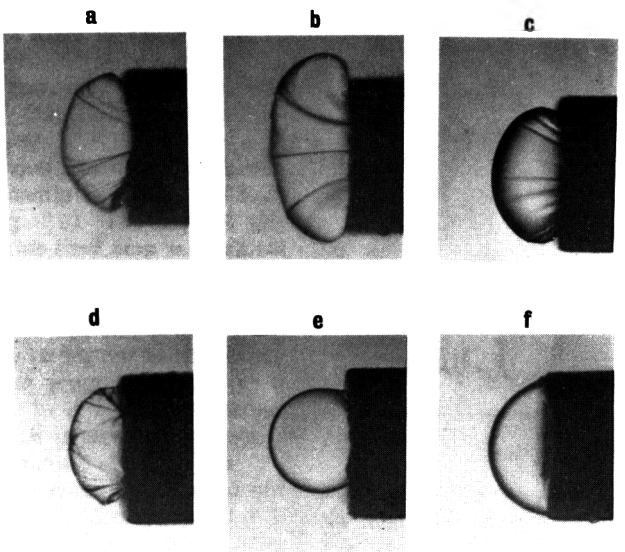

Fig. 1—Photomicrographs of oil globules suspended in solutions of (a) 1% salt-soluble protein of cow meat; (b) 1% salt-soluble protein of beef hearts; (c) 0.5% protein as sodium caseinate; (d) 2.5% gum acacia; (e) 2.5% protein of soy sodium proteinate; and (f) 1.0% propylene glycol alginate. (×100)

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between oil and water phases may contribute, as an additive factor, to overall stability of emulsions prepared with interfacially-active substances. The elasticity and mechanical strength of the films and the effects of pH, ionic strength and concentration of substance in interfacial film formation remain to be evaluated.

REFERENCES

Becher, P. 1965. "Emulsions: Theory and Practice," 2nd ed. Reinhold Publishing Corp., New York.

Bikkerman, J.J. 1958. "Surface Chemistry: Theory and Applications." Academic Press, New York.

Borchert, L.L., Greaser, M.L., Bard, J.C., Cassens, R.G. and Briskey, E.J. 1967. Electron microscopy of a meat emulsion. J. Food Sci. 32: 419.

Cockbain, E.G. and McRoberts, T.S. 1953. The stability of elementary emulsion drops and emulsions. J. Colloid Sci. 8: 440.

Cumper, C.W.N. and Alexander, A.E. 1950. The surface chemistry of proteins. Trans. Faraday Soc. 46: 235.

Helmer, R.L. and Saffle, R.L. 1963. Effect of chopping temperature on the stability of sausage emulsions. Food Technol. 17(9): 115.

Meyer, J.A., Brown, W.L., Giltner, N.E. and Guinn, J.R. 1964. Effect of emulsifiers on the stability of sausage emulsions. Food Technol. 18: 1796.

Osipow, L., Birsan, S. and Snell, F.D. 1957. Emulsion stability. J. Am. Oil Chem. Soc. 34: 34.

Pearson, A.M., Spooner, M.E., Hegarty, G.R. and Bratzler, L.J. 1965. The emulsifying capacity and stabilizing ability of soy sodium proteinate, potassium caseinate, and nonfat dry milk. Food Technol. 19: 1841.

Shotton, E. and White, R.F. 1963. Stabilization of emulsions with gum acacia. In "Rheology of Emulsions," ed. Sherman, P. The MacMillan Company, New York.

Swift, C.E., Lockett, C. and Fryar, A.J. 1961. Comminuted meat emulsions—The capacity of meats for emulsifying fats. Food Technol. 15: 468.

Ms received 6/2/72; accepted 7/9/72.

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A Research Note

BIOCHEMISTRY OF TEA FERMENTATION: FORMATION OF t-2-HEXENAL FROM LINOLENIC ACID

INTRODUCTION

TRADITIONAL black tea manufacturing processes cause fresh green tea leaf to undergo conversion to black tea (Millin and Rustidge, 1967; Eyton, 1972; Sanderson, 1972). This process makes use of endogenous tea leaf enzymes and mechanical manipulation of the tea leaf to effect this conversion to black tea in 2 to 6 hr. A most important part of the tea conversion process is the formation of the characteristic aroma of black tea. An improved understanding of the chemistry by which black tea aroma is formed is sought for application in improving black tea manufacturing processes.

Studies have already shown that certain black tea aroma constituents are formed from amino acids (Popov, 1956; Nakabayashi, 1958; Co and Sanderson, 1970; Saijo and Takeo, 1970a, b) and from carotenes (Sanderson et al., 1971). The mechanism proposed (Co and Sanderson, 1970; Sanderson et al., 1971; Sanderson, 1972) for these chemical changes comprises the oxidation of black tea aroma precursors by oxidized tea flavanols which are formed enzymically during tea fermentation. This mechanism suggests that other compounds present in fresh green tea leaf can, and do, serve as black tea aroma precursors. This hypothesis has now been further tested using linolenic acid as a possible black tea aroma constituent precursor.

EXPERIMENTAL

Materials

U-^{14}C-Linolenic acid was purchased from International Chemical & Nuclear Corp., Irvine, Calif. Unlabeled linolenic acid was supplied by Pfaltz & Bauer, Inc., Flushing, N.Y.

Fresh frozen tea leaf was obtained from experimental tea plantings near Charleston, S.C. The frozen leaf was cryogenically milled to a uniform particle size of about 0.5–1.0 mm^2 in a hammer mill.

Headspace volatile analysis by radio-chemical gas-liquid chromatography

The procedures adopted have been described by Co and Sanderson (1970). In all cases, 4.0g of dried tea, or the equivalent amount of tea leaf, were used in the analyses.

Preparation of black tea augmented with linolenic acid

Cryogenically milled fresh green tea leaf was sprayed with 0.5g linolenic acid containing 4μC: U-^{14}C-linolenic acid in 10 ml diethyl ether. Subsequently, the tea leaf was warmed to room temperature and allowed to undergo tea fermentation for about 3 hr. During fermentation, the tea leaf was covered with a damp cheesecloth to maintain high humidity around the leaf. The fermented tea leaf was dried in a forced-draft oven at 180°F for about 30 min to reduce the moisture content to about 3%. The dried product was black tea.

RESULTS

FRESH GREEN tea leaf was converted to black tea in the laboratory under conditions which simulated the traditional black tea manufacturing process except that radioactively labeled linolenic acid was added to the fresh tea leaf. Samples of the tea leaf were taken at different points in the black tea manufacturing process to be analyzed for the presence of radioactive volatiles by a GLC-headspace analysis system. The results (Fig. 1) show that linolenic acid is transformed to *t*-2-hexenal during the fermentation step of the black tea manufacturing process and that *t*-2-hexenal is the only volatile compound formed from linolenic acid during this process. The formation of only one volatile reaction product is surprising in that we would expect all of the double bonds in linolenic acid to undergo oxidative change: Perhaps the other possible products are nonvolatile with the result that they were not detected in our study.

More *t*-2-hexenal was found in the tea leaves after fermentation (Fig. 1a) than after firing (Fig. 1b). The loss of *t*-2-hexenal during this final step in black tea manufacture is undoubtedly due to volatilization during the drying process. This driving off of the "green" aroma notes may well be very important to the formation of black tea aroma inasmuch as it apparently leads to a balance of aroma constituents which is more characteristic of black tea (Yamanishi et al., 1966).

Control experiments were carried out which show that the formation of *t*-2-hexenal is dependent on enzymic changes since no *t*-2-hexenal was formed when the tea leaf enzymes are inactivated by steaming. However, the experiments carried out do not allow us to choose between the possibility that a specific enzyme acted directly on linolenic acid or whether linolenic acid was oxidatively degraded by the action of oxidized tea flavanols.

Linolenic acid is quantitatively the most important fatty acid in tea leaf (Dr. L.J. Dukker, personal communication) and *t*-2-hexenal is one of the major constituents of tea aroma (Bondarovich et al., 1967; Müggler-Chavan et al., 1966; Takei et al., 1937, Van Romburgh, 1920; Yamanishi et al., 1965). Further, *t*-2-hexenal has a decided grassy odor which must have an effect on the overall flavor impression of a food when it is present. Accordingly, the formation of *t*-2-hexenal from linolenic acid during the manufacture of black tea must be of some

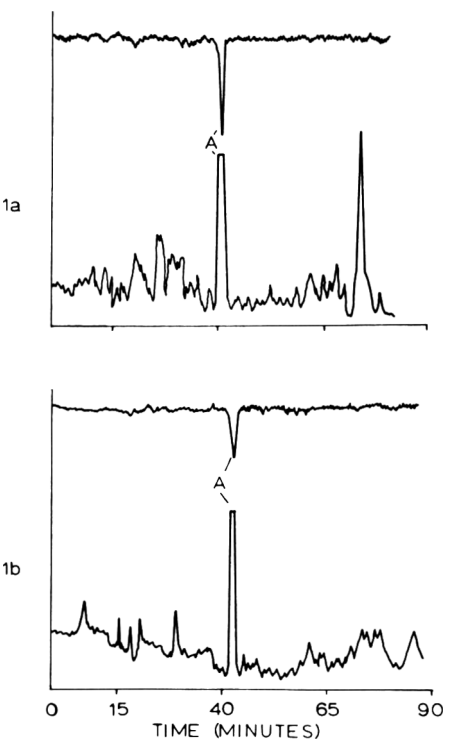

Fig. 1—Gas chromatograms of volatiles in tea leaf augmented with ^{14}-C-linolenic acid at various stages in the black tea manufacturing process. Upper trace shows response from radioactivity detector and lower trace shows response from flame ionization detector. (a) Tea leaf after fermentation; (b) Tea leaf after firing, i.e., as black tea. Peak A identified as t-2-hexenal by retention time.

importance in determining the quality of the final product. It remains for future work to rationalize the conditions which maximize the formation and retention of black tea aroma.

It is noteworthy that Major and Thomas (1972) have shown that *t*-2-hexenal is formed from linolenic acid in Ginkgo leaves.

REFERENCES

Bondarovich, H.A., Giammarino, A.S., Renner, J.A., Shephard F.W., Shingler, A.J. and Gianturco, M.A. 1967. Some aspects of the chemistry of tea. A contribution to the knowledge of the volatile constituents. J. Agr. Food Chem. 15: 36.

Co, H. and Sanderson, G.W. 1970. Biochemistry of tea fermentation: Conversion of amino acids to black tea aroma constituents. J. Food Sci. 35: 160.

Eyton, W.B. 1972. The chemistry of tea. Flavor Ind. 3: 23.

Major, R.T. and Thomas, M. 1972. Formation of 2-hexenal from linolenic acid by macerated Ginkgo leaves. Phytochem. 11: 611.

Millin, J. and Rustidge, D.W. 1967. Tea manufacture. Process Biochem. 2: 9.

Müggler-Chavan, R., Viani, R., Bricout, J., Reymond, D. and Egli, R.H. 1966. Sur la composition de l'arôme de thé. I. Helv. Chim. Acta 49: 1763.

Nakabayashi, T. 1958. Studies on the formation mechanism of black tea aroma. Part 5. On the precursor of the volatile carbonyl compounds of black tea. J. Agr. Chem. Soc. (Japan) 32: 941.

Popov, V.P. 1956. Oxidation of amino acids in the presence of tannins and polyphenoloxidase of tea. Biokhimiya 21: 383.

Saijo, R. and Takeo, T. 1970a. The production of phenylacetaldehyde from L-phenylalanine in tea fermentation. Agr. Biol. Chem. 34: 222.

Saijo, R. and Takeo, T. 1970b. The formation of aldehydes from amino acids by tea leaf extracts. Agr. Biol. Chem. 34: 227.

Sanderson, G.W. 1972. The chemistry of tea and tea manufacturing. In "Recent Advances in Phytochemistry," ed. Runeckles, V.C., Vol 5, p. 247. Academic Press, New York.

Sanderson, G.W., Co, H. and Gonzalez, J.G. 1971. Biochemistry of tea fermentation: The role of carotenes in black tea aroma formation. J. Food Sci. 36: 231.

Takei, S., Sakato, Y. and Ono, M. 1937. The essential oil of green tea. 9. Carbonyl compounds of black tea oil. Rikagaku Kenkyusho Iho 16: 773.

Van Romburgh, P. 1920. Unsaturated alcohol of essential oil of freshly fermented tea leaves. Proc. Kon. Ned. Akad. Wetensch. 22: 758.

Yamanishi, T., Kobayashi, A., Sato, H., Nakamura, H., Osawa, K., Uchida, A., Mori, S. and Saijo, R. 1966. Flavor of black tea. Part 4. Changes in flavor constituents during the manufacture of black tea. Agr. Biol. Chem. 30: 784.

Yamanishi, T., Kobayashi, A., Sato, H., Ohmura, A. and Nakamura, H. 1965. Flavor of black tea. Part 2. Alcohols and carbonyl compounds. Agr. Biol. Chem. 29: 1016.

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A Research Note

AN APPARATUS FOR MEASUREMENT OF CONTRACTILE PROPERTIES OF PORCINE SKELETAL MUSCLE

INTRODUCTION

THE TECHNIQUE of measuring the tension and time course of isometric muscle contraction has been applied to a number of experimental animals (Jewell and Wilkie, 1958; Buller et al., 1960; Close, 1964; Barnard et al., 1970; Mann and Salafsky, 1970). The electrical components for these different adaptations are similar, requiring a strain gauge, amplifier, oscilloscope and electrical stimulator. The type or design of the holding apparatus, however, varies with the experimental animal used. Obviously, the larger the animal and the greater the mass of the muscle to be tested, the larger and more durable the holding platform required.

Such a technique, as applied to porcine skeletal muscle research, would have many advantages, being useful in the study of fatigue, blood flow, blood electrolytes and metabolites and their effects on various physiological contractile parameters and how these effects might subsequently influence meat quality. With this purpose in mind, we have designed an apparatus and developed and tested a procedure whereby we can measure the contraction time (CT), one-half relaxation time (½ RT), net twitch tension/g wet weight muscle, net tetanus tension/g wet weight muscle and twitch-tetanus ratio in muscle of porcine animals weighing up to 100 kg.

EXPERIMENTAL

THE HOLDING APPARATUS is constructed of aluminum (Fig. 1) except for the base platform which is oak. An essential feature is that the force transducer can be moved through any axis necessary to align it as close as possible with the direction of the natural line of pull of the muscle. A brace is placed between the struts which hold the bone screws and the transducer holding mechanism. On the other side an angle brace is positioned between this mechanism and the platform. These braces give added rigidity and thus minimize any compliance which would detract from the requirement for isometric conditions.

Figure 2 is a schematic diagram of the recording system. The physiograph is the power supply. The strain gauge is a Grass FT10C isometric force transducer which was chosen because it can measure tension linearly (± 1%) up to 10 kg. The output of the transducer is amplified by a bridge preamplifier and then displayed on the oscilloscope, from which the event can be photographed. The time relay is used to standardize the duration of a tetanus; i.e., the impulse train during tetanus is timed to last 490 msec. The stimulator is used to excite the muscle via its nerve supply and also to trigger the electron beam sweep of the oscilloscope.

Experimentally, we have used porcine animals from age 1 day to 6 wk. Choice of the tibialis anterior was based on several prerequisites. The distal tendon was small in diameter

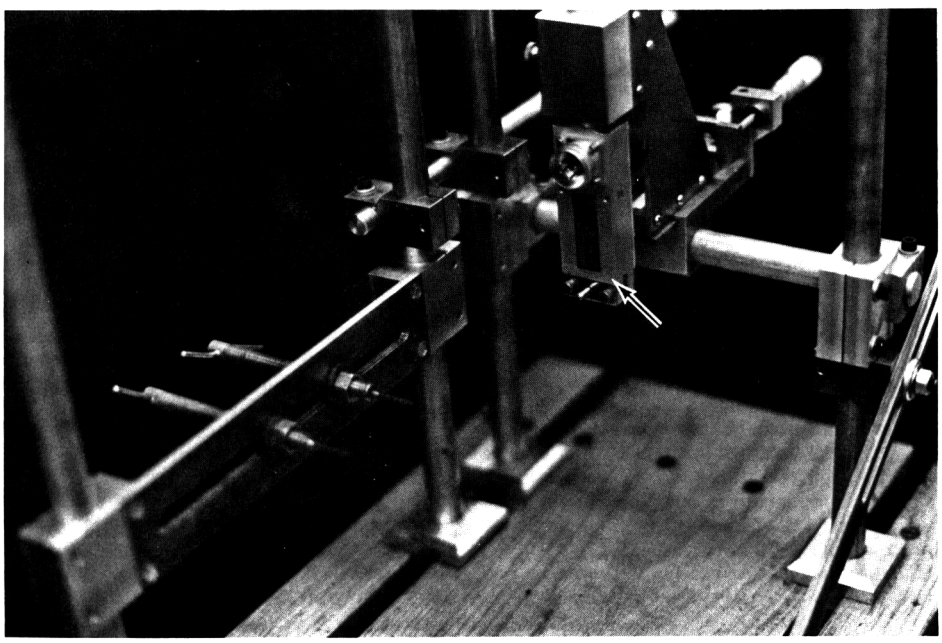

Fig. 1—Holding apparatus. Arrow indicates isometric force transducer. Note also the braces and bone screws.

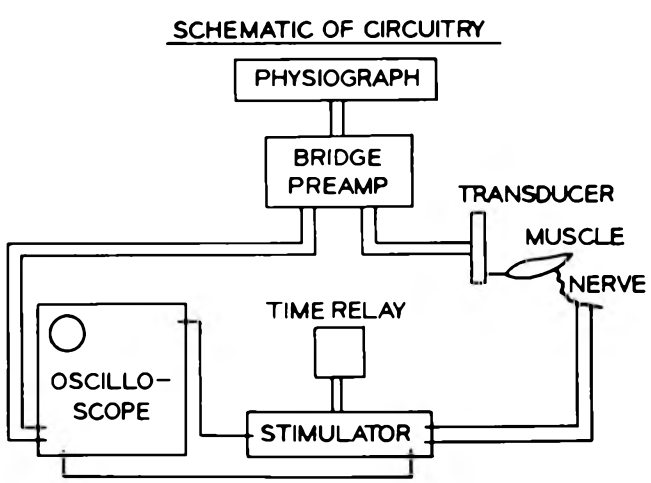

Fig. 2—Schematic diagram of electrical components.

Table 1—Physiological parameters of skeletal muscle from porcine and other animals

	Age (days)	CT (msec)	½ RT (msec)	Twitch tension/g muscle	Tetanus tension/g muscle	Twitch-tetanus ratio
Porcine						
Tibialis anterior	1	40	40	475	821	.58
	21	31	25	258	590	.43
Cat[a]						
Tibialis anterior	1	56	60			.23
	21	35	26			.35
Rat[b]						
Extensor digitorum longus	1	57	51			.53
Guinea pig[c]						
Gastrocnemius plantaris	adult	22.4	18.4	149	849	.17

[a]Mann and Salafsky (1970)
[b]Close (1964)
[c]Barnard et al. (1970)

which mechanically made it much easier to make a secure connection between the isometric force transducer and tendon. The muscle and its nerve supply were easily isolated. Finally, this muscle has been used by previous researchers allowing for a basis of comparison. From the porcine muscles that satisfy these criterion the tibialis anterior was arbitrarily chosen.

Animals were anesthetized intraperitoneally with sodium pentobarbital, and the tibialis anterior muscle was isolated by dissection leaving it attached at the proximal end. Care was taken to preserve the nerve and blood supply to the muscle. The common peroneal nerve was exposed and ligated. Screws were inserted into the distal end of the femur and the distal ends of the tibia and fibula. These screws in turn were bolted to the holding apparatus so that the pig was in a supine position and the tibialis anterior was in a horizontal position. The distal tendon was severed and connected by silk suture and metal wire to the transducer.

Throughout an experiment, the nerve and muscle were covered with saline soaked gauze. 200-watt light bulbs positioned over the tibialis anterior and chest were used to maintain the rectal temperature near 38°C and the muscle surface near 37°C. As shown by Gordon and Phillips (1953), to make comparative estimates, temperature control is important because the contraction time has a rather high temperature coefficient.

Stimulation via the common peroneal nerve was achieved by using supramaximal monophasic pulses of 0.2 msec duration. Resting length was established after the procedure of Buller et al. (1960). Contraction time and one-half relaxation time were measured according to Close (1964). Optimal frequency of tetanic stimulation was that frequency at which the tension was maximal 200 msec after onset.

After recordings were completed, the tibialis anterior was excised, freed of any surrounding connective tissue and weighed.

RESULTS & DISCUSSION

WHILE IT would be unlikely that the data from porcine tibialis anterior exactly paralleled data from muscles of other species, our results for porcine CT and ½ RT are in reasonable agreement with the same experimental data for cat tibialis anterior (see Table 1).

Futhermore, the frequency of stimulation necessary to achieve tetanus in the tibialis anterior muscle of the 6-wk old pigs was determined to be approximately 125 impulses/sec. This value agrees well with the value (120/sec) found by Gordon and Phillips (1953) for adult cat tibialis anterior.

Thus it is concluded that the technique for measuring muscle contraction isometrically as adapted for use on porcine skeletal muscle has been achieved in the sense that our data on porcine muscle are comparable to the experimental data for other species that have been obtained by different researchers.

REFERENCES

Barnard, R.J., Edgerton, V.R. and Peter, J.B. 1970. Effect of exercise on skeletal muscle. 2. Contractile properties. J. Appl. Physiol. 28: 767.

Buller, A.J., Eccles, J.C. and Eccles, R.M. 1960. Differentiation of fast and slow muscles in the cat hind limb. J. Physiol. (London) 150: 399.

Close, R. 1964. Dynamic properties of fast and slow skeletal muscles of the rat during development. J. Physiol. (London) 173: 74.

Gordon, G. and Phillips, C.G. 1953. Slow and rapid components in a flexor muscle. Qtr. J. Exp. Physiol. 38: 35.

Jewell, B.R. and Wilkie, D.R. 1958. An analysis of the mechanical components in frog's striated muscle. J. Physiol. (London) 143: 515.

Mann, W.S. and Salafsky, B. 1970. Enzymatic and physiological studies on normal and disused developing fast and slow cat muscles. J. Physiol. (London) 208: 33.

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A Research Note
SHELF LIFE STABILITY AND ACCEPTANCE OF FROZEN PACIFIC HAKE (Merluccius productus) FILLET PORTIONS

INTRODUCTION

THE STANDING STOCK of Pacific hake in waters off Oregon and Washington is estimated to range from 609.5–1206 thousand short tons with a sustainable yield of 174–349 thousand short tons (Alverson and Larkins, 1969). Hake of the Pacific coast are regarded generally as a trash fish having flesh of poor keeping quality. Only small amounts are used for animal food. Hake of the genus *Merluccius* are valuable human food in the USSR, Chile, Spain, Argentina and South Africa (FAO, 1967). This species of fish has considerable potential for filling the expanding domestic need for low-cost fish blocks (Dassow et al., 1970). This investigation was carried out to evaluate the acceptance and frozen shelf-life characteristics of portions prepared from frozen hake fillet blocks.

EXPERIMENTAL

ICED ROUND FISH were obtained from local commercial sources one day post-extraction. The fish were filleted, exposed to the various treatments and frozen at −30°F in 1 × 3.75 × 21-in. ID stainless steel trays. One day post-processing the frozen blocks were sawed into approximately 0.5 × 3.5 × 3.75-in. portions, vacuum sealed in moisture-vapor proof film and held at −15°F prior to evaluation.

Antioxidant was applied to each individual fillet in an atomized spray of a water miscible solution containing 1g of antioxidant solution (0.75g BHA, 0.75g BHT, 13.5g propylene glycol, 10.0g Tween 20) in 50 ml of water. Sodium tripolyphosphate (TPP) was aplied in a similar manner as a 10% aqueous solution. Based upon the weight added to three replicate lots of fillets, antioxidant additions ranged from 0.0013–0.0015% and TPP from 0.004–0.005%.

Flavor panels and 2-thiobarbituric acid (TBA) analysis (Yu and Sinnhuber, 1957) were conducted at 0, 3, 6, 9 and 12-months storage. For flavor testing, portions were battered and breaded in the frozen state using standard commercial products. The breaded portions were deep-fat fried from the frozen state at 375°F for 6–7 min. and served hot in coded cups to judges seated in individual booths. Trained flavor panels judged portions for texture, juiciness, flavor and overall desirability on an intensity scale ranging from 9, "liked extremely," to 1, "disliked extremely." All data were analysed by analysis of variance and the significance of means tested by the least significant difference (LSD) method.

RESULTS & DISCUSSION

FROZEN STORAGE did not adversely affect the texture of hake fillet portions (Table 1). Mean scores at the five evaluation times did not vary significantly ($P < 0.05$).

The application of antioxidants and polyphosphates did not appear to improve shelf-life with regard to juiciness. Mean scores varied significantly ($P < 0.05$) only at the 6 and 12 month storage periods. On the 6 month storage tests a preference was shown for portions treated with a combination of antioxidant and polyphosphate, but this preference was not sustained on the 9 or 12 month tests. After 12 months storage, the juiciness of portions treated with antioxidant was least preferred.

Mean panel scores for flavor and over-all desirability varied significantly ($P < 0.05$) at 0-storage time with an equal preference indicated for control portions and those treated with antioxidant. Scores for portions treated with polyphosphate alone or in combination with antioxidant were significantly ($P < 0.05$) lower. After 12-months storage, flavor and over-all desirability mean scores did not vary significantly ($P < 0.05$).

Application of antioxidants reduce the level of oxidative rancidity occurring in frozen fillet portions during the 12-month storage period (Table 2). The small difference in the level of oxidative rancidity as measured by TBA-analyses between control portions and those treated with antioxidant was not readily

Table 1—Flavor panel scores for hake fillet portions stored at −15°F

Flavor factor	Treatment	Mean score [d] 0-mos	3-mos	6-mos	9-mos	12-mos
Texture	Control	6.85	7.62	7.62	7.62	7.96
	Antioxidant	7.08	7.65	7.28	7.48	7.80
	TPP	7.33	7.58	7.10	7.90	7.60
	A + TPP	7.07	7.23	7.13	7.88	7.27
		NSD	NSD	NSD	NSD	NSD
Juiciness	Control	7.95	7.13	6.68[a]	7.10	7.40[a]
	Antioxidant	7.40	7.32	6.70[a]	6.95	6.15[b]
	TPP	6.23	6.97	6.18[a]	7.18	7.17[a]
	A + TPP	6.58	7.23	7.80[b]	7.22	7.55[a]
		NSD	NSD		NSD	
Flavor	Control	7.38[a]	6.78	7.13	7.23[ab]	7.37
	Antioxidant	7.48[a]	7.37	6.90	7.85[bc]	6.65
	TPP	6.33[b]	7.15	7.07	7.92[c]	6.75
	A + TPP	6.23[b]	7.40	7.10	7.10[a]	7.05
			NSD	NSD		NSD
Over-all desirability	Control	6.95[a]	6.53	6.55	6.43	6.65
	Antioxidant	7.05[a]	7.02	6.33	6.73	5.90
	TPP	5.47[b]	6.62	6.40	6.88	6.13
	A + TPP	5.53[b]	6.88	6.53	6.60	6.27
			NSD	NSD	NSD	NSD

[a,b,c]Average scores in a column with same exponent letter did not vary significantly ($P < 0.05$) from each other: NSD: no significant ($P < .05$) difference
[d]n = 30; Range of scores: 9, "liked extremely" to 1, "disliked expremely"

Table 2—TBA-values[a] for hake fillet portions stored at −15° F

Storage time (mos)	Control	Anti-oxidant	TPP	A + TPP
0	0.46	0.99	1.95	0.78
3	1.30	0.71	2.45	1.22
6	3.07	1.16	2.55	0.65
9	2.42	1.44	2.10	0.45
12	1.64	0.73	3.54	1.22

[a]Mg malonaldehyde/kg

apparent from flavor panel scores. The application of TPP appeared to produce a slightly pro-oxidative effect. Application of antioxidants in combination with TPP retarded this development of oxidation.

The results of this investigation indicate that fish portions prepared from frozen blocks of Pacific hake fillets possess a relatively high degree of acceptance and frozen shelf-life stability when stored under vacuum in moisture-vapor proof film. The application of antioxidants and TPP to portions packaged in this manner does not appear to favorably enhance frozen shelf-life characteristics. The degree of acceptance and shelf-life stability observed seems to indicate a good potential for utilization of this species for human food.

REFERENCES

Alverson, D.L. and Larkins, H.A. 1969. Status of knowledge of the Pacific hake resource. Calif. Mar. Comm., Cal COFI Rept., 13: 24.

Dassow, J.A., Patashnik, M. and Koury, B.J. 1970. Characteristics of Pacific hake, Merluccius productus, that affect its suitability for food. In "Pacific Hake," p. 127. U.S. Fish Wildlife Serv. Circ. 332.

FAO. 1967. "Catches and Landings, 1966." Yearbook Fish. Statistics 22: 406.

Yu, T.C. and Sinnhuber, R.O. 1957. 2-Thiobarbituric acid method for measurement of rancidity in fishery products. Food Technol. 11: 104.

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