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›CIENCE and ENGINEERING

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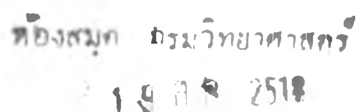

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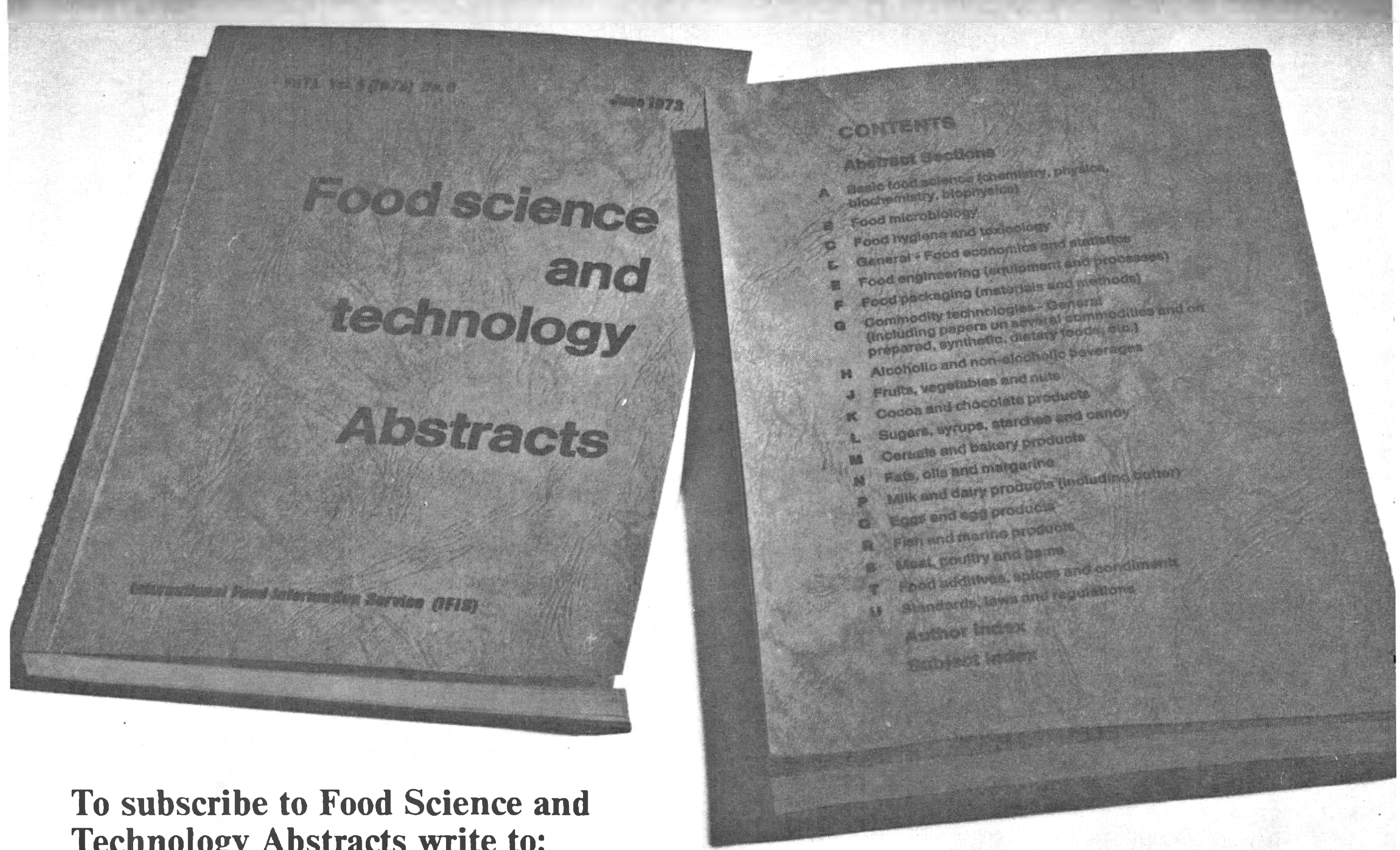

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ABSTRACTS:
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EXPERIMENTAL EVALUATION OF MATHEMATICAL AND COMPUTER MODELS FOR THERMAL PROCESS EVALUATION. A.A. TEIXEIRA, C.R. STUMBO & J.W. ZAHRADNIK. *J. Food Sci.* **40**, 653–655 (1975)–This paper presents results from laboratory assays on thiamine in samples of pea puree that had received carefully controlled thermal processes in a still cook retort. The percent thiamine retention for each of these processes based on the experimental assay results was then compared to predictions of thiamine retention obtained with mathematical and computer methods developed by the authors for identical processing conditions. A comparison of the results showed good agreement between assay determinations and mathematical and computer predictions, well within confidence intervals established by a statistical data treatment of the assay results and expected error with the mathematical and computer methods.

COMPUTER SIMULATION OF VARIABLE RETORT CONTROL AND CONTAINER GEOMETRY AS A POSSIBLE MEANS OF IMPROVING THIAMINE RETENTION IN THERMALLY PROCESSED FOODS. A.A. TEIXEIRA, G.E. ZINSMEISTER & J.W. ZAHRADNIK. *J. Food Sci.* **40**, 656–659 (1975)–A computer model was used to predict the effects on the level of thiamine retention produced by various time-varying surface temperature policies of equal sterilizing capacity with respect to a given heat resistant bacterium for a standard sized container. A second application of the model was made to study the effects of various container geometries of equal volume on the level of thiamine retention for both constant and time-varying surface temperature policies of equal sterilizing values. The maximum thiamine retention observed from the investigation of variable surface temperature policies represented no more than a 5% improvement over the level obtained with a conventional constant-temperature process. However, as much as 88% improvement in the level of thiamine retention was observed with policies of constant temperature and short time when applied to container geometries that promoted a more rapid heat transfer to the center of the container.

YIELDS AND SOLIDS LOSS IN STEAM BLANCHING, COOLING AND FREEZING VEGETABLES. J.L. BOMBEN, W.C. DIETRICH, J.S. HUDSON, H.K. HAMILTON & D.F. FARKAS. *J. Food Sci.* **40**, 660–664 (1975)–During blanching, cooling and freezing vegetables can either gain or lose water, or lose solids. This work investigated how each operation affected the yield and total solids of carrots, peas, green beans, broccoli, cauliflower, lima beans and Brussels sprouts. The work also demonstrated how air cooling with blancher condensate sprayed on the vegetables reduced the total effluent due to blanching and cooling and increased the solids retention of the vegetables as compared to water cooling. 3-lb samples were steam blanched, cooled by water immersion or air flow, and frozen in an air blast freezer, using experimental techniques which simulated commercial practice. Vegetables and effluents were weighed after each operation. Total solids analyses were done on the raw and frozen vegetables and the blanching and cooling effluents, which were also analyzed for total organic carbon.

CHANGES IN THE ASCORBIC AND DEHYDROASCORBIC ACID CONTENTS OF FRESH AND CANNED BEANS. A. MARCHESINI, G. MAJORINO, F. MONTUORI & D. CAGNA. *J. Food Sci.* **40**, 665–668 (1975)–The enzymatic assay of ascorbic and dehydroascorbic acids performed on 15 cultivars of green beans, cultivated under the same agronomic conditions and canned with two different treatments (8 min at 124°C and 25 min at 116°C) revealed no significant difference in the AA and DHA contents of the beans a few days after canning, or after 6 months' storage at room temperature. The result appears to be dependent on other factors such as pH of the brine, presence of metal ions, oxygen level, etc., rather than on the heat treatments given. The total vitamin content obtained from the general means decreased by 73% in the canned beans after heat treatment, and 81% after 6 months' storage. The quantity of AA in the tissue of beans after 6 months' storage is significantly higher than the quantity of AA determined some days after canning. The phenomenon may be explained by the reduced condition in the cans, which also affects the disappearance of the DHA. Finally, the enzyme ascorbic oxidase is highly specific for the assay of the ascorbic acid in green beans.

INFLUENCE OF POST-HARVEST STORAGE TEMPERATURES AND SOAKING ON YIELD AND QUALITY OF CANNED MUSHROOMS. R.B. BEELMAN & F.J. McARDLE. *J. Food Sci.* **40**, 669–671 (1975)–Temperature of post-harvest storage significantly affected the yield and quality of canned mushrooms. As storage temperature was increased, canned product yields increased but quality of the canned product generally deteriorated. Mushrooms stored for 18 hr following harvest at 2, 12 and 22°C increased in yield, compared to unstored controls, as much as 3.6%, 7.5% and 9.5%, respectively. When treatments involving post-harvest storage coupled with water-soaking operations were employed, even greater yields were attained and quality deterioration was reduced. Yield increases of up to 24.2% greater than a control process were attained. However, the optimum process involved soaking mushrooms 20 min in water, storing at 12°C for 18 hr then vacuum soaking prior to blanching (SSV-Process). The yield increase, compared to the control, was 15.3% and quality was comparable to that of mushrooms processed after 18 hr storage at 2°C which is a common commercial practice. Higher storage temperatures significantly increased both the water-binding capacity (WBC) and water-holding capacity (WHC) of mushrooms during processing. Both WBC and WHC correlated highly with canned product yield.

EFFECT OF OZONE FUMIGATION ON CROP COMPOSITION. E.L. PIPPEN, A.L. POTTER, V.G. RANDALL, K.C. NG, F.W. REUTER III, A.I. MORGAN JR. & R.J. OSHIMA. *J. Food Sci.* **40**, 672–676 (1975)–A survey was made of the effect of exposing cabbage, carrot, corn, lettuce, strawberry and tomato plants to two levels of ozone on nutrients in crops harvested from these plants. Nutrients determined include nine metals and the "nonmetals": vitamin C, riboflavin, niacin, β-carotene, thiamine, solids, nitrogen, fiber, ash and carbohydrates. In general, the quantity of nutrients in crops from the ozone fumigated plants and in crops from plants grown in carbon-filtered air were comparable. Ozone appeared to have had little effect particularly on the quantity of metals in these crops. However, in the "nonmetal" category, significant differences occurred in 24 of 52 sets of data and some definite compositional trends correlated with ozone treatment were evident. Significantly decreased solids were found in three of the crops exposed to ozone. Since a trend in solids content establishes a similar trend in other components, total solids would seem to be a particularly significant indicator of the influence of ozone on crop composition. Eight exceptions to the rule that solids trends establish compositional trends were observed and they are of particular interest because they logically reflect the influence of ozone on crop composition. An outstanding example is the definite upward trend of vitamin C in corn exposed to ozone, a trend which goes counter to the downward trend of solids in this corn.

CHEMICAL AND THERMAL EFFECTS ON PARATHION RESIDUES ON SPINACH. T.E. ARCHER. *J. Food Sci.* **40**, 677–680 (1975)–Thiophos® Parathion 4 E.C. was applied to spinach in the field 14 days before harvest at 1/2 and 1 lb/acre active ingredient by hand spray equipment. The spinach was sampled initially and at harvest and analyzed for parathion and related compounds. The sprayed spinach contained initially at the 1/2 lb rate 26.2 ppm and at the 1 lb rate 58.4 ppm total parathion residues. The residues at harvest were reduced to approximately 0.264 ppm and 0.474 ppm, respectively. The harvested spinach was cooked similarly to household cooking by boiling for 15 min in

water broth. The spinach was also cooked in 1% solutions of brine, sodium bicarbonate, brine plus sodium bicarbonate, 4% acetic acid and 4% acetic acid plus 1% brine to determine the relative effects on the pesticide residues. Cooking the spinach in the bicarbonate broths was more effective for reducing the parathion levels than cooking in the other broths. The possibility exists that residue losses occur on the cooked spinach both by volatility and chemical decomposition.

EFFECT OF TEMPERATURE AND PACKAGING ATMOSPHERE ON STABILITY OF DRUM-DRIED PINTO BEAN POWDER. D.G. GUADAGNI, C. DUNLAP & S. KON. *J. Food Sci.* **40**, 681–683 (1975)–The stability of air and nitrogen packs of commercially produced drum-dried pinto bean powder stored at 50, 70 and 90°F was determined from sensory data obtained with trained panelists using the duo-trio test. Air packed samples were stable for 5.8, 9.2 and 15.6 wk at 90, 70 and 50°F, respectively. Nitrogen packing increased stability of the powder at these same temperatures about 4- to 5-fold. The temperature coefficient for each 10°F increment was approximately 1.3–1.4 for both air and nitrogen packs in the range of 50–90°F. Preparation of the powder into soup containing standard commercial flavoring ingredients masked the off-flavor developed during storage.

EFFECTS OF CUCUMBER SIZE AND BRINE COMPOSITION ON THE QUALITY OF DILL PICKLES. S.I. NIEMELÄ & J.J. LAINE. *J. Food Sci.* **40**, 684–688 (1975)–Analysis of variance has been applied in connection with factorial arrangement of treatments to study interactions and main effects of cucumber size, brine salinity, glucose, alum and acetic acid addition on taste, consistency, appearance and bloater frequency of 48 experimental lots of genuine dill pickles. Instability of fermentations in the low-salt brines was judged to cause brine additives to have more influence at low salinities. Addition of 0.2% alum to the brine seemed to stabilize acid production and consistency, and to compensate the effect of glucose. Considering all quality criteria, the best dill pickles can be expected from brining largish cucumbers in slightly acidified brine of approximately 6% salt content (2–3% after equalization) with about 0.2% alum.

SALT FREE ACIDULANT STORAGE OF PICKLING CUCUMBERS. J.L. SHOUP, W.A. GOULD, J.R. GEISMAN & D.E. CREAN. *J. Food Sci.* **40**, 689–691 (1975)–The feasibility of storing cucumbers in acid solutions without the addition of sodium chloride was investigated in an attempt to reduce bloating and alleviate some of the problems incurred with spent brine disposal. Large sized cucumbers of mixed cultivars were covered with acetic acid or combinations of acetic with either lactic or citric acid. Potassium sorbate 0.1% by weight was added to the solutions. The cucumbers were stored in sealed 32 gal fermentation containers and the headspace air flushed with nitrogen. Bloating was decreased by almost 50% in an acetic acid solution compared to a salt brine control after 8 months. Acetic acid stored cucumbers were 3 lb higher in pressure resistance measured with the USDA fruit pressure tester compared to a salt brine control. Flavor analyses indicated that cucumbers processed in solutions containing food-grade acidulants were acceptable.

EFFECT OF ADDED ALUM ON THE QUALITY OF BRINED ROYAL ANN CHERRIES. D.V. BEAVERS, C.H. PAYNE & R.F. CAIN. *J. Food Sci.* **40**, 692–694 (1975)–The effect of including alum [$AlK(SO_4)_2$] in the initial brine for Royal Ann cherries was evaluated for certain brined cherry quality factors. Increasing amounts of added alum significantly reduced the brine shrink, decreased the total loss, and lowered the equilibrated brine pH. No significant differences were shown for percent unpitted fruit, pits, pitting loss, soft fruit, texture and solution pockets. In some instances, significant differences due to maturity were shown. Cherries were colored with FD&C Red No. 3 and 4. With Red No. 3, cherries containing more than 2% added alum could not be satisfactorily colored due to bleeding. No difficulty was encountered when coloring with Red No. 4.

EFFECT OF DIFFUSION RATE ON DRAINED WEIGHT OF CANNED GRAPEFRUIT SECTIONS. I.J. KOPELMAN, S. MIZRAHI & M. KOCHBA. *J. Food Sci.* **40**, 695–697 (1975)–The effect of the diffusion rate (varied through the sugar gradient) on the final drained weight (FDW) of canned grapefruit segments was studied in a well-controlled system. Results confirmed the rate-dependence of the FDW which was higher for gradual addition of sugar compared with single time addition. Theoretical analysis attributed the rate-dependent FDW to the interference of the fast moving water with the inflow of sugar into the segments. Control of sugar release rate can be obtained through the use of hard candy type amorphous sugar tablets added to the canned grapefruit segments. This method has been demonstrated to increase the FDW.

FACTORS INFLUENCING IMPREGNATION OF APPLE SLICES AND DEVELOPMENT OF A CONTINUOUS PROCESS. M.W. HOOVER & N.C. MILLER. *J. Food Sci.* **40**, 698–700 (1975)–Vacuum impregnation of apple slices was improved by raising the temperature of the impregnating medium to slightly below the "flash point" for the vacuum used. The degree of impregnation was also influenced by the amount of sugar in the impregnation medium. As the sugar increased, there was a responding decrease in impregnation. The amount of impregnation increased rapidly as the degree of vacuum increased. Only a slight improvement in impregnation occurred with an increase in time at peak vacuum beyond about 30 sec. A continuous vacuum system for impregnating fresh apple slices is described. The system utilizes either a syphon technique or a vaneless pump to transport the product and impregnation medium up through the unit into an elevated cyclone-vortex vacuum chamber and then down an impregnation column.

PAPAYA PUREE AND CONCENTRATE: CHANGES IN ASCORBIC ACID, CAROTENOIDS AND SENSORY QUALITY DURING PROCESSING. H.T. CHAN JR., M.T-H. KUO, C.G. CAVALETTO, T.O.M. NAKAYAMA & J.E. BREKKE. *J. Food Sci.* **40**, 701–703 (1975)–Ascorbic acid (AA), carotenoids and sensory quality were measured on samples from different stages in the preparation of papaya puree concentrate. About 5.5% of the original AA was destroyed during pulping in preparation of the puree; an additional 14% was lost during vacuum concentration. Changes in carotenoids were characterized by a hypsochromic shift which intensified during the processing sequence. The shift was attributed to the acid-catalyzed isomerization of 5,6-monoepoxycryptoxanthin to 5,8-monoepoxycryptoxanthin. Concentration did not affect flavor quality and had little or no effect on aroma quality of a beverage product.

EFFECT OF POST-HARVEST TEMPERATURE ON QUALITY, CAROTENOIDS AND ASCORBIC ACID CONTENT OF ALPHONSO MANGOES ON RIPENING. P. THOMAS. *J. Food Sci.* **40**, 704–706 (1975)–Storage of Alphonso mangoes at ambient temperature (26–32°C) was found to favor the development of typical organoleptic qualities and flesh carotenoids, on ripening. Low temperature (7–20°C) storage of preclimacteric fruits for periods ranging from 16–23 days adversely affected the development of the above qualities even when fruits were subsequently ripened under ambient temperatures. Storage at low temperature resulted in 20–70% reduction in the total carotenoids content of the flesh on ripening, the lowest being in fruits continuously held at low temperatures. Regardless of temperature, carotenes always exceeded xanthophylls, the former accounting for 60–70% of the total flesh carotenoids. Ascorbic acid retention on ripening was maximal in low temperature stored fruits while prolonged storage at low temperatures caused a net synthesis of ascorbic acid.

DEGRADATION PRODUCTS FORMED IN CANNED SINGLE-STRENGTH ORANGE JUICE DURING STORAGE. J.H. TATUM, S. NAGY & R.E. BERRY. *J. Food Sci.* **40**, 707–709 (1975)–Canned single-strength orange juice, stored 12 wk at 35°C, formed many degradation products, 10 of which were isolated and identified. Three had developed in concentrations above their taste thresholds; viz. α-terpineol, 4-vinyl guaiacol and 2,5-dimethyl-4-hydroxy-3(2H)-furanone. These three compounds when added to control juice caused odors and flavors very similar to those of stored juice.

EFFECTS OF PREPARATION AND MILLING ON CONSISTENCY OF TOMATO JUICE AND PUREE. P.G. CRANDALL & P.E. NELSON. *J. Food Sci.* 40, 710–713 (1975)–Samples of two tomato cultivars were processed into juice and purec using three manufacturing methods. Con-

sistency measurements were taken with the efflux pipette, Ostwald, Stormer and Bostwick viscosimeters. Half of each sample was further processed using a high speed rotary mill designed for cell fragmentation. Interactions among the methods of heating, additional mechanical shear treatment, and method of consistency measurements were shown. Data showed that the degree of increase in consistency results more from method of preparation than from cell fragmentation. Under these experimental conditions, it was found that the measuring instrument must be specified before one can determine which method of preparation to use.

THE INFLUENCE OF SPAGHETTI EXTRUDING, DRYING AND STORAGE ON THE SURVIVAL OF Staphylococcus aureus. D.E. WALSH & B.R. FUNKE. *J. Food Sci.* 40, 714–716 (1975)–Dough was inoculated with *Staphylococcus aureus* and processed into spaghetti. The average population of *S. aureus* ATCC No. 12600 in the dough was 1.6×10^7 cells/g. Extrusion reduced the population to 7.2×10^6 cells/g. However, extrusion temperature and auger speed within the practical ranges for spaghetti processing did not significantly (5% confidence level) influence *S. aureus* population level. The drying of spaghetti in the usual 35°C range resulted in a sevenfold increase in the population of *S. aureus.* Ambient storage, on the other hand, resulted in a decrease of the *S. aureus* population in dry spaghetti. For spaghetti with 4.9×10^7 and 1.5×10^7 cells/g of *S. aureus* strain 12600 and S-6 respectively, no enterotoxin development was detected.

COCONUT SKIM MILK AS AN INTERMEDIATE MOISTURE PRODUCT. R.D. HAGENMAIER, C.M. CATER & K.F. MATTIL. *J. Food Sci.* **40,** 717–720 (1975)–Water extraction of comminuted coconut meats gives coconut milk, which can be centrifuged to give coconut skim milk. Liquid concentrates were prepared by direct evaporation of coconut skim milk, or by reconstitution of the spray-dried product. Objective of the research was to evaluate the concentrate as an alternative to spray-dried coconut skim milk. Concentrate with 30% moisture had water activity of 0.74 and viscosity of 10,000 centipoise, and was stable to bacteria growth at 23°C storage. Other storage conditions were also investigated. Mold growth was controlled with 0.1% sorbic acid added. Problems with darkening of color and fat oxidation were encountered, but overall results suggest that intermediate moisture coconut skim milk is a possible product form.

QUALITY OF FOODS AFTER COOKING IN 915 MHz AND 2450 MHz MICROWAVE APPLIANCES USING PLASTIC FILM COVERS. G. ARMBRUSTER & C. HAEFELE. *J. Food Sci.* **40,** 721–723 (1975)–Foods were cooked in 915 MHz and 2450 MHz microwave appliances with and without Saran Wrap plastic film covers. Quality of cooked foods was evaluated by sensory methods, measurements of uniformity of doneness, cooking losses and vitamin content. Cooking times, convenience and hazards were also determined. Use of plastic film covers resulted in improved textural characteristics and more uniformity of doneness. Cooking times were also shorter. Temperature measurements in cooked foods revealed that film covers promoted faster heating and more even distribution of heat. No hazards were encountered when film was used. Few significant differences in cooking losses and vitamin content were observed.

A LABORATORY PROCEDURE FOR THE PRESSURE PROCESSING OF FLEXIBLE POUCHES. O. GUEDEZ & R.P. BATES. *J. Food Sci.* **40,** 724–727 (1975)–Systems representing foods which heat by conduction and convection were packed in polyester-aluminum-nylon 11 laminate pouches and retort processed at 15 or 25 psi steam (121 or 131°C) to an F_0 of 10 min. Minor retort modification permitted processing without overriding air pressure, except during cooling. With particular attention to proper pouch filling and sealing, processing could be conducted without pouch failure and was also applicable to polyester-aluminum-polyolefin laminate and (at 15 psi only) to transparent oven film pouches. A threefold reduction in conduction processing time at 121°C was effected, compared to a 303 can. The conduction process time at 131°C was approximately half that at 121°C. This technique is a practical means for evaluating the feasibility of pouch processes and for small-scale sample preparation.

AN AUTOMATED CONTINUOUS PROTEIN ANALYZER: MODIFICATION OF THE LOWRY METHOD. M.E. ANDERSON & R.T. MARSHALL. *J. Food Sci.* 40, 728–731 (1975)–An automated continuous protein analyzer is described. Six purified proteins–casein, egg albumin, bovine serum albumin, Supro 610 (a modified soy), soy protein N and gluten–were tested in concentrations (achieved by logarithmic dilution and concentration) which ranged from 0–250 μg/ml. Absorbency was characteristic of individual proteins and the degree of hydration of each. Curves depicting absorbency vs concentration were curvilinear, but, since time was constant, the same intensity of color developed for each concentration of each protein.

RUPTURE AND PROTEIN EXTRACTION OF PETROLEUM-GROWN YEAST. S.D. CUNNINGHAM, C.M. CATER, K.F. MATTIL & C. VANDERZANT. *J. Food Sci.* **40,** 732–735 (1975)–Since the yeast material studied exists as whole yeast cells and the yeast cell wall is highly indigestible in human beings, the cell wall must be ruptured if an efficient extraction of the intracellular proteins is to be achieved. Rupture methods evaluated include: homogenization, sonic oscillation, stone-milling, grinding, rapid gas decompression and freeze-thawing. The effect of a cell rupture technique was measured by the protein released from the cell, microscopy and the cell's reaction to Gram's stain. Cell rupture techniques were also evaluated for their feasibility in industrial scale application. Single-stage, multiple homogenization effects a 2–3-fold increase in protein release. Untreated cells tend to clump together and an increase in protein release after treatment is attributed to clump disruption rather than cell rupture. After homogenization, an exhaustive alkaline extraction, followed by isoelectric precipitation produced a yeast protein concentrate (70% protein) with approximately 42% of the nitrogen being recovered as the protein concentrate. Amino acid analysis of the yeast protein concentrate indicated a high lysine content.

THE FUNCTIONALITY OF BINDERS IN MEAT EMULSIONS. R.M. LAUCK. *J. Food Sci.* 40, 736–740 (1975)–The functionality of commercially available sausage binders, containing significant amounts of protein as partial replacements for meat in imitation frankfurters, was investigated. Meat emulsions were formulated for least-cost with a linear program. The meat emulsions were prepared in the laboratory by chopping meat and other ingredients, in a metal Omni-Mixer cup which was cooled in ice water. A sample of the emulsion was cooked to 79°C and the free water and fat expressed into a volumetric cylinder for a measure of emulsion stability. The binders which were studied included partially delactosed whey, whey protein concentrate, dried sweet whey, "lactalbumin," nonfat dried milk and soy isolate. Optimum finished emulsion temperature for the all-meat frankfurters was about 14°C although ENR-EX®, a binder derived from partially delactosed whey, performed best at 20–25°C. Pilot plant studies indicated that only ENR-EX® successfully replaced some of the beef in an all-meat frankfurter control formula. Meat emulsions prepared and cooked in the laboratory were much more predictive of results in the pilot plant than were model systems which involved titration of protein dispersions with liquid fat.

EFFECT OF PORK HEARTS, ADDITIVES AND pH ADJUSTMENT ON PROPERTIES OF MEAT LOAVES. P.A. HWANG & J.A. CARPENTER. *J. Food Sci.* 40, 741–744 (1975)–The use of pork hearts in meat loaves significantly increased cooking shrinkage and decreased panel scores for texture (firmness) and overall acceptability. Of the protein additives used, only nonfat dry milk (NFDM) decreased cooking shrinkage; whereas loaves containing peanut grits (PG), peanut flour (PF) and soy protein concentrate (SPC) were not different from control loaves. The use of isolated soy protein (ISP) resulted in greater shrinkage than the control samples. Cooking shrinkage decreased as the pH of the meat batters was increased from pH 5.0 to pH 8.0. The concentration of hematin decreased as the pH was adjusted toward 8.0. The concentration of salt-soluble proteins within treatment increased as pH increased (5.0 to 8.0), but the water-soluble protein content within treatment remained essentially unchanged.

QUALITY CHARACTERISTICS OF BROILED AND ROASTED BEEF STEAKS. O.M. BATCHER & P.A. DEARY. *J. Food Sci.* 40, 745–746 (1975)–Quality characteristics of roasted and broiled beef steaks (Semimembranosus muscle) cooked to internal temperatures of 60 and 71°C were compared. Steaks required twice as much time but less than half the

electrical energy to reach an internal temperature of 60 or 71°C when roasted in hot air than when broiled at the same temperature under direct heat. Yields of cooked meat and palatability scores were higher for roasted steaks than for broiled steaks. Panel members noted differences in palatability characteristics of steaks roasted to 60 and 71°C but did not observe such differences in steaks broiled to the same temperatures. Objective measures of press fluid and shear force verified panel evaluations of juiciness and tenderness. Total solids content of the cooked steak was related to panel scores for mealiness and for juiciness.

CHARACTERISTICS OF CONVENTIONALLY AND HOT-BONED BOVINE MUSCLE EXCISED AT VARIOUS CONDITIONING PERIODS. C.L. KASTNER & T.S. RUSSELL. *J. Food Sci.* **40,** 747–750 (1975)–The objective of this study was to evaluate the yield (percent loss), tenderness, flavor and color of bovine muscles held at 16°C and excised at 6, 8 or 10 hr postmortem (hot boning) as compared to muscles held at 2°C and excised at 48-hr postmortem (cold boning). 15 choice and good grade heifers, ranging in weight from 367–501 kg, were assigned to one of the three postmortem holding periods. Hot-boned halves were consistently lower in percent loss than their cold boned counterparts, but statistically different ($P < 0.10$) at only the 10-hr holding period. The differences between overall shear force means were statistically significant ($P < 0.05$) at only the 6-hr holding period. Even though statistical differences were observed between corresponding color reflectance parameter means, a color panel evaluation of the same samples revealed no statistically detectable differences ($P > 0.05$). Flavor panel evaluations indicated that no statistical differences ($P > 0.05$) existed between hot- and cold-boned samples. These data indicate that fabrication at 8 hr postmortem can yield a product of acceptable organoleptic qualities and yield.

THE REMEDIAL AND PREVENTATIVE EFFECT OF DIETARY α-TOCOPHEROL ON THE DEVELOPMENT OF FISHY FLAVOR IN TURKEY MEAT. L. CRAWFORD, M.J. KRETSCH, D.W. PETERSON & A.L. LILYBLADE. *J. Food Sci.* 40, 751–755 (1975)–It was found that about 200 mg/kilo α-tocopherol acetate afforded optimum prevention of fishy flavor in turkeys fed concomitantly with as much as 2% tuna oil. Withdrawal of tuna oil and beef fat substitution in the diet for 2 wk before slaughter effected some decline in fishiness but the addition of α-tocopherol acetate to the beef fat diet significantly accelerated the decline of fishiness in breast meat. Injection of α-tocopherol (170 mg per injection) 72, 48 and 24 hr before slaughter was as effective as feeding α-tocopherol acetate in the reduction of fishiness in thigh meat and skin. The same trend was noted for breast meat although not statistically significant.

DISCOLORATION IN PROCESSED CRABMEAT. A Review. D.D. BOON. *J. Food Sci.* **40,** 756–761 (1975)–Five types of discoloration in processed crabmeat are reviewed: (1) blue discoloration, caused by a reaction involving crab blood; (2) browning, from the Maillard reaction; (3) black discoloration from metallic sulfides; (4) the oxidative discoloration of frozen crabmeat; and (5) discoloration from diffusion of pigments. Emphasis is on blue discoloration. Proposed reaction mechanisms and methods to prevent discoloration are given. 117 references are cited.

IDENTIFICATION OF THE MAJOR VOLATILE COMPONENTS OF BLUEBERRY. T.H. PARLIMENT & M.G. KOLOR. *J. Food Sci.* **40,** 762–763 (1975)–The volatile constituents of high-bush blueberries (*Vaccinium corymbosum*), obtained by distillation-extraction of the fruit, were separated by gas chromatography and subjected to infrared and mass spectral analysis. The predominant feature of this fruit is the variety and concentration of the six-carbon compounds which include both saturated and unsaturated alcohols and aldehydes such as hexanal, trans-2-hexenal, hexanol and the trans-2- and cis-3-hexenols. Also identified are a number of terpene alcohols including the geraniol isomers, alpha-terpineol and linalool, as well as ethyl acetate, ethyl isovalerate, limonene and some commonly occurring alcohols. Relative concentrations are given and the organoleptic importance of the identified components is discussed.

THERMOBACTERIOLOGY OF CANNED WHOLE PEELED TOMATOES. G.K. YORK, J.R. HEIL, G.L. MARSH, A. ANSAR, R.L. MERSON, T. WOLCOTT & S. LEONARD. *J. Food Sci.* **40,** 764–769 (1975)–This research was undertaken to define conditions and procedures necessary to obtain microbial stability in canned whole peeled tomatoes. Spore concentrations were determined for bacteria usually present in unprocessed canned tomatoes and capable of germination and growth in tomatoes, namely *Bacillus coagulans* and butyric acid anaerobes. The effects of pH, heat treatment and spore concentration on the ability of spores to germinate and grow in tomato juice were studied to determine a safe number of spores which may survive in a can without spoiling the product. It was determined that an Integrated Sterilizing value ($I.S._{212}^{27}$) of 1.56 min provides a safe process at pH 4.3 or below.

IMPORTANCE OF ENZYME INACTIVATION PRIOR TO EXTRACTION OF SUGARS FROM PAPAYA. H.T. CHAN JR. & S.C.M. KWOK. *J. Food Sci.* **40,** 770–771 (1975)–Sugars were extracted from fresh papaya, separated by thin-layer chromatography and identified as fructose, glucose and sucrose. Quantitative analysis by gas chromatography showed sucrose to be the predominant sugar, constituting 48.3% followed by glucose 29.8% and fructose 21.9%. An invertase enzyme was detected in papayas which catalyzed the inversion of sucrose according to first-order kinetics with a rate constant of 0.261 min^{-1}. The presence of invertase might explain the conflicting low values previously reported by other workers.

SUGAR COMPOSITION AND INVERTASE ACTIVITY IN LYCHEE. H.T. CHAN JR., S.C.M. KWOK & C.W.Q. LEE. *J. Food Sci.* 40, 772–774 (1975)–Sugars were extracted from lychee, separated by thin-layer chromatography, and identified as fructose, glucose and sucrose. Quantitative analysis by gas-liquid chromatography showed sucrose to be the predominant sugar, constituting 51.1% of the sugars, followed by glucose 30.1% and fructose 18.8%. An invertase enzyme was detected in lychee which had a pH optimum of 2.6.

CHARACTERIZATION OF ANTHOCYANINS IN FIG (Ficus carica L.) FRUITS. A.A. PUECH, C.A. REBEIZ, P.B. CATLIN & J.C. CRANE. *J. Food Sci.* **40,** 775–779 (1975)–The anthocyanin pigments in the skin of ripe Mission fig fruits were extracted in methanolic 1% HCl and purified by paper chromatography. The major pigment comprising 75% of the total anthocyanin pigment was identified as cyanidin 3-rhamnoglucoside. Cyanidin 3,5-diglucoside (11%), cyanidin 3-monoglucoside (11%) and pelargonidin 3-rhamnoglucoside (3%) were also present. The anthocyanin in the pericarp and pedicels of the drupelets (seeds) of the fruit appeared identical to cyanidin 3-rhamnoglucoside in the skin.

TASTE THRESHOLDS OF FATTY ACID ESTERS IN RAISINS AND RAISIN PASTE. D.G. GUADAGNI, A.E. STAFFORD & G. FULLER. *J. Food Sci.* **40,** 780–783 (1975)–Commercial mixtures of ethyl oleate used to enhance the drying rate of grapes had much lower taste thresholds than similar mixtures of methyl oleate in raisin paste. The usual fatty acid ester impurities found in these mixtures had much higher thresholds than ethyl oleate. In three of the four commercial mixtures, thresholds of the known components accounted for the threshold of the mixture. Thresholds of high purity methyl and ethyl oleate prepared from the same source of oleic acid confirmed the lower threshold for ethyl oleate. Therefore, it is clear that methyl oleate would be less likely to give off-flavor problems than equivalent amounts of ethyl oleate in processed raisins.

STRUCTURAL FUNCTIONS OF TASTE IN THE SUGAR SERIES: GUSTATORY PROPERTIES OF ANHYDRO SUGARS. C.K. LEE & G.G. BIRCH. *J. Food Sci.* **40,** 784–787 (1975)–Studies of the stereochemical basis of sweetness in sugar molecules are limited by the availability of suitable model substances and by their intrinsic stability, ring size and shape. Previous reports in this field educed molecular patterns which elicit sweetness and which are related to hydrophilic or hydrophobic bonding ability. Chemical modification of sugar molecules at selected sites alters bonding properties and allows conclusions about stereospecific moieties within a sugar ring which elicit the sweet response. β-D-fructopyranose exists in the $_4C^1$ conformation and can be converted

to a stable derivative when water is intramolecularly eliminated. Anhydro sugars are formed under conditions of pyrolysis when foods are subjected to high temperature, pressure and acidity, thus their sensory properties are of fundamental interest. Accordingly this paper describes some structural functions of taste in a number of anhydro derivatives.

COMPUTER DERIVED PERCEPTUAL MAPS OF FLAVORS. H.R. MOSKOWITZ & E. VON SYDOW. *J. Food Sci.* **40,** 788–792 (1975)–Profiles of the flavor of four juices (cranberry, blueberry, grape, apple) with different levels of added sucrose (in cranberry and blueberry juice) were obtained from panelists, and analyzed by the method of multidimensional scaling. Panelists evaluated these juices using 22 aroma descriptors and 10 taste descriptors. The multidimensional scaling approach embedded both descriptor terms and juices into a two dimensional geometrical space, and simultaneously revealed relations among descriptors, relations among juices, and relations between descriptors and juices. An additional multidimensional scaling procedure, developed specifically to analyze individual differences (INDSCAL) revealed that for the evaluation of grape juice panelists utilized two major 'dimensions,' good-bad and relevant vs irrelevant. Panelists agreed on the use of good-bad descriptors, but differed in the way they conceptualized insistent vs vague attributes. The same analysis revealed that the panelists evaluated the flavor of apple juice consistently across a 16-day period. The methods provide a new, powerful approach for understanding of panelist's responses to the perceptual characteristics of flavor.

EFFECTS OF AMYLOLYTIC ENZYMES ON "MOISTNESS" AND CARBOHYDRATE CHANGES OF BAKED SWEET POTATO CULTIVARS. W.M. WALTER JR., A.E. PURCELL & A.M. NELSON. *J. Food Sci.* **40,** 793–796 (1975)–Baked roots of six sweet potato cultivars were evaluated organoleptically at harvest, after curing and at intervals during storage. The activity of α- and β-amylase enzymes was measured on the raw roots at the time of the sensory evaluations. Alpha amylase was significantly correlated with intensity of "moistness" in the baked roots after curing and storage for 6 wk. Correlations were not significant between "moistness" scores and β-amylase activity. The percent of the starch converted into maltose by baking declined slightly as storage times of raw roots increased. There was no direct relationship between β-amylase activity and maltose production. As storage time increased, α-amylase activity of the raw roots increased. Concurrently the dextrin content increased and molecular size of the total dextrin extract decreased when the stored roots were baked. Intrinsic viscosity, a property of the molecular size, total dextrin extract and starch content after baking were highly correlated with sensory "moistness" scores. This indicates that starch converted by α-amylase is important in defining differences in varietal organoleptic properties.

FLAVOR QUALITY AND STABILITY OF POTATO FLAKES: EFFECTS OF ANTIOXIDANT TREATMENTS. G.M. SAPERS, O. PANASIUK, F.B. TALLEY & R.L. SHAW. *J. Food Sci.* **40,** 797–799 (1975)–Alternative methods of antioxidant addition to potato flakes including Tenox 4 and 5 (BHA + BHT in corn oil and alcohol, respectively) emulsions and sprays, and new antioxidant treatments for flakes using quercetin, caffeic acid and Tenox 5 were investigated. Sensory evaluations and analyses for antioxidants and volatile oxidation products were performed on air-packed flake samples during storage. Initial antioxidant concentrations in flakes were higher with Tenox 4 than with Tenox 5 treatments. Small storage losses of BHT but not BHA occurred with all treatments. The method of antioxidant addition had little effect on flake storage stability. Quercetin and caffeic acid individually were ineffective as antioxidants, but quercetin exhibited possible synergism with Tenox 5.

RELATIONSHIP BETWEEN WATER ACTIVITY AND WATER BINDING IN HIGH AND INTERMEDIATE MOISTURE FOODS. E. KARMAS & C.C. CHEN. *J. Food Sci.* **40,** 800–801 (1975)–The relationship between water activity and water binding was studied using differential scanning calorimetry and electric hygrometry. Glycerol, for example, reduced water activity significantly but did not possess water-binding properties. Sodium caseinate, on the other hand, was found to be a good water binder but did not reduce water activity to the levels necessary in intermediate moisture foods. Water binding and water activity for protein-glycerol combination had a significantly negative correlation. No significant correlation was found between water activity and water binding in food model systems.

INFLUENCE OF WATER ACTIVITY ON GROWTH AND ENTEROTOXIN FORMATION BY Staphylococcus aureus IN FOODS. J.A. TROLLER & J.V. STINSON. *J. Food Sci.* **40,** 802–804 (1975)–Previously published data pertaining to the inhibition of staphylococcal enterotoxin production in low a_w laboratory media have been confirmed in two experimental food systems. Enterotoxin A production by *S. aureus* 196E was prevented at a_w 0.93 and enterotoxin B production by *S. aureus* S-6 was prevented at a_w 0.91 in shrimp slurries. Growth rates, maximal total numbers and toxin levels relative to cell yield were also suppressed as a function of a_w level. Similar results were obtained in potato doughs adjusted to various a_w levels.

GELATION AND THICKENING PHENOMENA OF VEGETABLE PROTEIN PRODUCTS. S.E. FLEMING, F.W. SOSULSKI & N.W. HAMON. *J. Food Sci.* **40,** 805–807 (1975)–The gel forming and viscosity characteristics of flours, concentrates and isolates from oilseed and legume seeds were investigated by heating 10% protein slurries in sealed containers at 90°C for 45 min. The heating treatment caused marked but variable increases in slurry viscosity. Only one soybean isolate developed a firm gel with high viscosity. Although sunflower protein did not form a gel, fababean and field pea concentrates developed gels with medium viscosity characteristics, and were somewhat less elastic than that produced by the soy isolate. A soft gel was developed by soybean flour after treatment by pH-activation. The albumin and globulin protein fractions were isolated from soybean and sunflower flour and from fababean and field pea concentrate. While the albumin fractions showed no thickening ability, the globulins from soybean, fababean and field pea produced gel structures of variable consistency to support the theory that gelation occurs in the globulin fraction.

OXIDATION OF PEA LIPIDS BY PEA SEED LIPOXYGENASE. M. HAYDAR, L. STEELE & D. HADZIYEV. *J. Food Sci.* **40,** 808–814 (1975)–In contrast to fresh green peas, which had two major lipoxygenases, the pea seed kept in cold storage for more than 6 months retained the activity of only one enzyme. This lipoxygenase was extracted and purified by ammonium sulfate fractionation followed by Sephadex G150 gel filtration and DEAE-cellulose column chromatography. The enzyme had a distinct elution profile from DEAE-cellulose, showed an inverse dependence of activity on enzyme concentration, gave a broad pH activity profile with an optimum around pH 7.0 and had a similar response to free linoleic acid and trilinolein. In addition, the enzyme was strongly inhibited by the presence of Ca^{2+} ion. Finally, electrophoresis in sodium-dodecylsulfate gels, after the enzyme was treated with dithiothreitol, gave a molecular weight of 106,000 Daltons. These data suggested that the storage resistant pea seed lipoxygenase corresponds to the recently characterized soybean isoenzyme lipoxygenase-3. In model systems consisting of pea neutral or polar lipid constituents, the enzyme catalyzed the oxidation of the neutral lipid fraction and, to a slightly lesser extent, the pure pea seed triglycerides. Upon preincubation of the triglycerides with lipase only a slightly increased oxidation rate was obtained. The phosphatidylcholine, phosphatidylethanolamine and diphosphatidylglycerol isolated from peas were poor substrates for lipoxygenase. However, their oxidation rate increased greatly after their incubation with snake venom phospholipase. Monogalactosyldiglycerides and digalactosyldiglycerides isolated from pea chloroplasts were also poor substrates for the enzyme and, like phospholipids, were rendered good substrates only upon preincubation with snake venom galactolipase. Thus, the storage resistant pea lipoxygenase seems to oxidize the free unsaturated fatty acids, discriminating against the acids bound in polar lipids, but not against those in triglycerides.

EFFECT OF GAMMA-IRRADIATION ON RED GRAM (Cajanus cajan) PROTEINS. S.P. NENE, U.K. VAKIL & A. SREENIVASAN. *J. Food Sci.* **40,** 815–819 (1975)–The effect of radiation processing on red gram proteins has been studied. Total proteins and amino acid profiles are not appreciably changed, but an overall increase of about 15% in free amino acid levels has been observed in irradiated (1 Mrad) samples. Studies on

stepwise in vitro digestibility by pepsin, trypsin and erepsin indicate increased susceptibility of irradiated red gram proteins to enzyme action. This can be attributed to the degradation of proteins to smaller molecular weight entities. Red gram trypsin inhibitor exhibits high thermostability and radioresistance up to 3 Mrad dose levels.

BOUND PHENOLIC ACIDS IN Brassica AND Sinapis OILSEEDS. A.B. DURKEE & P.A. THIVIERGE. *J. Food Sci.* 40, 820–822 (1975)–Paper and thin-layer chromatographic procedures indicated that sinapic acid was the major bound phenolic acid in *Brassica* and *Sinapis* oilseed meal. Other bound acids were: *p*-coumaric acid, *p*-hydroxybenzoic acid, caffeic acid and traces of ferulic acid. The seed coats contained small quantities of these acids, but additionally protocatechuic acid and vanillic acid as bound forms. Seed meal prepared from white mustard (*Sinapis alba*) contained *p*-hydroxybenzyl-glucosinolate, identified through products obtained after alkaline and acid treatment of the extract. This glucosinolate did not occur in the *Brassica* seed meals. Chlorogenic acid and sinapine, bound forms of caffeic and sinapic acid respectively were actually identified in *Brassica campestris* seed meal.

PROTEIN PRODUCTION FROM CRUDE LACTOSE BY Saccharomyces fragilis. Continuous Culture Studies. P. VANANUVAT & J.E. KINSELLA. *J. Food Sci.* 40, 823–825 (1975)–Continuous cultivation of *Saccharomyces fragilis* on crude lactose under varying conditions revealed that the optimum dilution rate and optimum lactose concentration were 0.18 per hr and 2% respectively under constant conditions of pH 5.0, 30°C, aeration rate of 1 VVM and agitation of 700 rpm. Under these conditions, the yields of yeast and protein were 72.55 and 29.20% respectively. Reduction of chemical oxygen demand (COD) was 61.2%. Productivity was 2g of yeast dry weight per liter of medium per hr. *S. fragilis* cells cultivated under these conditions contained 40% protein and 10% nucleic acids.

GROWTH OF FUNGI AND BOD REDUCTION IN SELECTED BREWERY WASTES. L.J. SHANNON & K.E. STEVENSON. *J. Food Sci.* 40, 826–829 (1975)–Four yeast and four mushroom cultures were inoculated into three brewery wastes, Grain Press Liquor (GPL), Trub Press Liquor (TPL), and Fermentation Sludge Liquor (FSL), to determine the ability of the fungi to grow in the wastes and produce microbial protein. The greatest cell yields and reductions in BOD were obtained in TPL; yields of 27.7 g/liter dry weight and 4.85 g/liter protein were obtained when *Calvatia gigantea* was grown in TPL, with a concomitant 56.1% reduction of the BOD. Cell yields and reduction of BOD were significantly lower in GPL and FSL, except *C. gigantea* reduced the BOD of GPL by 56.2%.

GROWTH OF Calvatia gigantea AND Candida steatolytica IN BREWERY WASTES FOR MICROBIAL PROTEIN PRODUCTION AND BOD REDUCTION. L.J. SHANNON & K.E. STEVENSON. *J. Food Sci.* 40, 830–832 (1975)–*Calvatia gigantea* and *Candida steatolytica* were inoculated into two brewery wastes, Grain Press Liquor and Trub Press Liquor, to determine their ability to reduce the biochemical oxygen demand of the wastes and produce microbial protein. Both of the wastes were buffered with 3 g/liter potassium phosphate, pH 6.0, and supplemented with 0.05, 0.10 and 0.15% nitrogen using $(NH_4)_2SO_4$ or Fermentation Sludge Liquor, another brewery waste, as the N-source. Supplementation with N significantly increased the cell yield, protein content and BOD reduction. Protein yields of up to 14.0 and 5.6 g/liter, and maximum BOD reductions of 75.0 and 54.5% were obtained using *C. gigantea* and *C. steatolytica*, respectively.

ROLE OF THE LYSINE, TYROSINE AND TRYPTOPHAN RESIDUES IN THE ACTIVITY OF MILK LYSOZYMES. B.A. FRIEND, R.R. EITENMILLER & K.M. SHAHANI. *J. Food Sci.* 40, 833–836 (1975)–Selective modification of the lysine, tyrosine and tryptophan residues in bovine milk lysozyme (BML) and human milk lysozyme (HML) was carried out to study the role of these amino acids in both the lytic and chitinase activity of the enzymes. Acetylation of the lysine residues reduced the activity of both BML and HML toward cell walls of *Micrococcus lysodeikticus* but did not alter their chitinase activity. It appears that the lysine residues function only in contributing to the total basicity of the enzymes and do not function directly in the enzymatic activity. Modification of the tyrosine residues with tetranitromethane reduced the activity of the milk lysozymes toward both substrates, but there was no direct relationship between the numbers of tyrosine residues modified and the loss of enzymic activity. Oxidation of the tryptophan residues with N-bromosuccinimide and inhibition studies with N-acetyl glucosamine and histamine indicated that tryptophan is involved in the substrate binding site of HML but not of BML.

STABILITY OF L-ASCORBATE 2-SULFATE AND L-ASCORBATE IN WHEAT FOODS AND MILK. S.F. QUADRI, Y.T. LIANG, P.A. SEIB, C.W. DEYOE & R.C. HOSENEY. *J. Food Sci.* 40, 837–839 (1975)–L-Ascorbate 2-sulfate has been found to be more stable than L-ascorbate during preparation of several cereal products. Recoveries of L-ascorbate 2-sulfate and L-ascorbate from bread, pancakes and extruded product ranged from 86–96% and 0–35%, respectively. In simulated pasteurization of milk, 100% L-ascorbate 2-sulfate was recovered, whereas, 80% L-ascorbate was recovered. The sulfate derivative was also more stable than L-ascorbate in milk stored at 10°C.

YIELDS FROM CHYMOTRYPSIN AND LYSOZYME UNDER FLUCTUATING TEMPERATURE TREATMENTS. A.C.M. WU, R.R. EITENMILLER & J.J. POWERS. *J. Food Sci.* 40, 840–843 (1975)–Chymotrypsin and lysozyme were shown to produce higher yields under fluctuating temperature treatment than at constant mean temperature in the temperature regions below their optimum. By comparing the accumulating yields with the calculated yields during temperature cycling, overshoot and undershoot phenomena were observed. The results indicated that the enzymes showed inverse compensation when subjected to the fluctuating temperature treatment. Above the optimum temperatures, the fluctuating temperature treatment created lower yields. The main reason was that the increment of inactivation due to the temperature fluctuation overshadowed the increment of activation. Statistical analysis of the data showed that at suboptimal temperatures greater amplitude of fluctuation and cycling-down led to greater product yields; whereas, frequency of fluctuation had no significant effect. Near the optimum temperature, cycling-down, greater amplitude of fluctuation and slower frequency of fluctuation led to lower yield.

AN IMPROVED DYED AMYLOSE FOR PLANT α-AMYLASE ASSAY. T.M. DOUGHERTY. *J. Food Sci.* 40, 844–846 (1975)–An improved dyed amylose was prepared which permits determination of plant α-amylase in much less time than required with established methods. The precision, sensitivity and convenience of the assay were demonstrated with tobacco and sweet potato enzymes. The substrate, which was treated with $CaCl_2$, is more sensitive than similar commercially available products and showed linearity with sweet potato α-amylase up to an absorbance of 0.70 units.

EFFECTS OF POLYPHOSPHATES ON THE FLAVOR VOLATILES OF POULTRY MEAT. CH.S. RAO, B.C. DILWORTH, E.J. DAY & T.C. CHEN. *J. Food Sci.* 40, 847–849 (1975)–Addition of polyphosphates enhanced the meaty aroma of canned poultry meat. Hydrogen sulfide and methyl mercaptan concentrations were found to be higher for the polyphosphate-treated samples and were proportional to the amount of polyphosphates added. The reverse situation was observed for both the saturated and the unsaturated carbonyl volatiles. Ammonia and malonaldehyde were also lower for the treated samples than for the control samples. A similar but much greater effect of the polyphosphates on volatile flavor compound production was obtained during the cooking process that consisted of refluxing for 13 hr. Polyphosphates significantly increased the yields of sulfur-containing volatiles and decreased the carbonyls. The reduction in yields of carbonyls was greater in meat than in depot fat samples.

POSTMORTEM GLYCOLYSIS IN PRERIGOR GROUND BOVINE AND RABBIT MUSCLE. R.H. DALRYMPLE & R. HAMM. *J. Food Sci.* 40, 850–853 (1975)–Prerigor ground bovine longissimus and sternomandibularis and rabbit white muscles were examined to determine the postmortem changes in the levels of glycolytic metabolites. The major metabolite changes were a decrease in glycogen and a build-up of lactate and

glucose. Some differences in glycolytic rate and metabolite levels were found between the different ground muscles sampled. From the flux of metabolites it was evident that phosphofructokinase plays a major role in postmortem glycolytic control. The conversion of glycogen into metabolites and the level of total metabolites were generally stoichiometric during the postmortem period studied.

STABILITY OF RAINBOW TROUT (Salmo gairdneri) MUSCLE LYSOSOMES AND THEIR RELATIONSHIP TO RIGOR MORTIS. R.C. WHITING, M.W. MONTGOMERY & A.F. ANGLEMIER. *J. Food Sci.* **40**, 854–857 (1975)–Rainbow trout (*Salmo gairdneri*) white muscle was extracted at various times postmortem into a nuclear-debris pellet, a lysosomal pellet and a supernatant containing soluble enzymes. The lysosomal pellet initially contained 23% of the catheptic and α-glucosidase activity. This percentage decreased to 15% by the end of rigor mortis with small subsequent changes, indicating that approximately one-third of the enzymes was released. Isolated lysosomes rapidly responded to changes in osmotic pressure, salt, pH and temperature conditions but subsequently were relatively stable. Increasing sucrose concentrations and decreasing incubation temperatures increased their stability. Minimum cathepsin release occurred near pH 6. Fish saline had little protective effect but NaCl and $CaCl_2$ appeared to promote enzyme-membrane binding.

STABILITY AND ACCEPTANCE OF INTERMEDIATE MOISTURE, DEEP-FRIED CATFISH. J.L. COLLINS & A.K. YU. *J. Food Sci.* **40**, 858–863 (1975)–Ocean catfish flesh was infused with solutions of sorbitol, other water activity (Aw) depressing agents, an antimycotic and flavor enhancers, then deep fried to yield Aw's of 0.76 and 0.8. Samples held 4 mo at 37.8°C had no change in Aw and color, lower moisture content and pH, and increased thiobarbituric (TBA) values. Samples had no detectable microorganisms after 4 mo. During storage samples became tougher, drier and developed a less desirable flavor and lower overall acceptance. Sauce increased acceptance scores. Samples of 0.8 Aw had higher moisture, pH, color purity, and TBA values; and were softer, more moist, more acceptable and had a more desirable flavor.

EFFECTS OF SULPHYDRYL BLOCKING ON THE THINNING OF EGG WHITE. T. BEVERIDGE & S. NAKAI. *J. Food Sci.* **40**, 864–868 (1975)–The changes which occur in albumen and ovomucin during aging of fresh and sulphydryl (SH)-blocked albumen were evaluated by viscometry and ultracentrifugation. Albumen exhibited decreasing apparent viscosity with shearing time, described by an equation $A = A_O - n \log t$ where t is time in seconds, A is the shear stress in dynes · cm^{-2}, A_O is a constant reflecting the initial shear stresses as shear begins and n is a constant reflecting the rate of structural breakdown. The value A_O was found useful as an index of albumen thinning. $KBrO_3$ treatment gave slightly lower values of A_O wherease KIO_3 gave higher values compared to an untreated control. SH levels were unaffected by $KBrO_3$ but KIO_3 reduced them about 8%. Blockage of up to 25% of the SH groups with $HgCl_2$ or p-chloromercuribenzoate resulted in a reduction of the rate of change of A_O with incubation time; however, more extensive blockage caused increases in A_O suggestive of aggregation reactions. Ultracentrifugation of ovomucin isolated from SH-blocked albumen showed that the relative increase in α-ovomucin which occurs on aging untreated albumin was inhibited.

EFFECTS OF ADDING 2% FREEZE-DRIED EGG WHITE TO BATTERS OF ANGEL FOOD CAKES MADE WITH WHITE CONTAINING EGG YOLK. E.A. SAUTER & J.E. MONTOURE. *J. Food Sci.* **40**, 869–871 (1975)–Angel food cakes were used to evaluate effects of 2% by wt of freeze-dried egg white (FDEW) in counteracting yolk contamination in egg white for angel food cakes. Cake batter was prepared according to the formula: egg white 122g, sugar 125g, cake flour 45g, cream of tartar 1.8g and NaCl 0.6g. Control cakes were made with fresh egg white without added yolk. Cakes were baked at 170°C for 27 min then inverted, cooled and volume determined by seed displacement. Volume of control cakes averaged 364 ml. Cakes from egg white having 0.1% yolk averaged 334 ml and volume of cakes decreased as yolk content increased. Egg white containing 0.75% yolk resulted in average cake volume of 200 ml. Addition of 2% FDEW resulted in cake volume similar to controls when added to egg white containing up to 0.3% yolk and significant improvement in cake volume at all levels of yolk tested. In general, overall acceptability ratings by the taste panel followed volume measurements but cakes containing FDEW usually were considered as slightly less tender than control cakes.

FATTY ACIDS IN THE TISSUES OF FOUR GENERATIONS OF MALE AND FEMALE RATS FED SEVERAL FOOD FATS WITH AND WITHOUT ADDED CHLORINATED HYDROCARBON INSECTICIDES. M. ADAMS, F.B. COON & C.E. POLING. *J. Food Sci.* **40**, 872–878 (1975)–To determine the influence of the prolonged feeding of high levels of fat with and without added insecticides on tissue fatty acids, male and female rats were fed for four generations diets containing 20% unheated, heated, and lightly hydrogenated cottonseed oil, lard and heated lard, soybean oil, and hydrogenated vegetable oil shortening. Insecticides did not influence tissue fatty acids. Generation differences suggest a slow adaptation to high fat intakes. Sex related differences in adult livers were influenced by composition of dietary fat. Maternal diet influenced similarly tissue fatty acids of weanlings and adults. Tissue linoleic and oleic acids reflected intake. Stearic acid levels were little influenced by intake. The ability of the body to exert considerable control over tissue fatty acids was apparent.

FACTORS AFFECTING THE RELATIVE BIOLOGICAL VALUE OF FOOD GRADE ELEMENTAL IRON POWDERS FOR RATS AND HUMANS. M.D. PENNELL, W-D. WIENS, J. RASPER, I. MOTZOK & H.U. ROSS. *J. Food Sci.* **40**, 879–883 (1975)–Two types of elemental iron powders, electrolytic and reduced, were separated into several particle-size fractions by elutriation with nitrogen. For two fraction sizes (7–10 μm and 20–26 μm) the relative biological values (RBV) of electrolytic iron for rats were higher than those of hydrogen reduced iron, the difference in RBV being significant for the 20–26 μm fractions. Similar differences in RBV between the two types of elemental iron were obtained with human subjects in studies based on the relative absorption of iron after ingestion of 100 mg doses of iron of ferrous sulfate (standard) and test samples. Processing of a rice cereal product enriched with electrolytic iron did not affect the RBV of the supplemental iron for rats.

OBJECTIVE ASSESSMENT OF MEAT JUICINESS. P.E. BOUTON, A.L. FORD, P.V. HARRIS & D. RATCLIFF. *J. Food Sci.* **40**, 884–885 (1975)–Samples of meat, both fresh and aged cooked at different temperatures were used to determine the applicability of objective methods to the measurement of juiciness. The moisture lost in cooking and the amount of juice extracted by high speed centrifugation of cooked meat samples were both measured and expressed as a percentage of the original raw meat weights. In meat of normal pH (5.4–5.8) both of these factors were highly correlated ($r \geq 0.90$, $n = 200$) with juiciness assessed subjectively.

THE ARMOUR TENDEROMETER AS A PREDICTOR OF COOKED MEAT TENDERNESS. D.R. CAMPION, J.D. CROUSE & M.E. DIKEMAN. *J. Food Sci.* **40**, 886–887 (1975)–The purpose of the present experiment was to assess the predictive value of Armour tenderometer (AT) measurements for certain carcass and organoleptic traits for beef carcasses that varied greatly in body composition. Marbling score alone accounted for 10% of the variation in taste panel tenderness. The additional variation accounted for by the AT measurement in a multiple regression equation ($R^2 = 0.14$) is of questionable practical importance even though the usefulness of the AT was increased when used at constant marbling score or in conjunction with marbling.

EFFECT OF SODIUM PYRUVATE ON THE TEXTURE OF FROZEN STORED COD FILLETS. V.D. TRAN. *J. Food Sci.* **40**, 888–889 (1975)–Cod fillets were treated by dipping in 10% sodium pyruvate solution prior to freezing and storage at −23°C for up to 180 days. At various times, the treated fillets and controls were thawed, cooked in steam, and evaluated for organoleptic texture by a panel of 8–10 trained judges. Results showed that the pyruvate treatment not only prevented texture toughening but also had a tenderizing effect upon the stored cod fillets.

PRODUCTION OF CHEDDAR-LIKE CHEESES VIA NONFERMENTATIVE pH MANIPULATION. J.R. ROSENAU, J.C. ANDERSON & H.A. MORRIS. *J. Food Sci.* **40**, 890–891 (1975)—A process is presented for the continuous production of Cheddar-like cheese through the production of a coprecipitate by the addition of heat and acid to 2% fat milk. The coprecipitate is further processed by pressing and extrusion to modify its textural properties. Rennet is not used. Provision for the addition of flavors and other food additives has been included so that a wide variety of product identities is possible. The heat denaturable whey proteins are incorporated into the product giving an increased yield over conventional systems. The product can be packaged into its final form at the point of manufacture and marketed without aging.

IDENTIFICATION AND DETERMINATION OF SUGARS IN SOUR SOP, ROSE APPLE, MOUNTAIN APPLE AND SURINAM CHERRY. H.T. CHAN JR. & C.W.Q. LEE. *J. Food Sci.* **40**, 892–893 (1975)—Sugars in soursop (*Anona muricata*) rose apple (*Eugenia jambos*), mountain apple (*E. malaccensis*), and Surinam cherry (*E. michelli*) were extracted, separated by thin-layer chromatography, and identified as fructose, glucose and sucrose. Gas liquid chromatography (GLC) of the trimethylsilylated sugars confirmed their presence. Quantitative analysis by GLC using myo-inositol as an internal standard gave the following fructose, glucose and sucrose contents: soursop, 1.80%, 2.27%, 6.51%; rose apple, 1.96%, 3.00%, 1.81%; mountain apple, 1.49%, 2.06%, none detected; Surinam cherry, 1.07%, 1.37%, 1.38%.

DETERMINATION OF TOTAL ASCORBIC ACID IN VEGETABLES FROM ALCOHOL SLURRIES. V.G. RANDALL, E.L. PIPPEN, A.L. POTTER & R.M. McCREADY. *J. Food Sci.* **40**, 894–895 (1975)—Ethanol slurry of vegetable tissues provided a single sample from which several nutrients could be analyzed. Comparative studies on total ascorbic acid content were conducted from meta-phosphoric acid slurry and from ethanol slurry of 10 frozen vegetables, to determine the suitability of ethanol as an extractant of ascorbic acid. Results show ethanol is suitable as a slurrying medium from which total ascorbic acid may be determined in frozen vegetables.

A SIMPLE METHOD FOR EMULSION END-POINT DETERMINATIONS. W.H. MARSHALL, T.R. DUTSON, Z.L. CARPENTER & G.C. SMITH. *J. Food Sci.* **40**, 896–897 (1975)—In an effort to improve and simplify emulsion end-point determinations, a visual technique was developed which employs colored oil as the discontinuous phase. The oil to be emulsified was colored with a biological stain (0.3g/liter of Oil-Red-O) and subsequently utilized in a model system consisting of a Waring Blendor (operated at full speed) and a Sage Tubing Pump (operated at 77.3% max rate). Oil was delivered to the top of the emulsion at the rate of 0.5 ml/sec. End-point determinations (n = 420) were made with colored or noncolored oil using longissimus muscle tissue (2.0, 10.0 and 18.0g of muscle slurry, made up to 50 ml in 1M NaCl), soy protein isolate, soy protein concentrate, soy flour and blood serum protein. The use of colored oil was associated with reduced quantities of oil required to reach the emulsion end-point for all of the proteins except soy protein concentrate. Use of colored oil also significantly lowered the standard deviations for end-point determinations for all of the proteins except soy protein concentrate. The decreased volume of oil required and the reduced variability of end-point determinations generally obtained with the use of colored oil are most likely the result of increased visibility of the colored oil droplets as they coalesce just prior to inversion, thus providing a definitive end-point when inversion takes place. Without the use of colored oil, the droplets must become larger to be visualized causing the observed end-point to be less distinct, oil addition to be prolonged and emulsification capacity of the protein to be overestimated.

Errata Notice

- J. Food Sci. 40(1): 50–52 (1975), A.S. Arafa and T.C. Chen: "Effect of vacuum packaging on microorganisms on cut-up chicken and in chicken products." On page iv (Abstract), line 4; page 50 (Experimental), line 12; and page 52 (Results & Discussion), line 2: Change "1.5 mil polyethylene" to read "3 mil polyester/polyethylene laminate."

- J. Food Sci. 40(3): 569–575, J.L. Etchells, H.P. Fleming, L.H. Hontz, T.A. Bell and R.J. Monroe: "Factors influencing bloater formation in brined cucumbers during controlled fermentation." On page 569, Author listing: Change the initials of Monroe from R.S. to R.J.

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JFS APPLIED SCIENCE and ENGINEERING

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EXPERIMENTAL EVALUATION OF MATHEMATICAL AND COMPUTER MODELS FOR THERMAL PROCESS EVALUATION

INTRODUCTION

HAYAKAWA (1969) presented a comparison of thiamine retentions in thermally processed foods determined by laboratory analysis with predictions made by the method of Ball and Olson (1957) and with predictions made by his recently-developed method employing new dimensionless parameters. Both of these methods were applicable to thermal process evaluations with respect to thiamine retention because they integrated the destruction effect of the temperature history at all points throughout the container. Timbers and Hayakawa (1967) also noted that Stumbo (1953) had developed a mathematical method to integrate the lethal effects on bacteria, of a thermal process at all points in the container, but was unable to obtain meaningful results when he attempted to calculate thiamine retentions with the method. This was as might have been expected since the method was developed for microbial sterility evaluation only. Jen et al. (1971) modified the original Stumbo method to obtain one that would apply equally well to both thiamine and bacterial inactivation, or to the inactivation of any other thermally vulnerable factor exhibiting first order inactivation kinetics.

Teixeira et al. (1969) developed a numerical computer model to simulate the thermal processing of canned foods, that would simultaneously predict the lethal effect of a heat process on bacteria and its destructive effect on thiamine.

The purpose of this paper is to present experimental evidence to support the accuracy and validity of both Stumbo's modified mathematical method and the computer model of Teixeira et al. (1969) for thermal process evaluations with respect to thiamine retention.

PROCEDURE

THE FOOD PRODUCT chosen for this study was pea puree because it was among the food products for which thiamine destruction rates were reported (Felliciotti and Esselen, 1957), and because peas by nature are usually relatively rich in thiamine (c.f. USDA, 1963). The puree was prepared from frozen, sweet peas purchased from a local food market. The peas were rapidly thawed by placing them in boiling water for 2 min. They were then blended in a Waring Blendor with sufficient water to obtain a puree containing 20% additional water by weight.

Heat penetration tests, employing nonprojecting plug-in thermocouples (Ecklund, 1949), were conducted to determine the thermal diffusivity and the heating parameter f_h of the pea puree. The heat penetration tests were carried out simultaneously with three 603 × 700 cans and three 303 × 406 cans, using a constant retort temperature of 250°F.

The two different can sizes were used in order to observe the effect of the expected conduction error from the shorter thermocouples in the smaller containers, and to compare this observation with the conduction errors reported by Segmiller and Stumbo (1957) for similar container sizes. The f_h parameter was determined for each can by applying a log-linear regression analysis to the data points along the straight-line portion of the heating curve taken directly from the recording potentiometer. The thermal diffusivity for the pea puree was then calculated by an equation (Olson and Jackson, 1942) relating thermal diffusivity with container dimensions and the f_h parameter for the 603 × 700 cans. The larger cans were used because the central j value agrees closely with the theoretical j value of the asymptote. A "corrected" value for the f_h in the smaller containers was calculated by entering this thermal diffusivity value, along with the inner dimensions of the 303 × 406 can, in the same equation.

Having determined the thermal properties of the pea puree, a number of thermal processes were specified to be modeled by the mathematical and computer methods, and to be applied in a pilot plant retort for obtaining processed samples for laboratory assay of thiamine. Four different retort processes were specified by choosing different

Table 1—Description of retort processes prescribed for thiamine assay comparisons[a]

Process number	Retort temperature (°F)	Process time (min)
1	250	85.0
2	240	136.0
3	260	65.0
4	T: 260, 250, 240, 230; t: 20, 40, 60, 87	87.0

[a] All processes have equal sterilizing value with respect to spores of B. stearothermophilus.

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retort temperatures and corresponding process times to make them all equivalent with respect to their sterilization capacity for spores of *Bacillus stearothermophilus*, having heat resistance characterized by D_{250} = 4.00 and z = 18. Constant retort temperatures of 240°F, 250°F and 260°F were specified for three of the processes. The required retort heating and cooling times to achieve a five log-cycle reduction in any initial spore population were calculated by Stumbo's (1965) method of process determination, and checked with the computer model. The fourth process was specified by a step-increasing retort temperature beginning with 230°F, then 240°F, 250°F and 260°F with 20 min between each step change. The process time was determined by the computer model on the basis of achieving the same five log-cycle reduction in spore population required of all four processes. A description of each of these processes with times and temperatures is shown in Table 1.

Since Felliciotti and Esselen (1957) did not establish thiamine destruction curves for a temperature of 250°F, it was first necessary to convert the D-value from one of the temperatures they used to a D-value at 250°F. For pea puree, Felliciotti and Esselen (1957) reported a D-value of 202.3 min at 246°F, which is equivalent to a D-value of 165.6 at 250°F. The thiamine retention for these processes was computed using destruction rates of D_{250}-value of 165.6 and z-value of 46.

The "thiochrome method" for thiamine assay described by the Association of Vitamin Chemists (1951) was used in this study. All five procedural steps outlined were required, including purification with thiochrome Decalso in base exchange tubes. In order to experimentally determine the thiamine retention associated with any one process, the assay procedure was conducted simultaneously on six individual samples of pea puree. Three of the samples were taken from cans that had been retort processed in the pilot plant. The other three samples (controls) were taken directly from the freshly prepared batch of pea puree immediately, prior to canning. Sufficient assay solution was prepared from each sample to permit four replicate readings on the Coleman photofluorometer plus one blank determination for each sample. This resulted in a total of 12 replicate readings to represent the thiamine concentration in the three processed samples, and 12 to represent the thiamine concentration in the three control samples.

When the assays were completed, the replicate readings were treated statistically to determine standard deviations and confidence limits on the mean values. The percent thiamine retention was then calculated directly from the mean values of the scale readings for the processed samples and control samples, since the meter deflections on the photofluorometer were linearly proportional to the thiamine concentrations for the range of concentrations that were used.

RESULTS & DISCUSSION

A SUMMARY of results from the heat penetration tests is shown in Table 2. Note that the observed f_h for the 303 X 406 cans was nearly 6 min shorter than the "corrected" value calculated with the thermal diffusivity obtained from the f_h for the larger number 603 X 700 cans. This difference in the f_h for the smaller container agrees closely with the observations made by Segmiller and Stumbo (1957), who found that the f_h measured from normal heat penetration data on such a container size was approximately 6 min shorter than a value which they obtained from a thermocouple whose lead-in wires and receptacle were protected from the high-temperature steam atmosphere. In this same way, they found a comparatively minimal conduction error in the normal heat penetration data for the larger 603 X 700.

Results from the thiamine assays revealed variations among replicate readings by as much as five scale deflections on the

Table 2–Results from heat penetration tests on pea puree in number 10 cans and 303 X 406 cans

Can size	f_h (min)	Thermal diffusivity (in.^{2}/min)
Number 10	170.5	0.0158
303 X 406	41.0	0.0182
303 X 406 (corrected)	46.9	0.0158

Table 4–Error sensitivity of computer model showing effect of perturbations in governing input variables on predicted thiamine retention

Process	Type of perturbation	Percent thiamine retention	Expected error
#1 (250°F) 85 min	Unperturbed condition	49	0
	Lower retort temp by 1/2°F and shorten process time by 1/2 min.	50	+1.0
	Lower thermal diffusivity by 0.001 in.^{2}/min	50.5	+1.5
	Lower D-value by 10%	52	+3.0
	Combined increase	55	+6.0
	Combined decrease	43	–6.0
#3 (260°F) 65 min	Unperturbed condition	49	0
	Combined increase	55	+6.0
	Combined decrease	42	–7.0

Table 3–Precision of experimentally determined thiamine retentions from a statistical data treatment of the laboratory assay results

Process	Samples	Mean value of meter readings	Blanks	90% Confidence interval on mean values	Percent thiamine retention	90% Confidence interval on % retention
1	Control	21.58	2.5	± 0.53	50.7	± 2%
	Process	12.17	2.5	± 0.13		
2	Control	13.83	2.7	± 0.93	42.3	± 6%
	Process	7.46	2.8	± 0.32		
3	Control	15.21	2.5	± 1.18	51.3	± 9%
	Process	9.54	3.0	± 0.46		
4	Control	12.87	2.5	± 0.47	53.5	± 6%
	Process	8.54	3.0	± 0.49		

Table 5–Comparison of thiamine retention in 303 X 406 cans of pea puree between Stumbo's method, computer model of Teixeira, and laboratory assay determinations for various thermal processes

Process number	Process description	% Thiamine retention by: Stumbo's method	Computer model	Lab assay
1	85 min at 250°F	48.7	49.2	50.7
2	136 min at 240°F	41.0	41.6	42.3
3	65 min at 260°F	47.3	48.8	51.3
4	Step-increase policy (Described in Table 1)		50.2	53.5

photofluorometer in some cases. Through the application of the t distribution, 90% confidence intervals of mean thiamine contents were estimated (Wilson, 1952).

Having established the confidence intervals for each of the mean values, the meter readings were then translated into percent thiamine retention for each process. This was accomplished by subtracting the blank readings from each mean value and taking the ratio of this difference for the processed samples over that for the control samples for each process. The 90% confidence interval on the final percent thiamine retention was then established by calculating the ratio twice more in each case using the maximum mean value of processed samples over the minimum mean value of control samples, and then the minimum mean value of processed samples over the maximum mean value of control samples. A concise presentation of these results for all four processes is shown in Table 3.

Actual thiamine retentions would agree with mathematical or computer predictions only if the value of each physical parameter, each rate constant, and every processing condition were precisely equal to each respective value supplied as input data to the computer model or mathematical calculation. In biological systems, it is unlikely that such precision can often be obtained. In order to adequately compare computer predictions to experimental results, the error sensitivity of the computer model was determined by estimating the variation that could be expected in the computer prediction from realistic perturbations in the input parameters that would chiefly govern the prediction of thiamine retention. For example, it was recognized that an error of ± 1/2 Fahrenheit degree in retort temperature could remain undetected throughout the process. Likewise, the process time could be controlled only to within ± 1/2 min. Variations of as much as 2 min for the value of f_h are commonly observed among replicate heat penetration tests. This would correspond to a variation in the calculated thermal diffusivity of ± 0.001 in.^{2}/min. A study of the thiamine destruction rate data in Felliciotti and Esselen (1956) revealed that the D-value might vary by as much as 10%. Table 4 was constructed to show the effect of each of these perturbations on the predicted thiamine retention for process number 1, followed by the extreme cases in which all perturbations were combined to alter the predicted thiamine retention in one direction and then in the other. The effect of the combined perturbations are also shown for process number 3 in which the highest retort temperature of 260°F was used. The results show a maximum possible variation of ± 6% in the thiamine retention predicted by the computer model for the remote possibility that all errors are compounded in the same direction.

In addition to comparing computer predictions with laboratory analysis, thiamine retentions were also calculated by Stumbo's modified method reported by Jen et al. (1971). This method, however, applied only to the three thermal processes in which the retort temperature was held constant.

The results from all three methods are compared in Table 5. Agreement between calculated values and laboratory assay results is, in all cases, well within the ± 6% expected error, and confirms the validity of both the computer model developed by Teixeira et al. (1969) and Stumbo's method as modified by Jen et al. (1971). A similar confirmatory comparison was reported by Jen et al. (1971) which included the assay determinations reported by Hayakawa (1969) and predictions of percent thiamine retention by the methods of Ball and Olson (1957), Hayakawa (1969), Teixeira et al. (1969) and Jen et al. (1971).

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COMPUTER SIMULATION OF VARIABLE RETORT CONTROL AND CONTAINER GEOMETRY AS A POSSIBLE MEANS OF IMPROVING THIAMINE RETENTION IN THERMALLY PROCESSED FOODS

INTRODUCTION

THE EFFECT OF the heat sterilization process for canned foods on the quality and nutrient retention of the food has been a major concern of food processors since Nicholas Appert first discovered the art of canning for food preservation in 1809. This concern has constantly prompted study into ways to reduce quality destruction in the sterilizing process. One result of this study has been the development of aseptic canning methods, which utilize the benefits of high temperature-short time processing. These methods, however, do not apply to solid-packed food products like canned meat and fish, in which the rate of heat transfer is very slow. The thermal processing of these products has been generally carried out with a constant retort temperature for a specified process time to insure some required sterilization. The notion that quality improvement might be possible with processes in which the retort temperature would vary as a function of time has always been dismissed because of the impractical experimental trial and error that would be involved.

Teixeira et al. (1969) developed a computer model that was shown to accurately simulate the heat processing of a solid conduction-heating material with constant thermal properties in the shape of a finite cylinder. By integrating through space and time, the model could predict the effects of any given set of process conditions on any number of thermally vulnerable factors that may be present in such material as long as the reaction kinetics for each of these factors were independent and could be described mathematically. In this study, the model was used to simulate the thermal processing of a canned food product in order to predict the effects on the level of thiamine retention produced by various surface temperature policies of equal sterilizing value. The purpose of this investigation was to discover whether or not any significant improvement in the nutritional value of a canned food product could be realized by permitting the surface temperature to vary as a function of time while insuring the sterilization capacity of the process.

A study of the basic kinetics has shown that both the destruction rate of bacteria and the degradation rate of thiamine accelerate with increasing temperature, but that this accelerating effect is much greater on the bacterial kinetics than on the thiamine kinetics, Ball (1938), and Joslyn and Heid (1963). Consequently, it has been shown that improved thiamine retention can be achieved with a high temperature-short time policy when the material can be heated rapidly. The shorter process time permitted by the accelerated lethal rate prevents the thiamine degradation from proceeding very far even though this degradation occurs at a faster rate, c.f. Ball (1938), Joslyn and Heid (1963), Everson et al. (1964) and Luh et al. (1969).

It has also been shown, however, that when high temperature-short time policies have been applied to conduction-heated canned foods, the resulting thiamine retention is lower, Teixeira et al. (1969). This was a consequence of the physical size and thermal properties of the material being heated. Because regions near the surface of the container must be exposed to the higher temperature sufficiently long to permit adequate sterilization at the center, the accelerating effect of the higher temperature on the rate of thiamine degradation in these regions is such that the reduction in process time is not sufficient to compensate for it. The rationale for this investigation was based on the hypothesis that it could be possible to adjust the degree to which each of these opposing effects would predominate by manipulating the surface temperature as a function of time.

Because the can size was expected to be a strong factor that would limit the response of interior temperatures to any variable control action on the surface, a second application of the model was made to study the effects of various container geometries of equal volume on the level of thiamine retention for both constant and time-varying surface temperature policies of equal sterilizing value.

PROCEDURE

IN ORDER TO LIMIT the scope of this investigation, the assumption has been made that any practical application of variable surface temperatures in the thermal processing of canned foods would be achieved with the use of saturated steam under pressure, either as a variable retort control, or by passing the food container from one chamber to another held at different steam temperatures. Because of the low thermal diffusivity and large container size of most canned foods, the time of exposure to any given surface temperature would have to be of at least several minutes duration in order to affect the interior temperature distribution. With exposure times of this magnitude, it was reasoned that surface temperatures above 265°F should be avoided to prevent excessive degradation in the outer regions of the container. The lower limit for the surface temperature was taken as 225°F because at lower temperatures there would be little or no lethal effect on thermophilic spores while nutritent degradation would continue at a significant rate.

Because of its widespread use, the container size chosen for this study was that of a number 2 can, which measured 3-7/16 in. in diameter by 4-9/16 in. in height. The thermal diffusivity of the food material that was modeled in this study was taken as 0.0143 in^2/min and has been found to be representative of most conduction-heated foods, Olson and Jackson (1942).

Spores of *Bacillus stearothermophilus* were taken as the food spoilage organism of greatest concern in this study. The destruction rate data for these organisms, as reported by Stumbo (1965), were given by a D-value of 4 min at 250°F and a z-value at 18 F°. The sterilizing effect of all the processes defined in this investigation was held fixed as a constraint, and was defined by a five log-cycle reduction of the initial spore population in the container. In thermal processing terminology, this lethal effect would be given by an F_S-value of 20 as defined by Stumbo (1965).

According to Felliciotti and Esselen (1957), thiamine in pork has a D_{246}-value of 178.6 and a z-value of 46. These were chosen for the thiamine destruction rate data used in this study.

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In order to establish a definition for "improved thiamine retention," a standard thermal process was accepted as a reference standard. This "standard process" was described as the process time required to achieve a five log-cycle reduction in spores of *B. stearothermophilus* in a number 2 can with a thermal diffusivity of 0.0143 in²/min at a constant surface temperature of 250°F. A hot-fill initial temperature of 160°F was assumed for all processes throughout the investigation.

Theoretically, there were an infinite number of ways in which the surface temperature could vary between the upper and lower limits specified. However, three general types of behavior patterns were chosen to classify nearly all of the possibilities. These were sinusoidal functions, ramp functions, and step functions. The method of search was to investigate, systematically, surface temperature policies in each of these categories. The purpose of investigating sinusoidal policies was to determine whether or not there would be some benefit to a policy in which the surface temperature reversed itself periodically between upper and lower limits. The amplitude was fixed so that the surface temperature oscillated between 265°F and 225°F at various frequencies. The lower limit for the range of frequencies studied was determined by noting the process time for the standard process, and choosing a frequency that would permit a one-half sine wave within that time. The upper limit was established by noting that frequency above which the results were no different than when the surface temperature was simply held constant at the mean value of the sinusoid. The investigation began with a frequency of one-half cycle per hour, then one cycle per hour, two cycles per hour, and so on. This same sequence was then repeated with a 90° shift in phase angle to investigate a corresponding series of cosine functions. The process for each policy was determined by repeated computer calculations in a search routine to find the time at which cooling should begin in order to achieve the required five log-cycle reduction in spore population at the end of cooling.

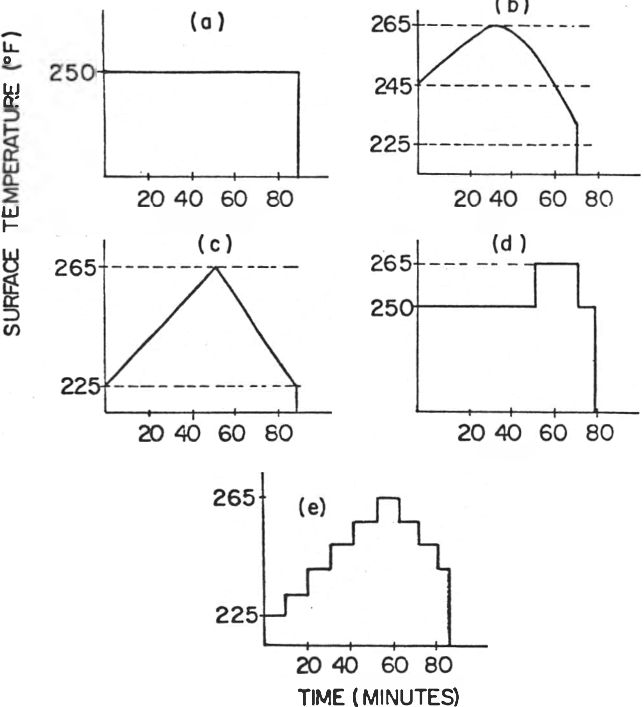

Fig. 1–Description of variable surface temperature policies showing maximum thiamine retention.

A preliminary investigation of possible ramp functions for the surface temperature was restricted to rising and falling ramps of different but constant slopes in which the surface temperature increased linearly from the lower limit to the upper limit, and then decreased linearly to the point at which cooling should begin for the required sterilization. The sequence of investigation consisted of locating the apex of the ramp functions at different points in time within the process time (Refer to the lower portion of Fig. 2). Once the "best" ramp function had been found from this investigation, attempts were made to obtain further improvement by investigating nonlinear ramp functions like exponentials and quadratics in the neighborhood of this "best" ramp.

Additional investigations with step functions were based on information obtained from the results of the previous investigations with sinusoids and ramps. Successive trials with step functions were made on the basis of information obtained from all previous results.

Limiting conditions were also specified for the investigation of various container geometries. In order to be consistent with practical considerations of can filling and sealing methods, and to accommodate the provision for a cylindrical geometry in the computer model, the investigation of container geometries was restricted to a right circular cylinder of various height-to-diameter ratios enclosing a constant volume equal to that of a number 2 can. The range of height-to-diameter ratios, L/D, was chosen between a low value of 0.1 corresponding to a container height of 0.75 in., and a high value of 13.7 corresponding to a container height of 20 in. Although a specific volume has been chosen for purposes of example, the findings given in this paper are likely applicable to cans of any volumes.

The surface temperature was held constant at 250°F to permit a direct comparison of thiamine retentions with the standard thermal process previously defined. The process time for each case was, again, determined on the basis of achieving the required sterilization at the end of cooling. Beginning with the dimensions of a number 2 can, process determinations were made for lower values of L/D corresponding to decreasing container heights of 3, 2, 1 and 0.75 in., respectively. Higher values of L/D were chosen corresponding to decreasing container radii of 1.5, 1.25, 1.00 and 0.75 in., respectively.

In this manner, it was possible to calculate the thiamine retention for thermal processes of equal sterilizing value on each of the container geometries specified, and to report an optimum geometry among these for which the thiamine retention was greatest. Additional improvement in thiamine retention was then found by applying higher constant surface temperatures to this optimum geometry.

Table 1–Maximum thiamine retention observed from investigations of various surface temperature policies including process times required to insure equal sterilization

Surface temp policy (see Figure 1)	Process time (min)	Thiamine retention (%)
Standard process Figure 1 (a)	89	41
Best sinusoidal function Figure 1 (b)	70	41
Best combination of ramps Figure 1 (c)	88	43
Best single square wave Figure 1 (d)	79	41
Best sequence of steps Figure 1 (e)	84	43

RESULTS & DISCUSSION

A SYNOPSIS of the results from the investigation of variable surface tempterature policies is presented in Table 1 showing the maximum thiamine retention observed with the optimal policy in each category, along with the process time required in each case to insure equal sterilization for all policies. Figure 1 has been included to present a graphical description for each of these policies. It should be noted that the policies producing the highest level of thiamine retention seem to favor a general configuration in which the surface temperature gradually increases from the lower limit to the upper limit approximately midway through the process time, followed by a gradual decrease to the point at which cooling should begin. Figure 2, showing the results from the investigation of linear ramp functions, clearly reveals this trend.

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The results show that the maximum thiamine retention observed was only two percentage points greater than that for the standard process, representing only five percent improvement. Since Felliciotti and Esselen (1957) reported deviations in their laboratory assay of thiamine within ± 5% of the mean, the maximum thiamine retention observed from this investigation cannot be accepted as any indication of significant improvement.

From what is understood of the relative reaction kinetics involved, it could be reasoned that maximum thiamine retention would favor a temperature policy such that every point throughout the container would experience a high temperature-short time process. However, since the driving force required to raise the temperature of the food must occur at the boundary, the temperature at any interior point can respond only in accord with the mechanics of heat conduction; and the rate of this response is severely limited by the depth of food material to which the heat energy must penetrate, and the low thermal diffusivity characteristic of most foods.

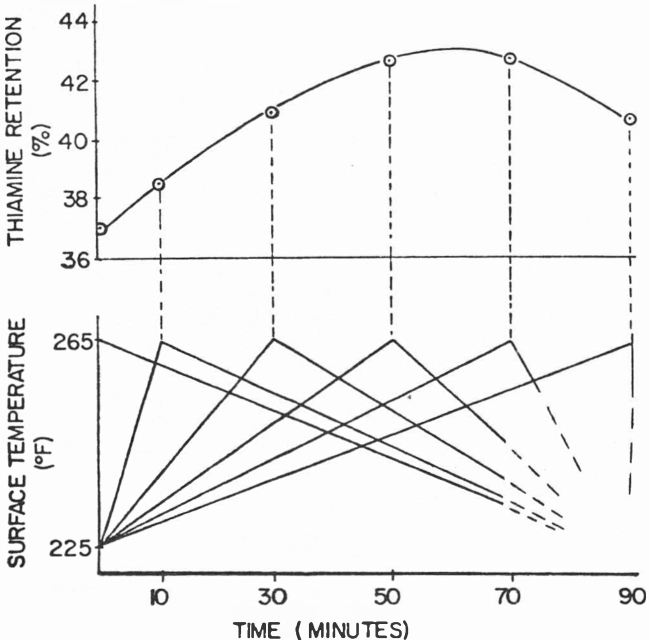

Fig. 2–Results from investigation of linear ramp functions, showing percent thiamine retention versus individual ramp policies.

Although the thermal diffusivity is a physical constant describing the thermal properties of the food, the depth of food material through which the heat energy must penetrate can be treated as a control variable by adjusting the container geometry. The results from the computer investigation of various container geometries, as described in the procedure, are presented graphically in Figure 3 as percent thiamine retention vs. L/D on a log scale. Note that the standard-size number 2 can is represented by one of the lowest points on the curve. The corresponding container dimensions, surface area, and process times, are listed in Table 2.

The results show that for an L/D of approximately 0.1 (flat disk), the level of thiamine retention when compared with a number 2 can, had increased from 41% to 68% with a decrease in the process time required from 90 min to 30 min. Improvement in the same order of magnitude was also observed at the opposite extreme with L/D greater than 10. These results were obtained with thermal processes in which the retort temperature was held constant at 250°F.

Using the flat disk geometry (L/D = 0.1), various surface temperature policies were investigated, and the results are shown in Table 3. Although an appreciable increase in the thiamine retention was observed with the linear ramp function, maximum retention was obtained with the high temperature-short time policy. This can be explained from the fact that the improved thiamine retention was a result of the shorter process time made possible by the accelerating effect of higher temperature on the bacterial kinetics. Since the system geometry in this case would permit rapid heating, it follows that little would be gained from a variable surface temperature policy other than that in which the surface temperature is held constant at the upper limit.

Table 2–Container dimensions, surface area, process time and percent thiamine retention corresponding to various L/D ratios for containers of equal volumes

L/D (No. 2 Can)	L (in.)	D (in.)	Surface area (in.²)	Process time (min) at 250° F	Thiamine retention (%)
0.096	0.075	7.8	113.9	30	68
0.143	1.00	7.5	98.5	38	63
0.495	2.00	4.8	66.4	75	45
0.767	3.00	3.9	61.1	90	40
1.270	4.20	3.3	60.6	89	41
1.710	5.12	3.0	62.4	83	43
2.960	7.40	2.5	67.9	68	49
5.760	11.50	2.0	78.5	52	56
13.750	20.60	1.5	100.6	38	63

Table 3–Effect of various surface temperature policies on a flat disk geometry with L/D = 0.1

Policy description		Percent thiamine retention
Standard	T; 250; 30 t	68
HT–ST	T; 265; 13.5; t	77
Ramp	265; 225; 27 t	74

In addition to the quality improvement that could be expected with these various geometries, there may also be certain economic advantages from the reduced process times required, as shown in Table 2. These advantages, however, might be offset by increased container costs relative to the greater surface area required in the various geometries.

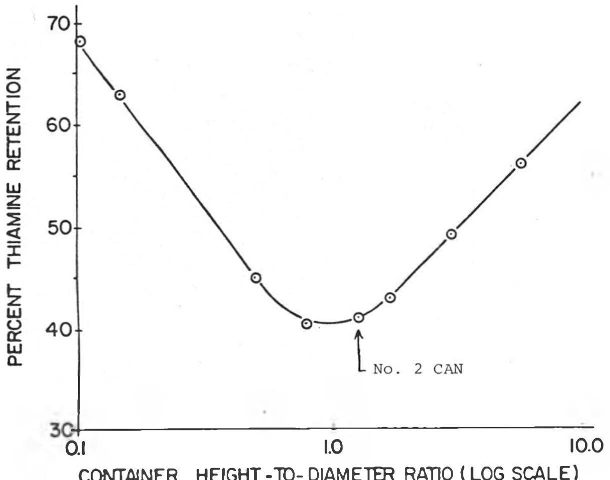

Fig. 3–Results from investigation of various container geometries, showing percent thiamine retention versus height-to-diameter ratio for constant volume.

CONCLUSIONS

BECAUSE of the limitations to rapid heat conduction imposed by the container dimensions and low thermal diffusivity, the use of time-varying surface temperature policies in the thermal processing of conduction-heated foods in standard sized containers does not appear to offer any significant advantages with respect to increasing the level of thiamine retention in the processed food. However, it has been shown that variations in container geometry which permit a more rapid heating of the product can be very effective in achieving a significant improvement in the nutritional value of thermally processed foods. These considerations would tend to support further development in the use of flexible packaging in thermal processing. Some of the new concepts in flexible packaging involving laminated materials of plastics and foils lend themselves readily to geometric configurations of thin cross-sections that would tend to promote rapid heat transfer.

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YIELDS AND SOLIDS LOSS IN STEAM BLANCHING COOLING AND FREEZING VEGETABLES

INTRODUCTION

CONCERN about the water pollution produced by vegetable freezing plants and about the nutritional quality of these products makes it important to know how blanching and cooling affect waste water and the yield and solids content of the vegetables. There have been many reports on the effects of blanching and cooling on solids and nutrient loss in vegetables (Lee, 1958; Hohl et al., 1947; Wager, 1946; Cruess, 1946). Most of the previous work used the ratio of soluble solids to insoluble solids or alcohol insoluble solids to determine the solids loss from vegetables taken from a processing line, or experiments were done under small scale laboratory conditions. In order to obtain accurate results that could be applied to commercial processing, the experiments reported here were done under carefully controlled laboratory conditions, but in such a way that they simulated the essential features of commercial processing. The results show how the yield and solids in a number of vegetables were affected by steam blanching, water cooling, air cooling using either blancher condensate or water spray, and freezing. Furthermore this work shows that air cooling using blancher condensate significantly reduced the organic waste load of the blanching and cooling effluents as compared to water cooling and that this procedure produced frozen vegetables with a higher proportion of their original solids.

The organic load of waste water, and the corresponding amount of solids lost from the vegetables, for steam and water blanching has been reported in a National Canners Association study (1971) and in a review article by Lee (1958). Research in recent years has been directed toward the problem of reducing blancher effluent, since it is a major contributor to the organic waste load of most fruit and vegetable processing plant liquid effluents (National Canners Assoc., 1971; Ralls et al., 1972a, b; Bomben et al., 1973, 1974; Brown et al., 1974; Lund, 1974).

Air cooling has been advocated for many years (Joslyn, 1942; Cruess, 1946; Moyer and Holgate, 1947; Coffelt and Winter, 1973), and in the past several years some frozen food plants have begun using air cooling in order to eliminate the large amounts of water used for flume cooling (Smith and Robe, 1973). Data on the effect air cooling has on the yield of cooled vegetables and on the amount of effluent and COD produced are very limited (Moyer and Holgate, 1947; Bomben et al., 1973; Brown et al., 1974). It was reported recently that the condensate produced in steam blanching, instead of water, can be sprayed on vegetables when air cooling (Brown et al., 1974). This cooling procedure gave no adverse effects on the quality of frozen green beans when compared to those cooled in water.

EXPERIMENTAL

PROCEDURES, which were a reasonable approximation of full-scale processing, were designed to measure accurately the initial and subsequent weight of product and effluent at the various stages of processing in order to determine the gain or loss of vapor, liquids or solids. The experimental technique gave reproducibility of weights and total recovery of product and effluents by reducing to a minimum the unmeasurable loss of liquids or solids adhering to the surface of belts and trays that are commonly used in commercial processing. Light weight trays (1–4 lb) holding 3 lb of vegetables were used for quick handling and rapid weighing to obtain accurate weight changes for each processing step. The overall experimental procedure is summarized in the chart shown in Figure 1.

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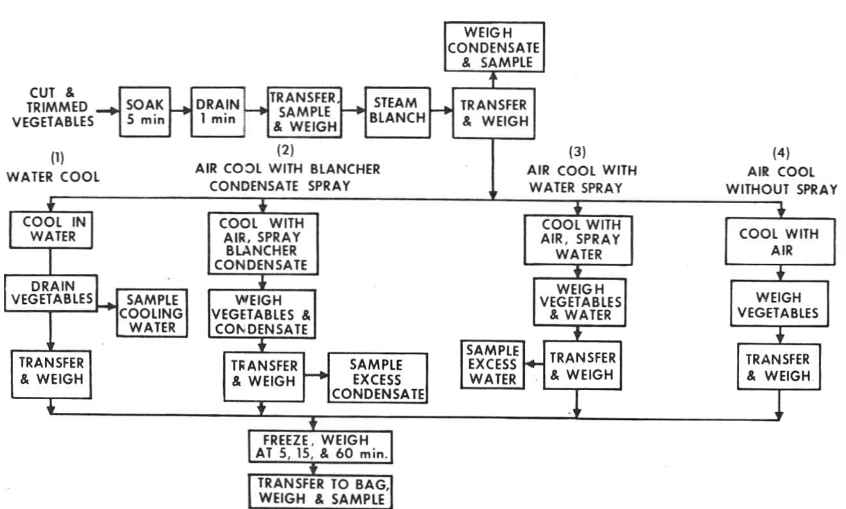

Fig. 1—Experimental procedure for measuring weight changes in blanching, cooling and freezing, using four different methods of cooling.

Table 1—Description of raw vegetables and processing conditions

	Carrots	Peas	Green beans	Broccoli	Cauli-flower	Lima beans	Brussels sprouts
Raw Vegetable Description	3/8 in. dice	Size No. 4, 5, 6 Rogers Bros. small variety	1 in. crosscut Galagreen var.	6 X 1 in. spears	2 in. flowerets	Field run Kingston var.	1-1/2 X 2 in. to 1 X 1-1/4 in. Jade var.
Blanching time (min)[a]	1.0	1.5	2.0	3.5	3.5	3.0	6.0
Cooling time (min)	2.0	2.0	2.0	3.0	3.0	3.0	6.0
Avg temp after water cooling[a] (°C)	25–27	23–24	27–28	23–27	26–28	27	28–29
Avg temp after air cooling w/condensate spray (°C)	–[b]	24–25	29–31	–[b]	32–36	23–24	31–35
Avg temp after air cooling w/water spray (°C)	27–29	24–25	28–31	27–29	32–36	22–25	31–33
Avg temp after air cooling w/o spray (°C)	–[b]	27–28	35	29–32	–[b]	23–25	33–37

[a] Cooling water temperature 18–21°C
[b] Not measured

Broccoli, carrots and cauliflower were obtained from a local wholesale produce market, and they were held at 1.1°C until they were trimmed and cut without washing. Peas, green beans, lima beans and Brussels sprouts were obtained from a local freezing plant prior to blanching, transported with ice in an insulated container, and stored at 1.1°C until used. The size and variety of the vegetables used are shown in Table 1.

Just prior to blanching the vegetables were submerged in running water for 5 min and transferred to a perforated tray where they were shaken briefly and allowed to drain for 1 min. After draining, the vegetables were transferred to a 16-1/2 × 24 × 1/2 in. dry perforated tray. Two 150–200g samples were removed and frozen for later total solids analysis. The underside of the tray was dried, and the tray and vegetables were weighed to within 0.001 lb to determine the net weight of vegetables before blanching. The tray was loaded with about 3 lb of vegetables to give a 1.1 lb/ft² loading. All yields, solids loss and effluents were calculated relative to this weight. The perforated tray containing the weighed vegetables was placed in a solid tray (17-1/2 × 25-1/2 × 1 in.) that had been preheated in the steam blancher and wiped dry, thereby reducing to a negligible amount the condensate produced by this tray during subsequent blanching (Fig. 2). The perforated tray had small bolts attached to it holding it 3/8 in. above the base of the solid tray, allowing room for steam penetration below the vegetables and for the steam condensate to drain away from them.

The trays with the vegetables were placed in the steam chamber of a tunnel blancher (Fig. 2) and held for the blanching times shown in Table 1. After blanching, the vegetables in the perforated tray were pulled out of the steam chamber and immediately transferred to another dry solid tray (17 × 25 × 1 in.) and weighed to get an accurate weight of the blanched vegetables. The solid tray containing the blancher condensate was wiped dry on the underside and weighed immediately after blanching. Transferring and weighing the vegetables and effluent took approximately 15 sec. The blancher condensate was transferred from the tray to a graduated cylinder with the aid of a squeegee, and it was either bottled for total solids analysis or used in the air cooling as described below. The perforated tray from which the vegetables had been transferred was also weighed to determine the amount of condensate adhering to it.

Cooling of the product immediately after blanching was carried out in four ways: (1) water cooling; (2) forced air cooling with fog spraying of blancher condensate; (3) forced air cooling with fog spraying of water; or (4) forced air cooling without any spray. For water cooling, the vegetables were dumped in five times their weight in water and gently stirred for the times shown in Table 1. The vegetables were next dumped on a perforated tray and allowed to drain for 1 min; a sample of the cooling water was taken for total solids and TOC (Total Organic Carbon) analyses. After draining, the vegetables were transferred to a dry tray and weighed. When the vegetables were air cooled, they were kept in the solid tray used for weighing after blanching and put in a parallel flow air cooler, shown in Figure 3 (air velocity = 700 ft/min, wet bulb temperature = 13–18°C). Midway through the cooling period the air flow was stopped, and blancher condensate was fog sprayed onto the product with an atomizer. (Spraying took about 30 sec.) Air was then passed over the product for the remainder of the cooling time. The solid tray with the product and excess condensate was then weighed. The product was transferred to a dry perforated tray, and the effluent was bottled for total solids and TOC analyses. The perforated tray containing the cooled vegetables was wiped on the underside and weighed. When air cooling was done with a water spray or no spray the procedure was identical to that above except that in the former, distilled water instead of blancher condensate was used in the atomizer, and in the latter no spray at all was used. After each method of cooling, a thermometer was placed in a mound of vegetables on the tray, and the average temperature was read after 1 min. These temperatures are also shown in Table 1. The cooling times, which are shown in Table 1, were the times needed for the vegetables to reach an average temperature of 27–32°C when air cooled with blancher condensate spray. It

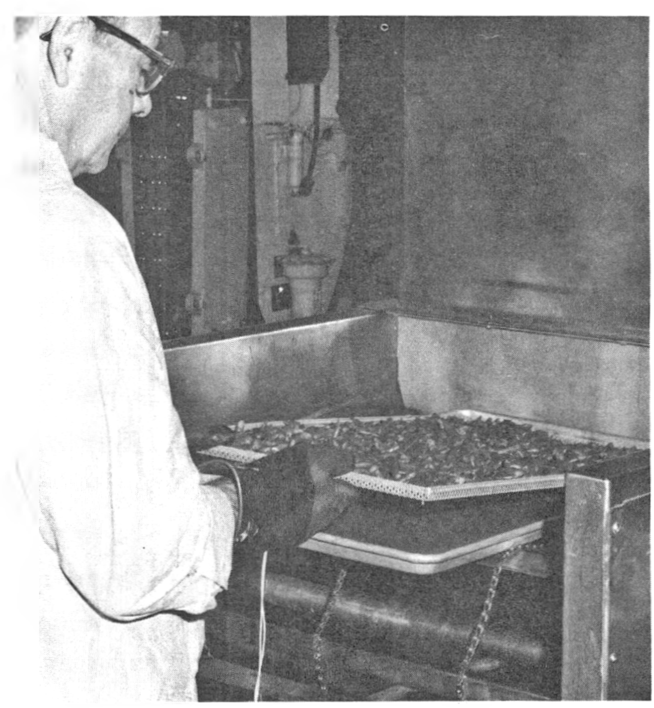

Fig. 2—Photograph of equipment used for blanching.

was arbitrarily decided to keep cooling times the same for all cooling conditions.

Freezing of the cooled vegetables was done by putting the vegetables on the perforated tray into an air blast freezer at −29°C and an air velocity of 500 ft/min. For green beans, lima beans, and Brussels sprouts, the blast freezer was not available, and the vegetables were frozen in front of the circulating fan in a −23°C cold box. The vegetable tray was weighed after 5, 15 and 60 min to determine evaporative loss during freezing. After an hour in the blast freezer, the frozen vegetables were transferred to a polyethylene bag in a low humidity room to reduce any moisture condensation on the frozen vegetables from the ambient air. The vegetables in the bag were weighed to determine the final weight of the frozen vegetables, exclusive of any ice on the tray. Three 150–200g samples of the frozen product were put in polyethylene bags and kept frozen for later total solids analysis.

Total solids analyses of vegetables and effluents were done by AOAC method 20.010 (AOAC, 1965). The total solids of the water cooling effluent had a blank subtracted for the initial cooling water. The TOC of the effluent samples was estimated using a Beckman Total Carbon Analyzer (Model 915) (APHA, 1965), and these values were converted to chemical oxygen demand (COD) by multiplying TOC by 2.67, the stoichiometric ratio.

RESULTS

TABLE 2 shows the solids content of the raw, soaked and drained vegetables and the yields and solids loss of blanching, freezing and the four cooling methods. All yields were based on the raw vegetable weight after soaking and draining:

$$\text{Blanched, cooled or frozen \% yield} = 100 \times \left[\frac{\text{wt of blanched, cooled or frozen vegetable}}{\text{wt of soaked and drained raw vegetable}}\right]$$

The solids loss was also based on soaked and drained raw vegetables:

$$\text{\% solids loss in blancher or cooler} = 100 \times \left[\text{Blancher or cooler effluent in lb/lb vegetable}\right]\left[\frac{\text{\% Total solids in blancher or cooler effluent}}{\text{\% Total solids in soaked and drained vegetables}}\right]$$

The solids loss expressed in this way gave the solids lost as compared to those originally present in the raw vegetable. This was felt to be a crude measure of the nutrient loss from the vegetables. The combined solids loss was the total of the solids lost in blanching and cooling.

Table 3 shows the amount and COD of the blancher and cooler effluents. The COD gave a measure of the effluent waste load produced by these operations. This table also compares the total amount of waste water and COD produced in the blanching and the different kinds of cooling.

Table 4 shows the effect of different cooling methods on the freezing weight loss. These data were calculated from the difference between the initial and final weight of the tray of vegetables after 1 hr of freezing.

The means and confidence limits shown in these tables are calculated from different numbers of replications. The blanching yields and effluents had from 11–27, the blanching solids loss and COD had from 6–15, and the cooling and freezing yields, the cooling solids loss and COD had from 3–7.

DISCUSSION

FROM TABLE 2 it is seen that most of the vegetables lost weight in blanching. Peas had the largest weight loss (11.3%), and only Brussels sprouts had a gain in weight (1.8%). Apparently for most vegetables the cell damage resulting from heating reduces the amount of liquid within the tissue. Brussels

Table 2–% Yield and % solids loss (with 95% confidence limits)

% Total solids in raw vegetable	Carrots 11.6 ± 0.2	Peas 18.4 ± 0.2	Green beans 9.2 ± 0.10	Lima beans 36.3 ± 0.2	Broccoli 9.4 ± 0.2	Cauliflower 7.98 ± 0.06	Brussels sprouts 12.74 ± 0.12
Blanching							
Yield after blanching	95.0 ± 0.4	88.7 ± 0.6	92.6 ± 0.3	96.5 ± 0.5	97.8 ± 0.3	96.8 ± 0.4	101.8 ± 0.3
Solids loss	2.2 ± 0.2	2.5 ± 0.4	1.66 ± 0.07	0.46 ± 0.03	1.9 ± 0.1	2.1 ± 0.1	1.16 ± 0.05
Water cooling							
Yield after cooling	95.0 ± 0.6	91.7 ± 0.6	94.3 ± 0.9	100.6 ± 1.2	103.8 ± 1.5	101.9 ± 2.6	113.1 ± 1.3
Yield after freezing	91.3 ± 0.9	88.7 ± 0.4	91.7 ± 0.9[a]	98.0 ± 0.9[a]	100.6 ± 1.7	99.0 ± 2.5	109.7 ± 1.3[a]
Solids loss	9.6 ± 0.9	2.2 ± 0.3	2.24 ± 0.24	1.00 ± 0.08	4.7 ± 0.8	3.1 ± 1.2	4.9 ± 0.3
Combined solids loss	11.8 ± 1.1	4.7 ± 0.7	3.9 ± 0.3	1.5 ± 0.1	6.6 ± 0.9	5.2 ± 1.3	6.1 ± 0.4
Air cooling w/condensate							
Yield after cooling	93.9 ± 1.2	87.7 ± 0.7	92.0 ± 1.1	93.9 ± 0.5	96.4 ± 1.7	95.2 ± 1.4	99.4 ± 0.6
Yield after freezing	91.5 ± 1.9	84.0 ± 0.6	89.6 ± 1.3[a]	91.6 ± 0.6[a]	93.7 ± 1.3	92.7 ± 1.9	97.3 ± 0.9[a]
Solids loss[d]	2.1 ± 0.7	1.8 ± 0.2	1.06 ± 0.14	0.38 ± 0.09	1.0 ± 0.4	1.3 ± 0.3	0.44 ± 0.06
Air cooling w/water spray							
Yield after cooling	93.7 ± 1.4	88.7 ± 0.6	92.7 ± 0.2	93.9 ± 0.5	97.1 ± 0.8	95.6 ± 1.6	100.2 ± 0.5
Yield after freezing	91.2[b]	85.2 ± 0.4	90.3 ± 0.4[a]	91.7 ± 0.4[a]	95.7 ± 1.7	92.8 ± 1.7	97.9 ± 0.5[a]
Solids loss	2.2[b]	0.9 ± 0.1	0.65 ± 0.21	0.38 ± 0.09	0.58 ± 0.13	0.6 ± 0.1	0.24 ± 0.07
Combined solids loss	4.4[b]	3.4 ± 0.5	2.3 ± 0.3	0.84 ± 0.12	2.5 ± 0.2	2.7 ± 0.2	1.4 ± 0.1
Air cooling w/o spray							
Yield after cooling	88.5[b]	82.8 ± 1.8	87.8 ± 0.5	89.5 ± 1.9	91.5[b]	—[c]	95.1 ± 0.7
Yield after freezing	—[c]	80.6 ± 1.4	86.2 ± 0.6[a]	87.6 ± 1.8[a]	89.3[b]	—[c]	93.2 ± 0.7[a]
Solids loss	—[c]	—[c]	0.54 ± 0.14	0.25[b]	—[c]	—[c]	—[c]
Combined solids loss	—[c]	—[c]	2.2 ± 0.2	0.71[b]			

[a] Frozen in cold box rather than blast freezer.
[b] Insufficient data for calculating confidence limit.
[c] Not measured.
[d] Also combined solids loss

sprouts may be an exception because the voids between the leaves entrap condensate.

Depending on the heat capacity of the vegetable (Lewin, 1962) the condensate produced by steam blanching can be calculated to be 9.0–11.5 lb/100 lb of vegetable, assuming the average temperature rise in the vegetable to be 49°C and taking the heat of evaporation of steam as 970 BTU/lb. Thus, the sums of the yield of the blanched vegetables in Table 2 and the weight of effluents per 100 lb of vegetable in Table 3 can be calculated, and they can be compared to those obtained experimentally (Table 3). For most of the vegetables, the experimental and calculated values agree reasonably well.

Except for lima beans, the amount of solids lost in blanching by all the different vegetables was about the same. The lima beans have a higher proportion of insoluble solids than the other vegetables, and as a result they lost less total solids by leaching, a fact also reported by Hohl et al. (1947). However, the COD of the lima bean effluent shown in Table 3 was not correspondingly reduced, since lima beans had a much higher total solids than did the other vegetables. Peas had the highest COD because they had the highest proportion of soluble solids and a relatively high total solids.

All vegetables, except carrots, gained weight relative to the blanched weight when water cooled, and lima beans, broccoli, cauliflower, and Brussels sprouts regained more than the original weight of the raw vegetable.

Water cooling was accompanied by a large amount of waste water and loss of solids for all the different vegetables. In all cases, the solids loss was larger than that of blanching, and it was significantly higher than that of any of the other cooling methods. The amount of water chosen for the simulated flume cooling was based on achieving a temperature of 27°C in the cooled vegetable. The ratio of 5 lb of water to 1 lb of vegetable was lower than that used by most commercial firms for fluming blanched vegetables. Processors have reported by private communication ratios anywhere from 7–30.

No significant differences in yields for any of the vegetables were observed between air cooling with either condensate or water spray. However, the vegetables air cooled without any spray showed a substantially lower yield in all cases, and they generally had a higher temperature after cooling (Tables 1 and 2).

Solids loss, effluent, and COD were each lower for water spray cooling than condensate spray cooling, but the solids loss and COD for the combination of blanching and cooling effluents were much higher (Tables 2 and 3). Thus the blancher condensate left a significant portion of the solids it contained on the vegetables when it evaporated during cooling.

In those experiments done in an air blast freezer (carrots, peas, broccoli and cauliflower) the vegetables were weighed after 5 and 15 min of freezing. It was observed that 60–80% of the weight loss occurred in the first 5 min, and no significant loss was found for any of these vegetables beyond 15 min of freezing.

Table 4 shows the freezing weight loss for some vegetables was affected by the different methods of cooling. Brussels

Table 3—Blanching and cooling effluents (in lb/100 lb raw vegetable with 95% confidence limits)

Heat capacities (BTU/lb °F)[a]	Carrots 0.93	Peas 0.82	Green beans 0.91	Lima beans 0.73	Broccoli 0.92	Cauliflower 0.93	Brussels sprouts 0.88
Blanching							
Effluent	11.7 ± 0.4	19.1 ± 0.4	15.0 ± 0.3	11.3 ± 0.6	11.9 ± 0.3	14.1 ± 0.5	10.4 ± 0.4
COD	0.23 ± 0.02	0.457 ± 0.009	0.154 ± 0.004	0.181 ± 0.012	0.179 ± 0.012	0.154 ± 0.011	0.161 ± 0.007
Effluent + blanched veg (calc)	106.7 (111.5)	107.8 (110.1)	107.6 (111.3)	107.8 (109.0)	109.7 (111.4)	110.9 (111.5)	111.2 (110.9)
Water cool							
Effluent	493 ± 7	496 ± 3	493 ± 4	496 ± 1	486 ± 7	493 ± 5	497 ± 1
COD	1.08 ± 0.11	0.48 ± 0.03	0.26 ± 0.03	0.56 ± 0.08	0.54 ± 0.03	0.29 ± 0.06	0.70 ± 0.02
Air cool w/conden spray							
Effluent	4.8 ± 0.3	9.1 ± 1.8	5.0 ± 0.5	4.0 ± 1.0	4 ± 2	5.8 ± 0.9	2.4 ± 0.5
COD	0.25 ± 0.02	0.33 ± 0.04	0.099 ± 0.012	0.16 ± 0.04	0.10 ± 0.03	0.09 ± 0.02	0.07 ± 0.01
Air cool w/water spray							
Effluent	5.2 ± 1.6	8.4 ± 1.4	6.6 ± 0.9	4.2 ± 0.6	4.0 ± 0.7	5.5 ± 0.7	2.2 ± 0.2
COD	0.094[b]	0.17 ± 0.02	0.06 ± 0.02	0.14 ± 0.01	0.06 ± 0.02	0.043 ± 0.009	0.032 ± 0.007
Air cool w/o spray							
Effluent	—[c]	—[c]	1.0 ± 0.2	1.1 ± 0.4	0.005[b]	—[c]	0.42 ± 0.12
COD	—[c]	—[c]	0.045 ± 0.015	0.095[b]	—[c]	—[c]	—[c]
Total waste water							
Blanch + Flume cool							
Effluent	505 ± 7	515 ± 3	508 ± 4	507 ± 2	498 ± 7	507 ± 5	597 ± 1
COD	1.31 ± 0.13	0.94 ± 0.04	0.41 ± 0.03	0.74 ± 0.09	0.72 ± 0.04	0.44 ± 0.07	0.86 ± 0.03
Blanch + Air cool w/cond							
Effluent	4.8 ± 0.3	9.1 ± 1.8	5.0 ± 0.5	4.0 ± 1.0	4 ± 2	5.8 ± 0.9	2.4 ± 0.5
COD	0.25 ± 0.02	0.33 ± 0.04	0.099 ± 0.012	0.16 ± 0.04	0.10 ± 0.03	0.09 ± 0.02	0.23 ± 0.02
Blanch + Air cool w/water spray							
Effluent	16.9 ± 2.0	27.5 ± 1.8	21.6 ± 1.2	15.5 ± 1.2	15.9 ± 1.0	19.6 ± 1.2	12.4 ± 0.6
COD	0.32[b]	0.63 ± 0.03	0.21 ± 0.02	0.32 ± 0.02	0.24 ± 0.02	0.20 ± 0.02	0.19 ± 0.01
Blanch + Air cool w/o spray							
Effluent	—[c]	—[c]	16.0 ± 0.5	12.4 ± 1.0	12.0[b]	—[c]	10.8 ± 0.5
COD	—[c]	—[c]	0.36 ± 0.02	0.28[b]	—[c]	—[c]	—[c]

[a] Lewin (1962)
[b] Insufficient data for confidence limit
[c] Not measured

Table 4—Weight loss in freezing as affected by the different cooling methods (with 95% confidence limits)

	Carrots	Peas	Green beans[a]	Lima beans[a]	Broccoli	Cauliflower	Brussels sprouts[a]
Freezing wt loss after water cooling	2.7 ± 0.1	2.3 ± 0.1	2.2 ± 0.2	2.5 + 0.5	2.7 ± 0.1	2.8 ± 0.4	2.9 ± 0.1
Freezing wt loss after air cool w/cond spray	2.7 ± 0.1	2.2 ± 0.1	2.2 ± 0.2	1.9 ± 0.3	2.3 ± 0.3	2.4 ± 0.4	2.2 ± 0.1
Freezing wt loss after air cool w/water spray	2.8 ± 0.1	2.4 ± 0.2	2.2 ± 0.3	2.0 ± 0.2	2.5 ± 0.1	2.6 ± 0.2	2.1 ± 0.1
Freezing wt loss after air cool without spray	—[c]	1.8[b]	1.7 ± 0.1	1.8 ± 0.2	2.4[b]	—[c]	1.8 ± 0.1

[a] Frozen in cold box rather than blast freezer
[b] Insufficient data for determining confidence limit
[c] Not measured

sprouts showed a significantly higher freezing weight loss when flume cooled than when air cooled by any of the methods used. The vegetables tended to show a lower freezing weight loss when air cooled without any spray as compared to the other methods of cooling. Apparently some of the water gained in the simulated flume or from the sprays was readily evaporated during freezing, but even though some of these freezing weight loss differences are statistically significant, they are probably of no practical significance, especially in view of the larger weight changes produced in blanching and cooling.

The lower yields obtained with air cooling as compared to water cooling would seriously hinder a frozen vegetable processing plant from taking advantage of the reduced COD of air cooling. Since most frozen vegetables are sold on the basis of total weight, these lower yields amount to expensive losses of production. In this work only air cooled carrots gave a yield equal to that of water cooling. Corn-on-the-cob is sold by number rather than weight, and therefore this vegetable can be air cooled without an economic penality for loss of weight. Since peas and lima beans typically go into a brine solution for quality grading after cooling, they could probably be air cooled without a loss of yield, but the disposal of the organic waste load in the brine is even more of a problem than its disposal in the flume water. Unless the frozen vegetable industry adopts a different method of marketing the majority of its products, it is doubtful whether air cooling will ever be widely used as a means of reducing waste water load.

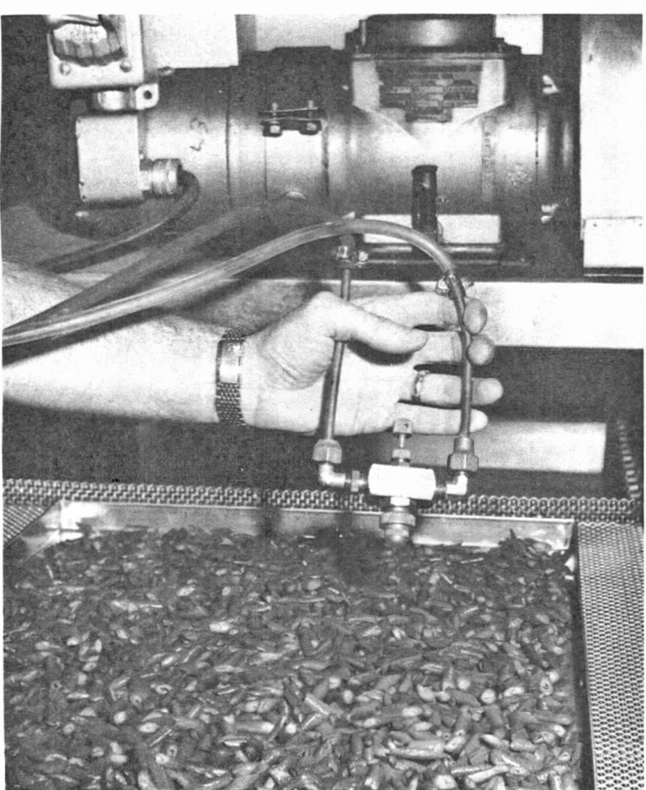

Fig. 3—Photograph of equipment used for air cooling.

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CHANGES IN THE ASCORBIC AND DEHYDROASCORBIC ACID CONTENTS OF FRESH AND CANNED BEANS

INTRODUCTION

THE PRESENT STUDY involved green beans, a widely consumed product which generally presents difficulties in assaying the ascorbic acid (AA) because of the low vitamin C content and the presence of interfering substances which increase during the preservation processes and after thermal treatment (Freed, 1966). Changes in the AA and dehydroascorbic acid (DHA) contents in 15 varieties of green beans grown under the same cultivation conditions, subjected to two different technological treatments, and stored at room temperature for 6 months were studied.

The enzymatic method developed on green beans was used during this research and was checked by spectrophotometric enzymatic assay in order to evaluate the possible presence of interfering substances and the sensitivity of the method itself.

MATERIALS & METHODS

Green beans

15 cultivars were cultivated in 5 × 5m plots; the distance between the rows was 0.50m with 55 plants/m². Summer gathering was carried out almost simultaneously for each plot and the beans were divided according to variety and brought to 4°C. Analysis of the AA and DHA was then performed within 48 hr (t = 0).

Canning

The beans were canned separately on the day of collection, under the following conditions: washing, topping and tailing with an IMC machine, followed by blanching for 1 min in water at 95°C. The products were cooled and filled into 75 × 107 mm cans with an epoxy-phenol internal lacquer coating. 200g of blanched beans and sufficient brine were added to each can. Half of the samples were sealed and sterilized at 124°C for 8 min and the other half at 116°C for 25 min. Cooling was done with water under compressed air.

The AA and DHA contents of the canned green beans were analyzed several days (t = 1) after canning and then after 6 months' storage at room temperature (t = 2).

Preparation of the samples and analytical methods

A sample (50g) of fresh or canned beans drained of brine by filtration, was homogenized for 3 min (Braun Knife Homogenizer) with 50g of 6% HPO_3 solution containing 0.0025M disodium EDTA. The homogenate was then treated as described in a previous paper (Marchesini et al., 1974). Filtered brine (5 ml) was analyzed without any treatment. The AA and DHA were determined by the enzymatic method of Marchesini (1972) and Marchesini et al. (1974). The figures refer to 100g of fresh weight or drained beans and to 100g of brine.

Table 1—Recovery of ascorbic acid in fresh and canned green beans

	Enzymatic method				Spectrophotometric method					
Green beans	Extract (ml)	Added AA (mcg)	Δ mm[a]	AA found (mcg)	Extract (ml)	Added AA (mcg)	Δ O.D.[b] at 245 μm	AA found (mcg)	Δ O.D.[b] at 265 μm	AA found (mcg)
		352	35	–						
		704	70	–						
fresh	2	–	11	110	0.02	–	0.025–0.026	1.1	0.048–0.050	1.0–1.1
	4	–	22	220	0.04	–	0.050–0.054	2.2–2.3	0.100–0.110	2.1–2.2
	2	352	46	462	0.02	10	0.275–0.278	11–11.5	0.528–0.530	11–11.5
	4	176	39	396	0.04	10	0.300–0.310	12–12.5	0.580–0.585	12–12.5
canned and stored for 6 month	2	–	5	50	0.02	–	0.017–0.019	0.5	0.024–0.026	0.5–0.5
	4	–	10	100	0.04	–	0.034–0.040	1.1	0.048–0.050	1.0–1.1
	2	352	40	302	0.02	10	0.262–0.265	10–10.5	0.504–0.508	10.0–10.5
	4	176	27	276	0.04	10	0.280–0.284	11–11.5	0.520–0.521	11.0–11.5

[a] Δ mm = difference between the total oxygen in the cell and the oxygen remaining after the enzymatic reaction, measured with Clark's electrode. This difference is stoichiometrically proportionate to the AA concentration. The following proportion is applied in calculating the AA concentration of the extract: $AA_{std\ soln} : mm_{corresponding} = (AA)X_{unknown} : mm_{found\ with\ extract}$

[b] Δ O.D. = Variation in the optical density due to the enzymatic oxidation of the AA in the extract.

A slight change was made in the assay of DHA. After reduction of the acid with homocysteine, about 10 mg of iodoacetic acid were added to the reaction cell to react with the residual homocysteine. The modification permitted obtaining the exact dosage of DHA.

Finally, the assays were always tested with increased quantities of extracts and liquids, with or without the addition of AA. The analysis was performed without changing the calibration of the Clark's electrode between the two indices–total oxygen or zero oxygen. By this technique the oxygen up-take was stoichiometrically proportional to the quantity of ascorbic acid present in the extract, quite independent of the total oxygen in the cell. At the same time, the AA content of some cans of beans was determined by the spectrophotometric enzymatic method of Marchesini et al. (1970). The extract was purified of pigments and proteins with Sephadex G25 SF. Portions were brought to pH 4 and 6 and the optical density of the first was read at 245 μm and of the second at 265 μm. The quantity of AA in the extract was obtained from the variation in the O.D. using a calibration curve prepared with pure AA.

The quantity of iron, copper and tin ions was assayed on some cans, using a Perkin Elmer model 300S atomic absorption spectrophotometer, and the oxido-reduction potential was measured with Plink Co. equipment. The quantity of oxygen was measured with the Clark's electrode.

Analysis of the data

The data were processed with an IBM CII 6006 computer. The means, RMS (standard deviation) and mean percentages were evaluated. The correlation between the data for AA and DHA of the beans at zero time and those at times 1 and 2 were also evaluated, using the coefficient of linear correlation "r." The value of 0.5 was taken as an index of positive correlation (Lison, 1958).

RESULTS

THE ENZYMATIC ASSAY of AA in fresh and canned green beans, using Clark's electrode, was compared with the spectrophotometric enzymatic method. Table 1 gives the results obtained from the enzymatic comparison.

Table 2 lists the AA and DHA values of fresh and canned green beans for times 0, 1 and 2, along with the values of the means, RSM and means of the percentage ratios. This table shows that the quantities of AA and DHA vary greatly in the cultivars considered. The RSM indicates variations of 30–50% compared to the mean values. It is also seen that the mean values of the vitamin content of canned beans (cooked 8 min at 124°C and 25 min at 116°C) and stored for times t = 1 and t = 2, show no significant differences, not even in the distribution of the vitamin between drained beans and brine.

A high percentage mean of DHA compared to AA is seen at time 1. At time 2, the DHA has almost completely disappeared while the AA appears to have increased–25.12% against 21.24% (the values of the general solid plus liquid means). The values of the general means show, however, a progressive fall of the total vitamin C content with time (t_1 = 26.21 and t_2 = 19.11). It can be seen from Figure 1 that a positive correlation exists between the initial values of the AA at time 0 and the AA values in both drained beans and brine at time 2. This correlation was not found for drained beans at time 1, while it is uncertain for brine. The time 2 data are excellently correlated between themselves. No positive correlation has been found for DHA.

Finally, the vitamin decay in time can be represented by an exponential curve of type $Y = Ae^{-Bt} + C$. For calculating the constants A, B and C in function of time t (days of preservation t = 0, t = 1, t = 180), the decay curves were made to pass through the general mean values (drained beans + brine) deducible from Table 2. Figure 2 gives the numerical results and the relative graphs.

Assay of the copper, iron and tin in the brine in the cans gave the following results expressed in ppm: Fe = 5.8; Cu = 0.79; and Sn = 1.45. The oxido-reducing potential measured at pH 5.70 (pH of the liquid in the cans) gave the following value: RH 8.70. The concentration of oxygen in the cans was 6×10^{-2} μM/ml.

DISCUSSION

THE AA VALUES of green beans given in Table 1, obtained with spectrophotometric enzymatic assay at two different pH values, and with the enzymatic method using Clark's method

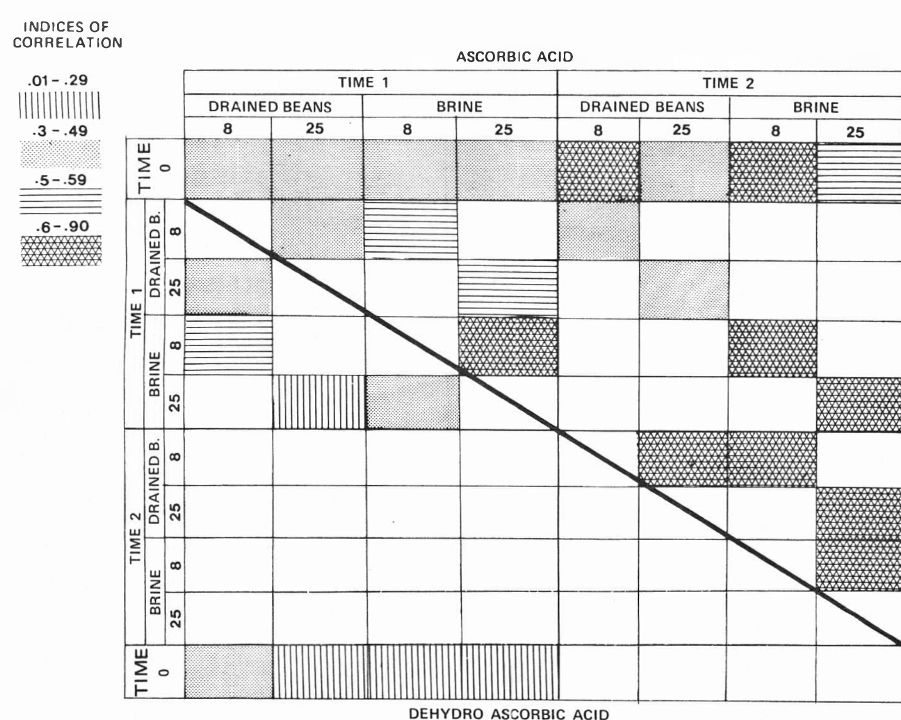

Fig. 1–Indices of correlation of the data of the AA and DHA in green beans between times 0, 1 and 2. Values above 0.49 indicate a positive correlation.

are considered coincident. The result confirms that, within the limits of the analytical sensitivity of the two different methods, the enzyme, ascorbic oxidase, acts specifically with its substrate present in the green beans extract. In fact, it is difficult to think of an interfering substance with an absorption spectrum at pH 4 and 6 coinciding with that of AA, and with a reaction with oxygen having the same stoichiometry as that of AA. The results obtained with the two methods are thus equivalent, excluding the presence of inhibitors or reducing agents.

Processing of the cans of green beans at different temperatures and times causes a decrease in the AA and DHA content, but with no significant difference found between the two treatments. This result appears to vary with the pH of the brine, presence of metal ions, and oxygen level, which influences the stability of the AA rather than the quantity of heat received by the beans (Khan and Montell, 1967; Spanyar and Kevei, 1963). The vitamin decay curves of canned and preserved green beans show a course very similar to that of an exponential of e^{-t} type. They reveal the disappearance of DHA and a slight increase in AA. Statistically speaking, this increase cannot be considered absolutely certain in view of the great variations in the vitamin content of the cultivars examined. Certainly, the quantity of AA present in the cans does not decrease during storage (Cook, 1974).

Table 2—AA and DHA of drained beans and in brine (mg/100g)

	Time	0[a]	1[a]				2[a]			
			Drained beans		Brine		Drained beans		Brine	
Vitamin	Cult.[c]	Fresh	8[b]	25[b]	8[b]	25[b]	8[b]	25[b]	8[b]	25[b]
AA	1	14.07	.00	3.91	.93	3.75	3.88	1.76	3.90	.35
	2	11.22	1.17	2.34	5.86	6.33	4.23	4.02	3.73	4.52
	3	21.20	3.52	5.47	3.91	7.43	5.33	8.56	5.33	8.12
	4	24.60	.00	3.12	4.49	3.52	6.00	3.80	5.00	4.75
	5	11.04	3.52	3.91	3.12	4.69	2.04	3.01	2.55	3.01
	6	13.68	4.69	3.52	5.86	7.04	4.80	8.04	4.85	6.53
	7	14.04	1.56	3.52	1.17	.97	4.84	2.01	3.38	2.01
	8	19.36	1.76	5.28	1.05	2.46	2.13	1.76	2.66	1.32
	9	11.22	4.92	3.41	4.26	2.13	2.98	4.40	2.64	4.01
	10	14.04	1.95	2.34	2.93	1.95	3.50	4.26	2.60	2.13
	11	21.12	2.81	1.40	5.28	4.22	4.40	3.20	3.50	5.53
	12	21.12	2.11	1.76	2.99	3.52	4.02	5.02	4.52	5.53
	13	27.42	5.28	7.39	9.32	8.97	11.70	9.90	11.25	9.90
	14	17.59	2.81	1.40	2.99	2.46	5.02	6.03	5.02	6.43
	15	13.36	2.11	2.11	1.58	1.76	.80	.35	1.00	.35
	MEAN[d]	17.00	2.54	3.39	3.71	4.08	4.37	4.40	4.12	4.29
	RSM[e]	5.01	1.57	1.61	2.19	2.29	2.37	2.63	2.23	2.71
	R. Avg[f]	100	16.4	20.9	22.8	25.0	25.5	26.3	24.0	25.0
DHA	1	7.03	1.95	3.12	.46	2.81	.00	.00	.00	.88
	2	7.74	4.69	3.52	1.64	1.87	.00	.00	.00	.00
	3	4.20	3.52	2.34	3.91	.00	.00	.00	.00	.00
	4	7.03	2.73	2.73	2.61	3.52	.00	.00	.75	.00
	5	2.64	1.95	2.34	.78	1.17	.00	.00	.00	.00
	6	5.28	3.12	3.52	2.81	2.34	.00	2.01	.00	.00
	7	5.58	4.30	5.47	2.73	1.36	.00	.00	.96	.00
	8	7.07	1.76	.00	2.46	2.99	.00	.00	.00	.00
	9	5.58	3.41	2.13	1.06	2.13	.42	.00	1.07	.00
	10	9.85	2.73	1.56	.97	1.95	.00	.00	.00	.00
	11	10.52	5.28	2.11	3.52	.73	.00	.00	.00	.00
	12	5.28	2.11	2.46	2.99	2.28	.00	.00	.00	.00
	13	7.92	2.11	3.16	.35	.52	.00	.00	1.45	.00
	14	5.62	1.66	3.52	2.28	2.46	.00	.00	.00	.80
	15	6.32	.70	.35	.35	.17	.80	.35	.80	.00
	MEAN	6.51	2.80	2.55	1.92	1.75	0.08	0.15	0.33	0.11
	RSM	1.96	1.22	1.29	1.16	1.02	0.22	0.50	0.50	0.29
	R. Avg	100	46.1	44.4	32.9	28.7	1.4	2.9	5.2	1.8
G.M. (AA + DHA)[g]		100	26.21				19.11			

[a] Times 0, 1 and 2 relate respectively to collection, several days later, and after canning and storing at room temperature for 6 months.
[b] The indices 8 and 25 refer to two different heat treatments: 8 min at 124°C and 25 min at 116°C.
[c] The numbers 1 to 15 relate to the following varieties: Jolanda, Astro, Top Crop, Arian, Rodeo, Early Harvest, Orbit, Tender Crop, Pico, Processor, Contender, Wade, Saxa, Constant and Corene.
[d] MEAN = Means of the initial data
[e] RSM = Standard deviations for the initial data (Root mean square)
[f] R. Avg = Means of the percentage ratios x(t)/x(o) (ratio average)
[g] G.M. (AA + DHA) = general means

The analytical method has been controlled also by means of correlations. The time 0 and time 2 data are clearly positive and thus the repeatability of the data is certain. With regard to the weak correlation with time 1, due to the different oxidation of AA, several variables can be called into play, such as the oxygen in the tissues and that remaining in the can, which exert an effect before and during heat treatment (Malakar, 1963; Mapson, 1972; Marchesini et al., 1970, 1974; McMillan and Todhunter, 1946). The increase and/or stability of the AA in the cans could result from the reduction of small quantities of DHA due to reducing compounds forming during the heat treatment and remaining unaltered during storage: RH = 8.7 (amino acids, sulphurated compounds, glucosamines, H_2S, inorganic reducing agents) and finally, from the low oxygen content in the cans (Bauernfeind and Pinkert, 1970). The study revealed the varieties richest in vitamin C and those most suitable for canning–Top Crop, Arian, Contender, Wade and Saxa. The enzymatic method was found to be extremely sensitive and accurate.

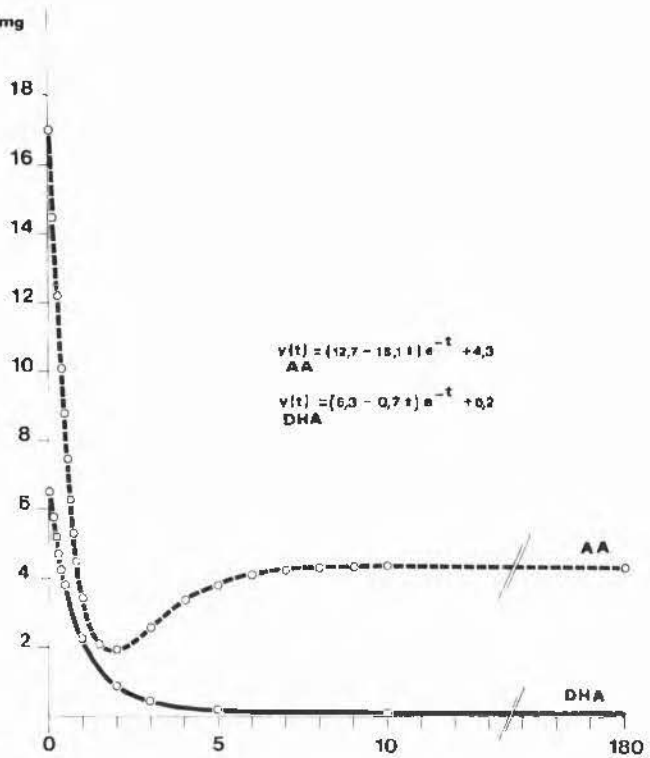

Fig. 2–Decay curves of AA and DHA for canned green beans, room temperature storage.

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INFLUENCE OF POST-HARVEST STORAGE TEMPERATURES AND SOAKING ON YIELD AND QUALITY OF CANNED MUSHROOMS

INTRODUCTION

METHODS to reduce shrinkage or increase the yield of mushrooms during canning operations are of economic importance to mushroom processors. Coale and Butz (1972) reported that a 5% reduction in shrinkage could increase the revenue of mushroom processors by as much as 20%.

Several experiments conducted in this laboratory have demonstrated that post-harvest storage of mushrooms prior to canning increased the canned product yield (decreased shrinkage) compared to mushrooms processed immediately following harvest. McArdle and Curwen (1962) reported reductions in shrinkage (yield increases) of 2.9–4.4% when mushrooms were held 24–48 hr at 2°C prior to processing.

Parrish et al. (1974) also reported greater yields of canned mushrooms following post-harvest storage. The increased yields were attributed to the greater water-holding capacity of the mushrooms that developed during storage. Beelman et al. (1973) demonstrated that canned product yield increases exceeding 9% could be attained by soaking mushrooms in water for 20 min (Soak I), storing at 2°C for 18 hr, and soaking in water again for 2 hr (Soak II) prior to blanching (PSU-3S-Process). Subsequently, McArdle et al. (1974) reported that a vacuum-soaking process (SSV-Process) could replace the second soaking operation of the PSU-3S-Process. When vacuum soaking was applied alone, yield increases of about 5% were obtained, but when vacuum-soaking and storage treatments were combined, yields were even greater than those attained using the PSU-3S-Process.

Beelman et al. (1973) speculated that chemical changes such as loosening of protein structure might occur in mushroom tissues during post-harvest storage resulting in greater water retention in mushrooms during processing, hence, increasing canned product yields. This led to the hypothesis that higher storage temperatures could possibly increase the rate of such reactions, thereby increasing canned product yields of mushrooms. This study was conducted to investigate the effect of post-harvest storage temperatures on yields of canned mushrooms and to determine the effects of such treatments on certain quality attributes of the canned product.

EXPERIMENTAL

Raw product

Cultivated mushrooms, *Agaricus bisporus* (Lange) Sing, were used in all four experiments in this report. Mushrooms were selected from uniform lots of known strain from commercial production facilities. Immediately after harvest the mushrooms were transported to the laboratory (within 4 hr) in insulated containers cooled with ice. Mushrooms with tightly closed veils and cap diameters ranging from 2.8–4.1 cm were selected and the stems hand trimmed to within 1 cm of the cap. The trimmed, button mushrooms were pooled, accurately weighed into 1 kg experimental samples (initial wt) and placed in numbered Kraft bags. Four bagged samples were selected at random for each treatment.

Processing conditions

All factors influencing canned product yield and quality, other than those under study, were controlled by standardizing processing conditions and comparing treatment results with those of a normal (control) process. Four repetitions of each treatment were performed. All samples were spray washed, blanched to a center temperature of 77°C by immersion in boiling water, cooled 2 min in cold water, drained and weighed. These mushrooms were filled into plain tin cans (211 × 212) using a fill weight of 120–130g depending upon the weight of the blanched sample. A 20-grain NaCl tablet was added to each can. The cans were filled with boiling water and closed using a can closer equipped with an automatic head-spacer (1 cm) and steam-flow closure. Thermal processing was conducted in a still retort at 121°C for 20 min (as currently recommended by the National Canners Assoc.). The canned products were held for 3 wk at ambient temperature prior to evaluation of the product.

Treatments involving storage but no soaking operation were washed after storage to avoid a storage-soaking interaction on yield. Storage operations were conducted in controlled-environment chambers set at specified temperatures and approximately 90% R.H. The PSU-3S-Process was conducted as described previously (Beelman et al., 1973), and treatments involving vacuum soaking (SSV) were conducted as described by McArdle et al. (1974).

Measurement of WBC and WHC

The water-binding capacity (WBC) and water-holding capacity (WHC) of mushrooms processed using the PSU-3S-Process and SSV-Process with different storage temperatures were determined in one experiment. WBC was a measure of weight gain due to water absorption during Soak II (PSU-3S-Process) or vacuum soaking (SSV-Process) and was calculated as follows:

$$\text{WBC (\%)} = \frac{\text{wt after soak II or vacuum soak} - \text{wt after storage}}{\text{wt after storage}} \times 100$$

WHC was defined as a measure of the ability of mushrooms to retain its own or added water during blanching and thermal processing. It was determined as follows:

$$\text{WHC (\%)} = 100 - \left(\frac{\text{wt after soak II or vacuum soak} - \text{drained wt}}{\text{wt after soak II or vacuum soak}} \times 100\right)$$

Canned product evaluation

The canned product yield was determined by the following formula:

$$\text{\% canned product yield} = \frac{\text{Drained wt}}{\text{Initial wt of raw product}} \times 100$$

Drained weights were determined from the pooled sample of all cans within each treatment repetition after the sample was drained for 2 min in a perforated stainless steel tray. Yields determined by this method were independent of any loss of weight that occurred during storage. Color, total solids and incidence of mushrooms with exposed veils of the canned products were determined as reported previously (Beelman et al., 1973).

Statistical analysis

In each experiment four repetitions of each treatment were conducted. An analysis of variance was performed using a completely randomized design. Mean separations were performed using the modified least significant difference procedure of Waller and Duncan (1969) with a k value of 100.

RESULTS & DISCUSSION

MUSHROOMS (golden white strain) were held at three different storage temperatures for 18 hr prior to processing (see

Table 1). Increased storage temperatures were found to have a dramatic effect on the yield and quality of canned mushrooms. Compared to the unstored control, significant yield increases of 2.1, 5.3 and 9.1% were obtained when mushrooms were held for 18 hr at 2, 12 and 22°C, respectively. Yield increases approximately doubled with each 10°C increase in temperature. This supports the hypothesis that chemical changes in the mushroom tissue might be responsible for this phenomenon. As expected, the Agtron color values of the canned mushrooms decreased significantly with increased storage temperature especially at 22°C (lower Agtron values indicate darker, less desirable mushrooms). The number of mushrooms with exposed veils increased greatly at 22°C. Large numbers of such mushrooms would present some problems in commercial processing operations. They would need to be diverted to the "stems and pieces" line when fancy buttons or sliced buttons are being processed.

It is a common commercial practice in many mushroom canneries to store mushrooms overnight at about 2°C before processing. The data from this experiment suggest that yield increases due to storage could probably be doubled without excessive loss in product quality by increasing storage temperatures from 2 to 12°C. However, care would need to be exercised to insure that temperature could be controlled at 12°C in commercial quantities of actively respiring mushrooms.

Another experiment was conducted where mushrooms (white strain) were stored at the same three temperatures and in addition were held for 24, 48 and 72 hr prior to processing (see Table 2). Yields of canned mushrooms were increased through 48 hr of storage at each temperature and were generally greater at higher storage temperatures. However, at 12°C yield was not increased between 48 and 72 hr and actually decreased during the same period when mushrooms were stored at 22°C. Agtron color values dropped with both increased time and temperature of storage. Color values were particularly low after 72 hr at 12°C and both 48 and 72 hr at 22°C. The incidence of mushrooms with exposed veils also increased with extended storage times at both 12°C and 22°C but were not affected by increased storage time at 2°C. From a quality standpoint, post-harvest storage of mushrooms beyond 24 hr at 22°C or 48 hr at 12°C appears infeasible. However, the results of this experiment indicate that increases in canned product yield due to post-harvest storage could be nearly doubled by storage at 12°C for 24 hr compared to 3 days storage at 2°C without excessive loss of product quality.

The influence of storage temperatures on mushrooms (cream strain) processed using the PSU-3S-Process compared to dry storage was also investigated (see Table 3). In this experiment sodium sulfite (1.6g/l) was added to the water of the Soak I operation of the PSU-3S-Process to investigate its effectiveness in controlling the color deterioration of mushrooms stored at higher temperatures. Compared to the unstored control, yield increases due to storage at the different temperatures were similar to those attained previously (Table 1). However, with the PSU-3S-Process yield increases nearly double those of the corresponding dry storage treatments were attained (up to 15.7%). The color of mushrooms processed by the PSU-3S-Process was also lighter (more desirable) compared to corresponding dry storage treatments at the same temperature. The sulfite apparently was effective in inhibiting the enzymatic browning reaction that occurs during storage especially at high temperatures. Also, the PSU-3S-Process has been shown to diminish the color deterioration that occurs during blanching and thermal processing since some tissue oxygen is replaced by water during soaking (Beelman et al., 1973). The PSU-3S-Process also resulted in a higher incidence of mushrooms with exposed veils. This was also observed previously by Beelman et al. (1973).

The effect of storage temperature on yield and quality of canned mushrooms (cream strain) processed by dry storage, PSU-3S-Process and the SSV-Process were all compared in the last experiment (see Table 4). The trends observed with the dry storage and the PSU-3S-Process treatment were similar to those obtained in the previous experiment (see Table 3). When the SSV-Process was applied using the different storage temperatures, the yields obtained were comparable to the PSU-3S-Process except at 22°C where a significantly higher yield was observed with SSV-Process. Also, better color and a lower inci-

Table 1—Influence of 18 hr of post-harvest storage at three temperatures on yield and quality of canned mushrooms

Treatment	Canned product yield (%)	Yield increase (%)	Agtron color (% Reflectance)	Exposed veils (No./kg)
Control (no storage)	69.6 D[a]	—	35.2 A	0.0 B
Dry storage at 2°C	71.7 C	2.1	27.6 B	0.0 B
Dry storage at 12°C	74.9 B	5.3	24.7 C	4.0 B
Dry storage at 22°C	78.7 A	9.1	19.9 D	35.0 A

[a] Means in a column followed by the same letter are not significantly different with k = 100.

Table 2—Influence of 24, 48 and 72 hr of dry storage at three different temperatures on yield and quality of canned mushrooms

Treatment	Canned product yield (%)	Yield increase (%)	Agtron color (% Reflectance)	Exposed veils (No./kg)
Control (no storage)	68.0 H[a]	—	43.4 A	1.5 DF
24 hr at 2°C	69.7 G	1.7	36.6 B	0.3 G
48 hr at 2°C	70.1 G	2.1	34.8 C	0.8 EF
72 hr at 2°C	71.5 F	3.5	29.4 D	0.5 EF
24 hr at 12°C	74.5 E	6.5	29.8 D	7.0 E
48 hr at 12°C	78.3 CD	10.3	23.4 E	18.3 D
72 hr at 12°C	78.0 D	10.0	15.9 F	34.8 B
24 hr at 22°C	78.8 BC	10.8	23.9 E	26.0 C
48 hr at 22°C	80.1 A	12.1	15.3 F	57.3 A
72 hr at 22°C	79.2 B	11.2	11.2 G	60.3 A

[a] Means in a column followed by the same letter are not significantly different with k = 100.

Table 3—Influence of dry storage and the 3S-Process using three different storage temperatures on the yield and quality of canned mushrooms

Treatment	Canned product yield (%)	Yield increase (%)	Agtron color (% Reflectance)	Exposed veils (No./kg)
Control	64.3 F[a]	—	35.2 C	0.5 E
Dry storage at 2°C	67.0 E	2.7	29.5 D	0.8 E
Dry storage at 12°C	70.8 CD	6.5	28.0 D	0.8 E
Dry storage at 22°C	71.4 C	7.1	24.8 E	16.0 B
3S-Process at 2°C	71.6 C	7.3	42.3 A[b]	3.8 D
3S-Process at 12°C	78.1 B	13.8	39.3 B[b]	12.3 C
3S-Process at 22°C	80.0 A	15.7	34.2 C[b]	33.3 A

[a] Means in a column followed by the same letter are not significantly different with k = 100.

[b] Color values influenced by 1.6g/l of sodium sulfite used in Soak I water of 3S-Process.

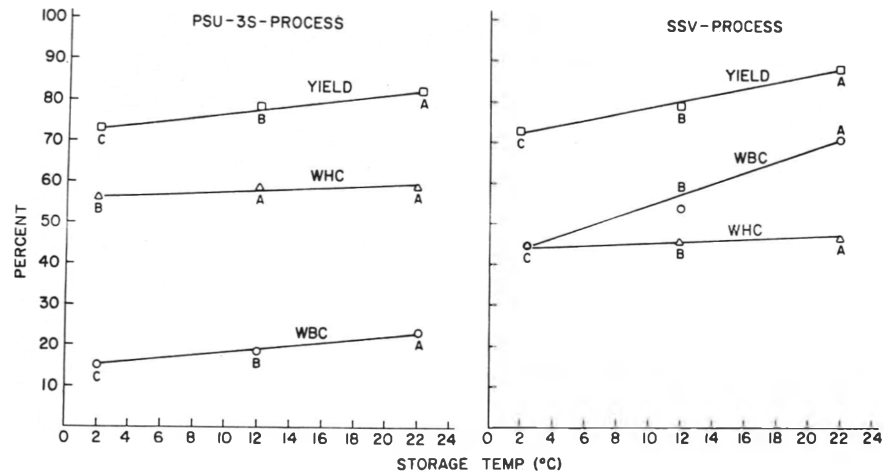

Fig. 1—Influence of storage temperature on WBC and WHC of mushrooms treated with the PSU-3S-Process and the SSV-Process (points on a line followed by the same letter are not significantly different with k = 100).

dence of mushrooms with exposed veils were found with the SSV-Process at each temperature. McArdle et al. (1974) reported better color and lower numbers of mushrooms with exposed veils with the SSV-Process compared to the PSU-3S-Process when a 2°C storage temperature was used.

The results of the total solids analyses (Table 4) demonstrated that as canned product yields increased, the solids content of the mushrooms decreased (or water content increased), indicating that increased yields were probably due mostly to the increased water retention. Beelman et al. (1973), McArdle et al. (1974) and Parrish et al. (1974) have all reported similar trends concerning water retention and yield increases.

From a practical standpoint, the SSV-Process with a 12°C storage temperature would appear to be the treatment with the most potential for commercial use. A yield increase of 15.3% compared to the control was attained with this treatment. This increase in yield or quality of the canned product was not significantly greater than that attained with the PSU-3S-Process at the same storage temperature, but the SSV-Process was shown to be more efficient in regard to time requirement during processing (McArdle et al., 1974).

Table 4—Influence of dry storage, 3S-Process and SSV-Process on yield and quality of canned mushrooms as affected by different storage temperatures for 18 hr

Treatment	Canned product yield (%)	Yield increase (%)	Total solids (%)	Agtron color (% Reflectance)	Exposed veils (No./kg)
Control	64.1 H[a]	–	10.4 A	43.4 A	0.0 C
Dry storage at 2°C	67.7 G	3.6	9.9 B	32.6 D	0.0 C
Dry storage at 12°C	71.5 F	7.4	9.6 BC	28.0 E	0.5 C
Dry storage at 22°C	74.0 D	9.9	9.5 CD	24.8 F	11.8 B
3S-Process at 2°C	73.0 DE	8.9	9.5 C	39.3 B	0.0 C
3S-Process at 12°C	79.2 C	15.1	8.8 E	33.9 CD	1.8 C
3S-Process at 22°C	82.6 B	18.5	8.3 F	31.3 D	16.0 A
SSV-Process at 2°C	72.6 EF	8.5	9.0 DE	40.9 AB	0.3 C
SSV-Process at 12°C	79.4 C	15.3	8.3 F	36.3 C	0.5 C
SSV-Process at 22°C	88.3 A	24.2	7.6 G	32.5 D	8.8 B

[a] Means in a column followed by the same letter are not significantly different with k = 100.

Water-binding capacity (WBC) and water-holding capacity (WHC) of mushrooms processed by the PSU-3S-Process and SSV-Process (Table 4) were determined and plotted with the corresponding canned product yields in Figure 1. With mushrooms processed by the PSU-3S-Process, both WBC and WHC increased significantly with temperature. Both WBC ($r = 0.91$) and WHC ($r = 0.96$) correlated highly with yield. Evidently, higher storage temperature increased the ability of mushroom tissue to absorb more water during soaking and the ability of the tissues to retain more of the water during processing, thereby increasing canned product yields. When the SSV-Process was used, similar trends were observed except that the WBC was much greater and WHC lower at each temperature. This would be expected since in vacuum soaking greater amounts of water are forced into the mushrooms. Apparently a point is reached where more water is forced into the tissues than can be retained under stress (blanching and thermal processing). However, WBC ($r = 0.98$) and WHC ($r = 0.91$) still correlated highly with yield when the SSV-Process was used.

The results of these experiments give additional evidence that some chemical changes occur in mushrooms during post-harvest storage which increases yields of canned mushrooms by increasing the WBC, WHC or both during processing. Investigations regarding the nature of these changes are currently underway in this laboratory.

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EFFECT OF OZONE FUMIGATION ON CROP COMPOSITION

INTRODUCTION

FOLLOWING the disclosure by Haagen-Smit et al. (1952) that ozone is formed in Los Angeles type (photochemical) smog, some of the deleterious effects of ozone on plants have been well established. Most of the investigations into the effect of ozone on plants have dealt with the nature of visible plant injury, measurement of the decrease in plant size, determination of the decrease in crop yield, and injury mechanisms.

Conceivably, the deleterious effects of ozone (and of air pollutants in general) could extend into the area of crop composition and could thus, for example, render crops from plants grown in polluted air nutritionally inferior. Since relatively little is known about the effect of air pollutants in this area a survey of the effect of polluted air and of the effect of two specific air pollutants, ozone and PAN (peroxyacetyl nitrate), on crop composition was undertaken at the Western Regional Research Laboratory in cooperation with the University of California at Riverside.

This report describes results of a survey of the composition of cabbage, carrots, corn, lettuce, strawberries and tomatoes harvested from plants grown in: (1) clean air (carbon-filtered air); (2) clean air fumigated with a low ozone level; and (3) clean air fumigated with a high ozone level. The two ozone levels used (approx 200 and 350 ppb O_3) represent levels commonly observed in the air in Southern California in summer and fall (Treshow, 1970). Items determined quantitatively include five vitamins, solids, nitrogen, fiber, ash carbohydrate (calculated) and up to nine metals.

Results of a similar survey in which plants were fumigated with PAN will be reported later.

EXPERIMENTAL

Plant growth

Plants were grown at the Statewide Air Pollution Research Center, University of California, Riverside, Calif. All species were grown in 2-gal pots in a soil mix of equal parts peat, loam and redwood shavings with a premixed nutrient supplement (Table 1). Each pot was thinned from four to a single seedling after emergence and 30 pots selected for uniformity from a total of 50. Three 10-pot treatments were then randomly selected.

Plants were grown in an evaporatively-cooled glass house containing air filtered through activated carbon. Day temperatures normally ranged between 27–32°C; night temperatures between 16–21°C. Plants were rotated weekly to assure equal exposure to variations in light and temperature within the house. A weekly nutrient supplement of 1 liter Hoagland's (1920) solution was given each plant during growth.

Ozone fumigations were monitored by a Mast Model 724–2 ozone meter calibrated regularly using the 2% KI standard method. Ozone was generated by an Orec Model 03BE-O ozonator and introduced into a 24 × 9 × 8.5 (ft) fumigation glasshouse using the cooling system ducting. Plants were rotated within the glasshouse before each fumigation to standardize exposure conditions.

The duration of fumigations varied among species due to logistical problems in moving large, staked plants such as tomatoes and corn (Table 2). Total exposure time varied among species from 3.59 to 1.62% of plant life.

Harvests were taken at specific stages of growth: corn at 21 days after silking; tomatoes at the "pink" stage of maturation when first color was observed; carrots, lettuce and cabbage at the plant life times shown in Table 2; strawberries ranging from first color to full maturity. All samples were quick-frozen in liquid nitrogen or dry ice immediately after harvesting and stored at –25°C until analyzed.

Sampling of crops

A portion of the crop representing a given treatment and weighing about 200g was partially thawed, broken up with a wooden mallet, if necessary, and weighed into a blender. An equal weight of absolute ethyl alcohol was added and the sample was blended to form a slurry. The alcohol slurries were stored in capped glass containers at about 0°C until subsamples were withdrawn for analyses (analyses were usually carried out within a week after slurries were prepared). The slurry was stirred continuously, to insure uniform suspension of solids in it, whenever a portion was being weighed for an analysis.

Solids

A portion of the slurry (about 20g) was pipetted into a weighed glass evaporating dish and the weight of the slurry determined. Most of the alcohol was first evaporated on a steam bath, the sample was then taken to apparent dryness in a forced draft oven at 70°C, and drying was completed in a vacuum oven at 70°C for 16 hr.

Total nitrogen

About 20g slurry was pipetted into a weighed evaporating dish and slurry weight was determined. After evaporating the slurry nearly to dryness on a steam bath, the residue was washed with water into a Kjeldahl flask and nitrogen was determined by the usual Kjeldahl procedure (AOAC 2.042, 1965).

Crude fiber

About 20g of slurry was weighed into an evaporating dish and taken to apparent dryness on a steam bath. The residue was washed into a beaker with the hot sulfuric acid and fiber was determined in the usual way (AOAC 22.038, 1965).

Ash

About 20g of slurry was weighed into a Vycor crucible. After evaporating the slurry nearly to dryness on a steam bath, it was taken to dryness in a forced draft oven at 70°C, carbonized under an infrared heater, and placed in a muffle at 550°C overnight, etc. as described elsewhere (AOAC 29.012, 1965)

Table 1–Contents of soil mix per cubic yard of unsteamed soil

Major constituents	
Sandy loam	9.3 cu ft
Peat moss	9.3 cu ft
Redwood shavings	9.3 cu ft
Chemical supplement	
Single super phosphate	2.0 lb
Calcium carbonate lime	4.0 lb
Dolomite lime	5.0 lb
Potassium sulphate	4.0 oz.
Potassium nitrate	4.0 oz
Isobutylidene diurea	26.0 oz.

Metal determination

Solids (300 mg) from determination of the solids content of samples were pelletized, and metals in the pellet were determined by energy dispersive X-ray fluorescence spectroscopy as described elsewhere (Reuter and Raynolds, 1974).

Carbohydrate

Carbohydrate was calculated as follows: Carbohydrate, g/100g = [solids (g/100g) – (g protein + g fat + g ash + g fiber)]/100g. Protein was calculated by multiplying N × 6.25. Fat was not determined in our samples, so we used fat values reported elsewhere (Watt and Merrill, 1963) for these calculations.

Table 2–Ozone fumigation data

Plant	Ozone (avg conc) Low level (ppb)	Ozone (avg conc) High level (ppb)	Avg frequency (times/wk)	Avg duration of each fumigation (hr)	Total exposure time Low level (hr)	Total exposure time High level (hr)	Plant life (days)	Ratio of total exposure time to plant life Low level (%)	Ratio of total exposure time to plant life High level (%)
Cabbage, Kopenhagen	200	330	1.5	6.0	74	72	105	2.94	2.86
Carrots, Emperator 58	200	350	3.0	2.5	63	73	116	2.26	2.62
Corn, sweet, Golden Jubilee	210	360	3.0	2.5	49	45	71	2.88	2.64
Lettuce, Prizehead (bronze)	200	330	3.0	6.0	50	40	58	3.59	2.87
Strawberries, Tioga	200	340	1.5	6.5	79	77	198	1.66	1.62
Tomatoes, H-11	200	350	3.0	2.5	99	97	116	3.56	3.48

Table 3–Average metal values determined by X-ray fluorescence spectroscopy and significance of differences between these values for crops harvested from plants fumigated with ozone

		Average quantity (mg) per 100g fresh weight																	
	Ozone fumigant (approx conc)	Cabbage			Carrots			Corn			Lettuce			Strawberries			Tomatoes		
Metal	(ppb)	Avg quantity (mg)	sig[a]	n[b]	Avg quantity (mg)	sig[a]	n[b]	Avg quantity (mg)	sig[a]	n[b]	Avg quantity (mg)	sig[a]	n[b]	Avg quantity (mg)	sig[a]	n[b]	Avg quantity (mg)	sig[a]	n[b]
Calcium	0	44.6	a	4							79.8	a	3						
	200	41.0	a	4							73.8	a	3						
	350	48.1	a	4							93.4	a	2						
Copper	0	0.116	a	4	0.120	a	2				0.106	a	3	0.067	a	2	0.037	a	1
	200	0.156	a	4	0.090	b	2				0.053	a	2	0.058	a	4	0.050	a	3
	350	0.135	a	4	0.072	b	3				0.073	a	2	0.051	a	1	0.054	a	3
Iron	0	0.489	a	4	4.83	a	3	0.79	a	9	6.53	a	3	1.22	a	5	0.247	a	5
	200	0.451	a	4	6.03	a	3	0.89	ab	10	2.20	a	2	1.34	a	5	0.251	a	5
	350	0.519	a	4	7.03	a	3	1.05	b	10	7.98	a	2	2.60	b	5	0.198	a	5
Lead	0	0.043	a	4							0.089	a	3	0.098	a	5	0.071	a	2
	200	0.038	a	4							0.056	a	2	0.092	a	5	0.049	a	3
	350	0.012	b	4							0.078	a	2	0.070	a	4	0.043	a	2
Manganese	0	0.097	a	4	0.213	a	3				0.491	a	3	0.216	a	5	0.100	a	4
	200	0.134	a	4	0.340	a	2				0.283	a	2	0.278	a	4	0.108	a	4
	350	0.129	a	4	0.217	a	3				0.486	a	2	0.245	a	4	0.105	a	4
Potassium	0	238.0	a	4				273.0	a	9	677.0	a	3						
	200	285.0	a	4				284.0	a	10	702.0	a	2						
	350	406.0	b	4				258.0	a	10	701.0	a	2						
Rubidium	0							0.54	a	9				0.067	a	5	0.280	a	5
	200							0.56	a	10				0.054	a	4	0.288	a	5
	350							0.27	b	10				0.056	a	3	0.220	a	5
Strontium	0	0.128	a	4	0.267	a	3				0.344	a	3	0.120	a	5			
	200	0.142	a	4	0.297	a	3				0.274	a	2	0.093	a	5			
	350	0.141	a	4	0.340	a	3				0.393	a	2	0.099	a	5			
Zinc	0	0.101	a	4	0.793	a	3	0.60	a	9	0.287	a	3	0.173	a	5	0.144	a	5
	200	0.124	a	4	0.560	b	3	0.71	b	10	0.249	a	2	0.183	a	5	0.141	a	5
	350	0.145	a	4	0.537	b	3	0.84	c	10	0.477	a	2	0.148	a	5	0.146	a	5

[a] Small letters indicate whether values differ significantly at the 5% level. If the same letter occurs in a pair being compared the two do not differ significantly. Conversely, if no two letters in a pair being compared are the same the two values differ significantly. Boxed-in data sets indicate at least one significant difference occurred in that set.

[b] n = number of observations (or replications) entering into the average value.

β-Carotene

The method of Knuckles et al. (1972) was used.

Vitamin C (ascorbic acid)

Ascorbic acid in corn was determined by the 2,6-dichlorophenol-indophenol photometric procedure using the xylene extraction method to overcome a turbidity problem (Freed, 1966). In this case, the corn was slurried directly into the metaphosphoric acid solution instead of being first slurried with alcohol.

Vitamin C, total

The requisite weight (x g) of stirred alcohol slurry was weighed into a 100 ml volumetric flask, x ml of 10% HPO_3 was added, the mixture was diluted to the mark, mixed, and filtered by gravity (E & D, 513 fluted paper). Total ascorbic acid was determined in the bromine-oxidized filtrate by the 2,4-dinitrophenylhydrazine procedure of Roe and Kuether (1943) and Roe and Oesterling (1944) as adapted and described by Freed (1966).

Thiamine (vitamin B_1)

Thiamine was determined by the thiochrome method as described by Freed (1966). For extraction, the required amount of stirred alcohol slurry was weighed into a 100 ml volumetric flask, 60 ml of 0.1N HCl were added, and the flask was heated 1 hr in a steam-heated water bath (max temp 85°C). Each sample was treated with Takadiastase enzyme and all sample extracts were purified on the cation exchanger, Decalso. Hot eluant and a 50 ml eluant volume were used to insure a more consistent and nearly quantitative recovery of thiamine from the resin as described by Pippen and Potter (1974).

Riboflavin (vitamin B_2)

This vitamin was determined microbiologically by the method of Johnson (1948).

Niacin

Niacin was determined microbiologically by the method of Sarett et al. (1945).

Table 4—Average analytical values of nutrients and significance of difference between these values for crops harvested from plants fumigated with ozone

	Ozone fumigant (approx conc)	Average quantity per 100g of fresh weight																	
		Cabbage			Carrots			Corn			Lettuce			Strawberries			Tomatoes		
Item determined	(ppb)	Avg quantity	sig[a]	n[b]	Avg quantity	sig[a]	n[b]	Avg quantity	sig[a]	n[b]	Avg quantity	sig[a]	n[b]	Avg quantity	sig[a]	n[b]	Avg quantity	sig[a]	n[b]
Solids (g)	0	6.63	a	4	14.5	a	3	28.7	a	10	5.29	a	3	7.90	a	5	7.01	a	5
	200	7.09	a	4	14.6	a	3	27.1	b	10	4.90	a	2	7.84	a	5	6.87	a	5
	350	8.38	b	4	12.3	b	3	26.2	c	10	5.81	a	2	8.20	a	5	5.16	b	5
Nitrogen (g)	0	0.20	a	2	0.32	a	3	0.43	a	10	0.23	a	3	0.08	ab	5	0.11	a	5
	200	0.22	a	2	0.31	a	3	0.44	a	10	0.21	b	2	0.09	a	5	0.12	a	5
	350	0.34	a	2	0.31	a	3	0.52	b	10	0.25	c	2	0.07	b	5	0.11	a	5
Fiber (g)	0	0.68	a	2	Not det.			Not det.			0.53	a	3	0.53	a	5	0.73	a	5
	200	0.77	b	2	Not det.			Not det.			0.47	a	2	0.53	a	5	0.72	a	5
	350	0.97	c	2	Not det.			Not det.			0.55	a	2	0.56	a	5	0.53	b	5
Ash (g)	0	0.47	a	2	Not det.			Not det.			1.20	a	3	0.44	a	5	0.36	a	5
	200	0.52	a	2	Not det.			Not det.			0.99	a	2	0.46	a	5	0.38	ab	5
	350	0.74	b	2	Not det.			Not det.			1.33	a	2	0.44	a	5	0.42	b	5
Carbohydrate (g)	0	3.40	a	4	Not det.			23.6	a	10	1.90	a	3	5.9	a	5	5.0	a	5
	200	4.39	b	4	Not det.			22.0	b	10	1.78	a	2	5.7	a	5	4.9	a	5
	350	4.87	b	4				20.6	c	10	2.21	a	2	6.3	a	5	3.3	b	5
β-Carotene (mg)	0	1.09	a	4	4.2	a	3	0.071	a	5	0.88	a	3	Not det.			0.14	a	5
	200	1.42	a	4	6.6	a	3	0.050	b	5	0.96	a	2	Not det.			0.17	a	5
	350	1.18	a	4	5.9	a	3	0.051	b	5	0.90	a	2	Not det.			0.14	a	5
Vitamin C (mg) (Ascorbic acid)	0	Not det.[c]			Not det.			10.2	a	10	Not det.			Not det.			Not det.		
	200	Not det.			Not det.			10.3	a	10	Not det.			Not det.			Not det.		
	350	Not det.			Not det.			10.9	b	10	Not det.			Not det.			Not det.		
Vitamin C (mg) (Total)	0	41.0	a	4	13.4	a	3	11.7	a	10	15.2	a	3	34	a	5	33.4	a	5
	200	45.0	a	4	10.5	a	3	12.2	b	10	14.0	a	2	36	a	5	33.9	a	5
	350	64.8	b	4	11.0	a	3	13.1	c	10	18.3	a	2	38	a	5	25.3	b	5
Thiamine (mg)	0	0.035	a	4	0.084	a	3	Not det.			0.034	a	3	0.020	a	5	0.105	a	5
	200	0.039	a	4	0.078	a	3	Not det.			0.033	a	2	0.019	a	5	0.090	b	5
	350	0.051	b	4	0.080	a	3	Not det.			0.040	b	2	0.020	a	5	0.070	c	5
Riboflavin (mg)	0	0.039	a	4	0.038	a	3	Not det.			0.078	a	3	0.066	a	5	0.018	a	5
	200	0.043	a	4	0.035	a	3	Not det.			0.074	a	2	0.070	a	5	0.017	a	5
	350	0.060	a	4	0.028	a	3	Not det.			0.089	a	2	0.074	a	5	0.017	a	5
Niacin (mg)	0	0.19	a	4	0.57	a	3	Not det.			0.44	a	3	0.23	a	5	0.018	a	5
	200	0.21	a	4	0.81	b	3	Not det.			0.38	a	2	0.26	b	5	0.017	a	5
	350	0.27	a	4	0.76	b	3	Not det.			0.51	a	2	0.27	b	5	0.017	a	5

[a] Small letters indicate whether values differ significantly at the 5% level. If the same letter occurs in a pair being compared the two do not differ significantly. Conversely, if no two letters in a pair being compared are the same the two values differ significantly. Boxed-in data sets indicate at least one significant difference occurred in that set.

[b] n = number of observations (or replications) entering into the average value.

[c] Not det.: Not determined.

RESULTS & DISCUSSION

IN GENERAL, the quantity of nutrients in crops from the ozone fumigated plants were comparable to those from crops grown in carbon-filtered air (see Tables 3 and 4). Hence, exposure of these plants to ozone did not result in a wholesale loss of these nutrients.

In the metal results (Table 3) significant differences occurred in only 8 of 38 data sets. Furthermore, these significant differences show little if any pattern or trend that correlates with ozone treatment. Therefore, for the most part these data (Table 3) show that the quantity of metals in these crops was not influenced by the ozone fumigation.

The solids of a fresh fruit or vegetable represents the sum of its nonvolatile components. Hence the maxim: a trend in total solids should also normally establish a similar trend for its constituents. For example, one would expect corn kernels relatively high in solids to be also relatively high in carbohydrate, vitamins, etc. This maxim seems to have prevailed fairly well in this survey since 11 out of the 20 significant differences in nonmetal constituents followed trends set by solids (see Table 5). Total solids, therefore, would seem to be a particularly significant indicator of the influence of ozone on crop composition. In this survey, ozone seems to have significantly lowered the solids content of three crops, had no significant effect on two crops, and resulted in increased solids in one crop (Table 5).

However, exceptions to the maxim that solids trends establish composition trends are perhaps the best indicators of the effect of ozone on crop composition. The exceptions are of particular interest because they logically reflect some variable in plant treatment and the only variable designed into this survey was exposure to ozone. The most impressive exceptions are compositional trends which go counter to solids trends. Five of these occurred involving niacin in carrots, vitamin C (as ascorbic acid), total vitamin C and nitrogen in corn, and ash in tomatoes (Table 5). Also the two significant differences in lettuce composition and the two in strawberries represent exceptions of a different type in that they occurred in spite of there being no apparent ozone effect on the solids content of these crops (Table 5). Probably not all of these exceptions reflect changes in crop composition as a result of ozone, but they do suggest that possibility and represent focal points for further investigations. Some of these exceptions will be discussed in greater detail.

In contrast to the metal results, the nonmetal nutrients (Table 4) show at least one significant difference in 24 of the 52 data sets and some trends are evident. Hence, ozone had a significant effect on the quantity of some of the nutrients in some of these crops.

Corn is the outstanding example of trends, for not only does every item determined show a trend but it also had a larger number (5–10) of analytical replications than any other crop. If we let the 28.7g of solids/100g corn in the control (Table 4, zero ozone) represent 100%, it is evident that corn solids decreased 5.6% and 8.7% in the low and high level ozone samples respectively and carbohydrate, as expected, follows this trend. β-Carotene also shows a downward trend with exposure to ozone (Table 4). Nitrogen, on the other hand is significantly higher in the high level ozone corn, an example of a trend counter to the solids trend. Perhaps this is a result of there being less solids and/or carbohydrate in the corn kernel to dilute the nitrogen. Total vitamin C values in corn also show a definite trend upward. This trend is particularly impressive since it goes counter to the corn solids trend. Furthermore, this increase in vitamin C in corn exposed to ozone was also apparent when vitamin C was determined in its reduced form, ascorbic acid (see vitamin C, reduced, Table 4). Apparently one way the corn plant reacts to an oxidative atmosphere is to increase the level of the reducing agent, ascorbic acid, in the kernel.

Cabbage exposed to ozone, in contrast to all other crops, showed a significant increase in solids and this trend also exists for all other components in cabbage that showed a significant difference (Table 4). The remarkable 58% increase in vitamin C in cabbage exposed to the high level ozone is about twice the 26.4% increase in solids in this cabbage. Hence cabbage, like corn, may respond to ozone by increasing its vitamin C content.

The small amount of lettuce available severely restricted the number of replications on this crop (there were only 2 to 3, Table 4). There were two significant differences involving nitrogen and thiamine but these show no definite trends.

The composition of strawberries appears to have been only slightly, if at all, affected by ozone fumigation (Table 4). A significant difference occurred in nitrogen values, but no trend is evident. Niacin is significantly higher in the ozone-treated berries as was the case for carrots.

Some consistent trends are evident in the tomato data in which six of the 10 data sets show significant differences (Table 4). Solids are significantly down (26% less than the control) in tomatoes from the high ozone treatment. This downward trend in solids apparently sets trends for fiber (down 27%), vitamin C (down 24%) and thiamine (down 33%).

Although this survey indicated that ozone does not have a major and generally deleterious impact on crop composition, it did indicate some areas where ozone influenced crop composition; these seem worthy of further investigation. Of particular interest is the indication that ozone reduces the solids content of some crops and thereby provides a mechanism for a general across-the-board loss of nutrients. Also of interest is the possibility disclosed here that some plants react to ozone by producing increased amounts of the reducing agent, ascorbic acid. Studies are continuing on the effect of both specific air pollutants and of ambient (smoggy) air on crop composition.

Table 5—Composition trends compared to solids trends in crops from ozone-fumigated plants (compiled from data in Table 4)

		Number of significant differences in composition (excluding solids)		
Crop	Solids trend	Total	Following solids trend	Counter to solids trend
Cabbage	Up	5	5	0
Carrots	Down	1	0	1[a]
Corn	Down	5	2	3[b]
Lettuce	None	2[d]	0	0
Strawberries	None	2[d]	0	0
Tomatoes	Down	5	4	1[c]
Totals:		20	11	5

[a] Niacin
[b] Nitrogen, vitamin C (as ascorbic acid), and vitamin C (total)
[c] Ash
[d] Exceptions to solids trend: 2 + 2 + 5 = 9

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CHEMICAL AND THERMAL EFFECTS ON PARATHION RESIDUES ON SPINACH

INTRODUCTION

PARATHION has been used for the control of aphids and other insects on spinach. The recommended rate of application is 1/2 lb actual material per acre not less than 14 days before harvest.

Investigators have previously studied the residue levels of parathion on field-treated spinach and the effect of frozen storage on these residues (Beckman and Thornburg, 1965), the removal of parathion from spinach by home and commercial preparative procedures (Lamb et al., 1968), the effect of heat processing and storage on pesticide residues in spinach and apricots (Elkins et al., 1972), and the effect of a phosphate buffer and magnesium carbonate on quality attributes of cooked green vegetables including spinach (Clydesdale et al., 1971). Although parathion levels were determined in several of the above investigations, most of the other possible related compounds were not determined.

The purpose of the present investigations was to determine quantitatively the fate of the levels of parathion and related compounds on spinach subjected to specific chemical and thermal treatments during cooking procedures. The chemical treatments selected were chosen to observe the specific thermal and chemical effects such as salts, acids and bases on spinach when compared to the usual procedures of cooking spinach in the presence of only table salt and water.

EXPERIMENTAL

Spinach plots

Rows of spinach 44 in. wide and totaling 150 ft in length were divided into three 50-ft sections. A 50-ft section (Plot A) upwind from the other two 50-ft sections was established as a control plot. A second plot (Plot B) was established for spraying with the recommended rate of parathion at 1/2 lb active ingredient per acre, and a third plot (Plot C) was sprayed with double the recommended rate of parathion or 1 lb per acre. The spinach was at the stage of maturity of two weeks before harvest. The first sampling of the control plot was taken before spraying the other plots. The other two plots were also sampled (approx. 500g) immediately after spraying. All sampled spinach was immediately extracted for quantitative analysis. At the harvest sampling, 14 days after pesticide application, all the spinach from each plot was completely harvested, composited, and subsamplings were extracted for analysis.

Spray application

The spinach was sprayed with Thiophos® 4 lb E.C. using a Hudson Climax® 6335 Simplex Sprayer, 8.5-liter capacity equipped with a Hudson 149-403 spray control valve and a nozzle extension. A 0.95 cm i.d. flexible neoprene, teflon-lined rubber tubing for chemical inertness was attached between the pressure tank and the Roto-Spray Nozzle®.

Parathion applied

The parathion applied was in the formulation of American Cyanamide Co. designated as Thiophos® Parathion 4 E.C. (1 gal contains 4 lb parathion).

Active ingredients: Parathion (O,O-diethyl O-p-nitrophenyl phosphorothioate) 46.7%; Xylene-Range aromatic hydrocarbon solvent 47.6%; and Inert ingredients 5.7%.

Plot B (1/2 lb active parathion/acre) was sprayed as evenly as possible with 1 gal of water containing 0.971g of parathion using the equipment described above and Plot C (1 lb active parathion/acre) was evenly sprayed with 1 gal of water containing 1.94g of parathion.

The formulation was found to contain by analysis and calculated as the parathion active ingredient equivalent 95.4% parathion, 3.89% aminoparathion, 0.12% paraoxon, 0.11% O,S-diethyl-O-p-nitrophenyl phosphorothiolate, 0.05% O,O-diethyl-S-p-nitrophenyl phosphorothiolate, 0.43%-nitrophenol and 0.01% O,O-diethyl phosphorothioate.

Sample extraction

25g spinach were extracted by refluxing in 250 ml of a solvent mixture containing 5% isopropanol-95% benzene (V/V) for 30 min. The refluxed sample was cooled in an ice bath and the solvent was filtered through Whatman #1 filter paper. The extraction was repeated for a total of three refluxes and the filtered solvent was pooled and stored for cleanup and analysis. The extracted plant material was discarded. All solvents used in the experiment were reagent grade freshly distilled prior to use. Extraction efficiencies were studied with fortified control samples at 1.0–0.1 ppm fortification.

Diazomethane preparation and methylation of the sample extract

The laboratory preparation of diazomethane is detailed on the label of the Diazald® reagent bottle obtained from the Aldrich Chemical Co., Inc., 940 W. St. Paul Ave., Milwaukee, WI 53233.

The extractives from 10g of spinach (300 ml solvent extract) were concentrated in vacuo at 50–60°C to approximately 1 ml and the volume was quantitatively adjusted to 5 ml with n-hexane. The extractives equivalent to 5g of spinach were added to 5 ml of etheral diazomethane solution at room temperature and allowed to stand for at least 15 min prior to sample cleanup.

Sample cleanup

A glass column 2.54 × 25.00 cm with a solvent reservoir at the top was packed with a plug of glass wool, 10g anhydrous Na_2SO_4, 20g of Florisil (activated at 270°C for 3 hr) and 10g anhydrous Na_2SO_4. N-pentane (100 ml) was allowed to flow to the column top by gravity flow, and the solvent was discarded after the column was washed. The methylated sample was added to the column in 25 ml of pentane followed by two 25-ml aliquots of pentane washes. The sample was eluted from the column with 390 ml of 30% diethyl ether-70% pentane (V/V) and with 390 ml of 25% methanol-75% benzene (V/V) after changing the column receiver between solvents. The eluates were collected and analyzed separately by gas-liquid chromatography (GLC) or in combination with thin-layer chromatography (TLC) after concentration in vacuo at 50–60°C to approximately 1 ml and transferred and adjusted to appropriate volumes in 6.5 ml MacKay-Shevky-Stafford sedimentation tubes. Parathion, aminoparathion, S-phenyl parathion, diethyl phosphorothioate and methylated p-nitrophenol were eluted from the column with the ether-pentane solvent. Paraoxon and the methylated diethyl phosphate were eluted with the methanol-benzene solvent. A separate spinach extract aliquot was cleaned up on the Florisil column which was eluted only with the 25% methanol-75% benzene solvent (V/V) for the detection of the S-ethyl parathion. All recoveries of samples cleaned up ranged quantitatively from 70–90%. Samples were analyzed both with and without cleanup. The cleanup procedure facilitated analysis by GLC using the electron capture detector as well as by TLC. An additional advantage of cleanup was the separation of the compounds into groups with the separate eluting solvents as described above.

Gas-liquid chromatography (GLC)

The gas chromatograph (Aerograph Model 200) was equipped with a thermionic phosphorus detector with a cesium bromide pellet. The glass coiled 8 ft column was packed with an equal mixture of 10% DC 200 and 15% QF-1 on 60–80 mesh Gas-Chrom Q column support resulting in a final concentration 5% DC 200 and 7.5% QF-1. The carrier gas flow (N_2) was 20 ml/min. The detector and injector temperatures were 200°C. All parathion and related compounds were quantitated on this

column with the exception of p-nitrophenol. The temperatures at which the following compounds were detected are: (1) parathion, 210°C; (2) aminoparathion, 210°C; (3) paraoxon, 210°C; (4) diethyl phosphorothioate, 150°C; (5) O,O-diethyl-S-p-nitrophenyl phosphorothiolate, 210°C; (6) O,S-diethyl-O-p-nitrophenyl phosphorothiolate, 210°C; (7) diethyl phosphate, 150°C. Diethyl phosphorothioate and diethyl phosphate were detected as the methylated compounds by GLC. A Varian-Aerograph Model 1200 gas chromatograph equipped with an electron capture detector and a 6 ft glass column packed with 5% SE-30 and 5% Dow 710 fluid on 60/80 mesh Chromosorb W acid washed and silylated, a carrier gas flow (N_2) of 30 ml/min and a column temperature of 140°C was utilized to quantitate the p-nitrophenol as the methylated compound. The detector and injector temperatures were 200°C. Quantitation of all compounds was accomplished by measurement of peak areas with a polar planimeter and compared with reference standards. The ppm data are based on the spinach fresh weight calculations rather than on a dry weight basis since the percent moisture of the samples ranged from 86.5–89.2 with a mean of 88.0 ± 0.8, and this slight percent moisture variation would not significantly affect the residue data.

Thin-layer chromatography (TLC)

Glass plates 20 × 20 cm were coated 250μ in thickness with Silica Gel H containing 1% zinc silicate in a water slurry. The plates were air dried and activated in a warm air oven at 100°C for 30 min and stored in a desiccator prior to use. After spotting, the samples were developed for a 15 cm solvent travel in 20% acetone-80% n-hexane. The solvent was air dried and the spots visualized under ultraviolet light at 370 nanometers. The compounds in the detected spots were extracted from the plate in 3 ml of 25% methanol-75% benzene in a 10 ml volumetric flask with shaking for 5 min. The sample extracts were adjusted to appropriate volumes and aliquots were injected into the GLC for quantitation. Usually for convenience the compounds were taken from the plates for extraction into the solvent in two strips. The first strip ranging in R_f from 0–0.24 (0–3.9 cm) contained aminoparathion, paraoxon, diethyl phosphate and S-ethyl parathion, and the second strip ranging in R_f from 0.24–0.60 (3.9–9.0 cm) contained parathion, p-nitrophenol, S-phenyl parathion and diethyl phosphorothioate. The GLC procedure was able to resolve the compounds in these extracts efficiently.

Spinach cooking experiments

The final spinach sampling (harvest) was processed in the following manner to determine the effect on the parathion and related compounds residues. 50-g samples were washed with 240 ml of cold tap water and boiled for 15 min in 240 ml of tap water, water containing 2.4g sodium chloride, 10.5g acetic acid, 10.5g acetic acid plus 2.4g sodium chloride, 2.4g sodium bicarbonate and 2.4g sodium chloride plus 2.4g sodium bicarbonate. The cooked spinach was extracted as previously described. The tap water washes and the cooking broth were extracted in a separatory funnel by shaking for 4 min with 100 ml of hexane. The aqueous phase was adjusted to pH 4.0 with 1N HCl and extracted with 100 ml hexane. Finally, the aqueous phase was adjusted to pH 10.0 with 1N NaOH and extracted with 100 ml hexane. The hexane was pooled, passed through anhydrous sodium sulfate, concentrated in vacuo at 50–60°C and adjusted to volume. The samples were analyzed either by GLC or in combination with TLC after cleanup.

RESULTS & DISCUSSION

TABLE 1 contains the chemical structures and names of parathion and related compounds investigated.

Table 2 shows the parathion and related compounds levels

Table 1–Chemical structures and names of compounds

Structure	Name
$(C_2H_5O)_2P(S)$–O–C_6H_4–NO_2	O,O-Diethyl O-p-nitrophenyl phosphorothioate (Parathion)
$(C_2H_5O)_2P(O)$–O–C_6H_4–NO_2	O,O-Diethyl O-p-nitrophenyl phosphate (Paraoxon)
$(C_2H_5O)_2P(S)$–O–C_6H_4–NH_2	O,O-Diethyl O-p-aminophenyl phosphorothioate (Aminoparathion)
$(C_2H_5O)_2P(O)$–S–C_6H_4–NO_2	O,O-Diethyl S-p-nitrophenyl phosphorothiolate (S-Phenyl parathion)
$(C_2H_5S)(C_2H_5O)P(O)$–O–C_6H_4–NO_2	O,S-Diethyl O-p-nitrophenyl phosphorothiolate (S-Ethyl parathion)
HO–C_6H_4–NO_2	p-Nitrophenol
$(C_2H_5O)_2P(O)$–OH	O,O-Diethyl phosphate
$(C_2H_5O)_2P(S)$–OH	O,O-Diethyl phosphorothioate

Table 2–Levels[a] of parathion and related compounds when applied to field spinach in an emulsifiable concentrate formulation

Sample (day)	Parathion (ppm)[b]	Amino-parathion (ppm)[b]	Paraoxon (ppm)[c]	S-Ethyl parathion (ppm)[d]	S-Phenyl parathion (ppm)[d]	Diethyl phosphate (ppm)[b]	p-Nitrophenol (ppm)[b]	Total parathion residues (ppm)
				Control (unsprayed)				
1	0.034	<0.001	0.002	<0.010	<0.010	0.005	0.095	0.136
14	0.003	<0.001	<0.001	<0.010	<0.010	<0.001	0.008	0.011
				1/2 lb/acre active ingredient				
1	25.3	0.410	0.186	0.182	<0.010	0.006	0.172	26.2
14	0.206	<0.001	0.021	<0.010	<0.010	0.001	0.033	0.264
				1 lb/acre active ingredient				
1	56.3	0.905	0.252	0.432	0.042	0.039	0.453	58.4
14	0.365	0.006	0.082	<0.010	<0.010	0.002	0.019	0.474

[a] Ppm calculated on a fresh weight basis; no O,O-diethyl phosphorothioate found on the samples; percent moisture ranged from 86.4–89.2 with a mean of 88.0 ± 0.8.

[b,c,d] Ppm method sensitivity 0.001[b], 0.002[c], 0.010[d]

at the initial interval of spray application and at harvest on the control (unsprayed), 1/2 lb/acre and 1 lb/acre active ingredient samples. The tolerance level on spinach for parathion applied 14 days prior to harvest at the recommended rate of 1/2 lb active ingredient/acre is 1 ppm at harvest. Double the recommended rate was also applied to the spinach for comparison purposes. The total residues on the spinach were well within the tolerance level at harvest in both sets of spinach samples.

Table 3—Levels[a] of parathion and related compounds in cooked spinach and broth

Treatment	Parathion (ppm)	Paraoxon (ppm)	p-Nitrophenol (ppm)	Total parathion residue (ppm)
		Harvested spinach		
None	0.206	0.021	0.033	0.264
		Spinach — washed		
Tap water washed spinach	0.184	0.019	0.010	0.213
Tap water wash	0.014	0.004	0.034	0.052
		Cooked spinach		
Water	0.086	<0.001	0.012	0.098
Brine	0.068	0.013	0.016	0.098
Sodium bicarbonate	0.033	0.008	0.006	0.047
Brine plus sodium bicarbonate	0.058	0.004	0.043	0.105
Acetic acid	0.092	0.006	0.038	0.136
Brine plus acetic acid	0.069	0.003	0.028	0.100
		Cooking broth		
Water	0.024	<0.001	0.037	0.061
Brine	0.023	<0.001	0.026	0.049
Sodium bicarbonate	0.013	0.001	0.027	0.041
Brine plus sodium bicarbonate	0.022	0.002	0.051	0.075
Acetic acid	0.031	<0.001	0.060	0.091
Brine plus acetic acid	0.023	0.006	0.004	0.033

[a] Ppm calculated on a fresh weight basis; 1/2 lb/acre active ingredient applied 14 days before harvest; S-ethyl parathion, S-phenyl parathion, < 0.010; diethyl phosphorothioate, diethyl phosphate, aminoparathion in all treated sample, < 0.001.

Table 4—Levels[a] of parathion and related compounds in cooked spinach and broth

Treatment	Parathion (ppm)	Amino-parathion (ppm)	Paraoxon (ppm)	Diethyl phosphate (ppm)	p-Nitro-phenol (ppm)	Total parathion (ppm)
			Harvested spinach			
None	0.365	0.006	0.082	0.002	0.019	0.474
			Spinach — washed			
Tap water washed spinach	0.333	0.003	0.082	0.003	0.018	0.439
Tap water wash	0.020	0.002	0.011	<0.001	0.004	0.037
			Cooked spinach			
Water	0.141	0.002	<0.001	<0.001	0.011	0.154
Brine	0.191	0.002	<0.001	<0.001	0.008	0.201
Sodium bicarbonate	0.047	0.002	<0.001	<0.001	0.005	0.054
Brine plus sodium bicarbonate	0.070	0.002	<0.001	<0.001	0.003	0.075
Acetic acid	0.129	0.001	0.004	0.002	0.007	0.143
Brine plus acetic acid	0.137	<0.001	0.002	0.003	0.002	0.144
			Cooking broth			
Water	0.068	0.005	0.031	<0.001	0.009	0.113
Brine	0.090	0.005	0.005	<0.001	0.008	0.108
Sodium bicarbonate	0.022	0.010	0.002	<0.001	0.010	0.042
Brine plus sodium bicarbonate	0.019	0.003	<0.001	<0.001	0.009	0.031
Acetic acid	0.070	<0.001	0.024	<0.001	0.011	0.105
Brine plus acetic acid	0.034	<0.001	0.012	0.003	0.003	0.050

[a] Ppm calculated on a fresh weight basis; 1 lb/acre active ingredient applied 14 days before harvest; S-ethyl parathion, S-phenyl parathion, < 0.010; diethyl phosphorothioate in all treated samples, < 0.001

As explained previously, there were related compounds present in the Thiophos® formulation in addition to parathion. O,O-Diethyl phosphorothioate was not detected in either the controls or treated samples. The data represent average values for the duplicate plots.

Tables 3 and 4 contain data for the parathion and related compounds measured in the water washes of the spinach before cooking and in the cooked spinach and cooking broths for the 1/2 lb and 1 lb/acre applications of actual ingredient, respectively. Water washing of the uncooked spinach removed mainly aminoparathion, paraoxon and p-nitrophenol and reduced the total parathion residues by approximately 10.7% on the 1/2 lb rate and 8.8% on the 1 lb rate (Tables 3 and 4). Cooking the washed spinach for 15 min by boiling in water, brine, bicarbonate, acetic acid and their mixtures reduced the levels of parathion, paraoxon and p-nitrophenol on the cooked spinach by various amounts depending upon the cooking solution. For example, parathion at the 1/2 lb rate (Table 3) was reduced by cooking in water 53.2%, brine 67.0%, bicarbonate 82.0%, acetic acid 68.4%, brine plus bicarbonate 50.0% and acetic acid plus brine 62.5%. Paraoxon and diethyl phosphate were mostly removed below detection limits and approximately 33.3% of the p-nitrophenol was removed from the harvested spinach by washing and cooking. In the cooking broth 13.0%, 12.5%, 7.0%, 16.8%, 11.9% and 12.5%, respectively, of the parathion on the washed spinach was found and the aminoparathion, diethylphosphate, diethyl phosphorothioate, S-ethyl parathion, and S-phenyl parathion levels were not detectable. The paraoxon disappeared or dropped to very low levels on the cooked spinach and broth samples. The p-nitrophenol levels in the cooking broths on the average were approximately 77.2% of the initial levels as determined by the amounts found in the washed spinach and water washes. The cooked treated spinach contained approximately 54.5% of these amounts. Similar results were obtained for the 1 lb rate (Table 4). As listed above for the 1/2 lb rate, 57.6, 42.6, 85.8, 61.2, 78.9 and 58.8% of the parathion was removed, respectively, by the various cooking treatments. The possibility exists that losses occurred by volatility and chemical decomposition during cooking. Other compounds may have been present on the spinach, however, investigations were concentrated on the compounds listed in Table 1.

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EFFECT OF TEMPERATURE AND PACKAGING ATMOSPHERE ON STABILITY OF DRUM-DRIED PINTO BEAN POWDER

INTRODUCTION

A CONVENIENT dry powder from cooked beans, peas or lentils has been described (Morris, 1961). Subsequently, the process was studied on a pilot scale (Bakker-Arkema et al., 1967), and some storage studies were conducted on laboratory prepared powders (Boggs et al., 1964; Burr et al., 1969). Following these studies, bean powder from cooked pinto beans was produced on a limited commercial scale. As a result of this commercial activity, market and nutritional studies on this product were reported (White and Kon, 1972; Miller et al., 1973). The primary purpose of this study was to determine stability of the commercially prepared powder stored in air and under a nitrogen atmosphere at 50, 70 and 90°F.

MATERIALS & METHODS

Pinto bean powder

The powder was produced in a commercial plant in the Pacific Northwest from locally grown pinto beans, as described by Kon et al. (1974). The beans were thoroughly washed, soaked overnight at room temperature and cooked for 1 hr at 212°F in about four volumes of water. The cooked beans were slurried by pumping through a finisher. The slurry was applied to a commercial double drum drier (42 in. × 90 in.) set at 60 psi with a clearance between drums of about 0.018 in. The drying time was 18 sec, and the resulting powder had a moisture content of 6.2% and a bulk density of 0.34g/cc. The flakes prepared in this way were packed in boxes double lined with polyethylene bags and shipped to the laboratory. Some of the powder was repackaged in hermetically sealed cans under an atmosphere of nitrogen, and some was air packed.

Representative samples of both air and N_2 packs were placed at −20, 50, 70 and 90°F immediately after packaging. The samples at −20°F served as controls for those stored at the higher temperatures. At periodic intervals, samples of both N_2 and air packs were removed from storage and compared with −20°F controls to determine stability at the higher temperatures. All N_2 pack samples were checked for O_2 content with a Beckman O_2 analyzer. The probe from the analyzer was fitted to a special air tight can adapter and puncturing device. The connected apparatus was pressed on the can lid, and the O_2 content of the headspace atmosphere was directly read from the instrument. The average O_2 content of N_2 packed samples was 0.5% with standard deviation of 0.2%. Samples containing more than 1% O_2 were discarded.

Sensory evaluation

The N_2 and air packed powder held under various time-temperature conditions was evaluated as flavored and unflavored soup. The unflavored soup was prepared by stirring 50g of powder in 200 ml of boiling water containing 0.5% NaCl until a smooth mixture was obtained. The mixture was then placed in a water bath thermostatically controlled at 160°F and served at this temperature. The flavored soup was prepared according to the following formula:

Ingredients	Grams
Water	420
Pinto bean powder	70
Shortening	3.5
Sodium chloride	2.0
Hydrolyzed vegetable protein	1.4
Monosodium glutamate	1.4
Onion powder	0.7
Bacon flavor	0.5

This mixture was brought to a boil, stirring constantly, and then placed in the water bath, as described above.

The soups were evaluated by a panel of judges who were screened for their ability and consistency in detecting off-flavor in a series of powders with varying degrees of oxidized off-flavor. From an initial group of 40, the regular panel was reduced to 18 panelists who demonstrated consistency in detecting off-flavors, once their individual threshold was reached.

The soups were presented to the judges as a duo-trio in which the N_2 sample held at −20°F was the labelled control, and one of the two coded samples was held at a higher temperature. In addition to indicating which sample in the coded pair matched the labelled control, the judges were required to check the sample with the freshest bean flavor. The soup was served in paper cups (approximately 1 oz) to judges in individual booths maintained at 74 ± 2°F and 50% RH by means of a pressurized air deodorizing and conditioning system. The booths were equipped with 7.5 watt green bulbs to eliminate the influence of possible color differences, and with running water to rinse between samples. One duo-trio was presented at each taste session, and each comparison was replicated twice. Control and stored samples were presented first in each pair an equal number of times to prevent first sample bias.

It was desired to determine stability of the powder at each of the temperature and packaging conditions in terms of weeks or days with the appropriate 95% confidence interval as a measure of variability. It is assumed that storage time at the higher temperatures (50, 70, 90°F) causes the production of breakdown products which form the basis of a gradually increasing stimulus difference from the control sample. It is, therefore, reasonable to treat the duo-trio data as a series of stimulus response curves with time as the stimulus axis, and percentage correct responses as the response axis. A further requirement for quantifying the sensory results in terms of confidence intervals is that the data plot as a straight line on log-probability paper. To accomplish this, the duo-trio data were corrected for chance to give a 0 to 100 detection scale by the following relation:

$$Pc = (Po - 50) \times 2$$

where Po = percentage correct responses in the duo-trio test, 50 is the percentage correct responses by chance and Pc can be regarded as the percentage of responses actually detecting the difference in stimulus between samples. If the data in this form plot as a straight line on log-probability paper, they meet the requirements of the dose-response procedure described by Litchfield and Wilcoxon (1949). Figure 1 shows a plot of the corrected duo-trio data for air and N_2 packs of pinto bean powder stored at 50, 70 and 90°F. It is apparent that the data do plot as a reasonably good straight line and hence Pc_{50} (Pc = 50) corresponds to LD_{50} in dose response experiments. It is also evident that Pc = 50 when Po or the uncorrected duo-trio responses are equal to 75%. The advantages of this procedure over the usual methods is that all of the sensory data are utilized in determining the stable period and its 95% confidence interval.

RESULTS & DISCUSSION

Detection of off-flavor development

Figure 1 shows steadily increasing Pc values with storage time at these temperatures, indicating an increasing degree of off-flavor development in both air and N_2 packs. The curves

[1] Present address: Golden Grain Macaroni Co., 1111 139 Ave., San Leandro, CA 94578

for both packs are essentially parallel to each other for each of the temperatures, indicating that the panel was more or less equally sensitive to the increasing degree of off-flavor that

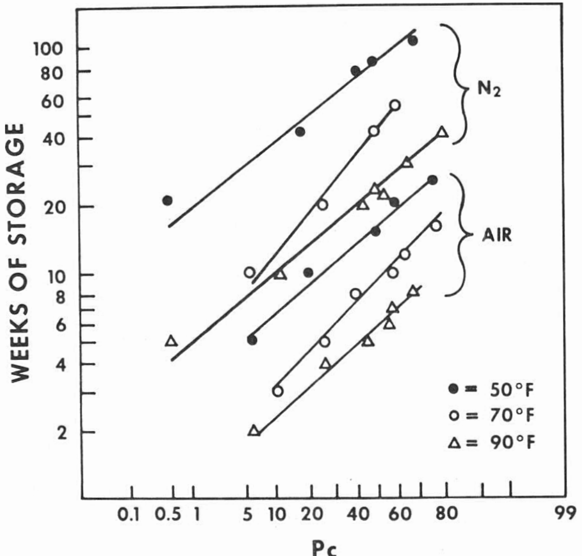

Fig. 1–Relation between off-flavor development as measured by Pc values, and storage time for air and N_2-packed samples of pinto bean powder. Off-flavor development is statistically significant when Pc = (Po – 50) X 2 = 50. Po = percentage correct responses in due-trio test.

Table 1–Direct comparisons between N_2 and air packs of pinto bean powder stored under the same time-temperature conditions

Temp (°F)	Time (wk)	Total judgments	Correct judgments	No. preferring N_2 pack
–20	0	32	15	17
–20	9	30	14	16
–20	18	26	15	13
–20	29	32	13	17
50	23	32	22*	27
70	18	28	23**	23
90	9	30	25**	26
90	29	30	18	20

* $P \leq 0.05$
** $P \leq 0.001$

Table 2–Effect of packaging atmosphere, temperature and flavoring on apparent shelf life of pinto bean powder

Temp (°F)	Packaging atmosphere	Shelf life in weeks as measured by Pc_{50} [a]	
		Plain soup	Flavored soup
50	air	15.6 (13.3–18.3)	56
50	N_2	83 (71–97)	>78
70	air	9.2 (7.6–11.1)	30
70	N_2	42 (34–53)	>57
90	air	5.8 (4.9–6.8)	30
90	N_2	23 (19.6–27)	57

[a] Pc_{50} = Pc = 50 when Po = 75%. Figures in parentheses represent 95% confidence intervals.

developed in these products. Thus, N_2 packing effectively delayed the onset of deteriorative autoxidative reactions or increased the induction period for such reactions, but once the reaction started, its progress, as measured by Pc values, appeared to be similar to that occurring in the air packs.

More direct evidence for the similarity of off-flavors developing in both air and N_2 packs is shown in Table 1. First, these data show that N_2 packing has no advantage at the control storage temperature of –20°F, since statistically significant differences were not observed between the two types of pack during 29 wk of storage at this temperature. Secondly, it is clear that N_2 and air samples stored at 50, 70 and 90°F for 23, 18 and 9 wk, respectively, were readily distinguishable from one another. The panel preferences in these comparisons were preponderantly in favor of the N_2-packed samples, indicating that off-flavors had developed in the air packs but not in the N_2 packs. This conclusively shows that N_2 packing effectively retarded off-flavor development at these temperatures. It should be noted, however, that the storage times were well beyond the point where the judges noticed differences between stored and control samples in the air packs, but the N_2 packs were still undistinguishable from their –20°F controls. Comparisons between N_2 and air packs held at the same temperature for times in excess of those required for off-flavor development in each type of pack (90°F for 29 wk) resulted in no significant flavor differences. This confirms the sensory data presented in Figure 1, which indicate that off-flavor development is similar in both types of pack, but is merely delayed by packing in an inert atmosphere.

Effect of added flavoring

The time required for 50% of the judgments to detect off-flavor (Pc_{50}), in both flavored and unflavored powder preparations held at 50, 70 and 90°F is shown in Table 2. The data for air packs indicate that stability of the powder is rather poor if unprotected from O_2. Off-flavors were significantly detectable after only 5.8, 9.2 and 15.6 wk at 90, 70 and 50°F, respectively. The value for 90°F agrees well with that reported by Boggs et al. (1964) for laboratory prepared pinto bean powder (6.1 wk). Nitrogen packing increased the time required to reach the same degree of off-flavor development approximately 4- to 5-fold. The 95% confidence intervals show a variability of ± 15–20% of the Pc_{50} values for both air and N_2 packs, indicating reasonably good reliability for the sensory data. The figures for the time required to cause significant differences in the flavored soups illustrate the large increases in apparent shelf life of the powder that could be obtained by merely masking the off-flavor with standard ingredients often added to soups of this type. Under these conditions even air packs stored at 90°F could be considered stable for 6 months or more, and those held at 50°F did not show significant changes from –20°F controls for more than a year. For the N_2 packs evaluated in the flavored formulation, apparent stability was in excess of a year at all temperatures. While evaluation of the powders as flavored soup indicates dramatic increases in apparent shelf life, it should be emphasized that these values could be significantly changed by variations in the flavoring formula. Since the added flavoring was introduced during preparation of the powder into soup, it obviously does not prevent off-flavor development, but clearly acts as a masking agent for the off-flavor which has developed during storage. Nevertheless, as a practical matter, it is clear that flavoring the powder with common standard commercial ingredients would maintain acceptability of the powders for reasonable storage periods at temperatures as high as 90°F.

Effect of temperature

Figure 2 shows the relation between temperature and stability as measured by the Pc_{50} value for unflavored soup. There appears to be a fairly good exponential relation between

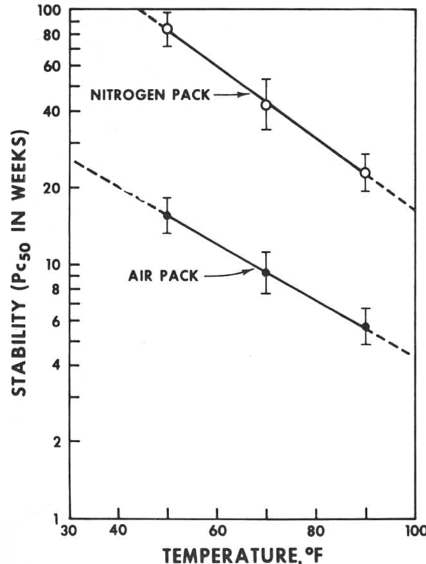

Fig. 2—Relation between storage temperature and stability of pinto bean powder as measured by Pc_{50} values. Pc_{50} = Pc when Po = 75% in the equation Pc = (po − 50) × 2. Brackets represent 95% confidence intervals for Pc_{50} values.

temperature and stability in the 50–90°F range. This is indicated by the essentially linear increase in stability with decreasing temperature when the data are plotted on semilogarithmic paper. As indicated by the 95% confidence intervals, the Pc_{50} values for each 10°F increment in temperature are statistically significant for both air and N_2 packs. The temperature coefficient for the off-flavor reaction may be expressed as:

$$q_{10°F} = \frac{Pc_{50} \text{ at } t°F}{Pc_{50} \text{ at } t + 10°F.}$$

The $q_{10°F}$ values for air and N_2 packs are approximately 1.3 and 1.4, respectively. These values indicate that the effect of temperature on off-flavor development was very similar in both types of pack, as indicated by the essentially parallel lines in Figure 2. Thus, the rate of off-flavor development is increased by 1.3–1.4 times for each temperature increase of 10°F. This is a relatively low temperature coefficient indicating that flavored soups made from the N_2 pack powders would probably be very acceptable, even after 6 months storage at 100°F.

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

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EFFECTS OF CUCUMBER SIZE AND BRINE COMPOSITION ON THE QUALITY OF DILL PICKLES

INTRODUCTION

MOST OF THE KNOWLEDGE about microbiological processes in cucumber curing has accumulated during studies of salt-stock fermentations. In our country, pickles resembling "dills," "Kosher-type dills" or "overnight dills" are greatly favored. Because most of the pickles sold are imported from the Soviet Union, and also Poland, these pickles have lately become known as "Russian pickles." Due to the unpredictability and perishability of low-salt pickles, few plants in Finland have ventured to start production of fermented dills. Correct recognition of the major factors influencing the course and outcome of microbiological processes in cucumber curing would aid in establishing better control over the processes, and this triggered our experiments. Review of the literature on salt-stock fermentations helped us limit the number of likely factors, but it was felt necessary to seek experimental verification because of the possible differences in both local conditions and in the high-salt and low-salt processes Due to the chosen design some hitherto untested combinations of factors came to be studied.

The course and outcome of spontaneous cucumber fermentations have been shown to depend on a number of factors, although not always in a predictable way. In this report the effects of several easily adjustable starting conditions on the taste, consistency, appearance and frequency of hollow cucumbers (bloaters) are evaluated. The factors studied were cucumber size, salt concentration, and addition of glucose, alum, and vinegar.

Early works on salt-stock and dill pickle fermentations abound with experiments on salinity effects. Within the range from 2 to almost 20% NaCl, it has been repeatedly observed that low salinity induces earlier acid production as well as higher final acidity (Fabian et al., 1932a, b; Jones et al., 1940; Pederson and Ward, 1949; Pederson and Albury, 1950; Jones and Harper, 1952; Etchells et al., 1961). However, the effect of salinity is not completely predictable, for variation in acid production has been observed even in parallel fermentations in identical conditions (Etchells et al., 1945; Pederson and Albury, 1956).

The studies on the influence of prior acidification of the brine seem to indicate that a slight amount of acetic or lactic acid might be beneficial through aiding establishing the proper fermentative flora at an early stage. High initial acidity is less advantageous. According to Jones et al. (1940) acidification of the brine at the start with 0.8% lactic acid virtually prevented subsequent spontaneous bacterial fermentation. Instead, it seemed to offer an opportunity for spoilage yeasts to multiply, and was therefore decidedly harmful. Etchells et al. (1966) found that slight acidification (0.01%) with lactic acid accelerated the onset of acid formation but had essentially no effect on the total amount of acid produced in pure culture fermentations of green olives. Fabian and Wickerham (1935) concluded that acidification with sufficient vinegar (0.054% acetic acid) to produce an initial pH of 4.6 was beneficial in dill pickle fermentations, although the conclusion seems rather arbitrary considering their experimental design. Etchells et al. (1964) found prior acidification with 0.24–0.38% lactic acid a good means of totally suppressing undesirable coliform and spore-bearing bacterial populations in brines with equalized salinity of about 5.5%.

The early investigators were unanimous in claiming that the addition of sugar to fermenting cucumbers is advantageous because it induces early acid production and high final acidity (Ref. Veldhuis et al., 1941a). According to a more recent view, based on convincing experimental evidence (Jones et al., 1940; Veldhuis et al., 1941a, b; Ragheb and Fabian, 1957), the addition of sugar does not seem to promote acid production even though it greatly increases the density of acid-producing bacteria. Undesirable effects–stimulation of yeast growth and increase in bloater frequency–are more likely results of sugar addition.

According to the available records, alum has been used only for firming and plumping cucumbers at later processing stages of pickle manufacture (Fabian et al., 1932a; Weiser, 1962) so that nothing is known about the effect of alum in fermentations.

Large and small cucumbers seem to react differently to the addition of salt. Fabian et al. (1932a) found that smaller cucumbers lost weight initially at a more rapid rate than large ones. Costilow and Fabian (1953a, b) were able to demonstrate that brines containing smaller cucumbers were richer in the essential nutrients for Lactobacilli than were the brines containing the larger sizes. This was partly due to more rapid diffusion of nutrients but also due to actually higher concentration of essential nutrients in the smaller cucumbers. For these reasons cucumber size might fundamentally influence the course of fermentations, and accordingly, the quality of the product. Another feature which seems rather directly influenced by size is the occurrence of bloaters (Jones et al., 1940; Pederson et al., 1964), although size seems by no means the only reason for bloating.

Bloaters are formed, according to Etchells et al. (1945, 1968), by the liberation and expansion of dissolved supersaturated gas in cucumber tissue. The gas itself may be produced by subsurface yeasts (Etchells et al., 1961), *Lactobacillus brevis* (Borg et al., 1955; Etchells et al., 1968), or *Aerobacter* (Etchells et al., 1945). The susceptibility of cucumbers to bloating seems to increase with size (Jones et al., 1940; Pederson et al., 1964) and to differ with the variety of cucumbers (Jones et al., 1954). Year-to-year variations in bloater frequency, reported by Pederson and Albury (1961), would thus seem almost inevitable due to chance variations in the spontaneous microbial flora as well as in the cucumber growth history. Etchells et al. (1945) observed considerable direct dependence of total gas evolution, and the structure of the gas-producing flora, on brine salinity which may in turn explain the increase in bloaters with salinity observed by Jones et al. (1940). It would thus seem that the percentage of bloated stock is among the least reliably predicted features in cucumber fermentations.

It is characteristic of most of the earlier studies that only one factor has been varied at a time, which makes it impossible

to study interactions between two or more factors. Considering the diverse and sometimes conflicting observations reviewed above, it seemed possible that interactions between factors might be found. To make such studies possible, we simultaneously varied five factors and based our experimental design on a completely balanced factorial arrangement of treatments to be analyzed by the analysis of variance.

EXPERIMENTAL

Packing

48 plastic containers of 4.5 liter capacity were packed with alternate layers of black currant leaves and freshly picked faultless cucumbers, with a layer of leaves uppermost. Dill weed and mustard seed were added. 24 of the containers were packed with mechanically sorted Size I (less than 4.5 cm diameter) and the other half with Size II (4.5–5.3 cm diameter) green cucumbers of pickling variety "Superb OE."

Preparation of brines

12 lots of brine with different chemical composition were prepared, starting from solutions of 6, 9, and 15% (w/v) sodium chloride. The brine lot of each different salinity was divided into 4 equal parts which received sufficient amounts of concentrated glucose and alum solutions to give the following combinations:

A = neither alum nor glucose added
B = 0.2% (w/v) alum, no glucose
C = no alum, 0.5% (w/v) glucose
D = 0.2% alum, 0.5% glucose

Each of the 12 different solutions was poured into 4 containers; two containing small and two large cucumbers. Before pouring in the brine, one container of each pair received 10 ml of 10% acetic acid, giving an approximate concentration of 0.05% acetic acid in the brine (0.02% per total volume).

The experimental design thus contained all the $3 \cdot 2^4 = 48$ combinations of:

Cucumber size, two levels (sizes I and II)
salinity, three levels (6, 9, and 15%)
glucose, two levels (0.0 and 0.5%)
alum, two levels (0.0 and 0.2%)
acetic acid, two levels (0.0 and 0.05%)

Fermentation

After filling, the containers were closed with tightly fitting lids, trying to avoid trapping air inside. Lacking experience with fermentations at outdoor temperatures in Finland, the fermentation was begun indoors at 22–24°C to ensure a good start. After 2 days the gas trapped between the cucumbers and the leaves was liberated. At this stage the containers were transferred to a temperature between 13–15°C and kept closed until about 1 month later. (This temperature is closer to the range expected normally during the harvest period.) Since the cucumbers after 1 month's fermentation appeared sufficiently cured, and as peroxidase activity (determined according to Jacobs, 1959) had sufficiently subsided, the fermentations were considered completed. Chemical and sensory analyses were made at this stage and the containers were subsequently stored at 0–4°C until determination of bloated frequency 12 wk from packing.

Chemical and sensory analyses

Brine acidity expressed as lactic acid, was determined by titrating with 0.1 N NaOH (AOAC, 1970). Brine pH value was measured potentiometrically.

A sample of 6–7 cucumbers was removed from each container and 3–4 were homogenized. Salinity of the homogenate was determined by titrating with $AgNO_3$ according to AOAC (1970). The dry matter was determined by weighing after drying a weighed sample overnight at 105°C.

The remaining three cucumbers were used for organoleptic evaluation by a panel of six experienced tasters. The evaluation panel noted the faults verbally. No scores were given. Appearance, structure, color, odor, and flavor were described without restrictions to the choice of words and a summary of evaluations was written down.

Bloaters

12 wk from packing all cucumbers were cut in two for determination of the degree of bloating. Three classes were recognized: "hollow" (includes the classes "balloon" and "lense" of Etchells et al., 1968), "small holes" ("honeycomb"), and "solid."

Analysis of data

Numerical results of chemical analyses were used as such, or after linear transformation, for the analysis of variance using conventional techniques appropriate for the fixed effects completely balanced $3 \cdot 2^4$ factorial design without replications.

The percentages of hollow stock were subjected to analysis of variance after angular transformation. χ^2 tests of bloater frequencies were performed with untransformed data.

The written descriptions of cucumber quality were interpreted as either positive or negative with regard to consumer acceptability. Four quality criteria were assessed separately: appearance, consistency, aroma and saltiness. The frequencies of positive and negative scores were used for comparisons of treatment effects by the χ^2 test.

Whenever a significant effect or a significant difference is claimed in the following, it is based on a statistical test and a minimum of 95% probability. Whenever significant interactions were found in the analysis of variance, the corresponding main effects were not tested against the residual variance but against the significant interaction.

RESULTS & DISCUSSION

A SUMMARY of all the statistically significant effects detected is presented in Table 1. In accordance with the earlier work on cucumber fermentation, the brine salt concentration came out as the most influential single factor in the experiment. Many of the simple effects observed agree with the present knowledge on cucumber fermentations.

A summary of average salinity effects (including the statistically nonsignificant) is presented in Table 2.

The inverse dependence of acidity on salinity is in agreement with earlier work reviewed in the introduction. The pH values instead did not vary according to the titratable acidity, which is also a previously known situation (Fabian et al., 1932a; Pederson and Albury, 1950, 1961). Pederson and Albury (1961) have shown that heterofermentation in general yields more titratable acid for a given pH, so the observed discrepancy may indicate a more heterofermentative type of activity in the 6% brine compared to the 9% brine.

The rising content of dry matter with increasing salinity is due to salt itself, as indicated by an almost equal rise in NaCl content of the cucumbers.

As a whole, large cucumbers received fewer negative comments regarding their appearance than the small ones. Out of 24 containers of each size class, the frequency of negative comments was 15 for the small cucumbers but only 8 for the large ones. This degree of asymmetry is statistically significant at the 95% level.

Table 1—List of statistically significant (95% level or higher) effects of starting conditions on cucumber quality and brine acidity as revealed by analysis of variance and χ^2 tests.

Quality parameter	Influenced significantly by:[a]
Brine total acid	Salinity (−), S X A interaction
Brine final pH	Salinity (+)
Cucumber dry matter content	Salinity (+), G X A and K X S interaction
Cucumber salt content	Salinity (−), Size (−), G X H X A interaction
Relative bloater frequency	Size (+), G X S interaction
Cucumber appearance	Size (+)
Cucumber consistency	Prior acidification (+)
Cucumber aroma	Salinity (−), Size (+)
Cucumber saltiness	Salinity (+)

[a] Signs (+) and (−) indicate the direction of correlations. The capital letters stand for the following main effects: S = brine salt concentration at start; A = alum addition; G = glucose addition; K = cucumber size; H = prior acidification.

Table 2–Summary of the effects of salinity on fermented dill pickle quality.

Brine salinity[b] (%)	Objective quality attributes[a]					Consumer acceptability (Positive scores out of 16)			
	Acid[c] (%)	pH	Cucumber NaCl (%)	Cucumber dry matter (%)	Bloaters[d] (%)	Appearance[e]	Consistency[e]	Aroma	Saltiness
6	0.81	3.70	5.0	2.6	11	10	10	13	16
9	0.75	3.60	7.2	4.5	11	9	7	6	2
15	0.33	4.10	9.4	6.7	26	6	6	4	0

[a] The "objective quality" figures are arithmetic mean values of 16 determinations from batches of equa salinity, although differing in other respects.
[b] Brine salinity at the start of the experiment before equalization
[c] Titratable brine acidity, calculated as lactic
[d] Figures in the column do not differ significantly due to strong interaction with glucose (See: "Effects on relative bloater frequency")
[e] Difference between column figures statistically nonsignificant

Cucumber aroma was even more significantly affected by cucumber size, as negative evaluation was given in 17 cases out of 24 for the small cucumbers but only 8 times for the larger cucumbers.

Interaction effects 1. Total acidity

Interpretation of the statistically significant (salinity) X (alum) interaction (Fig. 1) seems simple. The general rule of inverse relation between salinity and final acidity did not hold unless alum was also added to the brine. In other words, alum has helped stabilize acid production to the expected level; without alum acid production has failed at the lowest salinity. To continue the speculation, one can be fairly certain that *Lactobacillus plantarum* has had a dominant role in the fermentations in 6% brine with alum, because 0.96% average acidity was achieved (Pederson and Albury, 1956, 1961; Etchells et al., 1964, 1966). In 6% brines without alum less acid (0.67%) was produced, and the fermentations may thus have been of different nature. High-acid fermentations are less likely to evolve great quantities of gas because of the homofermentation; in the medium-acid fermentations gas-formers have a much higher probability to appear. In this case it would be well in line—according to the general hypothesis of Etchells et al. (1968)—to find more bloaters in the 6% brine without alum than with alum. This was actually found to be the case. Without alum there were 33 bloaters out of 231 cucumbers examined (14%), whereas with alum only 15 bloaters out of 215 cucumbers (7%) were found. The difference is statistically significant (χ^2 = 6.18, 1 d.f.). The mechanism by which alum could help stabilize acid production in low-salt brines must for the time being be left unexplained. Lacking direct microbiological observations it is not possible to decide whether alum has prevented acid-consuming yeasts from multiplying or whether the fermentation without alum failed to produce more acid in the first place.

Interaction effects 2. Cucumber dry matter

The (size) X (salinity) interaction is illustrated in Figure 2. The result means that large cucumbers were, on the average, significantly "wetter" than the small ones, and relatively more so at the highest salinity tested. This probably agrees with everyday experience.

The (alum) X (glucose) interaction on cucumber dry matter, illustrated in Figure 3, seems to indicate that these two

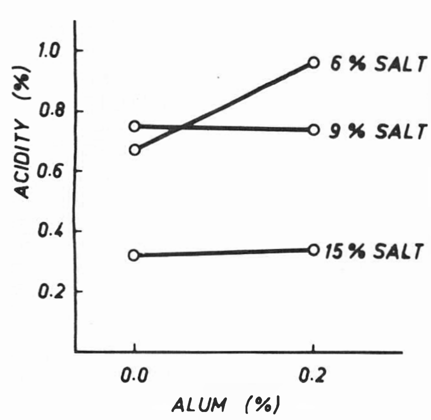

Fig. 1–The effect of alum addition on final brine acidity, expressed as lactic acid, at different starting concentrations of salt in the brine. Each point represents the mean of eight determinations with the same salt and alum treatment.

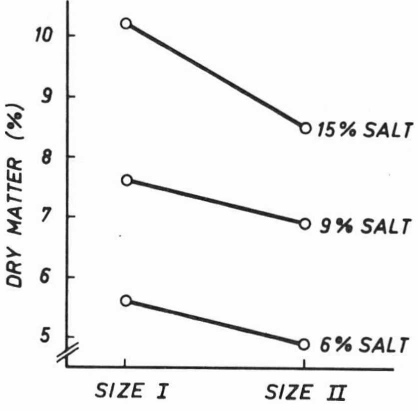

Fig. 2–Dependence of cucumber dry matter content on cucumber size and brine initial salinity. Each point represents the mean of eight determinations with the same salt content and cucumber size. Size I = less than 4.5 cm diameter; size II = 4.5–5.3 cm diameter.

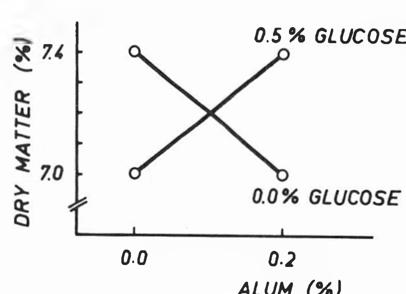

Fig. 3–Dependence of cucumber dry matter content on additions of alum and glucose. Each point represents the mean of 12 determinations with identical alum and glucose treatment.

additives have effects on dry matter content which oppose each other. It is not possible to determine whether alum compensates the effect of glucose or vice versa, but at any rate there is a significant competitive relation. Glucose addition alone tends to increase the wetness of cucumbers, but this tendency is cancelled by simultaneous addition of alum. The mechanism might be purely physical but it is equally possible that the finding reflects a complex effect of alum and glucose on the microbiological processes.

Interaction effects 3. Cucumber equilibrium salt content

The most complicated statistically significant relationship revealed by the analysis of variance was the second-order interaction of (glucose) X (alum) X (acidification) on the cucumber salt content. Due to difficulties of graphic presentation, this is shown in tabular form (Table 3). The cucumber salt content seems generally to increase with glucose and acetic acid additions so that the salt concentration remains the lowest if neither glucose nor acid is added and becomes the highest if both ingredients are added. This tendency is, however, further modified by alum in such a manner that if neither glucose nor acid, or both, are added alum seems to increase the salt content. If only one additive, glucose or acid, is used then alum decreases the salt content.

With the present knowledge of cucumber fermentations it is impossible to judge whether this finding has a rational interpretation. It is possible that this complicated relation is a mere random occurrence, although it is interesting to note that the factors concerned are partly the same which were above found to affect acid production and cucumber dry matter content.

Interaction effects 4. Relative bloater frequency

The known tendency of larger cucumbers to produce more bloaters (Jones et al., 1940; Pederson et al., 1964) was distinguishable in the form of a highly significant main effect of cucumber size on relative bloater frequency (Table 1). Out of 868 small cucumbers examined, 84 (9.7%) were found hollow, whereas 128 (25.6%) out of 483 large cucumbers were bloated.

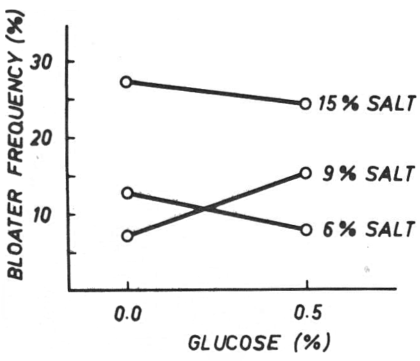

Fig. 4—The effects of glucose and salt on bloater frequency. Each point represents the mean of eight determinations with the same glucose and salt concentration.

Table 3—The interaction of glucose X alum X acidification. The figures represent the mean NaCl concentration (in per cent) of samples of cucumbers from six experimental fermentations with the same glucose-alum-acid treatment.

		No acid	Acid added
No Glucose	No alum	4.08	4.92
	Alum added	4.60	4.54
Glucose added	No alum	4.59	4.60
	Alum added	4.36	5.17

A more subtle relationship was also detected. Bloatiness has been attached to brine strength (Jones et al., 1940) but in our experiment the straightforward increase of bloater frequency by salt was so much modified by the presence or absence of glucose that the main effect did not even reach statistical significance. The analysis of variance was performed with transformed percentages (angular transformation) but the significant finding is here illustrated without transformation (Fig. 4).

CONCLUSIONS

THE AIM OF the experiment was to find the factors which most markedly influence dill pickle quality in general, and more specifically to find, if possible, the best choice of brine composition considering the overall consumer acceptability of the resulting fermented dill pickles. For practical reasons only a limited number of factors, selected mainly on the basis of salt-stock literature, could be tested. The principal conclusions were the following:

There is only one possible choice of brine salinity, close to 6%, otherwise the pickles will be judged too salty. This forced selection is both fortunate and unfortunate. Many characteristics (aroma, acidity, and possibly even appearance and consistency) are likely to be at their best in the same salinity range. Furthermore, bloater probability is low. However, low-salt fermentations tend to be precarious. Small additions of acid, glucose, or alum may influence the outcome in an unpredictable way.

In the light of the present experiment, alum may be recommended as a stabilizing agent in low-salt fermentations but the value of added sugar remains doubtful or at best unsettled. The decision whether to add glucose or not may never be a simple matter, for it no doubt depends among other things on the sugar content and skin texture of the cucumbers, which in turn may depend on a multitude of factors during the growth season. The knowledge about correlations between cucumber growth history and the course and outcome of fermentations is at present insufficient.

With cucumber size a compromise must be reached. Large cucumbers received more favorable comments on aroma and appearance but tended to produce more bloaters.

This experiment shed little light on the somewhat controversial matter of prior acidification of the brine, but on account of the positive effect on cucumber consistency acidification can be recommended.

With the experience gained from the analysis of the results, generally the most satisfactory dill pickles might be expected by brining largish cucumbers in slightly acidified 6–7% brine which additionally contains about 0.2% alum. However, with such a choice of cucumber size, aroma and appearance are given precedence at the risk of greater bloater frequency.

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SALT FREE ACIDULANT STORAGE OF PICKLING CUCUMBERS

INTRODUCTION

BLOATER DAMAGED PICKLES have caused serious economic losses to the pickle industry. The primary cause for this type of spoilage has been shown to be due to the production of gases, primarily CO_2, in the fermentation brines (Etchells et al., 1968). The microorganisms responsible for these gaseous fermentations have been isolated from numerous genera of yeasts (Etchells and Bell, 1950), *Aerobacter* (Etchells et al., 1945), and heterofermentative lactic acid bacteria (Etchells et al., 1968).

More recently, it has been demonstrated that the cucumber tissue itself and homofermentative lactic acid bacteria can be sources of bloater damage (Fleming et al., 1973a). Further dissolved CO_2 in the brines has been found to contribute to bloater development (Fleming et al., 1973b).

Texture is an important quality factor determining the consumer acceptability of brined cucumber pickles. It has been reported that the softening of cucumbers brined under commercial conditions is primarily the result of the hydrolytic action by a system similar in action to polygalacturonase and another system pectinesterase (Bell et al., 1950). Also it has been reported that cellulolytic enzyme systems may contribute to total softening (Etchells et al., 1955). Studies have shown that as purified pectinolytic enzymes were incorporated into cucumber brines of increasing salt strength, firmness increased as brine strength increased (Bell and Etchells, 1961). The influence of various organic acids such as acetic, lactic, citric, malic and oxalic on cucumber firmness has also been demonstrated (Bell et al., 1972).

Recently the concern for the environment has stimulated researchers to look for alternative methods for the disposal of spent brines without causing water pollution. A submerged combustion crystallizer has been demonstrated for separating salt from spent brines (Durkee and Lowe, 1973). An efficient method for recovering salt from the spent brine could be achieved by adjustment of the brine pH and filtration (Geisman and Henne, 1973). A procedure for salt-free storage of olives and other produce including pickling cucumbers which would not only eliminate some of the problems of brine disposal but also improve texture by combining acidity, food preservatives, and anaerobiosis to retard microbial spoilage has also been investigated (Vaughn et al., 1969).

The present work was undertaken to investigate the feasibility of storing cucumbers in salt-free acidulant solutions in an attempt to alleviate some of the problems normally encountered in the salt brining process.

MATERIALS & METHODS

Preparation of acidulant storage containers

Large size cucumbers (1-3/4–2-1/8 in. in diam) of mixed cultivars were washed and placed in 32 gal fermentation containers. Three acidulant solutions were prepared as follows: (1) acetic acid 4.4% by titration; (2) a 2:1 mixture by weight of acetic acid 4.4% and lactic acid 0.53%; (3) a 2:1 mixture by weight of acetic acid 4.4% and citric acid 0.52% as shown in Table 1. To each acidulant treatment 0.1% by weight potassium sorbate was added. The covering solutions were added to give a 60:40 ratio by weight of cucumbers to solution. The volumes for the cucumbers and acids were calculated to leave an 8-in headspace in the container. The cucumbers were weighted down with a 6 mil polyethylene plastic sheet fitted over the acidulant surface with the outer rim of the sheet folded up so as to hold water. Distilled water was then added to the top of the sheet to submerge the cucumbers. After 72 hr equilibration, the containers were sealed with a 1/8 in. plexiglass sheet cut to fit the top of the container. The seal was completed with high vacuum desiccator grease. The plexiglass tops were drilled and fitted with rigid plastic pieces and tygon tubing. The container construction allowed flushing with compressed gas and withdrawal of acidulant solution samples by siphoning. After sealing, the headspaces were flushed with nitrogen from a compressed gas cylinder for 5 min. Flushing was accomplished at frequent intervals throughout the experimental period.

A salt brining control treatment was prepared as described earlier. The cucumbers were covered with a 40° salometer brine. The equilibrated brine strength was maintained until a 0.6% titratable acidity (calculated as lactic) was attained at which time the brine strength was increased 2° salometer weekly to a holding strength of 50° salometer. All treatments were duplicated and held at 22°C.

Chemical analysis

The pH and percent titratable acidity (TA%) were measured with a Beckman pH meter (glass electrode) and by titrating with 0.1N NaOH to a pH of 8.1. The ml NaOH required was converted by calculation to percent acid expressed as acetic. The sodium chloride content was determined according to AOAC method 32.018.

Bloater damage evaluation

Cucumbers were cut longitudinally and examined for balloon, honeycomb, and lens-type bloaters (Etchells, 1973).

Evaluation of firmness

The pounds resistance to center puncture was measured with the USDA fruit pressure tester with a 5/16 in. tip (Etchells, 1973).

Preparation for flavor evaluation

At the 8-month analysis period, 8-lb samples of the acidulant and salt stock were removed (4 lb from each of the duplicate treatments). The salt stock was freshened whole at 22°C by soaking in an equal weight of tap water changing the water every 8 hr for a 24-hr period. The acidulant stock was sliced into 1/8 in. slices with a vegetable slicer and freshened in a similar manner as the salt stock.

After freshening, the whole cucumbers were sliced as previously described and all treatments were rinsed with tap water. The cucumber slices from all treatments were prepared as processed dills by filling 270g of sliced material into 16 oz jars. The salt brine control pickles were covered with a standard process dill brine containing vinegar (5% acid distilled), tap water, table salt, sugar, and dill emulsion (Seasoning Mills No. 20471). The acidulant stored cucumber pickles were packed

Table 1—Average pH and titratable acidity of treatment solutions[a]

Treatment	Initial		Equilibrated[b]		8 Months	
	pH	TA[c]	pH	TA[c]	pH	TA[c]
Salt control	–	–	–	–	3.3	0.48[d]
Acetic and lactic	2.7	3.1	3.3	2.0	3.4	1.9
Acetic and citric	2.7	3.1	3.3	2.2	3.4	2.0
Acetic	2.7	4.4	3.3	2.8	3.4	2.4

[a] Average of duplicate treatments
[b] After 72 hr
[c] Titratable acidity expressed as percent acetic acid
[d] Titratable acidity expressed as percent lactic acid

in the salt brine mentioned above, and in a no salt brine which contained the same ingredients except no salt was added. The final two cover media were prepared from the acetic acid tank storage solutions rather than fresh vinegar. The salt and no salt cover media contained the same ingredients as mentioned previously. Immediately after filling, all jars were capped with "Twist" off caps and pasteurized in a hot water bath to 74°C internal temperature for 15 min (Etchells and Jones, 1944). After a 2-wk equilibration period, the processed cucumber slices were presented to a taste panel consisting of 10 judges. The judges were instructed to rate the color, texture and flavor of the samples on a rating scale of 1 to 10, 1 being off, and 10 being perfect.

RESULTS & DISCUSSION

THE SALT BRINING PROCESS as used commercially allows cucumbers not only to be cured but also to be stored for significant time periods after the holding brine strength is achieved. The function of the salt in the process is to enable the desirable salt tolerant lactic acid microorganisms to ferment and produce the acid necessary for curing the cucumbers while inhibiting the growth of undesirable spoilage microorganisms. Nevertheless, certain microorganisms can withstand the brine process resulting in bloater damage and softening of the cucumbers.

Further, it has been shown (Vaughn et al., 1969) that olives and other produce including cucumbers could be stored in salt-free acidulant solutions with a chemical preservative under anaerobic conditions thus eliminating the need for salt and the subsequent disposal of the salt in the spent brine. It seemed reasonable to the present investigators that under conditions similar to those described earlier (Vaughn et al., 1969), bloater damage and cucumber softening could be reduced by inhibiting the primary microbial sources of this spoilage. Further, under these conditions, the advantages of the salt brine process allowing cucumbers to be held for significant periods of time may still be retained.

The initial, equilibrated, and 8-month storage pHs and percent titratable acidities (average of duplicate treatments) are presented in Table 1. It can be seen that the initial pH increased from 2.7 to 3.3 and in general the titratable acidity decreased by slightly over 1% after equilibration for all treatments. After 8 months storage the pH increased slightly and the titratable acidity decreased slightly. In the case of the salt brine control, the cucumbers were initially covered with a 40° salometer brine which after addition of adjustment salt equilibrated to 24° salometer. A 0.6% lactic acid was attained in approximately 3 wk and the 50° salometer strength after 17 wk.

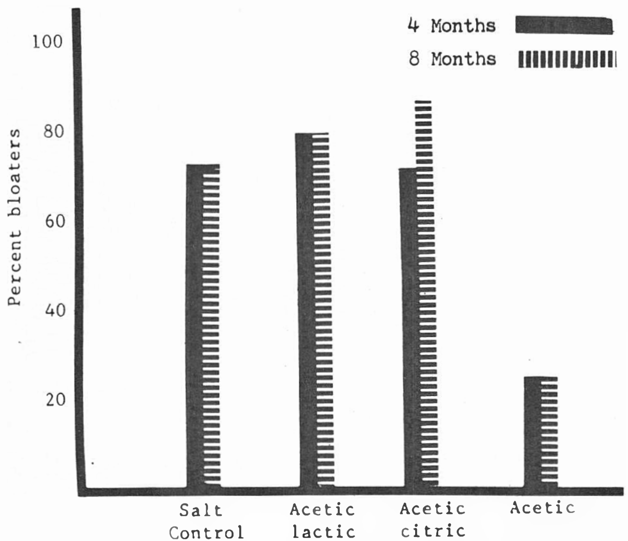

Fig. 1–Average total percent bloaters including balloons, lens, and honeycomb for duplicate treatments

Table 2–Average pressure test scores of salt and acidulant stock cucumber pickles[a]

Treatment	4 months[b]	8 months[b]
Salt control	14.3	14.7
Acetic and lactic	14.0	15.6
Acetic and citric	14.5	15.9
Acetic	15.7	17.7

[a] Pressure test scores in pounds measured with the USDA fruit pressure tester with a 5/16 in. tip (10 cucumbers from each duplicate treatment)

[b] Rating scale 20 lb = very firm; 16–19 lb = firm; 11–15 lb = inferior; 5–10 lb = soft; 4 lb and less = mushy. (Scale recommended for size 3–see text)

Bloater evaluation

The data indicating the total percent bloaters including balloon, honeycomb, and lens types for the 4- and 8-month analysis periods are presented in Figure 1 (average of duplicate treatments). The salt brine control treatments yielded values of 75% during both analysis periods. The combination treatments of acetic with lactic and citric acids resulted in 80 and 85% bloater damage respectively at the 4-month period and 80 and 75% respectively at the end of 8 months. The acetic acid treatment yielded 27.5% bloater damage during both analysis periods. The differences between the combination acidulant treatments and the salt control do not appear to be significant; however, with the acetic acid treatment there was almost a 50% reduction in total bloating during both periods relative to the salt control.

When analyzing the data presented in Figure 1, it should be taken into consideration that the data indicate total bloater damage from all three types regardless of severity. The data for the salt control do not mean 75% severe balloon bloaters and in general the bloater damage was moderate in all cases. As was indicated earlier, an attempt was made to reduce the primary microbial sources of CO_2 and thus bloating by storing the cucumbers in relatively high acid solutions with potassium sorbate under nitrogen in a sealed container. Nevertheless, a significant amount of bloater damage still occurred with the combination acidulant mixtures and to a lesser extent with the acetic acid treatment. It has been shown that the respiratory or fermentative activity of the cucumber tissue can be a source of CO_2 in pure culture fermentations with homofermentative lactic acid bacteria (Fleming et al., 1973a). Further, it was stated (Fleming et al., 1973a) that the degree of respiratory or fermentative activity probably varies with the physiological state of the fruit, storage conditions prior to brining, and the inhibitory effect of salt and acid diffusing into the tissue during brining. In this experiment, the highest acidulant concentration, acetic alone, may have been more effective in decreasing cellular respiration and therefore bloater damage than the acidulant combinations.

Firmness evaluation

The results for the pressure test scores for the salt and acidulant stock cucumber pickles at the 4- and 8-month storage periods are presented in Table 2 (average of duplicate treatments). As can be seen from the data, the acetic acid storage solution resulted in the only cucumber pickles in the firm category with a 17.7 lb average, 3 lb higher than the salt control after 8 months storage. The results reveal 1–2 lb increase in pressure test scores between the 4- and 8-month analysis periods for the acidulant storage treatments. The reason for this apparent increase is not known; however, the differences do not appear to be significant.

As indicated earlier, previous research has shown that cucumber softening can be a problem in low salt brines (Bell and Etchells, 1961). In this study no apparent cellulolytic enzyme softening was observed after 8 months. Also the results seem to indicate that acetic acid alone is superior to the combination acid treatments for firmness. This observation is consistent with previous studies showing that acetic acid is the best acidulant precluding the use of lactic, citric, malic, or oxalic in the manufacture of fresh pack pickles (Bell et al., 1972).

Flavor evaluation

Data from the flavor panel evaluation and chemical analysis of the salt and acidulant stock prepared as process dills are presented in Tables 3 and 4. The results show that for the salt-free acidulant storage treatments receiving a cover medium containing sodium chloride (salt), the average numerical rating for flavor ranged from 5.7 in the case of the acetic and citric combination to 7.8 for the acetic and lactic combination compared to 7.3 for the salt brining control. The panel's remarks indicated that the salt control and the acetic and lactic combination samples were good while the acetic and citric combination and acetic treatment samples were fair. Where no sodium chloride (salt) was used in the preparation of the cover medium, the flavor scores were reduced to the poor and fair categories for the acidulant storage samples with the judges indicating that the samples were flat, bland and poor. These data are consistent with earlier observations that when fresh pack dill pickle products were packed with low amounts of sodium chloride and increasing amounts of potassium chloride the flavor scores were poor and unacceptable (Bell et al., 1972).

Since the acidulant storage solutions were free from active fermentation, the acidulant solutions were clear relative to the salt fermentation brines. Therefore, it was decided to prepare a cover medium utilizing the acetic acid storage solution rather than fresh acetic acid (vinegar). The data in Table 3 indicate that the average numerical rating from the judges for flavor with the storage acid solution was 7.3. This was equivalent to the salt brine control. This implies that utilization of the tank solution may be an alternative to disposal.

With the obvious advantages of reducing bloater damage and disposing of lower salt brines, results of this study show that further research with acidulant storage may be justifiable. Further research should address potential problems with storage under these conditions. Microbiological data should be collected especially since, without an active fermentation, one would expect a significant amount of reducing sugar to remain in the storage solution. The curing rate is slower and the cured color is lighter. The processed slice color is lighter than the processed salt stock, the latter is more like fresh pack slices. Even though results of this study showed no softening upon storage, the potential for cellulolytic enzyme activity still remains. A possible answer to this problem could be to blanch the fruit prior to tanking.

Table 3—Evaluation of salt and acidulant stock prepared as process dill slices

Treatment	Equilibrated brine % NaCl[a]	Avg numerical rating[b] Color	Texture	Flavor	Panel remarks
Sodium chloride[c]					
Salt control	1.44	6.7	7.2	7.3	good
Acetic and lactic	0.63	7.2	5.9	7.8	good
Acetic and citric	0.65	5.9	5.3	5.7	fair
Acetic	0.64	6.3	5.7	6.6	fair
Acetic[d]	0.81	7.6	6.9	7.3	good
No sodium chloride[c]					
Acetic and lactic	0.0	5.7	5.7	3.8	flat, poor
Acetic and citric	0.0	6.8	6.9	4.4	bland
Acetic	0.0	6.5	6.9	4.6	bland
Acetic[d]	0.0	7.5	6.4	4.2	bland

[a] Calculated by AOAC (1970) method—see text
[b] Based on panel of 10 judges with a scale: 10 = perfect; 7, 8 and 9 = good; 4, 5 and 6 = fair; 2 and 3 = poor; and 1 = off
[c] Refers to packing cover mediums—see text
[d] Refers to acetic acid storage solution—see text

Table 4—Chemical analysis of the equilibrated brines for cucumber pickles prepared as dill slices

Treatment	pH	TA%[a]
Sodium chloride[b]		
Salt control	3.4	0.77
Acetic and lactic	3.4	0.82
Acetic and citric	3.3	0.87
Acetic	3.4	0.80
Acetic[c]	3.3	0.78
No sodium chloride[b]		
Acetic and lactic	3.3	0.80
Acetic and citric	3.3	0.83
Acetic	3.4	0.80
Acetic[c]	3.3	0.82

[a] Refers to percent titratable acidity expressed as acetic
[b] See footnote previous table
[c] See footnote previous table

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EFFECT OF ADDED ALUM ON THE QUALITY OF BRINED ROYAL ANN CHERRIES

INTRODUCTION

ROYAL ANN CHERRIES are harvested and placed in SO_2-Ca brine for a period of 6 wk to 2 yr to decolorize anthocyanin pigments (Jurd, 1964; Timberlake and Bridle, 1967), firm and preserve the fruit (Van Buren, 1965; Van Buren et al., 1967; Yang et al., 1966; Watters et al., 1963; Beavers et al., 1971; Payne et al., 1969) until they can be processed into maraschino or glace cherries.

During the brining process the cherries lose weight due to equalization with the brine. This loss, known as "brine shrink," is governed by the soluble solids of the fresh fruit, the ratio of brine to fruit, brine composition, among other factors, and is reported to range from 6–12% (Yang, 1968; Beavers et al., 1971). After the cherries are cured in the brine, they are machine pitted preparatory to further manufacture. The pitting loss (weight of pit and adhering tissue) may also vary from 6–12% depending upon fruit maturity, size and texture of the fruit as noted by Yang, 1968 and Beavers et al., 1971.

Added Ca^{++}, under conditions of controlled pH, will combine with the pectin and form insoluble Ca pectinate which firms the fruit (Beavers et al., 1971; Doesburg, 1965). Historically, alum $[AlK(SO_4)_2]$ has been occasionally added to the calcium bisulfite brine to increase fruit firmness (Cruess, 1958; Blumenthal, 1935). In an investigation of the effect of added hardening agents upon the firmness of brined cherries (Beavers et al., 1971) it was observed that there was an increase in yield (as exemplified by drained weight) at all levels of maturity with additions of alum to the fresh brine. This paper presents further information concerning the effect of added alum on the major quality factors of brined cherries.

MATERIALS & METHODS

IMMATURE, mature and overmature sweet cherries (*Prunus avium* L. cv. Royal Ann) were obtained through the courtesy of a commercial cherry briner. They were classified according to color: immature fruit was straw-yellow in color with only an occasional pink blush; mature fruit was predominately pink in color with a small amount of yellow showing on approximately 50% of the fruit; and overmature fruit was red in color throughout. The fruit was brined in the early, middle and late portions of the cherry harvesting season. 250 lb of cherries of each maturity level were brined in polyethylene coated 55-gal steel drums to which 240 lb of brine had been previously added. The base-brine (control) was 1.4% gaseous SO_2 in water (w/w) which was then partially neutralized with $Ca(OH)_2$ to pH 2.9. Experimental brines were made by addition of 0.5, 1.0, 2.0 and 3.0% $AlK(SO_4)_2$ (w/w) to portions of the control brine. There was no change in pH with additions of alum to the initial brine. Three replicate barrels of each of the control and the alum brines were packed on the three processing dates. The fruit was stored for 5 months and then evaluated for percent brine shrink (+ or –), pitting loss, total loss, pits, unpitted fruit, torn pitter holes in Oregon grade No. 3 (Anon., 1954), soft fruit, solution pockets, texture (expressed in gram compression force) and brine pH. The percent brine shrink, pitting loss, total loss and pits were calculated on the basis of the ingoing fresh fruit weight.

The brine shrink was determined by draining the cherries 5 minutes and calculating the weight difference between the ingoing and recoverable fruit weights (Anon., 1954). Following draining, the cherries were returned to the brine. Subsequently, a 15-lb subsample of the brined cherries of each lot was segregated. 300 additional cherries of each sample were visually examined under transmitted light for presence of solution pockets (Cain and Smith, 1968; Cameron and Westwood, 1968; Beavers et al., 1971) with the aid of a Nichols illuminator. Cherries with pockets less than 1/4 in. were not counted. Forty cherries were then measured for texture using the methods outlined by Beavers et al., 1970. The 15-lb sample of cherries was pitted on a Dunkley model SP pitter fitted with 26 mm cups. The pits were recovered and weighed. The pitted fruit was caught in a 1/8 in. mesh stainless steel basket (18 in. diameter × 10 in. high), drained 5 min and weighed. The pitting loss was computed from the latter weight and included losses due to pits, adhering tissue and liquid loss. The total loss was the additive loss due to brine shrink and pitting loss. The recovered pits were weighed in order to determine the percent pits. Cherries which were unpitted were segregated and weighed to determine percent unpitted fruit. The pitted fruit was then returned to the brine and subsequently graded by professional plant grading personnel for soft fruit and Oregon brine grade No. 3 torn pitter holes. The pH was determined on the equalized brine.

The data were analyzed by analysis of variance and regression lines drawn where appropriate.

RESULTS & DISCUSSION

Percent pits

The average percent pits in the immature, mature and overmature cherries were 8.5, 8.1 and 6.6%, respectively. The difference in percent pits between maturities is a reflection of the increase in the ratio of tissue/pit with maturity. Irrespective of maturity level progressive additions of alum resulted in an increase in percent pits. The average percent pits over all maturities was 7.4% in the control brine and 8.0% in that brine to which an additional 3% alum was added. The coefficient of determination, R^2, was 0.038 indicating that additions of alum resulted in no significant change in the percent pits. It was noticed during the pitting operations that there were very tightly adhering pieces of tissue attached to the pits in the samples with higher alum concentrations. The "stick-tight" tissue would account for the increase in pit weight.

Pitting loss

The percent pitting loss is a reflection of those losses incurred due to the pit, adhering flesh and liquid. The remainder is the total recoverable and usable tissue. The percent pitting loss in immature cherries slightly decreased with additional levels of alum to the control brine (12.0 vs 11.7%) while an increase was observed with mature (10.9 vs 12.3%) and overmature (9.9 vs 11.2%) cherries.

No significant interactions were found between maturity and alum for the percent brine shrink, total loss, and the equilibrated pH. Regression equations and the coefficient of determination R^2 were calculated for these factors.

Brine shrink

Brine shrink was significantly affected by maturity levels and very significantly decreased by the addition of alum to the

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control brine. In the control brine the percent brine shrink was −10.8, −8.1 and −13.3 for the immature, mature and overmature cherries, respectively. The additions of alum to the control brine resulted in a progressive and highly significant decrease in brine shrink. The regression line equation, and coefficient of determination as an average of the maturity levels are shown in Figure 1. The addition of alum to the brine had a particularly important effect on the brine shrink as indicated by the coefficient of determination (R^2 = 0.74).

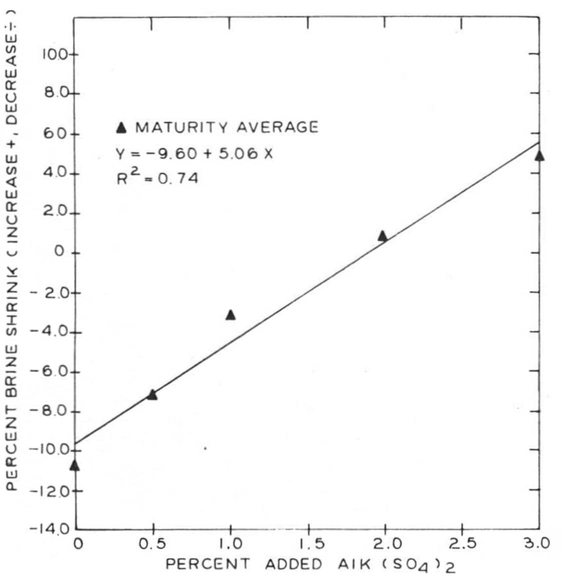

Fig. 1–Regression line for the brine shrink of sweet cherries (Y) as influenced by added $AlK(SO_4)_2$ (average of all maturity levels).

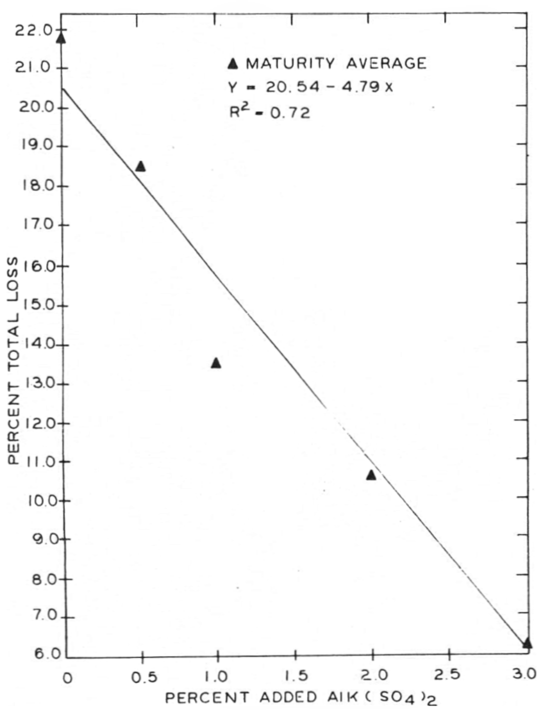

Fig. 2–Regression line for the effect of added $AlK(SO_4)_2$ on the percent total loss of brined cherries (Y) (average of all maturity levels).

Total loss

A highly significant decrease in total loss occurred with increasing levels of alum at all maturities (Fig. 2). In the control brine, the average total loss was 21.8%. The percent total loss was 18.5, 13.5, 10.6 and 6.2% when 0.5, 1.0, 2.0 and 3.0% alum, respectively, were included in the brine. The reduction in brine shrink was the main contributing factor in the decrease in total loss. This difference in yield is potentially of considerable importance, especially since the alum treatment had no adverse effect upon the quality factors during pitting, or upon development of soft or poor textured fruit.

Equilibrated brine pH

Increasing the concentration of alum resulted in a highly significant decrease in the pH of the equilibrated brine as shown in Figure 3. The pH of the initial brine was not effected by the alum additions. The pH of the equilibrated brine in the immature cherry group was significantly higher than that of the other groups. An increase of 1% alum decreased the final equilibrated pH by about 0.2 pH units. In the control brine, the pH was 3.39, 3.32 and 3.35 for the immature, mature and overmature cherries, respectively, and in brines with 3% alum additions, the pH was 2.82, 2.76 and 2.76.

The lowering of the pH with increase in alum concentrations did not have an adverse effect upon texture or the amount of soft fruit. However, at the higher alum concentrations the low pH (2.8) may have prevented maximum firming of the fruit by interfering with the Ca^{++} cross binding of the pectin (Doesburg, 1965). Van Buren (1967) found that prolonged storage at low pH caused pectin degradation and resultant softening of the fruit. Since increasing the level of added alum caused a decrease in pH of the equilibrated brines, it seems likely that upward adjustment of the pH of the initial brine containing added alum to bring the equilibrated brine pH to 3.1–3.3 should be considered.

Solution pockets

Although the occurrence of solution pockets did increase from 13.5% in the control to 21.6% in the 3% alum brine, the effect was not significant. This trend, which was also observed by Beavers et al. (1971) suggests that further study of this

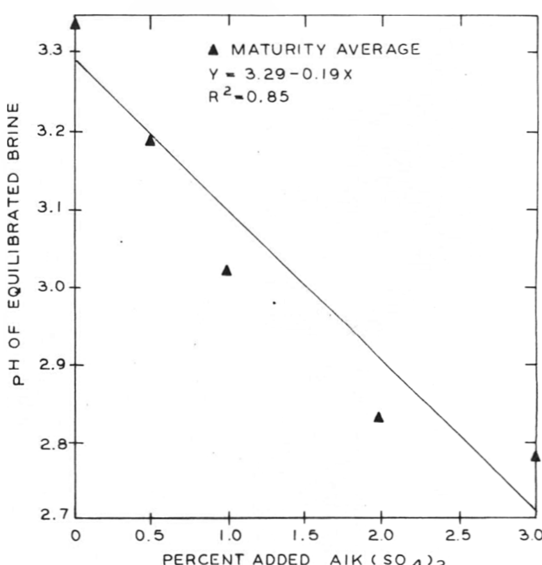

Fig. 3–Effect of added $AlK(SO_4)_2$ on the equilibrated pH of sulfurous acid brines for sweet cherries (Y) (average of all maturity levels).

effect should be made. The apparent effect upon solution pockets formation may be due to the almost instantaneous penetration of the cherry cuticle by the K^+ ion (Wilson, 1972) with the resultant increase in the osmotic pressure of the cherry.

Dye bleeding

Cherries brined in alum concentrations above 0.5% had a tendency to bleed when colored with FD&C Red No. 3. By increasing the times cherries are boiled for removal of initial brine constituents, increasing by half again the time of dye penetration and by boiling the dyed cherries at least twice for 30 min each in order to void excess dye, nonbleeding cherries were produced from alum brined cherries up to and including 2% added alum. Nonbleeding cherries were not obtained from the 3% alum treated cherries. Strict attention to procedural detail throughout the entire manufacturing process must be adhered to in the dying of alum treated cherries with FD&C Red No. 3 and this may tend to negate its use in commercial processing. The erythrosine colored alum treated cherries were more light stable than the colored control cherries, probably due to the formation of aluminum lakes. Alum treated erythrosine dyed cherries retained their color after exposure to normal light for 3 yr, whereas the untreated control became colorless in 4–5 months.

No difficulty was encountered in leaching the cherries or in coloring them with FD&C Red No. 4. The speed of dye penetration was uniform and complete when additional penetration time was allowed.

The percent unpitted fruit, soft fruit, No. 3 grade pitter holes, solution pockets and texture were shown to have little if any relationship to the additions of alum.

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EFFECT OF DIFFUSION RATE ON DRAINED WEIGHT OF CANNED GRAPEFRUIT SEGMENTS

INTRODUCTION

THE DRAINED WEIGHT is an important economic factor in canned grapefruit segments. The parameters relevant to its behavior were investigated among others, by Berezovsky (1970), Levi et al. (1969) and Mannheim and Bakal (1968). The proposed mechanism is mainly based on diffusion of solutes (sugar and acids) and solvent (water) [A comprehensive review on other canned fruits was given by Sterling (1959).], but there is no indication as to whether the final drained weight is governed by the final equilibrium in solute concentration alone (as would be the case when the species in the system are free to diffuse according to their respective bulk concentrations), or also by the rate of diffusion (as would be the case when there is mutual interference between them). This latter case was the subject of the present study, with the rate varied through the sugar gradient.

MATERIALS & METHODS

Materials

Grapefruit segments (lye-peeled) were drawn from a commercial production line prior to canning.

Sucrose:SO_2 (5% aqueous solution)—all analytical grade.

Amorphous sugar ("hard candy" type)—circular tablets (diameter approx. 60 mm, weight 90 ± 0.1g), supplied by courtesy of Elite Inc., Ramat-Gan.

Experimental procedure

Set-up (Fig. 1). A 150-g sample of grapefruit segments was placed in a plastic mesh bag (mesh size about 5 mm) inside an 800 ml glass jar with a plastic lid. The bottom of the bag was stretched around a stainless-steel ring in order to shield the segments during handling. The mouth of the bag was tied with a nylon string and the bag suspended from a crooked handle driven through the lid. All samples (preserved with 1000 ppm SO_2, based on total weight) were pasteurized in a 90°C water bath for 25 min and water-cooled immediately.

The syrup used was as to reach two final concentration levels: commercial (20° Brix) and extra-thick (35° Brix). Each series comprised two dosage variants: "spread," with the sugar added in seven equal portions over 14 days, and "lumped," with the whole amount added at the beginning. In an additional series, the sugar was introduced in the form of a hard candy, and released into the system continuously as it dissolved. In the model system a 1-in. diameter dialysis tube (A.H. Thomas, Philadelphia) containing 10% sucrose + 1% citric acid substituted the grapefruit segments in the 35° Brix final sugar concentration experiment.

Drained-weight determination (Fig. 1). The drained-weight pattern was determined by lifting the mesh bag periodically out of the syrup, suspending it (by the crook of the lid handle) from a hook provided at the bottom of a balance (Semi-analytical Sartorius model 2257, accuracy ± 0.005g), and weighing after a 30 sec delay allowed for draining. The drained-weight data in the diagram are averages of eight replicates, with standard error of about 0.6% final drained weight (FDW).

Theoretical considerations

We shall analyze a specific case where a canned fruit system can be simulated by a permeable membrane cell initially containing W_c and S_c moles of water and permeable solute, respectively. When such a cell is placed in a more concentrated syrup (containing W_s and S_s mole of water and sugar, respectively), the species will flow from syrup to cell in a rate determined by the following equation (for a system having a constant activity coefficients, typical for ideal or dilute solution):

$$\frac{ds}{dt} = K_s\, A\, \Delta N_s \qquad (1)$$

$$\frac{dw}{dt} = K_w\, A\, \Delta N_w \qquad (2)$$

where ds/dt, dw/dt are the solute and water flow rate, respectively; K_s, K_w are the solute and water permeability constant, respectively; A is the membrane surface area; and ΔN_s, ΔN_w are the sugar and water molar fraction difference. All molar fraction differences are taken from outer to inner solutions. In a two component system, $\Delta N_s = \Delta N_w$; therefore, dividing Eq (1) by Eq (2) one gets

$$\Delta W = \frac{K_w}{K_s}\, \Delta S \qquad (3)$$

where ΔS, ΔW are the total migrated solute and water, respectively

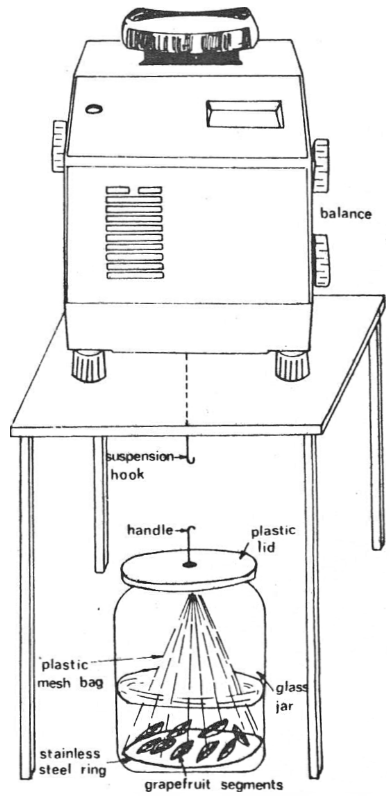

Fig. 1—Experimental set-up (schematic).

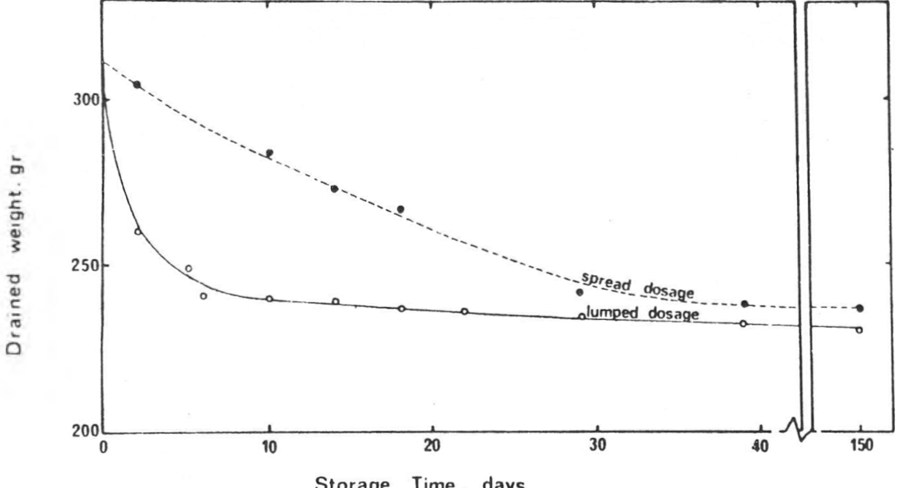

Fig. 2–Effect of sugar addition rate on drained-weight pattern–20° Brix final equilibrium.

(absolute value). Equilibrium is reached when the solute molar fraction is equalized throughout the system: namely,

$$\frac{S_c + \Delta S}{S_c + \Delta S + W_c - \Delta W} = \frac{S_T}{S_T + W_T} \tag{4}$$

where S_T, W_T are the total amount of solute and water in the system, respectively, ($S_T = S_c + S_s$; $W_T = W_c + W_s$).

Combining Eq (3) and Eq (4) and rearranging, one gets:

$$\Delta S = \frac{S_T W_c - S_c W_T}{W_T + S_T \frac{K_w}{K_c}} \tag{5}$$

Let us consider two hypothetical cases: (I) Two migrating components (sugar and water) with no mutual interference; and (II) Two migrating components where the fast moving solvent (water) interferes with the slow moving solute (sugar).

Case I. The decrease in drained weight can be expressed as follows:

$$\Delta G^I = \Delta W^I M_w - \Delta S^I M_s \tag{6}$$

where ΔG is the total decrease in drained weight; and M_w, M_s are the molecular weight of water and solute, respectively. The superscript notation indicates the respective case.

The value of ΔW^I and ΔS^I can be calculated according to Eq 3 and 5, respectively. By analyzing Eq 3, 5 and 6 one can see that the total change in drained weight depends only upon the system's initial and final concentration. Therefore, the FDW will not be dependent upon whether the solute (sugar) is added in a "lumped" or "spread" dosage. In other words, in such case the FDW is an equilibrium rather than rate-controlled phenomenon.

Case II. Interference of the fast moving solvent with the solute migration will lower the value of K_s in a manner inversely proportional to the water flow rate. Keeping all concentrations as in Case I, Eq 3, 4 and 6 yield:

$$\Delta S^{II} < \Delta S^I \tag{7a}$$
$$\Delta W^{II} > \Delta W^I \tag{7b}$$
$$\Delta G^{II} > \Delta G^I \tag{7c}$$

Interference of the fast moving solvent with the solute migration will reduce the FDW as compared to the FDW of the noninterference system (case I). The FDW will be affected, among other things, by the water flow rate itself–the higher the water flow the lower the FDW. Therefore, one can expect that the addition of solute in a lumped dosage will result in a lower FDW, as compared to the case where the solute is added in spread dosage.

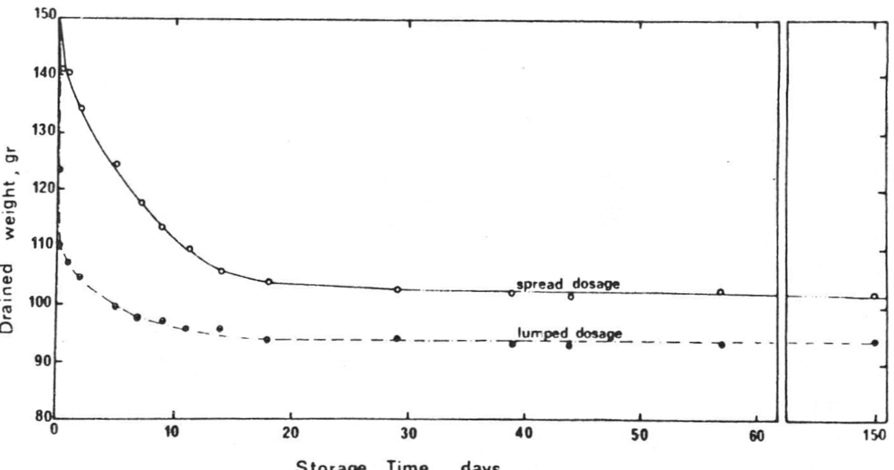

Fig. 3–Effect of sugar addition rate on drained-weight pattern–35° Brix final equilibrium.

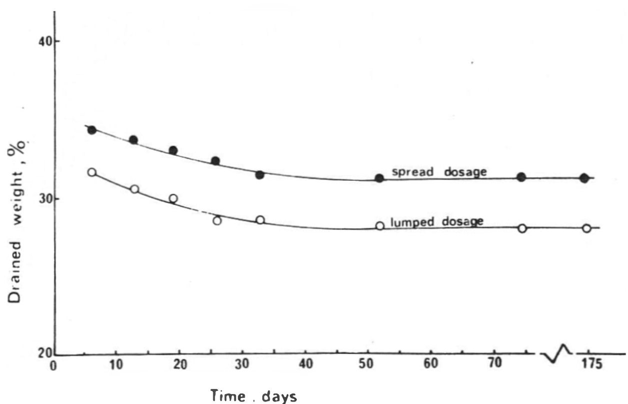

Fig. 4—Effect of sugar addition rate on drained-weight pattern—in model system.

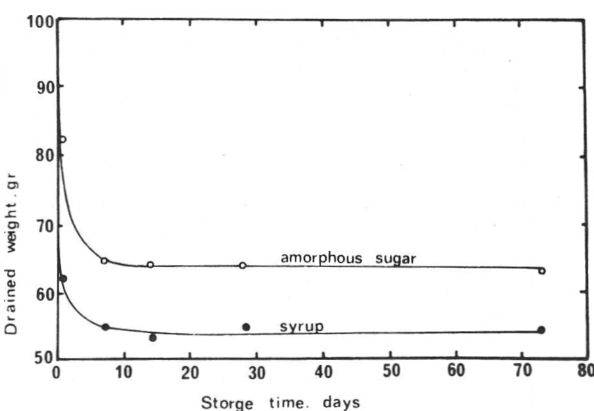

Fig. 5—Effect of controlling sugar addition rate on drained-weight pattern.

RESULTS & DISCUSSION

THE DRAINED WEIGHT PATTERN of grapefruit segments packed in syrup where the sugar was added gradually (spread dosage) and in a single lumped dosage (as practical in commercial packing) is given in Figure 2. The curves are seen to lack the "turning-point," typical of many canned fruits (Sterling, 1959; Berezovski, 1970), suggesting a system practically permeable to all solutes.

The rate effect was enhanced by using a higher sugar concentration (35°Brix final concentration, Fig. 3). In this case the difference in favor of the spread variant was about 15%. The same pattern is also seen for the all-permeable solutes model system (Fig. 4). The higher FDW obtained for the spread dosage (as compared to the lumped dosage) indicate a typical case II mechanism where the fast moving solvent interferes with the solute migration. The addition of a third component (citric acid) into the cell, although increasing the FDW does not alter the typical pattern of the interference mechanism. The increase of the FDW by the addition of the third component is attributed to the increase and decrease of sugar and water gradient, respectively. The similarity exists between the permeable cell model and the grapefruit segments system suggests that both belong to the same category of case II mechanism.

As noted, the rate-dependence of the FDW is due to mutual interference of the rapid water outflow and the inward migration of the sugar, both of which are governed by the sugar gradient. A very high rate of water outflow may result in (1) local dilution of the syrup at the syrup-segments interfaces, and (2) relative reduction of the inflow of the sugar—the net result being a lower FDW.

The results in Figure 5 indicate that a technological solution for controlling the slow release of sugar (added in the form of hard candy-type amorphous sugar) has been demonstrated to increase the FDW.

In conclusion, the FDW of a grapefruit segments system is flow-rate dependent. This dependency is due to the interference of the fast moving water with the slow migrating solutes. From a technological point of view, the FDW can be increased by controlling and maintaining a slow release of sugar into the syrup.

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FACTORS INFLUENCING IMPREGNATION OF APPLE SLICES AND DEVELOPMENT OF A CONTINUOUS PROCESS

INTRODUCTION

THE TISSUE of fresh apple slices is rather porous and may be easily impregnated with an aqueous solution (Archer, 1962; Collins and Wiley, 1967; Smock and Neubert, 1950; Wiley and Lee, 1970). For many years processors have utilized the vacuum impregnation technique for firming the tissue and improving the quality of canned and frozen apple slices (Holgate and Kertesz, 1948; Kitson and MacGregor, 1967; Smock and Neubert, 1950). The methods employed have generally been some type of batch process.

One commercial process that has been used extensively to improve the texture of apple slices consists of placing the freshly prepared apple slices into a stainless steel tank of sufficient strength to withstand a vacuum in the range of 27–29 in. of mercury. After the vacuum tank is filled to the desired level with apple slices, the chamber is evacuated to 26–28 in. of mercury for 2–8 min to remove the air from intercellular spaces of the apple tissue. Then the vacuum line is closed and steam is admitted. The steam condenses on the surface of the apple slices and in the intercellular spaces, thus partially excluding the air after the vacuum is broken. A variation of this process also used by processors consists of admitting an aqueous solution at the end of the vacuum period rather than steam. The aqueous solution may contain sugar, calcium salts, organic acid and sometimes a sulfurous salt or a combination of these ingredients. The latter method may have some advantage over breaking the vacuum with steam because it allows introduction of various additives into the apple slices to improve texture, flavor and color. On the other hand, the steam break system has the advantage of increasing the temperature of the product which may inactivate the enzyme systems.

This investigation was designed to study some of the factors that influence vacuum liquid impregnation and to develop an efficient and economical continuous impregnation system for carrying out the process.

MATERIALS & METHODS

APPLES of the Stayman variety ranging in size from 2-3/4 to 3-1/4 in. in diameter were used in the pilot plant portion of this study. The apples were sized, peeled, cored and cut into 12 equal size slices.

5-lb samples of apple slices were filled into a perforated stainless steel basket fitted with a perforated lid. The basket and contents were then submerged in the treating solution. Vacuums of 14, 21 and 27 in. of mercury were drawn on the container and its contents. It required approximately 20 sec for the vacuum to reach 27 in. The product was held under vacuum for periods of time ranging from 1 sec to 4 min. The vacuum was released at approximately the same rate of speed as the "come-up" time. The slices were allowed to remain in the liquid, after the vacuum returned to atmospheric pressure, for the same period of time that they were held at peak vacuum. The slices were then drained and weighed to determine the increase in weight due to impregnation with the solution. The weight gained by the apple slices was used as an index of the degree of impregnation obtained.

In studying the effect of solution temperature on impregnation, temperatures of 23, 27, 38 and 49°C were used. Except where specified otherwise in this manuscript, the temperature of the impregnating medium was held at approximately 23°C.

The impregnating solution contained sucrose at 0, 12, 24 and 36%. In addition to sucrose, 0.3 calcium chloride and 0.1% malic acid were used in all treating solutions.

The effect of post-vacuum pressure was studied. In the batch system the pressure was applied immediately after the vacuum was released while the slices were still submerged in the solution. Compressed air was used to raise the pressure in the tank to 15 psi.

Based on the results obtained in the batch pilot plant unit, two commercial size continuous units were designed and built. One of the commercial units utilized a syphon technique as shown in Figure 1 for introducing the apple slices and also for moving the liquid through the system. In the second commercial unit, a 4 in. vaneless-type product pump was installed for moving the apples and impregnating medium through the system. A sketch of the second unit is shown in Figure 2. The syphon unit (Fig. 1) was installed and operated for 1 yr at an apple freezing plant. The second unit (Fig. 2) was installed and operated successfully at a canning plant.

The upward and downward "legs" of the continuous units (Fig. 1 and 2) were made up of 4 in. i.d. fiberglass tubing. The cyclone-vortex vacuum chamber was constructed of 18 in. i.d. fiberglass tubing tapered at the bottom to fit the 4 in. downward "leg." The upward flow "leg" entrance into the vacuum chamber was located so that the liquid and product would enter the chambers tangentially.

In the syphon system (Fig. 1) the flow through was maintained by keeping the level of liquid in the product feed tank several feet above that of the outlet of the downward impregnation "leg" of the unit. A high capacity pump connected to the main liquid source tank was adjusted to maintain a flow of liquid into the vortex-feeder chamber equal to the flow through the whole system. A vacuum of 28–29 in. of mercury was maintained at the apex of the system by the use of a large water jet exhauster operated with a 5 hp centrifugal pump. Other suitable types of vacuum systems which would maintain the necessary vacuum under operating conditions could be used.

RESULTS & DISCUSSION

Pilot batch impregnation system

The results of various impregnation treatments on the percent weight gain in fresh apple slices utilizing a pilot plant size batch system are shown in Table 1.

A small increase in the weight of the slices occurred with time at peak vacuum of 27 in. As the peak vacuum time advanced, there was an increase in weight from 20.9% in 1 sec to 22.4% when held for 4 min. Over 50% of this increase in weight occurred within the first 30 sec. The relatively small gain in weight obtained as a result of longer time under vacuum was less than might be expected in view of the much longer vacuum holding time practiced by industry. It should be pointed out, however, that in this study the sugar concentration in the solution was perhaps higher than might be experienced under commercial conditions. Also, depending on the type commercial unit used, the effective net vacuum might be different under some conditions because of the difference in hydrostatic pressure in commercial units as compared to the batch system used in this study.

The temperature of the impregnating solution had a decided influence on the degree of impregnation. For instance, the weight of the apple slices increased from 22% at 23°C to 28.5% at 49°C. The boiling point or "flash point" of the liquid

at a given vacuum is the major limiting factor in the utilization of a hot solution for increasing the penetration of the liquid into the slices.

One problem that can occur when apple slices are firmed by vacuum impregnation with a calcium salt is the difficulty of obtaining the desired fill weight in the container. It was found that the problem could be minimized by subjecting the freshly impregnated slices to a short steam or hot-water treatment after impregnation and prior to packaging.

Operating the system under maximum vacuum is important to efficient impregnation. As may be noted in Table 1, there was a sharp increase in weight of the fruit as a result of increasing the vacuum. For instance, the percent gain in weight due to impregnation increased 40% when the vacuum was raised from 14 in. to 21 in. and 20% between 21 and 27 in.

The level of impregnation decreased as the sugar content in the liquid increased (Table 1). The amount of weight gained in apple slices from impregnation with varying levels of sugar declined from 27.1% when impregnated with water to 20.3% for those submerged in 36% sugar sirup. This represented a decline of approximately 25%. This decline is probably due primarily to the difference in viscosity of the solutions containing different levels of sugar. This statement is based on the observation that the addition of a small amount of pectin to acidified water also reduced the amount of impregnation similar to that which occurred when high percentages of sugar were used.

Continuous syphon system

In the operation of the continuous syphon impregnation unit the freshly peeled, cored and sliced apples are conveyed into the product feeder where they are sucked into the system by the action of the liquid vortex (Fig. 1). They are then conveyed up through the upward tube to the cyclone-vortex vacuum chamber where the air is removed from the apple slices. As they move downward through the impregnation "leg" back toward atmospheric pressure, the voids are filled with the impregnating medium. The outlet of the downward impregnation column of the system is located below the liquid level in the liquid-product separation tank. A screen is placed around the outlet tube and positioned so the impregnated apple slices are deposited on the conveyer screen to be conveyed out of the tank.

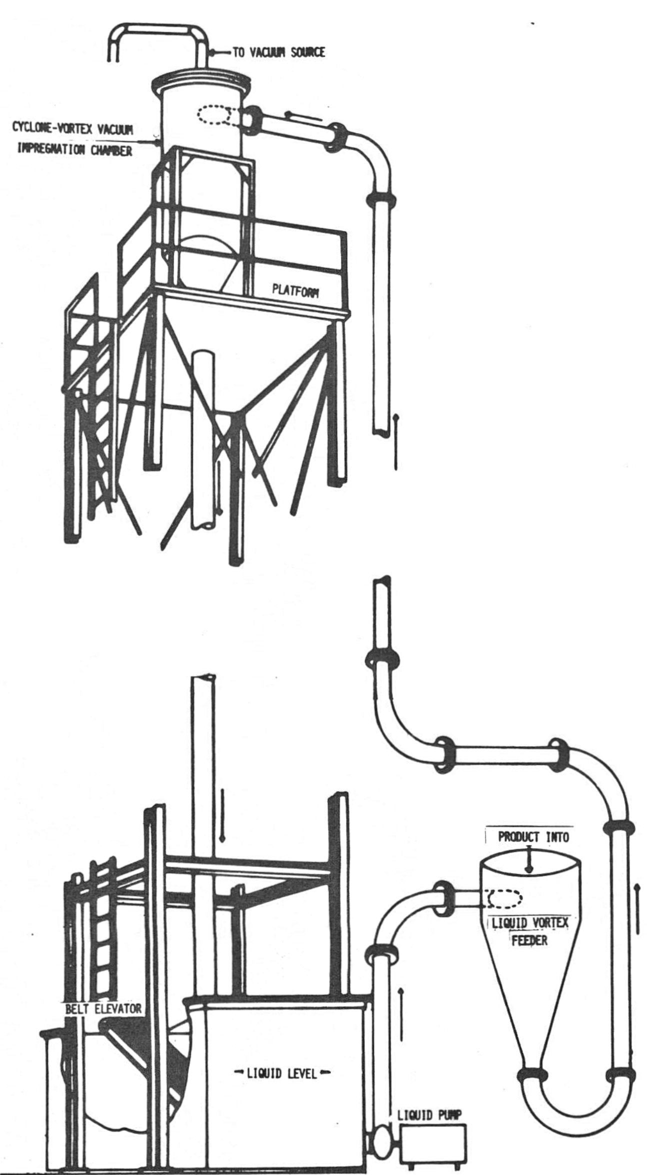

Fig. 1—Continuous syphon-vacuum impregnation unit.

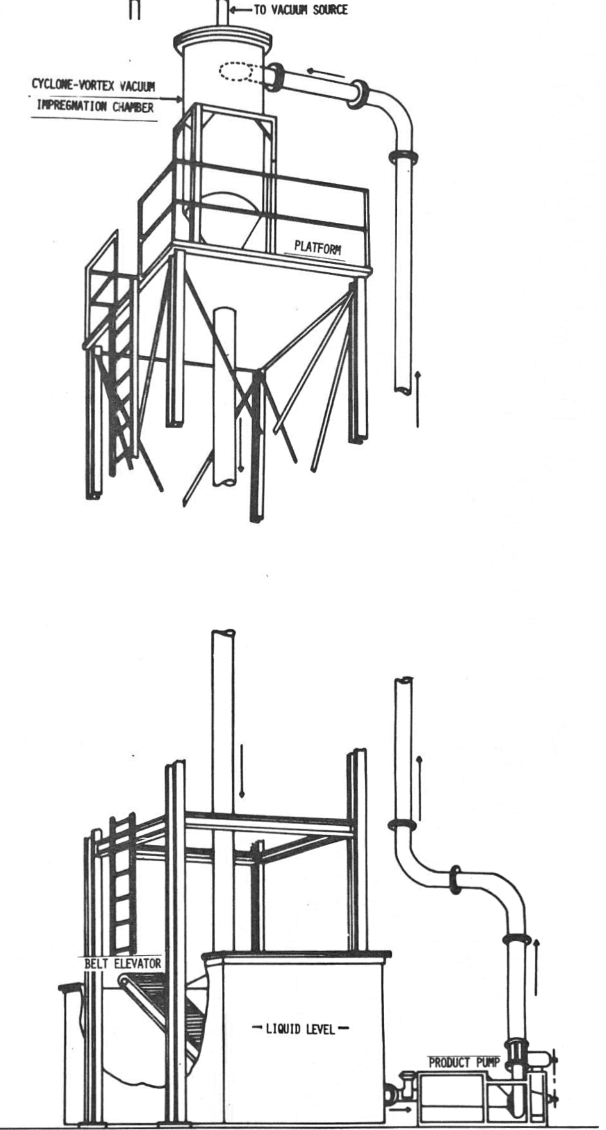

Fig. 2—Continuous vaneless pump-vacuum impregnation unit.

Table 1—The effect of various impregnation treatments on percent weight gained in fresh apple slices

Treatment[a]				%
Slices submerged in	Time	Vacuum (In. Hg)	Temp	Weight gained
24% sugar soln	1 sec	27		20.9
24% sugar soln	30 sec	27		21.8
24% sugar soln	1 min	27		22.0
24% sugar soln	2 min	27		22.2
24% sugar soln	4 min	27		22.4
24% sugar soln	1 min	27	23°C	22.0
24% sugar soln	1 min	27	27°C	23.0
24% sugar soln	1 min	27	38°C	25.9
24% sugar soln	1 min	27	49°C	28.5
24% sugar soln	1 min	14		13.1
24% sugar soln	1 min	21		18.3
24% sugar soln	1 min	27		22.0
water	1 min	27		27.1
12% sugar soln	1 min	27		24.5
24% sugar soln	1 min	27		21.9
36% sugar soln	1 min	27		20.3

[a] For details of treatment refer to Materials and Methods

Best results were obtained when the temperature of the impregnating liquid was maintained as high as possible consistent with the vacuum being used. The higher impregnating liquid temperature gave higher container fill weight which can be important if firming agents such as calcium chloride are used.

Very good results were obtained when the syphon unit was operated properly. However, the syphon unit had certain drawbacks associated with it. In the syphon system the amount of liquid and rate of flow through the unit is somewhat limited. Also, the height of the cyclone-vortex chamber above the liquid water level in the base tank is very critical. Stated in another way, the maintenance of a vacuum at the apex of the system high enough to cause a continuous flow of liquid through the syphon system is very important. If for any reason the critical vacuum level is lost, the syphon system ceases to function. As an example, based on a column of water the height of the input to the cyclone-vortex chamber above the water level in the feed tank should be approximately 32.7 ft for a 29 in. vacuum and 30.5 ft for a 27 in. vacuum. This is based on the formula: height in ft = vacuum (in. of mercury) × 1.13. A means of raising or lowering the height of the cyclone-vortex chamber to coincide with the vacuum being drawn is possible. However, the economics might rule it impractical under most conditions.

Mechanical pump-vacuum impregnation system

After gaining experience in the operation of the syphon unit, a second commercial size system was designed and operated. In this unit a vaneless product pump was used to maintain a flow of product and impregnation medium through the system (Fig. 2). This unit was operated a full season with excellent results. It possessed several advantages over the syphon system previously described. These were (a) the height of the vacuum chamber above the base water level was not critical; (b) better control over the rate of flow of product and liquid through the system was obtained; (c) a higher throughput was possible; and (d) a slight loss of vacuum was not as critical.

The design of the syphon impregnator and the vaneless pump units is similar except for the method of maintaining a flow through the system. In the syphon impregnation unit, a high volume centrifugal pump was utilized to maintain a differential head pressure in the input and output columns whereas a vaneless product pump was used for moving the product and liquid through the second unit.

Most vaneless product pumps have high capacity and the ability to raise a column of water to a higher level than is possible with the syphon system. Therefore, when a vaneless pump is used to transport the products through the system, the vortex-vacuum chamber may be installed several feet above the critical height for the particular vacuum being used.

Tests in both the experimental batch setup and in the commercial unit indicate some improvement in impregnation due to pressure treatments in the range of 12–15 psi applied immediately after vacuum impregnation. The small improvement obtained, however, probably would not justify the additional cost of such an installation.

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PAPAYA PUREE AND CONCENTRATE: CHANGES IN ASCORBIC ACID, CAROTENOIDS AND SENSORY QUALITY DURING PROCESSING

INTRODUCTION

THE NUTRIENT CONTENT of processed foods has become an important consumer concern and, with the advent of nutritional labeling, a concern of the processor. Development of new processing methods requires that attention be given to the retention of nutritional quality as well as other quality factors.

Fresh papayas are widely consumed in some tropical and subtropical areas and are being marketed in increasing amounts in the U.S. mainland and Japan. Wenkam and Miller (1965) reported that this fruit can be an excellent source of vitamins A and C. Yamamoto (1964) described the carotenoid composition of papaya, showing significant amounts of carotenoids with provitamin A activity. Recently, a method has been devised (Brekke et al., 1972) for making a high quality puree. This can be used for remanufacture into beverage products, jams, jellies and dairy products. The usefulness and economics of concentrated liquid-form products have been realized for many years, and exploratory work in this laboratory had indicated that puree concentrate might be considered as a commercial product outlet for papaya. The success of this would be predicated on retention of color, flavor and aroma quality as well as nutrient content in the concentrate. The study reported here delineates changes in ascorbic acid, carotenoids and sensory quality during puree processing and concentration.

EXPERIMENTAL

TWO LOTS of 3,000 lb each of processing-grade papayas (*Carica papaya*, var. 'Solo') were used. Processing-grade fruit is that which is sorted out from the fresh fruit packing operation; it is wholesome, nutritious fruit which fails to meet fresh market standards due to size, external blemishes, or irregular shape. The fruit was treated with hot water by the method of Akamine and Arisumi (1953) to reduce incipient rot. The fruit was sorted into greens, half ripes, full ripes and rots. Greens and half ripes were allowed to ripen at ambient temperature, full ripes were stored at 13°C, and rots were discarded.

Puree preparation

Papaya puree was prepared by an improved processing method (Brekke et al., 1972) (Fig. 1) and samples for chemical analysis were taken at steps 1, 2 and 3. The puree was stored in polyethylene-lined containers at 0°F for 2–3 months. An additional sample for analysis was taken after the frozen puree was allowed to thaw at room temperature for 24 hr prior to concentration.

Pretreatment

Concentrate was made with a vacuum evaporator (Centritherm CT-1B, Alpha Laval Corp.). Puree was treated with a pectinolytic enzyme (Pectinol 10-M, Rohm & Haas) to reduce its consistency before concentration. The enzyme was added to puree at the optimum temperature for the enzyme (50–56°C) at levels of 0.05–0.2%, and depectinization was allowed to proceed for 1.5–2.0 hr before concentration. Batches of 215–350 lb of puree were concentrated 2.4- to 3-fold. Operating parameters for the Centritherm evaporator were as follows: vapor temperatures 38–50°C, vacuum 27–29 in. Hg, condenser water temperature 24–27°C. Samples for analysis were withdrawn following concentration (step 5).

Ascorbic acid assay and ash content

The colorimetric method of Loeffler and Ponting (1942), with slight modifications, was used to assay ascorbic acid (AA). The method was modified by increasing the metaphosphoric-acid concentration to 3%. The degree of concentration was based on the ash content of puree and concentrate; ash was determined by the method of Lees (1968).

Alcohol-soluble color index

100 ml of 70% methanol were mixed with 40g of puree or 20g of concentrate and 1–2g of Filtercel (Johns Manville Co.) for 3 min. The mixture was filtered in vacuo through a layer of Filtercel on Whatman No. 2 filter paper.

The filtrate was brought to 200 ml, with 70% methanol. Absorbance was measured at 400 nm with a Spectronic 20 (Bausch & Lomb Co.) using 70% methanol as a blank. The alcohol-soluble color index was calculated by dividing the absorbance at 400 nm by the product of grams of sample and fold of concentration. Simple alcoholic extraction methods such as this have given results that correlated well with nonenzymatic browning in many food products (Maier and Schiller, 1960; Hendel et al., 1950; Stadtman, 1948; Nury and Brekke, 1963).

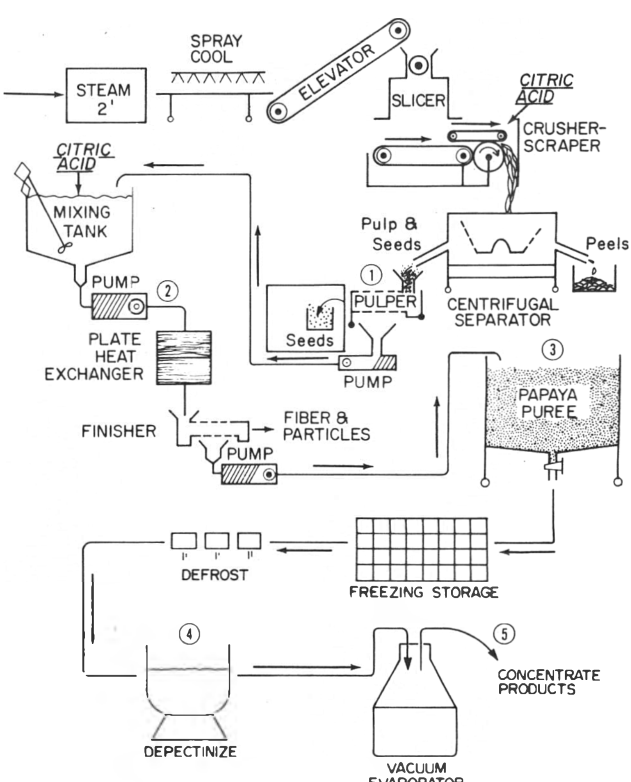

Fig. 1—Flow sheet for production of papaya puree and concentrate (Brekke et al., 1972).

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Carotenoids

Carotenoids were extracted from papaya puree and concentrates as follows: 30g of puree or 17g of concentrate were mixed with 5g Hyflo Supercel (Johns Manville Co.) and 75 ml of 70% methanol (v/v), and filtered in vacuo through Whatman No. 2. The filtrate was discarded. The residue was mixed with 75 ml of acetone-methanol (2:1) (v/v), filtered in vacuo through Whatman No. 2. The residue was extracted two more times with 75 ml of acetone:petroleum ether (1:1) (v/v). The extracts were transferred to a 500-ml separatory funnel containing 25 ml of 25% KOH in methanol (w/v), and allowed to stand for 0.5–1.5 hr. Partition was achieved by adding 75 ml of petroleum ether and 100 ml of 20% NaCl (w/v), and mixing gently. The hypophasic layer was discarded. The epiphasic layer was washed three times with water, passed through anhydrous Na_2SO_4, and made to 250 ml with petroleum ether. Absorption spectra in the visible region, 350–750 nm, were run with a Perkin Elmer 202 spectrophotometer. Total carotenoid values were calculated from the absorption maxima at 445 nm, using $E^{1\%}_{1\,cm}$ = 2370. The remainder of the carotenoid extracts were concentrated in vacuo for analysis by thin-layer chromatography.

Thin-layer chromatography (TLC)

Precoated sheets of cellulose (Polygram Cel 300, Mackerey-Nagel & Co., and Eastman Chromatogram 6064) were impregnated with coconut oil by dipping the sheets into a solution of 70g coconut oil to 1 liter of petroleum ether. The sheets were allowed to dry until free of petroleum ether. Carotenoid extracts in petroleum ether were applied as a band to the chromatograms with a micropipette. The chromatograms were developed in a solvent system of methanol:acetone:water (15:10:1) (v/v). After development, the separated carotenoids were cut into single bands, extracted from the cellulose powder with petroleum ether, and analyzed spectrophotometrically. Each carotenoid fraction was tested for 5,6-epoxides by adding a few drops of 0.1N HCl and observing the characteristic hypsochromic shift after 2–3 hr at 95°F. The quantity of 5,6-monoepoxidecryptoxanthin was calculated using the value $E^{1\%}_{1\,cm}$ = 2500 (Goodwin, 1965). Values in Table 2 represent the average of four or more replications.

Sensory evaluation

Product quality was evaluated by sensory evaluation of nectars prepared from concentrates and single-strength puree. The nectars contained 25% puree and were made up to 14° Brix by the addition of sucrose and water. Temperature of the samples at serving was 13°C. A 10-member trained taste panel first compared nectar prepared from concentrate with nectar prepared from the corresponding single-strength puree in a triangle test. This procedure was repeated for a second batch of concentrate. 36–40 judgments were obtained for each test. If a significant difference was found, further evaluation was conducted. Flavor and aroma quality were rated in a separate test in which the panel used a 7-point scale on which 7 = excellent, natural papaya aroma or flavor and 1 = unacceptable, extreme off-aroma or off-flavor. Judges evaluated the samples at four sessions giving a total of 36–40 observations for each product. Scores were then statistically treated by analysis of variance.

Table 1—Average ascorbic acid content[a] during puree processing and concentrate

Step (see Fig. 1)	Ascorbic acid[b] (mg/100g puree)
1	69.4
2	65.6
3	64.5
4	65.2
5	55.3

[a] Average of four to eight values
[b] Values connected by the same line are not significantly different.

Table 2—Changes in absorption spectra of papaya carotenoids

Sample	Ratio absorbance 425 nm/445 nm
Before pulping (step 1)[a]	0.889
After heating (step 2)[a]	0.958
Before concentration (step 4)[a]	0.991
After concentration (step 5)[a]	1.041

[a] See Fig. 1.

RESULTS & DISCUSSION

Effects of processing and concentration on ascorbic acid

Ascorbic acid was determined in two comparable batches of papaya during processing from the crushed stage to the concentrate. Average AA content is given in Table 1 for each step in the processing line (Fig. 1). The values for the concentrate have been adjusted to mg/100g of puree. Losses were significant during puree processing and concentration. The "t" test showed that significant losses occurred during pulping ($p = 0.05$) and during concentration ($p = 0.01$). The loss during pulping represented 5.5% of the initial AA. Incorporation of air during pulping probably contributed to this loss. Retention might be improved by incorporation of a deaeration process at this point. Loss in AA was greater, 14.3%, in the concentration step. Heat applied during this process probably contributed to destruction of AA. Retention might be improved by cooling the concentrate at this point. The total loss from crushed fruit to concentrate was 20.3%.

Effect of concentration on the alcohol-soluble color index

The absorption at 400 nm of alcohol extracts of puree and concentrates was used as an indicator of browning. The index increased from 0.314 in the puree to 0.502 in the concentrates, a 1.6-fold increase. This increase as well as the AA loss suggests that there was some heat damage during concentration. However, color change due to browning was not readily visible, and thus not considered a significant deteriorative change.

Effect of processing on carotenoids and provitamin A activity

Absorption spectra differed for total carotenoid extracts of fresh papayas, puree and concentrated puree. Absorption maximum for the total carotenoid of fresh papaya was at 445 nm with minor peaks at 469 and 425. After the acidification step in the processing of puree, step 2 (Fig. 1), the spectrum shifted with increased absorption at 425 nm and decreased absorption at 445 nm. The difference became pronounced after concentration when absorption at 425 nm was clearly the major peak.

The ratios of absorption at 425 nm to that of 445 nm of the carotenoids extracted from samples taken at various points in the processing line are shown in Table 2. The results show a definite hypsochromic shift in the absorption spectra: absorption increased at 425 nm, decreased at 445. The hypsochromic effect increased progressively with the processing sequence.

Separated carotenoids

Of the total carotenoids, about 15% cryptoxanthinmonoepoxide was detected in fresh papaya puree, 9.8% in processed puree, and none detected in the papaya concentrate. The isomerization of 5,6-monoepoxycryptoxanthin to 5,8-monoepoxycryptoxanthin under acidic conditions (pH 3.5), would explain the hypsochromic shift of the total carotenoid extract in the puree and concentrate samples. The hypsochromic effect due to the acid-catalyzed isomerization has been reported by Curl and Bailey (1954), Singleton et al. (1961) and Gortner and Singleton (1961) with carotenoids in other processed fruits.

The carotenoid composition for solo papaya has been reported by Yamamoto (1964). β-carotene and cryptoxanthin, carotenoids with provitamin A activity, were reported to be

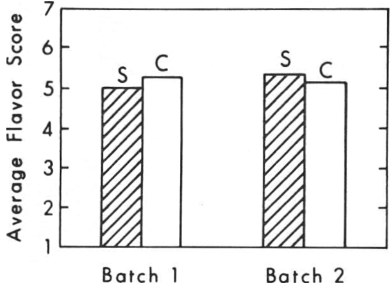
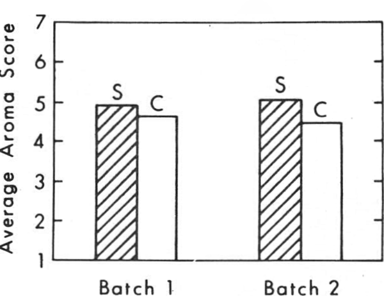


Fig. 2—Average flavor scores for nectars prepared from single-strength puree (S) and from puree concentrate (C).



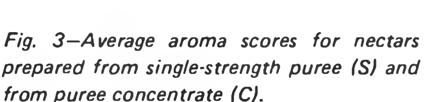


Fig. 3—Average aroma scores for nectars prepared from single-strength puree (S) and from puree concentrate (C).

4.8% and 38.9% of the total carotenoids, respectively. Cryptoxanthinmonoepoxide was reported to be 15.6%; it, too, may be a provitamin A depending on the position of the epoxide group. Since there is a question at this time on whether the epoxy group is 5,6, or 5′,6′ the provitamin A activity of this carotenoid is uncertain. However, the isomerization from the monoepoxy form to the furanoid form should not affect the provitamin A activity of the puree, because 5′,6′, epoxycryptoxanthin is not a provitamin A and the isomerization of 5,6-epoxycryptoxanthin would not affect the β-ionone (provitamin A) portion of the molecule.

Total carotenoids, as determined from the absorbance of carotenoid extracts at 445 nm, decreased from an initial value 2.83 mg% to a final value after concentration of 2.12 mg%. Because of the hypsochromic effects, such losses in total carotenoid values should not be construed as destruction of carotenoids and provitamin A, but rather as a change in composition. Measurements for total carotenoids have been frequently used as an approximation of a foods' provitamin A potential. In view of the the results from this study, changes in total carotenoid values should not be interpreted without first considering the composition of carotenoids. The presence of 5,6-monoepoxycarotenes and their inherent susceptibility to acid-catalyzed isomerization would be of special importance.

Sensory evaluation

Triangle tests on two separate batches of concentrates showed a detectable difference between nectars prepared from concentrate and those from single-strength puree ($p = 0.01$). However, when the importance of these differences was assessed by the flavor quality evaluations, the differences in flavor quality were not significant (Fig. 2). In nectar prepared from one batch of concentrate, aroma scored slightly lower than for that made from the corresponding single-strength puree ($p = 0.05$) (Fig. 3). In the same test on a second batch of puree, no significant aroma difference was detected. For nectars made from both concentrates and single-strength puree, flavor rated "good" (score of 5) or slightly higher on the scoring scale and aroma ratings were "good" or slightly lower.

SUMMARY

IN THE PROCESS of making a puree and a concentrate from papaya, small but statistically significant losses in vitamin C occurred. Changes in carotenoids were measurable, but the nutritional significance of this is uncertain. No obvious change in color due to heat-induced browning was seen. Flavor quality was not changed by concentration of the puree, and little or no change took place in aroma. It was apparent from this study that quality retention is satisfactory in low-temperature, low pressure evaporation of papaya puree to produce a concentrate.

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EFFECT OF POST-HARVEST TEMPERATURE ON QUALITY, CAROTENOIDS AND ASCORBIC ACID CONTENT OF ALPHONSO MANGOES ON RIPENING

INTRODUCTION

THE MANGO (*Mangifera indica* L.) is the most popular tropical fruit cultivated in India and among the various commercially important varieties, the Alphonso is particularly prized for its delicate aroma, flavor, taste and the attractive orange-yellow flesh color. The fruit is considered to be a rich source of vitamin C and provitamin A carotenoids (Hulme, 1971). According to Singh (1960) the vitamin A content of mango may be equal to that of butter. A comparative assessment of various tropical fruits have shown that mangoes possess maximum vitamin A activity (Czyhrinciw, 1969).

The mango being a tropical fruit is very susceptible to low temperatures and lengthy storage at temperatures low enough to delay ripening and senescence is out of the question (Hulme, 1971). The lowest temperatures for increasing the storage life of Indian varieties has been given as 10°C (Banerjee et al., 1934) or 6–7°C (Mathur et al., 1953) and the optimum temperature for ripening as 19–21°C (Singh and Mathur, 1952). However, studies conducted at this laboratory during the last three seasons have shown that storage of Alphonso mangoes at temperatures below 25°C resulted in loss of the typical aroma, flavor and flesh color on ripening.

Though several investigators have studied the qualitative and quantitative changes in the carotenoid pigments during ripening at tropical temperatures (Ramasarma and Banerjee, 1940; Ramasarma et al., 1946; Sadana and Ahmed, 1949; Chowdury, 1950; Jungalwala and Cama, 1963; John et al., 1970), little information is available on these changes during ripening of fruits previously held at low temperatures.

This communication reports the influence of post harvest temperature on quality, retention of ascorbic acid and formation of carotenoids in Alphonso mangoes on ripening.

MATERIALS & METHODS

MATURE, preclimacteric fruits, grown in Ratnagiri District, Maharashtra State, and a day old from harvest, were obtained through a local dealer. 40 fruits each were stored in cardboard boxes (20 fruits × 2 boxes) for varying periods at 7 ± 1°C, 15 ± 1°C, 20 ± 1°C and 29 ± 3°C (room temperature, RT). Fruits stored at low temperatures were withdrawn at different time intervals and allowed to ripen at RT after subjecting them to a hot-water dip treatment (50°C for 7 min). In separate studies it was observed that hot-water dip accelerates ripening and reduces fungal decay, whereas ethylene application accelerated ripening, but resulted in more spoilage. A portion of the fruits was also allowed to ripen at the respective storage temperatures without any external stimuli.

Fruits were analyzed soon after receipt in the laboratory and at eating ripe stage. Eating ripe stage was judged by color, texture, total soluble solids and taste.

Extraction of pigments

Extraction and phase separation of carotenoids were carried out according to the procedure of Tomes (1963). 20g of pulp tissue was extracted repeatedly with a cold acetone:n-hexane mixture (75:60 v/v) in a Sorvall Omnimixer and filtered through a sintered glass funnel using suction until the extracts were colorless. The pooled extracts were freed of acetone by repeated washings with distilled water and the hexane layer containing the total carotenoids was dried over anhydrous sodium sulfate.

A portion (25 ml) of the hexane layer was shaken with small volumes (10–15 ml) of 85% aqueous methanol to separate the free xanthophylls until the methanolic washings were colorless. The methanolic layers were pooled and made to a known volume.

To separate xanthophyll esters, the hexane upper layer was shaken overnight at room temperature in a rotary shaker with 25 ml of 20% KOH in 85% aqueous methanol. After separating the methanolic lower layer, the upper layer of hexane was re-extracted with aqueous methanol to ensure complete removal of hydrolyzed xanthophyll esters. The methanolic phase was pooled and made to volume. The hexane phase

Table 1—Effect of post harvest temperature on carotenoid content in the flesh of Alphonso mangoes on ripening[a]

Storage and ripening conditions			Carotenoids content mg/100g fresh pulp					
Low temp		Room temp						
Temp 0°C	Days	Days	Total carotenoids	Carotenes	Total xanthophylls	Free xanthophylls	xanthophyll esters	Carotenes: xanthophylls ratio
Initial (Green, hard unripe fruit)			0.955	0.601	0.354	–	0.335	60:40
Ripe fruits								
–	–	14	15.8 ± 0.8(100	9.6(100)	6.2(100)	1.0	5.2	61:39
20	16	2	7.9 ± 1.3(50)	4.3(45)	3.6(58)	0.47	3.1	54:46
20	22	–	8.1 ± 0.3(51)	4.9(51)	3.1(50)	0.24	2.7	61:39
15	16	7	11.7 ± 1.6(74)	7.1(74)	4.6(74)	0.44	3.6	61:39
15	23	6	7.4 ± 1.0(47)	5.3(56)	2.1(32)	0.44	1.5	72:28
15	43	–	5.4 ± 0.5(34)	3.6(37)	1.5(25)	0.29	1.2	66:34
7	16	7	12.4 ± 1.7(78)	8.1(84)	3.4(54)	–	–	–
7	23	6	8.1 ± 0.2(51)	5.9(62)	2.2(36)	0.48	1.4	70:30
7	43	–	4.7 ± 0.3(30)	3.1(32)	1.6(26)	0.17	1.3	65:35

[a] Data in parentheses indicate values as a percentage of room temperature ripened fruits.

Table 2—Effect of post harvest temperature on quality of Alphonso mangoes on ripening[a]

Storage and ripening conditions	Color	Aroma	Flavor	Texture	Overall acceptability
14 days at room temperature (RT)	8.5	8.0	8.5	8.0	8.0
16 days at 20°C + 2 days at RT	6.0	5.5	6.0	6.0	6.0
22 days at 20°C	5.5	5.0	6.0	6.5	6.0
16 days at 15°C + 7 days at RT	7.0	6.5	7.0	7.0	7.0
23 days at 15°C + 6 days at RT	6.5	6.0	6.0	7.0	6.0
43 days at 15°C	4.0	4.0	4.5	5.5	4.0
16 days at 7°C + 7 days at RT	7.5	7.0	7.0	7.0	7.0
23 days at 7°C + 6 days at RT	6.0	6.5	7.0	7.0	6.5
43 days at 7°C	4.0	4.0	5.0	5.0	4.0

[a] A score of 5.5 and above is considered acceptable.

containing carotenes was freed of methanol and alkali by washing with distilled water and dried over anhydrous sodium sulfate.

The relative concentrations of free xanthophylls, xanthophyll esters and carotenes were determined by absorbance at 436 nm in a Bausch & Lomb Spectronic 20 spectrophotometer and the amounts were calculated with reference to a standard graph based on β-carotene.

Ascorbic acid, titratable acidity, pH and total soluble solids were determined as described earlier (Thomas et al., 1971). All analyses were carried out in duplicate using two separate fruits and the mean values are reported. For organoleptic evaluation, fruits ripened under varying conditions (see Table 2) were sliced and placed for assessing flesh color, aroma, flavor and texture. A panel of seven judges scored on a 9-point Hedonic scale, from 1, extreme dislike, to 9, extreme liking. The final rating was obtained by averaging the marks, with 9 as the highest. A score of 5.5 and above was considered acceptable.

RESULTS & DISCUSSION

THE RELATIVE CONTENTS of total carotenoids, carotenes, xanthophylls and xanthophyll esters of mangoes stored and ripened at different temperatures are given in Table 1. An examination of the data shows there was a tremendous increase in carotenoid content on ripening and that the carotenoid development was maximal in fruits stored at room temperature (RT): approx 16 mg/100g fresh pulp, while fruits held at low temperatures (7–20°C) for 16–23 days and subsequently ripened at RT recorded 22–53% less carotenoids. Maximum inhibition of carotenoid formation on ripening occurred in fruits stored continuously at low temperatures, the respective values for 7°, 15° and 20°C stored fruits being 30, 34 and 51% of RT stored fruits. The optimal temperature for carotenoid formation and color development in citrus fruits was reported to be in the range 15–20°C (Wheaton and Stewart, 1973) while in tomatoes, temperatures above 30°C inhibited the synthesis of lycopene but not of β-carotene (Vogele, 1937; Tomes, 1963). Exposing tomatoes to temperature above 30°C did not have a permanent deleterious effect since fruit held at the high temperature and then returned to a low temperature could synthesize lycopene again. The results of the present study indicate that mangoes stored at low temperatures and subsequently ripened at room temperature failed to synthesize as much carotenoids as that of fruits held continously at room temperature. Goodwin (1954) has reported that high temperatures were not deleterious to the formation of carotenoids in mango. The fact that the carotenoid content of fruits stored at 7° and 15°C for 16 days and subsequently ripened at RT was 74 and 78% of fruits held at RT would suggest that flesh carotenoid formation in low temperature stored mangoes could be improved by removing the fruits to temperatures above 25°C while they are still in the preclimacteric state. Once ripening has been initiated during low temperature storage, subsequent removal to optimum ripening temperature did not compensate the loss in formation of carote-

Table 3—Effect of storage temperature on total soluble solids, pH, acidity and ascorbic acid content of Alphonso mangoes on ripening[a]

Storage and ripening conditions: Low temp — Temp 0°C	Low temp — Days	Room temp — Days	Total soluble solids	pH	Total titratable acids (ml N/10 NaOH/100g pulp)	Ascorbic acid (mg/100g pulp)
Initial (Green, hard unripe fruit)			7.0	2.87	435.0	88.0(100)
Ripe fruits						
–	–	14	15.00	4.57	30.3	27.9(32)
20	16	2	15.25	4.62	27.0	75.0(85)
20	22	–	12.25	4.58	21.9	83.6(95)
15	16	7	15.25	4.65	30.0	59.2(67)
15	23	6	13.00	4.62	30.0	97.5(111)
15	43	–	12.25	4.78	19.8	105.9(120)
7	16	7	15.50	4.62	31.9	79.5(90)
7	23	6	14.25	4.55	30.0	99.6(113)
7	43	–	14.25	4.88	27.1	121.8(138)

[a] Data in parentheses indicate values as percentage of initial content.

noids. Krishnamurthi and Subramanyam (1973) recently reported that the content of total carotenoids and β-carotene were very low in Alphonso manges ripened at 10°C, the amount of carotene being only 30% of fruits ripened at room temperature (28°C).

The data on the various carotenoid fractions revealed that regardless of the storage and ripening temperatures, carotenes always exceeded xanthophylls (54–72% carotenes as against 24–46% xanthophylls depending on storage temperature) which is in agreement with the earlier findings of Jungalwala and Cama (1963) and John et al. (1970) on Alphonso and Badami mangoes ripened under tropical temperatures. Similarly xanthophyll esters accounted for 70–87% of the total xanthophylls present which is in conformity with the general trend noticed in other fruits (Goodwin, 1965).

Apart from impairing the formation of carotenoids, low temperature storage was also found to decrease the aroma, flavor and overall organoleptic qualities of the fruits on ripening as compared to fruits stored continuously at RT (Table 2). Quality characteristics of fruits stored for 16 days at 7° and 15°C and subsequently ripened at RT were better than those held for longer periods at these temperatures. Fruits continuously held at lower temperatures though sweet to taste when ripe, were bland and atypical in terms of aroma and flavor. Rolz et al. (1971) observed that 'Mamey' mangoes stored at 8–12°C when removed to ambient temperatures, the ripening process was abnormal, accompanied by poor flavor and color, uneven ripening and overall bad appearence. Pattabhiraman et al. (1968) have reported that carotene present in the fruit seems to modify the aroma. In separate studies with different mango varieties, it has been observed that the flesh carotenoid content of the ripe fruit could be correlated with the aroma and flavor, varieties with naturally high content of carotenoids having more intense aroma and flavor than varieties with low carotenoids content. The present findings also show a possible correlation between carotenoid content and aroma and flavor of Alphonso mangoes as influenced by storage temperature. Recently, Bandyopadhyay and Gholap (1973) reported that the organoleptically evaluated aroma and flavor characteristics of Alphonso mango pulp could be correlated to the ratio of palmitic to palmitoleic acid content. It is likely that since the flesh color of mango fruits is due to lipid-soluble carotenoids, both the content and composition of carotenoids and lipids may influence the aroma and flavor in addition to the influence exerted by sugars, acids and tannins on the overall flavor of the fruit.

Storage temperature was also found to influence the content of ascorbic acid on ripening. Fruits stored at RT retained approximately 32% of the original ascorbic acid content at eating ripe stage while those held for 16 days at low temperatures and subsequently ripened at RT showed 67–90% retention (Table 3). In contrast, fruits stored for longer periods at low temperatures prior to removal to RT for ripening or those stored throughout at low temperatures recorded ascorbic acid levels higher than the initial, suggesting the possible synthesis under such conditions. Singh and Mathur (1953) have reported better retention of ascorbic acid in mangoes ripened at 19–21°C.

In conclusion, the present findings clearly indicate that storage temperatures below ambient (25°C) would adversely affect the development of typical aroma, flavor and carotenoid formation of Alphonso mangoes on ripening. This may be of significance when the fruits are to be stored for longer periods to make them available for fresh trade as well as for processing during out of season.

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DEGRADATION PRODUCTS FORMED IN CANNED SINGLE-STRENGTH ORANGE JUICE DURING STORAGE

INTRODUCTION

CANNED single-strength orange juice (SSOJ) when stored at warm temperatures, develops detrimental off-flavors. Citrus juice processors need methods to assure retention of high quality in consumer products. Analytical methods to assess storage or transit abuse would be helpful. To develop methods which could be used to check the quality of questionable samples at the point of purchase, the chemical constituents responsible for the off-flavors should be identified. Although some empirical methods may serve as indicators of quality, those based on actual constituents responsible for quality changes should be more reliable. Many theories have been proposed to explain the flavor instability of SSOJ but none has been fully substantiated. Off-flavors have been thought to originate primarily from peel oil constituents (Boyd and Peterson, 1945; Riester et al., 1945; Blair et al., 1950) and lipid constituents (Curl and Veldhuis, 1947; Swift, 1951). Blair et al. (1950) suggested that d-limonene, the principal component of peel oil, undergoes a series of hydration-dehydration reactions producing α-terpineol, 1,4-cineole and other products. Curl (1946) and Curl and Veldhuis (1947), however, indicated peel oil did not contribute to off-flavor development but that it masked some off-flavors. They proposed that the fraction responsible for off-flavors was the suspended matter of juice (cloud), predominantly lipids. A major limitation in many previous investigations was the lack of methods for isolation and identification of components responsible for off-flavors. Methods used to analyze dehydrated orange juice (Tatum et al., 1967; 1969) probably could be adapted to solve analytical problems associated with off-flavor detection in SSOJ.

Because of recent advancements in flavor chemistry and methodology, and the commercial importance of flavor stability in SSOJ, this investigation was initiated. The purpose of this study was to identify degradation products formed during high-temperature storage of SSOJ. Degradation compounds possessing malodorous properties were evaluated organoleptically. The information obtained might provide a basis for developing definitive quality testing procedures.

EXPERIMENTAL

SSOJ samples

Canned single-strength orange juice samples from several commercial plants were taken from the canning lines and placed in storage. Control samples were stored at −18°C and experimental samples at 35°C for 12 wk. Samples were extracted and analyzed as outlined below.

Extraction and solvent separation

To 1 liter of canned SSOJ, in a 1500 ml beaker, NaCl (100g) was added and the mixture stirred 5 min with a counter-rotating mixer. The juice was then divided between two 1-liter separatory funnels. Each portion was extracted 5 times with 200 ml methylene chloride for each extraction. The extracts were dried over anhydrous Na_2SO_4, combined and concentrated at 30°C on a rotary film evaporator. The material was transferred to a 2 ml volumetric flask for analysis by GLC. 100 μl samples were used for GLC analysis. Both control and experimental samples were prepared by this procedure. 2–3 liters of juice were extracted as above and concentrated for identification of all degradation products.

Chromatographic methods

Gas-liquid chromatography (GLC) using stainless steel columns was carried out with the following conditions: A 9-ft × 0.25-in. od Carbowax 20M packed column (20% on 60–80-mesh Gas Chrom P) was operated at a helium flow rate of 200 ml per min. The temperature was programmed as follows: Initially, 80°C for 6 min, increased to 130°C at 6 min, 135°C at 14 min, 140°C at 24 min, 155°C at 30 min, 180°C at 46 min, 190°C at 56 min, 200°C at 64 min and 220°C at 76 min. The maximum temperature was maintained for 2 hr and 10 min. All temperature increases were at the rate of 60°C per min, and all times were from the moment the sample was injected on the column.

The instrument employed was an F & M Model 810, equipped with a dual-flame dual column and a 5-to-1 effluent splitter. Samples were collected at the exit port which has a male luer lock with a hypodermic needle attached. Capillary tubes were placed over the needle and cooled with liquid nitrogen from below to condense the sample.

Standard curves were prepared for α-terpineol and 4-vinyl guaiacol as follows: α-terpineol (40 mg) in 2 ml ethanol (1), and α-terpineol (20 mg) plus 4 vinyl guaiacol (10 mg) in 2 ml ethanol (2). Aliquots of these samples were analyzed by GLC and curves drawn showing peak height vs μg.

Isolation of malodorous compounds by thick-layer chromatography (TLC)

A simple method was used to isolate 4-vinyl guaiacol and α-terpineol by TLC. An extract of aged juice was prepared (see Experimental) and streaked on thick layer plates (Silica Gel HF-254, 1 mm, Brinkmann Instruments, Inc., Westbury, N.Y.). Plates were developed in a paper-lined tank to the top of the plate using benzene-acetone 95:5 by volume. Plates were air dried and passed slowly under the nose, by one to four investigators. Areas on the plates which possessed objectionable odors were marked, scraped from the plate and eluted with benzene-acetone (75:25). The malodorous materials were concentrated and separated by GLC. The isolated peaks emerging from the exit port of the GLC possessed odors similar to those from the TLC plates. These were later identified as α-terpineol and 4-vinyl guaiacol by infrared (IR) and mass spectrometry (MS).

Spectrophotometric methods

The IR spectra were run neat or in KBr pellets on a Perkin Elmer 137 Spectrophotometer. The mass spectra were determined on a DuPont Model 21-490 Mass Spectrometer. All compounds reported were identified by GLC retention time, and by comparison of their IR and MS spectra with authentic samples.

Flavor evaluations

Flavor was evaluated by a 12-member experienced taste panel. Panelists were presented two trays, each containing three samples: either two experimentals and one control or two controls and one experimental. Each taster was requested to designate the sample that was different. Data were treated statistically by the method of Krum (1955). Specified compounds were added to control juice until a significant taste and aroma difference was noted. α-Terpineol, 4-vinyl guaiacol and 2,5-dimethyl-4-hydroxy-3(2H)-furanone were added to control juice as follows: α-terpineol and 4-vinyl guaiacol were added (with a 10 μl syringe) at room temperature while juice was stirred with a counter rotating mixer. The 4-vinyl guaiacol was added to 1 liter of juice and the desired amount of this juice used to add to a control to make experimental samples with desired concentrations. The furanone was diluted to 10 ml with water and then added as above. Generally, beginning concentrations were 1/2 to 1/10 the thresholds given in Table 2. In repeated tests concentrations were doubled until the flavor was obvious, then decreased in more narrow increments until the flavor threshold ranges were established.

Synthesis of compounds

Compounds that were not readily available were synthesized. Syntheses were carried out as follows:

4-Vinyl guaiacol was synthesized by the procedure of Shono et al. (1959) with several changes. When the intermediate ferulic acid was decarboxylated, the product was not steam distilled. Instead the reaction mixture was cooled and taken up in 500 ml benzene. This solution was washed six times with 10 ml conc HCl in 50 ml water to remove the quinoline. The mixture was dried (Na_2SO_4 anhydrous) and the solvent removed. The product was distilled at 0.1mm with an oil bath at 110°C (product temperature at 64°C), 15g ferulic acid yielded 6.3g of pure 4-vinyl guaiacol.

Cis- and trans-1,8-p-menthanediol. Rybinski (1968) and Guenther (1949). To 10g of α-terpineol which had been cooled in an ice bath was added 30 ml of 40% H_2SO_4. The mixture was stirred 4 hr at ice bath temperature, then neutralized with NaOH and extracted two times with 150 ml portions of diethyl ether. The etheral solution was dried with Na_2SO_4; at this point a product began to precipitate out. 100 ml of acetone was added and the flask shaken and the solution filtered. The extract was concentrated to an oil containing a solid. About 50 ml of diethyl ether was added before refrigeration. The trans isomer which precipitated at this point was recrystallized one more time from ether and twice from ethyl acetate, m.p. 154.8–155.5 reported m.p. 156–158°C. The solvent was evaporated from the mother liquor and ethyl acetate (30 ml) was added. Under refrigeration the cis product crystallized. After four crystallizations from ethyl acetate, the m.p. was 103–104.5°C, (reported m.p. 104–106).

RESULTS & DISCUSSION

THE 10 DEGRADATION COMPOUNDS isolated from canned SSOJ stored at 35°C for 12 wk are listed, with GLC retention times, in Table 1. Three of these compounds, viz. α-terpineol, 2,5-dimethyl-4-hydroxy-3(2H)-furanone and 4-vinyl guaiacol exhibited malodorous properties when isolated by GLC. When these three compounds were added separately to control juice (–18°C stored) flavor changes were detected at the concentration levels shown in Table 2.

α-Terpineol has been reported as a degradation product of aged orange juice (Kirchner and Miller, 1957; Rymal et al., 1968). Askar et al. (1973) reported that α-terpineol increases linearly with storage time in orange juice and suggested α-terpineol might serve as an indicator for predicting storage time of orange juice. Taste panel members at our laboratory described the odor of α-terpineol when added to fresh juice as stale, musty or piney.

4-Vinyl guaiacol is reported for the first time as a constituent of orange juice. This compound, reported in tomatoes by Buttery et al. (1971) could be the unidentified phenol isolated by Blair et al. (1950) from orange juice. When added to control juice, most taste panelists remarked that this compound imparted an "old fruit" or "rotten" flavor to juice.

2,5-Dimethyl-4-hydroxy-3(2H)-furanone is responsible for the pineapple-like odor of aged orange juice. The presence of this compound also is reported here for the first time in orange juice. Rodin et al. (1965) state that this is the principal flavor compound in pineapples. Its flavor was not objectionable to orange juice, but caused an unnatural flavor and aroma. The full orange-like aroma was depressed or masked when this compound exceeded its flavor threshold level ($\geqslant$ 0.05 ppm). These three compounds when collectively added to freshly processed juice gave an aged, off-flavor and aroma very similar to that observed in juice samples which had been stored at 35°C for 12 wk.

Following these results, three degradation products, viz α-terpineol, 4-vinyl guaiacol and cis-1,8-p-menthanediol, were monitored in commercial juice stored for 12 wk at –18 and 35°C. Table 3 shows that the levels of α-terpineol and 4-vinyl guaiacol in 35°C stored juice were well above their off-flavor threshold limits (Table 2). Cis-1,8-p-menthanediol did not affect flavor at the levels shown. This compound forms from α-terpineol by acid-catalyzed hydration (Blair et al., 1950) and may actually be a useful degradation product because it reduces the content of objectionable α-terpineol. Trans-1,8-p-menthanediol was also found in aged juice but at very low levels.

Five of the compounds listed in Table 1, furfural, 3-hydroxy-2-pyrone, 2-hydroxyacetyl furan, benzoic acid and 5-hydroxymethyl furfural were previously identified as degradation products in dehydrated orange juice (Tatum et al., 1967, 1969). The gas chromatographic peak corresponding to 2,5-dimethyl-4-hydroxy-3(2H)-furanone was contaminated with an unidentified compound (retention time 43 min, Table 1). One disadvantage to the gas chromatographic procedure employed to separate these degradation products was the time

Table 1—Degradation products found in canned SSOJ after 12 wk at 35°C

Compound	GLC retention time (min)[a]
Furfural	12.5
α-Terpineol	21.0
3-Hydroxy-2-pyrone[b]	41.0
2-Hydroxyacetyl furan[b]	41.0
2,5-Dimethyl-4-hydroxy-3(2H)-furanone[b]	43.0
Unidentified	43.0
Cis-1,8-p-menthanediol	47.5
Trans-1,8-p-menthanediol	52.0
4-Vinyl guaiacol	54.5
Benzoic acid	72.0
5-Hydroxymethyl furfural	75.0

[a] Retention times determined on 9 ft X 1/4 in. 20M column (20%)
[b] Resolved on 9 ft X 1/4 in. UCW-98 column (20%) (See Experimental)

Table 2—Concentration of degradation products causing detectable flavor change when added to SSOJ[a]

Compound	Conc (ppm)	Significance of difference
α-Terpineol	2.5	$p < 0.001$
	2.0	$p < 0.05$
4-Vinyl guaiacol	0.075	$p < 0.001$
	0.050	$p < 0.01$
2,5-Dimethyl-4-hydroxy-3(2H)-furanone	0.10	$p < 0.01$
	0.05	$p < 0.05$

[a] Controls stored at –18°C

Table 3—Amounts of three degradation products in three different samples of SSOJ after 12 wk at –18° and 35°C

Sample	Temp (°C)	Degradation product (ppm) α-Terpineol	4-Vinyl guaiacol	Cis-1,8-p-menthanediol
A	–18	0.1	trace	absent
	35	5.5	0.6	2.8
B	–18	0.8	0.07	absent
	35	3.4	1.3	1.7
C	–18	0.1	trace	trace
	35	3.4	1.6	1.2

required (see Experimental). In addition to the degradation product GLC peaks, six major peaks eluted after 5-hydroxymethyl furfural. These peaks contained long chain fatty acids resulting from hydrolysis of neutral lipids and phospholipids (Nagy and Nordby, 1970). They were collected, in toto, converted to methyl esters and identified (Nordby and Nagy, 1969). The major acids comprising over 90% were palmitic, palmitoleic, oleic, linoleic and linolenic.

Nootkatone, previously shown to be present in orange oil by MacLeod and Buigues (1964) was found in control and temperature-abused juices. This compound was of interest because of its grapefruit-like aroma and because aged juice is sometimes described as exhibiting a grapefruit-like aroma. Comparative examination of this compound in control and temperature-aged juice indicated that formation of this compound did not increase during storage. Apparently the grapefruit-like aroma of aged juice is not primarily due to the nootkatone level. The three compounds that affect flavor appeared to exhibit a synergistic effect on both taste and smell, as determined by the panel. The individual compounds had different quality aromas and flavors to those found in combination. The furanone compound, in combination with other degradation products, probably is responsible for the grapefruit-like aroma of stored juice. Adding this compound to juice produced an enhanced grapefruit-like aroma. Other unknown compounds probably form in juice and affect flavor. If present, they are in low concentrations and would have extremely low flavor threshold levels. The importance of these three compounds to juice off-flavor has been observed repeatedly with additional juice samples.

In conclusion three compounds have been isolated and identified that affect the flavor of canned orange juice. Two are reported for the first time as degradation products of orange juice. In the opinion of all panelists, the most detrimental compound appears to be 4-vinyl guaiacol. When this compound was added to control juice, it imparted an aged, malodorous property. When α-terpineol or 2,5-dimethyl-4-hydroxy-3(2H)-furanone was added to control juice each imparted its characteristic odor, but these odors were not judged by panelists to be as detrimental as that of 4-vinyl guaiacol. When all three compounds were added, the characteristic odor of aged or heat-abused juice became evident. Tests for these three compounds in orange juice might provide the basis for a definitive test of orange juice quality.

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EFFECTS OF PREPARATION AND MILLING ON CONSISTENCY OF TOMATO JUICE AND PUREE

INTRODUCTION

CONSISTENCY is an important quality factor of tomato products (USDA, 1953). Tomato products such as catsup with low consistency may be graded as unacceptable or sold at a lower price. Juice with proper consistency will have a smooth mouth feel (Hand et al., 1955), and its solids will not separate as readily from the serum (Robinson et al., 1956).

For more than 25 yr the literature has abounded with studies of the factors affecting consistency of tomato products. An early summary by Kertesz and Loconti (1944) showed that tomato juice consistency had considerable variation depending on character, size and proportion of the suspended particles and the serum consistency. Several researchers (McColloch et al., 1950; Luh and Daoud, 1971) have stressed consistency as it is affected by pectin content and pectic enzymes. Luh et al. (1954) have investigated the effect of different cultivars on consistency.

Heating raw tomatoes prior to or immediately after crushing inactivates the pectic enzymes. If these enzymes are not quickly inactivated, they can alter consistency (Kertesz and Loconti, 1944; McColloch et al., 1952). The method of heating has been shown to influence consistency (Kertesz and Loconti, 1944).

Consistency can also be altered by the extent of cell rupturing. Hand et al. (1955) found that additional mechanical cell fragmentation by a piston-type homogenizer resulted in an increase in consistency of tomato juice. Using a Waring Blendor, Whittenberger and Nutting (1957) confirmed these results and concluded that the ruptured cell walls are the building blocks of consistency. In another study using a Waring Blendor and a creamery homogenizer, Becker et al. (1972) observed that the amount of cell rupturing varied with the method of heating.

Hand et al. (1955) found that the apparent viscosity (gross consistency) of tomato juice is not an absolute value but varies with the method of measurement.

Progress in the understanding of the interrelationship of method of heating, cell fragmentation, and consistency has been hampered by the use of different or unspecified tomato cultivars, and in many cases there have been differences in the instrumentation and methods used to measure consistency. Equipment used to fragment the cells usually differed from equipment found in commercial practice. For these reasons there are varying and widespread conclusions among researchers (Luh et al., 1954; Hand et al., 1955; Whittenberger and Nutting, 1958; Becker et al., 1972).

This study was undertaken to determine the interactions that affect tomato product consistency. Included were the effects of method of preparation, cell fragmentation, and method of consistency measurement. Care was exercised to use commercially recognized cultivars and a mill currently used by industry.

EXPERIMENTAL

THREE LABORATORY SCALE processing operations were designed to simulate wide ranges of preheating temperatures. Red, ripe fruit of 'Chico III' and 'Centennial' tomato cultivars were randomly divided into separate 80-lb lots. Samples of each cultivar were processed into tomato juice and puree by modified hot, modified cold, and boiling break methods of preparation. Half of the juice and puree samples were subjected to intentional cell fragmentation using a commercial high speed rotary mill (Urschel Laboratories, Inc., Valparaiso, Ind.). The other half of the samples did not receive this mechanical shear treatment and served as a control.

Methods of preparation

In these experiments the samples were prepared by three different methods: a modified hot break, a modified cold break and a boiling break method. To eliminate confusion which could result regarding these terms, the methods of preparation used in this study are defined as follows.

Modified hot break method. In the modified hot break method of preparation, a small commercial extractor (Langsenkamp Co., Model 185S) was used to crush and express the tomatoes through a 3/8-in. screen. As the product was crushed, it was continuously heated to 93°C in an open, steam jacketed kettle. This process took about 2 min. The hot juice then passed through a 0.023-in. screen to remove the seeds and skin. No salt was added to any sample. The samples were canned in 303 × 406 enameled cans and heated in boiling water for 20 min, cooled rapidly under running water, and then stored at room temperature for later analysis.

Modified cold break method. In the modified cold break method the above process was altered by holding the crushed tomatoes at 21°C for 1 hr before heating. This allowed extended enzyme action in the product prior to heat inactivation (Luh et al., 1954; Whittenberger and Nutting, 1958; Becker et al., 1972).

Boiling break method. In the boiling break method, the tomatoes were pretreated prior to crushing. This is a modification of the procedures of Kertesz and Loconti (1944) and Wagner et al. (1969). 80 lb of each tomato cultivar were divided into forty, 2-lb samples, and placed in sealed plastic bags to prevent water contact with the fruit. The samples were heated in boiling water for 45 min to inactivate enzymes. During preliminary experiments, thermocouples inserted into the tomatoes indicated that 45 min were required to heat the entire fruit to 93°C. After being boiled, the bags' contents were poured into the extractor and then processed as the modified hot break method described above. The method of preparation was designed to provide a gentle in situ heat inactivation of enzymes with little change in the physical components of the fruit.

Puree was also prepared by the modified hot, modified cold, and boiling break methods. The crushed tomatoes at 5° Brix were concentrated in an open, steam jacketed kettle to 15° Brix. This took 30–40 min. The puree was then extracted through a 0.045-in. screen and subsequently handled in a manner similar to that of the juice.

Additional mechanical treatment

One-half of the juice and puree samples was treated mechanically by passing it once through a mill prior to canning. The mill consisted of 200 blades arranged in a circle around an impeller. The passage between two adjacent blades was 0.010 in. The impeller, driven by a 15 hp motor, forced the tomato product through the passages at 3600 rpm. The tomato cells were fragmented by a combination of cutting and impact (Urschel Laboratories Inc., 1971).

All samples were canned and stored at room temperature for 4 months before analysis. Data on ° Brix, pH, titratable acidity, serum consistency, and gross consistency were obtained on two cans from each sample.

Indices of quality

° Brix was measured with an Abbe refractometer and corrected to

25°C. Titratable acidity was measured after samples of juice or puree had been centrifuged at 13,000 × G and subsequently filtered through a Whatman No. 1 filter. Five ml of each sample were diluted with 30 ml of distilled water before titrating with 0.1N NaOH and the result expressed as percent anhydrous citric acid.

The serum viscosity of both juice and puree was determined by the use of an Ostwald viscosimeter. This Ostwald had a capillary that was 90 mm long with a 0.4 mm bore. Five ml of the centrifuged and filtered serum described above were used. Four readings were taken on each of the juice and puree samples. The standard flow time for 5 ml of distilled water at 25°C was 81.8 sec.

The gross consistency of the juice and puree samples was determined using an efflux pipette and a Stormer viscosimeter. A Bostwick consistometer was used only on the puree. The puree samples were diluted with distilled water to 7.5°Brix, making it possible to use the Stormer and efflux pipette to measure the gross consistency of the diluted puree. The procedure was similar to that of Luh et al. (1954), who found dilution was necessary to compare the Stormer and Bostwick, due to the inability of the Stormer to function on high total solids materials. The juice sample was unaltered. All sample temperatures were standardized to 25°C.

An efflux pipette was made from a 100 ml pipette according to the modifications of Wagner and Miers (1967) and standardized with this reference. The Stormer viscosimeter was used in a manner similar to the procedure described by Gould (1953a) with the following modifications: a 70-ml sample was placed in the sample cup and maintained at a constant temperature of 25°C. Seven driving weights at 5g intervals (60–90g) were used to drive the cylinder. The time to complete 100 revolutions of the rotating cylinder was determined for each driving weight. Four readings were taken for each of the seven weights. The flow characteristics of undiluted puree were also determined by the Bostwick consistometer. The procedure, similar to Gould (1953b), was altered so that the bottom of the trough was elevated an additional 12 cm at the gate end to increase the flow of the sample. This modification was necessary to cause the puree of high consistency to flow readily. The undiluted puree was allowed to flow for 30 sec at room temperature.

Statistical analyses

Data from °Brix, pH, titratable acidity, serum and gross consistency for each manipulated factor were tested in one- and two-way analyses of variance programs. The Newman-Keuls Sequential Range test was used to show significance at the 0.05 level (Anon., 1972).

RESULTS

THE RESULTS of the experimentation are divided into two categories: (1) those variables unaffected by the two manipulated factors: mechanical treatment and method of preparation; and (2) variables that were affected. The manipulated factors did not significantly affect the variables of °Brix, pH and titratable acidity in either the juice or puree (Table 1).

On the other hand, readings taken on the consistency of the juice and puree by the various procedures were significantly affected by the methods of preparation and/or mechanical treatment.

Tomato juice

Serum viscosity. Data obtained by the Ostwald viscosimeter showed that the method of preparation was the only significant factor contributing to the differences in serum viscosity. This was true for both cultivars. Table 2 shows the differences in the means of the Ostwald readings.

In the two cultivars tested, the boiling break method gave significantly higher readings than either the hot or cold break methods, indicating that the boiling break preserves or enhances those factors which contribute to a viscous tomato serum.

Whole juice consistency. The analysis of variance on the efflux readings of each tomato cultivar showed that the method of preparation was the only significant contributor to the observed differences. The boiling method yielded significantly higher efflux readings than either the modified hot or cold methods.

In addition to the analysis of variance, the means in Table 2 show juice prepared by the boiling break method without mechanical treatment had the highest consistency, but when mechanically treated, there was a significant drop in the efflux readings.

Neither mechanical treatment nor method of preparation significantly contributed to the observed differences in the Stormer readings. No significance was found when the time readings at a single weight were tested in a one-way analysis. For the Stormer readings a regression analysis showed the relationship among these readings. Driving weights were plotted linearly on the independent "X" axes against time plotted logarithmically on the dependent "Y" axes. A regression program showed a straight line relationship between these two factors. Significant differences found in the means of the slope and intercept are shown in Table 2. In the analysis of the Stormer readings, mechanical treatment decreased the slope and intercept of both tomato cultivars prepared by all three methods of preparation.

Table 1—Juice and puree variables not affected by manipulated factors[a]

Cultivar	Treatment	Mechanical	Juice Degrees Brix	Juice pH	Juice Titratable acidity (% Citric acid)	Puree Degrees Brix	Puree pH	Puree Titratable acidity (% Citric acid)
Chico III	Boil	–	4.9	4.40	0.341	15.0	4.26	0.461
		+	4.9	4.38	0.439	15.1	4.21	0.464
Modified	Hot	–	4.9	4.33	0.384	14.7	4.22	0.489
		+	4.9	4.34	0.330	14.8	4.15	0.507
Modified	Cold	–	4.9	4.33	0.373	14.8	4.22	0.501
		+	5.1	4.34	0.389	14.8	4.25	0.505
Centennial	Boil	–	5.1	4.39	0.491	15.0	4.35	0.477
		+	5.1	4.38	0.426	15.0	4.30	0.478
Modified	Hot	–	4.9	4.30	0.452	15.0	4.28	0.491
		+	5.0	4.31	0.459	15.1	4.28	0.500
Modified	Cold	–	4.9	4.29	0.473	15.1	4.27	0.482
		+	4.9	4.30	0.510	15.2	4.38	0.496

[a] Values are the mean of two readings. The experimental factors did NOT contribute significantly at the 0.05 level to the differences in these variables.

Table 2—Consistency measurements on tomato serum and juice[a]

Cultivar	Preparation	Mechanical treatment	Ostwald (sec)	Efflux (sec)	Stormer Slope	Stormer Intercept
Chico III	Boil	–	236.6a	143.4a	−2.6a	6.5a
		+	201.7b	86.4b	−2.6a	6.4a
Modified	Hot	–	119.6c	61.5c	−2.7a	6.4a
		+	119.5c	91.9b	−2.4a,b	6.0a
Modified	Cold	–	116.4c	54.1c	−2.4a,b	5.7a
		+	112.1c	58.1c	−2.0b	5.3a
Centennial	Boil	–	319.0a	184.0a	−2.6a	6.6a
		+	291.5b	105.8b	−2.6a	6.4a
Modified	Hot	–	166.5c	45.4c	−1.8b	4.7a,b
		+	175.2c	98.9b	−1.6b	4.4b
Modified	Cold	–	137.7d	93.7c	−2.6a	6.4a
		+	131.9d	109.4b	−2.3a	5.9a,b

[a] Values are the mean of four readings. Numbers not followed by the same letter are significantly different at the 0.05 level of significance.

Tomato puree

Serum viscosity. The data obtained by the Ostwald viscosimeter showed significant differences in the mean readings (Table 3).

The analysis of variance showed method of preparation and mechanical treatment both affected the serum viscosity significantly. For 'Chico III' the method of preparation was the most significant contributor to the variation in flow times. The interaction between methods of preparation and mechanical treatment was the second largest contributor, and mechanical treatment was the smallest contributor.

On puree serum prepared from 'Centennial' tomatoes the method of preparation was also shown to be the most significant contributor to the differences in Ostwald readings. Mechanical treatment was the second largest significant contributor, and the interaction between the methods of preparation and mechanical treatment contributed least.

The boiling method of preparation yielded a puree with an Ostwald value that was approximately four times as great as the Ostwald values of puree prepared by either the modified hot or cold methods. An elaboration on this point is contained in the discussion.

Table 3–Consistency measurements on puree serum and diluted puree[a]

Cultivar	Preparation	Mechanical treatment	Ostwald (sec)	Efflux (sec)	Stormer Slope	Stormer Intercept
Chico III	Boil	–	1045.7b	574.0a	–3.4a	8.7a
		+	1303.1a	410.0b	–1.7b	5.4b
Modified	Hot	–	242.8c	160.5d	–2.4a,b	6.6a,b
		+	194.7d	82.7f	–2.1b	5.5b
Modified	Cold	–	238.0c	205.0c	–2.8a,b	7.2a,b
		+	250.0c	126.5e	–1.9b	5.4b
Centennial	Boil	–	2298.1b	363.5b	–2.9a	7.6a
		+	2739.5a	532.8a	–2.6a	7.1a
Modified	Hot	–	496.8c	66.2c	–1.9b	5.0b
		+	510.3c	76.0c	–2.6a	6.5a,b
Modified	Cold	–	274.2d	76.2c	–1.9b	5.0b
		+	294.0d	79.9c	–1.9b	5.2b

[a] Values are the mean of four readings. Numbers not followed by the same letter are significantly different at the 0.05 level of significance.

Table 4–Bostwick consistency measurements of tomato puree[a]

Cultivar	Preparation	Mechanical treatment	Distance of flow in cm
Chico III	Boil	–	2.78c
		+	0.75a
Modified	Hot	–	4.65e
		+	3.89d
Modified	Cold	–	1.78b
		+	2.08b
Centennial	Boil	–	1.56b
		+	0.65a
Modified	Hot	–	8.03e
		+	6.09f
Modified	Cold	–	4.00d
		+	3.80c

[a] Values are the mean of at least two readings. Numbers not followed by the same letter are significantly different at the 0.05 level of significance.

Whole puree consistency. The analysis of variance of efflux readings showed that the method of preparation for 'Chico III' tomatoes was the most significant contributor to the differences in efflux readings. Mechanical treatment was the only other significant contributor. The method of preparation was also the most significant contributor to the differences in efflux readings of puree of 'Centennial' tomatoes. The interaction between the methods of preparation and mechanical treatment was the second largest significant contributor, and mechanical treatment did not contribute significantly.

The results of the differences in the means for the efflux readings are shown in Table 3. The 'Chico III' cultivar subjected to the boiling break method of preparation produced the highest consistency. Both of the boiling break samples, mechanically treated and untreated, had at least a twofold higher efflux reading than the other types of preparation. The 'Centennial' cultivar had correspondingly high efflux readings for the boiling break with at least a threefold drop compared to the other types of preparation. This points out the large effect that the boiling break had on the consistency of puree.

In the puree, as with the juice, there were significant differences in the intercept and slope of the Stormer regression line (Table 3). Mechanical treatment resulted in decreased slope and intercept of both tomato cultivars prepared by the boiling method.

The analysis of variance of the Bostwick readings on puree prepared from 'Chico III' tomatoes showed that the methods of preparation were the most significant contributor. The interaction between the methods of preparation and mechanical treatment was the second largest significant contributor, and the Bostwick readings were not significantly affected by mechanical treatment.

As for 'Centennial' tomatoes, the methods of preparation were the most significant contributor to the differences in the Bostwick readings. The second largest significant contributor was the interaction between mechanical treatment and the methods of preparation, and mechanical treatment was the smallest contributor. Both cultivars showed that puree prepared by the boiling break method with mechanical treatment had the highest consistency (Table 4). The boiling break puree, considering the mechanically treated and nontreated samples together, was more than twice as high in consistency as the other two methods of preparation.

DISCUSSION

WHITTENBERGER AND NUTTING (1957) found tomato juice consistency increased when the juice was forced through passages of small clearance. Therefore, one would expect samples forced through the small passages of a mill would likewise have a higher consistency than the untreated sample. However, this study did not unequivocally support these earlier findings. The data in this study show that mechanical treatment may increase consistency. However, the degree of increase depends largely upon the method of preparation. The data also show that differences in consistency are a function of the measuring instruments used.

The manipulated factors, methods of preparation and mechanical treatment, did not significantly affect the measurement of °Brix, pH and titratable acidity. This was true for the two cultivars tested and for both product forms. Only consistency was significantly affected.

The methods of preparation were the most significant contributor to the differences in the Ostwald, efflux, Stormer and Bostwick readings. The readings taken by these instruments support the hypothesis that the primary consideration in obtaining a high consistency is the method of preparation, not mechanical treatment. Considering all of the samples together, mechanical treatment significantly increased gross and serum consistency in 30% of the samples. 44% of the samples re-

mained the same, and 24% of the samples decreased in consistency due to the additional mechanical treatment.

Mechanical treatment decreases the serum viscosity of the juice prepared by the boiling break method. This finding supports similar findings by Becker et al. (1972) but not those of Whittenberger and Nutting (1957). Serum viscosity of the modified hot and cold break methods showed no significant change when mechanically treated, a finding similar to that of Whittenberger and Nutting (1957).

The current study shows that the efflux measurements on the juice of both cultivars prepared by the boiling break method significantly decreased when mechanically treated. Becker et al. (1972), using a preheat enzyme inactivation method, obtained decreased efflux readings upon blending the sample. However, using a preheat treatment, Whittenberger and Nutting (1957) obtained increased Brookfield readings when the samples were blended.

The consistency of the modified hot break juice, measured by the efflux, is significantly increased by mechanical treatment. Whittenberger and Nutting (1958) also found the consistency of hot break juice, measured by the Brookfield, to increase with mechanical treatment in a blender.

The efflux measurements on the modified cold break juice in our study show mechanical treatment had no significant effect on consistency. Yet, Becker et al. (1972) found an increase in consistency due to blending a cold break juice. Differences between these and earlier findings might be explained by varying experimental methods, especially the difference between using a blender and a commercial mill.

The four- to fivefold increase in the Ostwald readings due to the boiling method of preparation should be emphasized. Reasons for the large increase in the boiling break preparation may be theorized from existing research. McColloch and Kertesz (1949) found that pectin methyl esterase (PME) is present in the intact tomato fruit in large quantities and is strongly bound in the tissues, showing no activity in place. They showed that PME prepares the pectic substrate for polygalacturonase (PG) activity, and together they are responsible for decreased viscosities. Hobson (1962) tested the activity of tomato PG after maceration of the fruit and found it more than 10 times the activity of the other fruit tested. Thus, according to these researchers, pectic enzymes affect viscosity.

McColloch et al. (1952) have shown that the use of hot water blanches, immersing the fruit in 93°C water for 2 min, inactivates the pectic enzymes located near the surface of the tomato fruit. Because the center of the tomato is still not heated sufficiently to destroy the enzymes, these researchers stressed the importance of heating above 88°C immediately after crushing to inactivate remaining enzymes. They emphasized the difficulty in preserving 100% of the labile pectin even with blanching and the most rapid heating after chopping. They found that a short hot water blanch disrupted cells, leading to an increased enzyme activity since the fruit temperature was between 50–62°C; McColloch and Kertesz (1947) found the pectic enzyme activity greatly accelerated at these temperatures. Disruption of the membranes by heat may also lead to the release of acids or salts from the vacuole. Lineweaver and Jansen (1951) found pectinesterases of tomatoes to be activated by 0.15M NaCl and 0.03M $CaCl_2$ salts.

If one were to follow this line of thinking, the pectic enzymes should be able to attack the pectin during our boiling break method, thus lowering viscosity. However, this did not occur. There were large increases in the serum viscosity.

Luh and Daoud (1971) may offer some explanation for these differences. They found that serum viscosity increased at higher break temperatures and longer holding times. They offered two explanations for better pectin retention in these tomato pulps: (1) heat inactivation of pectic enzymes or (2) the solubilization of protopectin into water soluble pectin which could increase the serum viscosity. Thus the large increases in serum viscosity of our boiling break method of preparation may be due to inactivation of pectic enzymes throughout the tomato prior to crushing, or the long heat treatment may have increased the solubilization of protopectin. As pointed out by Reed (1966), however, extreme care must be taken in interpreting heating effects on enzymes in the natural environment. It is difficult to distinguish between the effect of heating on the enzyme and/or the heat's effect on the substrate.

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THE INFLUENCE OF SPAGHETTI EXTRUDING, DRYING AND STORAGE ON SURVIVAL OF *Staphylococcus aureus*

INTRODUCTION

A MAJOR CONCERN in food processing is to control the development of harmful microorganisms in foods. *Staphylococcus aureus* has been implicated in numerous outbreaks of food poisoning. The organism produces five immunologically different enterotoxins (types A, B, C, C_2 and D) (Bergdoll, 1972). Type A enterotoxin has most often been implicated in staphylococcal food poisoning according to Casman (1967).

S. aureus cells are sensitive to temperatures above 60°C. In a weak buffered solution, 90% reduction in the population of *S. aureus* occurred with heating for 2.5 min at 60°C (Walker and Harmon, 1966). The organism, however, in other media has exhibited heat resistance. Heat resistant strains of *S. aureus* have been isolated and the composition of the heating menstruum contributes to heat resistance of the organism (Baird-Parker, 1972).

Production of enterotoxin by *S. aureus* appears dependent on the strain of the organism and the conditions of the growth medium. Woodburn et al. (1973) showed that large amounts of enterotoxins were produced when *S. aureus* S-6 was incubated under still (non shaken) conditions such as a paste or gel where there was no active aeration. Accordingly, the colloidal systems found in many foods may be favorable to enterotoxin formations.

Scheusner and Harmon (1973) reported that enterotoxins are not produced in all foods which support *S. aureus* growth. There was no apparent relation between food type and enterotoxin production. However, pH was an influencing factor and no enterotoxin was detected in foods which were below pH 5.00.

Staphylococcal enterotoxins are heat resistant substances. Jamlang et al. (1971) reported that enterotoxin B retained much of its immunological activity after heating at 90°C and 100°C. Denny et al. (1971) reported that staphylococcal enterotoxin A remained active after 200 min of heating at 99°C. From these results it is doubtful that enterotoxins would be denatured by boiling of pasta foods.

Walsh (1972) reviewed the bacteriological aspects of pasta processing and indicated that pasta products are generally extruded and dried below pasteurization temperatures. Consequently, microorganisms can be isolated from even the most carefully processed pasta products.

In 1973 and 1974 an FDA laboratory found coagulase-positive *S. aureus* in 42 of 1,533 packages of macaroni collected from retail outlets (Leininger, 1974). They also reported that 1,417 noodle samples were tested and 79 contained *S. aureus*. The study included examination of finished goods and products at intermediate stages of production which were obtained from pasta factories. Of 350 factory drawn samples, 179 contained *S. aureus*. The study, however, was not a complete analysis of the industry. The purpose of the present research was to study the influence of spaghetti processing conditions on the growth of *S aureus* and to determine if enterotoxins were formed when inoculated ingredients were processed into spaghetti.

MATERIALS & METHODS

Cultures

S. aureus strain 12600 was obtained from the American Type Culture Collection, Rockville, Md. *S. aureus* strain S-6 was obtained from Dr. C. Genigeorgis of the University of California at Davis. The cultures were grown in nutrient broth at 37°C. Inoculum was separated from the broth by centrifugation. Sufficient inoculum (as determined by turbidity readings in a spectrophotometer) was added to pasta ingredients so that the mixture contained 1.0×10^7 to 1.5×10^7 cells per g (dry basis).

Preparation of inoculated spaghetti

Spaghetti ingredients consisted of commercial enriched semolina, spray dried whole egg solids and distilled water. To extrude spaghetti, a dough consisting of 1,580g semolina, 80g egg solids and 350 ml distilled water were blended 3 min in a Hobart C-100 mixer with a pastry knife. To prepare inoculated spaghetti dough, *S. aureus* inoculum was added with the water. Spaghetti was extruded according to the method of Walsh et al. (1971) with a continuous DeMaco pasta extruder which had an extrusion capacity of 11 kg per hr. The extruder processed spaghetti with vacuum mixing and auger extrusion conditions comparable to commercial practice. Each lot of spaghetti was extruded through a 1.5 mm Teflon spaghetti die. Auger speeds of 12, 20 and 30 rpm and extrusion temperatures of 35, 45 and 55°C were used. Spaghetti was dried at 35° for 18 hr in a laboratory pasta dryer described by Gilles et al. (1966). To dry spaghetti, the relative humidity (RH) in the drying chamber was decreased in a straight line gradient from 95% RH at the beginning to 60% at the end of the drying cycle. Wet spaghetti contained 31.5% moisture; the dry spaghetti contained approximately 12.5% moisture. Spaghetti was packaged in half-pound commercial spaghetti boxes and stored at room conditions. All data were expressed on a dry weight basis to compensate for moisture variation in the spaghetti and dough.

Enumeration of bacteria

Samples (25g) of dough, wet spaghetti or dry spaghetti were blended with 255 ml 0.1% Peptone Broth in a Waring Blendor at high speed for 2 min. The mixtures were allowed to settle for 1 min and samples were drawn 2 cm from the bottom of the blendor container. Serial dilutions were done as necessary in 0.1% Peptone Broth. To enumerate the staphylococci, a 0.1 ml volume of diluted sample was spread evenly on Baird-Parker Egg Yolk Tellurite plates with a sterile, bent glass rod. The plates were incubated for approximately 36 hr at 37°C, and typical black staphylococcal colonies were counted. For low populations of *S. aureus* (below 3,000 cells/g), the most-probable-number method of Scharf (1966) was used. Diluted 10 ml samples of suspension were added to 10 ml double strength Trypticase Soy 10% NaCl broth (DIFCO) and incubated 48 hr at 37°C. Aliquots (0.1 ml) were enumerated on Baird-Parker agar plates. Experiments were conducted in duplicate and a randomized complete block analysis of variance design was used to test for significance of the data.

Enterotoxin determinations

Enterotoxins were extracted from the dough, wet spaghetti and dry spaghetti and assayed by the gel-diffusion technique of Casman and Bennett (1969) and Casman (1967).

RESULTS & DISCUSSION

NONE of the ingredients, mixed dough, or spaghetti samples contained detectable *S. aureus* prior to inoculation. Populations of *S. aureus* were determined for each major step in

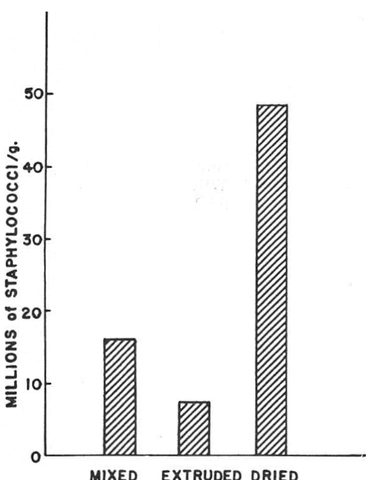


Fig. 1–Survival of Staphylococcus aureus 12600 for inoculated dough after each major spaghetti processing operation (extruded at 35°C, 20 rpm, 18 in. vacuum).

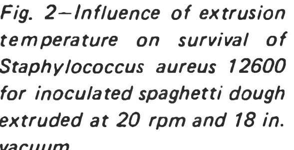
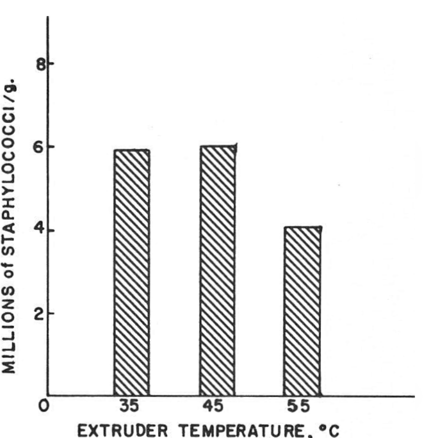


Fig. 2–Influence of extrusion temperature on survival of Staphylococcus aureus 12600 for inoculated spaghetti dough extruded at 20 rpm and 18 in. vacuum.



pasta processing. Figure 1 illustrates the influence of extrusion and drying on the population of *S. aureus* in spaghetti processing.

The inoculated dough contained an average *S. aureus* population of 1.6×10^7 cells/g. This is a contamination level far higher than would occur naturally but was necessary in order to follow any substantial decrease during processing. Extrusion decreased (5% confidence level) the population so that 7.2×10^6 cells/g were found in the extruded spaghetti prior to drying. With drying, a sevenfold increase (1% confidence level) in population occurred.

In drying spaghetti, the wet product is held at 35–40°C, 95% RH for 4 hr at an initial moisture content of 31.5%. The spaghetti dough has a pH near 6.0 and a water activity above $a_w = 0.95$ during the initial 4–8 hr of drying. Scott (1953, 1957) reported *S. aureus* growth in foods under similar conditions. Consequently, the conditions at the early stage of spaghetti drying appeared favorable to growth. No tests were done to determine populations for different periods in the 18 hr spaghetti drying cycle.

Figure 2 illustrates the influence of spaghetti extruding temperature *S. aureus* population. No significant change (5% confidence level) was noted over an extrusion temperature range from 35–55°C. At 35°C and 45°C, populations of 5.9×10^6 cells/g respectively were detected. A slight decrease in population occurred at 55°C where 4.1×10^6 cells/g were found. Doubtless, higher extrusion temperatures would further reduce the population. However, 55°C appeared to be the upper temperature limit for extruding spaghetti. Spaghetti extruded at 55°C had a soft cooked texture and exhibited excessive cooking losses (Walsh et al., 1971). Consequently, high temperature extrusion as a means of reducing staphylococcal populations in spaghetti appeared impractical.

Figure 3 depicts the influence of extrusion auger speed on *S. aureus* population. Although the slowest speed (13 rpm) showed the highest *S. aureus* population (9.4×10^6 cells/g), no general trend relating auger speed to population was apparent. At auger speeds of 20–32 rpm, 6.0×10^6 and 7.2×10^6 cells/g respectively were detected. Other research (Walsh, 1974) showed that *Salmonella typhimurium* survivals decreased as auger speed increased. However, a similar trend was not found for *S. aureus*.

The next phase of the study was to measure the survival of *S. aureus* during storage. Inoculated spaghetti was stored in half-pound boxes at ambient conditions (approximately 22°C, 60% RH). Initially an average of 4.9×10^7 cells/g of *S. aureus* were detected in the stored spaghetti (Fig. 4). When assayed after 41 days of storage, a population of 9.3×10^6 cells/g was found. At 61 days the population was 4.5×10^6 cells/g. After 179 and 209 days of storage only 550 and 88 cells/g respectively were found by most-probable-number methods.

Although the *S. aureus* viable population decreased rapidly

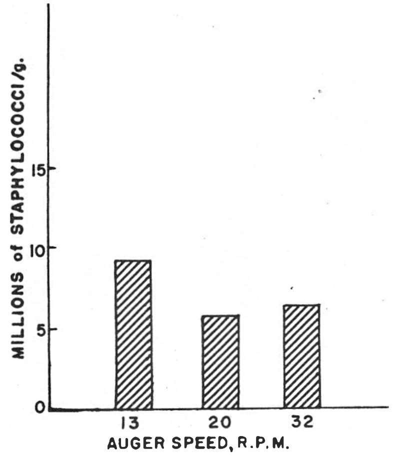
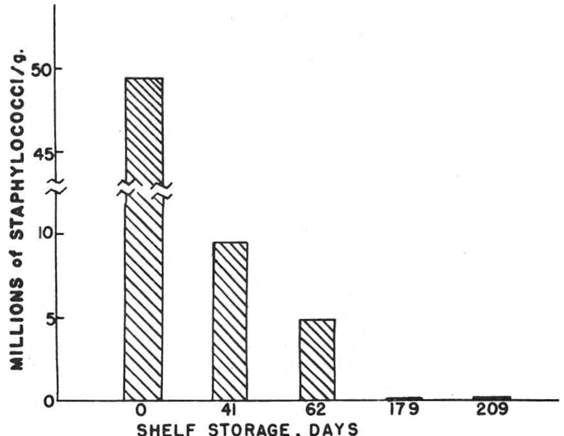


Fig. 3–Influence of extrusion rate on viability of Staphylococcus aureus 12600 (extruded at 35°C and 18 in. vacuum).

Fig. 4–Effect of ambient storage time on the viability of Staphylococcus aureus 12600 for inoculated dry spaghetti (179 and 209 days of storage showed 550 and 88 cells/g, respectively, by most-probable-number methods).



during spaghetti storage, enterotoxins which might be present would probably remain active. Enterotoxins are highly stable proteins (Jamlang et al., 1971). Consequently it is doubtful that shelf storage or boiling in preparation of spaghetti foods would destroy enterotoxins if they were present in the product.

These experiments were originally intended to follow changes in staphylococcal populations as processing progressed. However, the increases in population during the drying process suggested the possibility of toxin production. Spaghetti which developed a high population (4.5×10^7 cells/g) of *S. aureus* 12600 was submitted to the FDA Food Microbiology Laboratory, Washington, D.C. for enterotoxin analysis. No enterotoxins were detected. However, we had to consider the possibility that *S. aureus* 12600, although the neotype strain, might not have been an enterotoxin producing strain.

Therefore, in the subsequent attempts to establish whether or not enterotoxins were produced in pasta processing, *S. aureus* S-6, which is known to produce enterotoxins A and B (Woodburn et al., 1973), was substituted. Duplicate samples of inoculated dough were processed and dried as described earlier. All samples were frozen at −30°C and kept frozen until tested for toxin. Samples which showed the highest level of *S. aureus* entering dough mixture and the dried finished spaghetti contained 2.5×10^6 and 1.5×10^7 cells/g and failed to reveal the presence of either enterotoxin A or B.

The failure to establish enterotoxin production is confirmative of the report of Lee et al. (1974).

CONCLUSION

IN PROCESSING inoculated spaghetti, the extruding operation decreased the average population of *S. aureus* 12600 from 1.6×10^7 cells/g in the dough to 7.2×10^6 cells/g for the extruded spaghetti. *S. aureus* populations did not differ (5% confidence level) for spaghetti extruded over a temperature range from 35–55°C and auger speeds from 13–32 rpm. The drying operation, however, resulted in a sevenfold increase (1% confidence level) in the population of *S. aureus* for inoculated spaghetti. During ambient storage of dry spaghetti a rapid decrease in *S. aureus* population occurred. No enterotoxins were detected in spaghetti samples, even though populations of *S. aureus* strains 12600 and S-6 developed as high as 4.9×10^7 and 1.5×10^7 cells/g respectively.

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COCONUT SKIM MILK AS AN INTERMEDIATE MOISTURE PRODUCT

INTRODUCTION

THE AQUEOUS PROCESSING of coconuts consists of comminuting fresh coconuts, making aqueous extraction(s), and centrifuging the resulting oil-in-water emulsion (coconut milk) to obtain an oil phase and an aqueous phase called coconut skim milk. Various aqueous processing schemes have been investigated. In their description of a recently developed process, Dendy and Timmins (1973) give a good set of references on the subject.

The process used to prepare the coconut skim milk used in the present study was developed at the Food Protein Research and Development Center (Hagenmaier et al., 1973). In the Texas A&M process, there is the option of using the coconut water during the extraction step. When the coconut water is used, the composition of the coconut skim milk solids are changed as described by Hagenmaier et al. (1974).

In the process as used, the coconut milk was batch pasteurized (65°C for 15 min), centrifuged, and the coconut skim milk vacuum evaporated at 57°C, after which the moisture content was ca. 60%. The concentrate was either spray dried at 108°C outlet air temperature, or further concentrated with a laboratory rotary evaporator in 43°C water bath. Before pasteurization the pH was raised from ca. 6.2 to ca. 7.5. The purpose of the pH adjustment is to prevent protein coagulation, which is more likely to occur at low values of pH (Hagenmaier et al., 1974).

It has become apparent that elimination of the spray drying step would be economically advantageous, primarily because of the expense of spray drying the small amount (11 tons/day) of coconut skim milk solids produced in a plant of estimated optimum size. Therefore, a concentrated coconut skim milk is being considered as a final product (or possibly as a form suitable for shipment to a central drying facility, where the operation of economics-of-scale would permit lower-cost drying).

Gonzales et al. (1971) have previously looked at the stability of canned coconut skim milk concentrates, with 10–15% sugar added as a preservative. Their best preserved samples contained 15% sugar and 20% water, and had good organoleptic characteristics after eight weeks of storage, presumably at ambient temperature.

The purpose of the present work is to examine effects of pH, moisture content, inclusion of coconut water and other variables on the physical properties and storage stability of concentrated coconut skim milk as stored under non-sterile conditions.

MATERIALS & METHODS

THE COCONUTS USED were mature nuts imported from Honduras and Jamaica. The concentrate used for the bulk of the stability data (Tables 2–4, Fig. 3) refers to product rehydrated from spray-dried products. Spray-dried coconut skim milk did not contain the solids from coconut water. Before initiation of the stability experiment the dry (3% moisture) product, pooled from several preparations, had been stored at 23°C for 8 months. At the beginning of the storage experiment, the spray-dried product was reconstituted by mixing with water for ca. 1 hr, with heating in a water bath, during which the temperature of the mixture rose to 60°C. Sodium sulfite added to some batches in processing (in an unsuccessful attempt to produce lighter-colored spray-dried product) would make the pooled spray-dried samples 0.006% in sodium sulfite, if none had been oxidized.

For the storage stability data in Tables 2 and 3, and also Figure 3, the rehydrated concentrate was subdivided, sodium sulfite added as necessary, and the samples sealed in separate glass canning jars. The samples were stored at −20°C or +23°C. Separate jars were opened after 4 and 31 days for analysis. Because of the time required to prepare samples, it was not possible to analyze samples on day zero.

For the data in Tables 5 and 6, and Figures 1 and 2, the concentrate was prepared by direct evaporation. In preparation of product the coconuts were not pared, i.e., the dark testa or seed-coat was not separated from the white meats. The one exception is the reference to the lighter color of concentrate made from pared meats.

Viscosities were measured with a Brookfield viscometer, with cylindrical spindles. Three readings were taken over a 5-min interval and the average value used. The observations were made at 3 rpm, with sample temperature of 35°C.

Moisture contents were measured by toluene distillation for 2½ hr. Standard plate count conformed to AOAC (1970) methods, except that spread plates were used. Other microbiological methods were also 1970 AOAC methods. Values of pH were measured after mixing 1g of sample with 10g of boiled, distilled water.

Color measurements of reflected light were L, a, b values obtained with a Hunter color-difference meter, standardized on white. This instrument measures whiteness as L value on a 0–100 scale. Positive b value measures amount of blue, and positive a value measures redness. Negative a and b values were not encountered with the coconut samples.

Untrained panelists were presented with four samples each of product as beverage, at 13% solids. The four samples consisted of two cups of each of the two products being compared. Panelists were asked to pair identical samples and give 1–9 hedonic rating to each pair. Probability of accidental paring is 33%.

Equilibrium relative humidity was measured according to the method of Fett (1973). Protein solubility was measured at pH 7 and 23°C. Aqueous suspensions at 10% solids were adjusted to pH 7, mixed 30 min and centrifuged 15 min at 1,100 × G. Aqueous layer was decanted and analyzed by the Kjeldahl method.

For the mold growth experiments, unidentified wild mold was scraped off miscellaneous samples of coconut skim milk concentrate. The mold was suspended in lactose broth and plated out on potato dextrose agar. At commencement of the storage experiment one drop of suspension was mixed with 10g of concentrate. The sample jars were sealed to prevent moisture loss, but were opened periodically for a few minutes to maintain aerobic conditions. For all mold growth experiments, the concentrate was prepared by direct evaporation of coconut skim milk.

Measurement of free fatty acid, peroxide value, and nitrogen content conformed to AOAC methods. Titratable acidity was determined by titration to a phenolphthalein endpoint. Reducing sugars were determined as described by Triebold and Aurland (1963). Available lysine was determined by the method of Carpenter (1960).

Each reported observation is the average of at least two measurements.

Observed differences were judged for statistical significance by analysis of average differences and application of student's t. Where appropriate (in Tables 2 and 3) the data were treated as paired comparisons.

RESULTS & DISCUSSION

Physical properties

Concentrated coconut skim milk is a syrup with honey-like consistency and appearance at moisture contents of 25–40%. The color of concentrate is light brown, with typical L, a, b values of 30, 5 and 11 at 35% moisture both fresh and recon-

stituted samples. Typical color values for spray-dried product (at 3% moisture) are 81, 0.1 and 9. The whiteness of freshly rehydrated spray-dried product is sharply dependent on moisture content, with minimum L value occurring at ca. 35% moisture (data now shown).

Concentrate made from white, pared coconuts was much whiter than the product prepared from coconuts which retained the dark testa. Concentrate from pared coconuts (not spray-dried) had L value of 41 at 35% moisture. All other data in this article refer to products made from unpared coconuts.

The relationship between moisture content and water activity is presented in Figure 1, over a narrow range of moisture content. There is approximate linear dependence of water activity upon moisture content. At given water activity, samples are approximately 6% higher in moisture content if they include the coconut water solids. Higher moisture content of product with coconut water can be accounted for by the deliquescent nature of coconut water, which has 47% moisture at water activity of 0.85. The data presented in Figure 1 are similar to previously reported data (Hagenmaier et al., 1974), but are considered to be more accurate.

Viscosity of intermediate moisture coconut skim milk samples as a function of water-activity is shown in Figure 2, for concentrate with or without coconut water. Coconut water decreases viscosity. Over the narrow range of water activity investigated, the logarithm of viscosity shows approximate linear dependence upon water activity. Viscosity is expressed as a function of water activity, rather than moisture content, in order to emphasize the relationship between these two critical properties. Viscosity of 10,000 cp or water activity of 0.80 are considered limiting conditions because of anticipated processing problems at higher viscosity, and microbiological spoilage at higher moisture.

Viscosity is also quite dependent upon processing temperatures. The data in Table 1 report viscosity as a function of processing heat. Viscosities in Table 1 refer to concentrates with 32.5% moisture, prepared without coconut water. The viscosity in each case was determined from a graph of log viscosity vs. percent moisture. The data indicate that reconstituted spray dried samples have higher viscosity (presumably as a result of drying heat) than concentrate prepared by direct evaporation.

Additional experiments indicated that concentrated with 31–40% moisture (rehydrated from product spray-dried at 98°C) showed an average of 16% increase in viscosity after heating 30 min in a boiling water bath. This heat treatment caused only 0.6% decrease in protein solubility (not statistically significant at 95% level). These data indicate that for coconut skim milk, viscosity is more sensitive than protein solubility to heat effects. Because viscosity is so sensitive to heat effects, the viscosity values reported herein only relate to the present processing conditions.

Gelation was observed after rehydrated, spray-dried product (59% moisture) was placed in the boiling water bath. Gelation did not occur when samples of lower moisture content were heated. Gelled samples gave 30% decrease in protein solubility.

Storage stability

Samples of rehydrated, spray-dried coconut skim milk were prepared as described in the methods section. The experimental design was complete block, with two moisture levels (31% and 37%), two pH values (6.5 and 7.5), two storage temperatures (–20°C and +23°C), and with or without 0.03% added sodium sulfite. In addition, samples at 23°C (no sulfite) were stored in light and darkness. The results of this one experiment are summarized in Tables 2 and 3 and in Figure 3.

The samples stored at –20°C did not change in appearance with 1 month's storage. However, the concentrate stored at 23°C changed color, with a dark brown layer forming on the uppermost 2–3 mm. The data in Table 2 indicate that 23°C storage caused significant decrease in Hunter L, a, b values. The change in pH is also statistically significant, but was small.

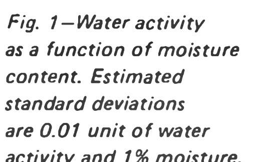
No statistically significant differences were noted by taste panel comparisons of fifteen panelists comparing product stored at 23°C for 1 month with fresh or frozen sample; only six correctly paired the stored samples. These six gave average ratings for 6.7 for stored product and 6.3 for control. The taste panel evaluations indicate that no gross changes in flavor occurred during 23°C storage.

The data in Table 2 suggest that no changes of any serious consequence occurred with 1 month's storage at –20°C. Observed changes were either too small to be of much importance or were not statistically significant.

Of particular importance in Table 2 is the data that reflects microbiological activity. The small pH change suggests (but does not prove) that little microbiological activity has occurred. The constant value of total aerobic plate count during 1 month's storage suggests that the product remained wholesome.

The microorganisms present in starting samples were for the most part unidentified. Analysis of initial spray-dried product indicated total aerobic plate count of 20,000/g, total coliforms of 7/g, and negative values for *Salmonella, E. coli, Staphylococcus aureus*, yeasts and molds. That moisture content is critical to maintaining stable microbiological levels was indicated by an auxiliary experiment: when water content was increased to 42% obvious spoilage occurred.

For further analysis of the variables affecting color change the "total color" change (ΔE) will be used. [$\Delta E^2 = \Delta L^2 + \Delta a^2 + \Delta b^2$ (Hunter, 1973).] The total color change from zero time



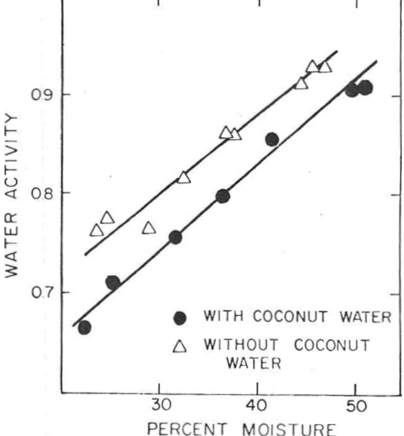


Fig. 1–Water activity as a function of moisture content. Estimated standard deviations are 0.01 unit of water activity and 1% moisture.

Table 1–Viscosity of concentrate as affected by processing heat

Max processing temp	Viscosity[b] (cp)	Protein solubility
Pasteurized at 65°C, but not spray-dried	9,000	78%
Pasteurized at 65°C, then spray-dried at 98°C[a]	17,000	76%
Pasteurized at 65°C, then spray-dried at 108°C[a]	87,000	78%

[a] Average outlet air temperature. The spray-dried product was reconstituted with water to form the 32.5% moisture product.
[b] At 32.5% moisture and 35°C, measured with Brookfield viscometer

can be examined if an assumption is made. The assumption is that average color values for samples in cold storage will be taken as equal to color of the rehydrated, spray dried coconut skim milk at zero time. This assumption is based on the fact that cold-stored samples showed no significant color change during storage. Zero time is taken as that time after dry product was mixed with water, pH adjusted, portioned into sample jars, and put into cold storage. These activities required ca. 8 hr.

Using averaged values for cold samples as zero time values, total color change at 23°C is indicated in Table 3. These data indicate that neither exposure to light nor added sulfite affected color change. However, color change at 31% moisture was only 34% of the change at 37% moisture, which suggests that the problem of color instability can be lessened by the use of low moisture content.

The effect of pH on color stability is more complex, and is shown in Figure 3. These data indicate that at pH 6.5 the samples darken faster (slope is steeper), although at pH 7.5 the samples were much darker after the 8 hr required for rehydration and sample preparation. The samples with higher pH experienced a big decrease in L value during the rehydration process (which involved heating to 60°C).

In a related experiment, the adverse effect of pH on whiteness was also noted. Solutions of coconut skim milk were adjusted to selected pH values, dried, and color of dry product observed. Samples of coconut skim milk at pH values of 6.1, 6.8 and 7.5 were spray dried to give product with L values of 83.6, 79.3 and 77.3. Freeze drying the samples immediately after pH adjustment gave similar results. These data suggest

Table 2–Changes occurring between 4 and 31 days of storage, for paired samples

Parameter observed	Initial value[a]	Observed change[b]	Change required for significance
Stored at −20°C			
Standard plate count (colonies/g)	14,000	+2,000	2,000
Moisture (%)	35.1	−0.1	2.5
Brookfield viscosity (cp)	93,000	+16,000	21,000
Titratable acidity (meq/g)	0.074	+0.002	0.011
pH	7.09	−0.04	0.04
Hunter L value	28.1	−0.2	1.2
Hunter a value	6.0	−0.1	0.17
Hunter b value	9.3	−0.02	0.27
Stored at +23°C			
Standard plate count (colonies/g)	12,500	+800	1,900
Moisture (%)	33.6	−0.2	1.1
Viscosity (cp at 35°C)	89,000	+8,000	10,000
Titratable acidity	0.076	+0.006	0.008
pH	7.05	−0.06	0.02
Hunter L value	24.6	−3.5	1.1
Hunter a value	5.8	−0.2	0.15
Hunter b value	8.2	−0.8	0.3

[a] Each reported initial value is the average of at least eight samples. Each reported change is the average change for at least eight pairs of samples, with one of pair analyzed after 4 days and one after 31 days.
[b] Average change required for significance at 95% level, calculated from t test for paired samples.

Table 3–Color change as affected by exposure to light, sulfite addition and moisture content, for paired samples

Treatment	E[a]	Number of paired samples
No added sulfite	3.9	
0.03% Na_2SO_3	3.9	
difference[b]	0.0	4
37% Moisture (a_w = 0.84)	5.9	
31% Moisture (a_w = 0.79)	2.0	
difference[b]	3.9	6
Exposed to light	3.9	
Stored in dark	3.9	
difference[b]	0.0	8

[a] Average total color change after 1 month of storage at 23°C
[b] A difference of less than 0.8 units is not significant at the 95% confidence level.

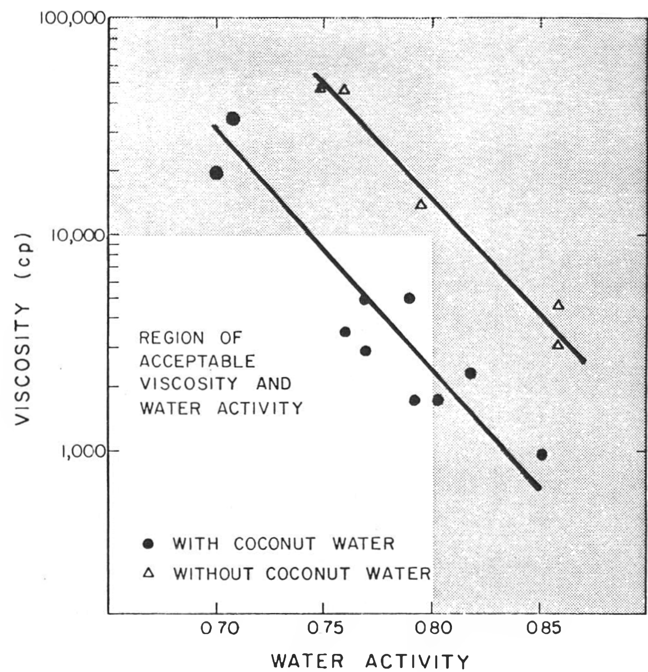


Fig. 2–Viscosity vs water activity. Viscosity and moisture contents were measured, and water activity was read from Figure 1. Samples were prepared by direct evaporation rather than reconstitution of spray dried samples. Viscosity was measured at 35°C; water activity at 23°C.

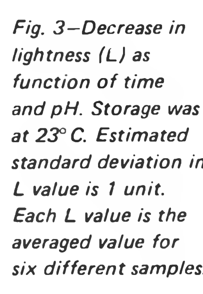


Fig. 3–Decrease in lightness (L) as function of time and pH. Storage was at 23°C. Estimated standard deviation in L value is 1 unit. Each L value is the averaged value for six different samples.

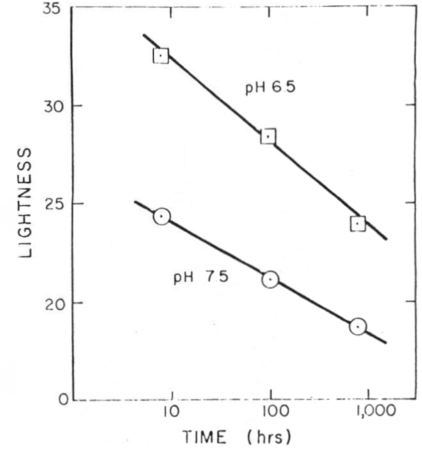


Table 4–Changes occurring during storage of coconut skim milk at 31–37% moisture

Parameter	Avg[a] initial value	Storage	Observed change	Change req for significance[b]
Protein solubility (%)	81	30 days, −20°C	−4	5
Protein solubility (%)	81	30 days, +23°C	−4	3
Reducing sugars, (%)	1.6	3 months, 23°C	+0.2	0.2
Available lysine (g/16g N)	3.9	3 months, 23°C	−0.3	0.4
Peroxide value (milli-equivalents/kg)	13	6 months, 23°C	+22	10
Free fatty acids (as % of oil)	3.3	6 months, 23°C	−0.1	0.7

[a] Initial value refers to spray-dried coconut skim milk
[b] Average change required for significance at 95% level, calculated from t test for significance, and based on average of at least two observations for both control and stored sample

Table 5–Effect of moisture contents on time elapsed until appearance of visible mold on samples of concentrate[a]

Moisture content	No. of samples	Average time (days)
28%	3	22
29%	3	29
31%	3	14
33%	2	11
35%	3	8
37%	5	8
39%	5	4

[a] Samples stored at 23°C. Concentrate includes coconut water solids and was made by direct evaporation. Mold count after inoculation was 80,000/g.

Table 6–Effects of variables on appearance of visible mold on inoculated samples of concentrate[a]

		Time[b] (days) until appearance of visible mold	
		Control (No sorbic acid)	Samples with 0.1% sorbic acid
35°C Storage			
pH	5.0	7	100+[c]
	5.9	7	100+[c]
	6.5	7	100+[c]
	7.2	7	19
23°C Storage			
pH	5.0	10	100+[c]
	5.9	10	100+[c]
	6.5	10	100+[c]
	7.2	10	16
4°C Storage			
pH	5.0	100+[c]	100+[c]
	5.9	100+[c]	100+[c]
	6.5	100+[c]	100+[c]
	7.2	100+[c]	100+[c]

[a] Coconuts were extracted with coconut water in making the concentrate. Made by direct evaporation (not reconstituted). Mold count after inoculation was 540,000/g.
[b] Each value is average for two samples
[c] No visible mold was observed at termination of experiment, which was after 100 days of storage.

that elevation of pH during processing be avoided if possible. Protein precipitation would best be avoided by avoiding excess heating, rather than by elevation of pH.

Other parameters related to storage stability are reported in Table 4. The observations reported in Table 4, unlike those in Tables 2 and 3, measure differences between spray dried coconut skim milk and stored concentrate. For control observations (Table 4 only) the samples were not rehydrated to form a concentrate before analysis. The stored samples were concentrates made by rehydration.

The data in Table 4 indicate that at 23°C storage reduction in both protein solubility and reducing sugars, and also increase in peroxide value, were significant at the 95% level.

Reduction in available lysine was significant at the 90% level. A reduction in available lysine would suggest that the color change was a Maillard browning.

The rise in peroxide value is considered a problem. Although the samples did not have a rancid odor, the peroxide value is probably approaching the danger point. Use of an antioxidant may be necessary.

Since the changes reported in Tables 2–4 refer to concentrates made by rehydration of spray-dried product, they do not necessarily give quantitative indications of changes that will occur during storage of concentrate made by direct evaporation. However, the data are assumed to give a valid picture of the potential problems to be expected upon storage of intermediate moisture coconut skim milk.

The most serious problem encountered upon storage of coconut skim milk concentrate was the growth of wild, unidentified mold on an occasional sample, although no growth was observed in the samples analyzed during the storage experiment described.

Samples of concentrate at moisture contents of 28 to 39% were inoculated with mold and the samples observed daily for visible mold growth. The results, shown in Table 5, indicate that mold will grow on contaminated samples at 28–39% moisture, although with slower rates at lower moisture content.

Prevention of mold contamination would of course solve the mold growth problem. However, this approach would probably involve use of expensive packaging, which would seem to defeat the purpose of considering an intermediate moisture product (the purpose being to economize).

Therefore, the use of sorbic acid as a mold inhibitor was examined. Because the normal, unadjusted pH of the concentrate (pH 6.0–6.5) is near the upper limit of effectiveness for sorbic acid, samples were prepared with different pH values. The results, shown in Table 6, indicate that at pH 5.0–6.5, sorbic acid (0.1%) is effective in controlling mold growth on concentrated coconut skim milk at 30% moisture. Refrigerated storage is also effective.

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QUALITY OF FOODS AFTER COOKING IN 915 MHz AND 2450 MHz MICROWAVE APPLIANCES USING PLASTIC FILM COVERS

INTRODUCTION

UNEVEN HEATING of foods during microwave cooking has been recognized as a problem by Copson (1962), Goldblith and Pace (1967) and Van Zante (1968). Both Fenton (1957) and Copson (1962) suggested that use of a cover would retain steam during cooking and minimize uneven heating. The purpose of this study was to examine the effect of use of plastic film covers during microwave cooking on the quality of cooked foods and to determine convenience and hazards associated with their use.

EXPERIMENTAL

A TOTAL of 37 foods, including appetizers, entrées, vegetables, desserts, breads and snacks were used in the study. Foods were prepared in three to four serving size units and cooked in 915 MHz (700 watt) and 2450 MHz (600 watt) appliances. Glass or glass ceramic containers were used; Saran Wrap film was used as the film utensil cover. For tests of hazardous characteristics associated with use of film utensil covers, Saran Wrap, Handi-Wrap and Stretch'n Seal films were used.

Quality-characteristics of appearance, color, fork texture, mouthfeel, moistness and flavor were determined by a sensory evaluation panel of seven experienced judges, using the paired comparison test.

Uniformity of doneness was determined in five foods by making temperature measurements in five locations in foods following microwave cooking in 915 MHz and 2450 MHz appliances. Cooking losses observed in two meats included total and drip losses using the method reported by Kylen et al. (1961). Reduced ascorbic acid, thiamine, and riboflavin content of two foods was measured. The method of Loeffler and Ponting (1942) was used for reduced ascorbic acid; the thiochrome method of the AVC (1966) for thiamine, and the fluorometric method of the AVC (1966) for riboflavin were used. Convenience and hazardous characteristics associated with the use of film covers in microwave cooking observed included time required to cook, evidence of oven spattering, rupture, burning, swelling, melting, and shrinkage of film during cooking, and ease of removal of film after cooking.

RESULTS & DISCUSSION

RESULTS of sensory comparisons of quality-characteristics of foods cooked with and without Saran Wrap film cover in 915 MHz and 2450 MHz microwave appliances are found in Tables 1 and 2. Most of the foods cooked in 915 MHz and 2450 MHz appliances with Saran Wrap covers were rated as better in one or more quality characteristics when compared with the same foods cooked without film cover. Quality characteristics which were better for the greatest number of different foods were mouthfeel when the 915 MHz appliance was used and fork texture when the 2450 MHz appliance was used. Flavor and color were also frequently noted as being better when a film cover was used. This indicated that improvement in textural quality of foods cooked with a Saran Wrap cover in both 915 MHz and 2450 MHz microwave appliances was perceptible to the taste panel.

Temperatures of five foods following 915 MHz and 2450 MHz microwave cooking with and without film cover are found in Table 3. Cooking times were the same for foods cooked with and without film covers. Use of Saran Wrap film had no effect on the temperature in the center of chicken when cooked in a 915 MHz appliance indicating the ability of this wavelength of microwave to penetrate foods deeply. In contrast, use of Saran Wrap covers made a large difference in the temperatures in other locations when chicken was cooked in both 915 MHz and 2450 MHz appliances, as well as in the center temperature in chicken cooked in a 2450 MHz appliance. In eggs, soup, peas and cake, Saran Wrap covers resulted in similar temperatures in the center of foods cooked in both 915 MHz and 2450 MHz appliances and except for peas cooked in the 915 MHz appliance resulted in similar small temperature ranges in other locations in foods studied. The temperature ranges observed, especially in chicken, would be sufficient for the product to be considered unevenly cooked.

Results of cooking losses observed in chicken and meatloaves cooked in 915 MHz and 2450 MHz appliances are found in Table 4. Use of a Saran Wrap cover during 915 MHz and 2450 MHz microwave cooking of chicken caused a significant ($P < 0.025$) reduction in total cooking losses in chicken. However, the use of a Saran Wrap cover had no statistically significant effect on total cooking losses in 915 MHz and 2450 MHz

Table 1—Sensory comparisons of quality of foods cooked with and without Saran Wrap cover in 915 MHz microwave appliance

	Quality characteristics rated better when cooked with cover by 50% or more of panel						
Foods	Appearance	Color	Fork texture	Mouthfeel	Moistness	Flavor	Overall pref.
Egg omelet	X	X		X	X	X	X
Haddock	X	X	X	X			
Macaroni-cheese		X	X				
Meatloaf	X	X		X	X	X	X
Pork loin	X	X				X	
Stuffed pepper	X	X	X		X		
Turkey	X	X		X	X	X	X
TV dinner			X	X	X	X	X
Corn chowder	X	X	X	X			
Vegetable soup			X	X			
Baked beans		X		X	X		
Corn on cob						X	
Potatoes au gratin		X	X	X	X	X	X
Baked apple	X		X	X	X	X	X
Cake				X	X	X	X
Cherry pie				X			
Coffee cake	X	X	X	X	X	X	X

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cooked meatloaves. Use of a Saran Wrap cover during 915 MHz and 2450 MHz microwave cooking of chicken had no significant effect on drip losses but meatloaves covered with a Saran Wrap cover showed significantly greater drip losses ($P < 0.001$) than meatloaves cooked in microwave appliances without Saran Wrap covers.

Mean values of reduced ascorbic acid and thiamine in peas and riboflavin in chicken after cooking in 915 MHz and 2450 MHz microwave appliances with and without Saran Wrap covers are found in Tables 5 and 6. Use of a Saran Wrap cover during 915 MHz and 2450 MHz microwave cooking had no significant effect on the reduced ascorbic acid, thiamine or riboflavin content of cooked peas and chicken.

Time savings were realized in selected foods as a result of use of Saran Wrap covers. Results of time measurements in foods cooked in 915 MHz and 2450 MHz appliances with and without use of Saran Wrap covers are reported in Table 7. Use of Saran Wrap covers resulted in reductions of total cooking times from 1–10 min.

Table 2—Sensory comparisons of quality of foods cooked with and without Saran Wrap cover in 2450 MHz microwave appliance

	Quality characteristics rated better when cooked with cover by 50% or more of panel						
Foods	Appearance	Color	Fork texture	Mouth-feel	Moist-ness	Flavor	Overall pref.
Bacon	X	X			X	X	X
Chicken	X	X	X	X		X	X
Eggs scrambled	X	X	X	X			
Egg omelet	X	X	X		X	X	X
Perch	X	X		X		X	X
Salmon							
Haddock				X	X		
Hamburger	X	X				X	
Macaroni-cheese	X	X	X	X	X	X	X
Meatloaf	X	X	X	X	X	X	X
Pizza			X	X			
Pork loin	X	X				X	
Shrimp	X		X	X	X	X	X
Spaghetti	X						
Stuffed pepper			X	X	X	X	X
Turkey	X	X	X	X	X	X	X
TV dinner	X	X	X	X	X	X	X
Chicken soup		X	X	X		X	X
Corn chowder	X	X	X	X		X	X
Vegetable soup	X	X	X	X		X	X
Baked beans	X	X	X	X	X	X	X
Baked beans/franks			X	X			
Corn on cob	X		X	X	X	X	X
Peas	X	X	X	X	X	X	X
Lima beans			X				
Potatoes au gratin			X	X	X	X	X
Spinach	X	X	X	X	X	X	X
Baked apple	X	X	X	X		X	X
Cake	X		X		X		
Apple pie		X				X	
Cherry pie							
Pudding		X	X				
Coffee cake maple	X	X	X	X	X	X	X
Coffee cake blueberry			X	X	X	X	X
Popcorn	X	X	X	X	X		X

Table 3—Temperatures of foods following 915 MHz and 2450 MHz microwave cooking with and without Saran Wrap cover

				Temperature	
Food	Cooking appliance	Film cover	Cooking time (min)	Center (°C)	Other positions (°C)
Chicken	915 MHz	Saran Wrap	7	92	76–94
	915 MHz	No cover	7	92	49–93
	2450 MHz	Saran Wrap	7	94	82–99
	2450 MHz	No cover	7	74	51–94
Eggs scrambled	915 MHz	Saran Wrap	3	94	80–85
	915 MHz	No cover	3	94	80–88
	2450 MHz	Saran Wrap	2	84	85–94
	2450 MHz	No cover	2	80	80–94
Chicken soup	915 MHz	Saran Wrap	5	62	71–85
	915 MHz	No cover	5	58	71–88
	2450 MHz	Saran Wrap	5	60	76–94
	2450 MHz	No cover	5	64	66–94
Peas	915 MHz	Saran Wrap	6	95	82–96
	915 MHz	No cover	6	96	49–94
	2450 MHz	Saran Wrap	6	99	82–99
	2450 MHz	No cover	6	99	94–99
Cake	915 MHz	Saran Wrap	5	88	80–88
	915 MHz	No cover	5	92	74–88
	2450 MHz	Saran Wrap	4	83	76–90
	2450 MHz	No cover	4	88	74–88

Table 4—Cooking losses in chicken and meatloaves following 915 MHz and 2450 MHz microwave cooking with and without Saran Wrap covers

			Cooking losses	
Food	Cooking appliance	Film cover	Total (%)	Drip (%)
Chicken	915 MHz	Saran Wrap	18	14
	915 MHz	No cover	24	13
	2450 MHz	Saran Wrap	18	11
	2450 MHz	No cover	24	9
Meatloaves	915 MHz	Saran Wrap	26	22
	915 MHz	No cover	25	17
	2450 MHz	Saran Wrap	23	9
	2450 MHz	No cover	27	7

Table 5—Reduced ascorbic acid and thiamine content of peas cooked in 915 MHz and 2450 MHz microwave appliances with and without Saran Wrap covers

Cooking appliance	Film cover	Reduced ascorbic acid (mg/100g)	Thiamine (mg/100g)
915 MHz	Saran Wrap	17.5	0.23
915 MHz	No cover	20.3	0.25
2450 MHz	Saran Wrap	18.8	0.22
2450 MHz	No cover	17.4	0.24

Table 6–Riboflavin content of chicken cooked in 915 MHz and 2450 MHz microwave appliances with and without Saran Wrap covers

Cooking appliance	Film cover	Riboflavin (mg/100g)
915 MHz	Saran Wrap	0.16
915 MHz	No cover	0.15
2450 MHz	Saran Wrap	0.16
2450 MHz	No cover	0.15

Foods cooked in the 2450 MHz appliance without film covers caused oven spattering. The foods included bacon, chicken, fish, hamburgers, lambchops, meatloaf, pork, steak, stuffed peppers, baked beans and baked apples. Use of Saran Wrap film covers during cooking reduced or eliminated oven spattering in these foods. No oven spattering was observed during 915 MHz microwave cooking of any foods.

Hazardous characteristics observed during cooking for 37 foods in 915 MHz and 2450 MHz appliances with film covers of Saran Wrap, Handi-Wrap, and Stretch'n Seal included rupture, burning, swelling, melting and shrinkage during cooking, and difficulty in removing the film after cooking. The number of occurrences of hazards for each film used were 60 out of 192 uses for Saran Wrap, 73 out of 75 uses for Handi-Wrap and 97 out of 30 uses for Stretch'n Seal. Using Friedman's test of significance, Saran Wrap exhibited significantly fewer ($P < 0.05$) hazards than did Handi-Wrap. Saran Wrap and Handi-Wrap each exhibited significantly fewer ($P < 0.001$) hazards than did Stretch'n Seal, which frequently showed swelling, melting and shrinkage, and was always difficult to remove after cooking.

Table 7–Time savings in 915 MHz and 2450 MHz microwave cooking with and without Saran Wrap covers

Food	Cooking appliance	Cooking time (min)		Time saved (min)
		Saran Wrap	No cover	
Beef stew	915 MHz	188	189	1
	2450 MHz	192	193	1
Chicken	915 MHz	7	9	2
	2450 MHz	7	10	2
Perch	2450 MHz	5	9	3
Lambchop	2450 MHz	4	6	2
Meatloaf	915 MHz	18	21	3
	2450 MHz	21	23	2
Pork loin	915 MHz	18	24	6
	2450 MHz	18	24	6
Spaghetti	2450 MHz	7	8	1
Turkey	915 MHz	70	70	0
	2450 MHz	68	78	10
Vegetable	915 MHz	25	25	0
soup	2450 MHz	25	20	5
Spinach	2450 MHz	3	4	1
Cake	915 MHz	5	6	1
	2450 MHz	4	5	1
Pudding	2450 MHz	5	6	1

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A LABORATORY PROCEDURE FOR THE PRESSURE PROCESSING OF FLEXIBLE POUCHES

INTRODUCTION

THE FLEXIBLE POUCH as a container for thermally processed low acid foods has a development history of about 20 yr. The prime mover in this effort has been the U.S. Army Natick Laboratories whose in-house and contract research has provided the major impetus and resulted in systems which meet military and space packaging requirements as well as having promise of commercial feasibility (Davis et al., 1972; Brody, 1971). Despite the pioneering successes in military feeding, industrial uses of retortable pouches seem more advanced in Europe and Japan than in the U.S. (Anon., 1973).

Although pouch laminate stock and the package seal are quite rugged at ambient temperatures, their strength is reduced considerably at process temperatures. During cooling, when the content temperature and consequent vapor pressure exceeds retort pressure, the pressure differential can cause seal failure or delamination. The presence of excessive head space gas accentuates pouch swelling and bursting (Whitaker, 1971). Thus overriding air pressure which compensates for internal pouch pressure is generally provided over the entire steam or hot water process cycle, and refinements for assuring reliable override in batch or continuous systems have been important contributions to pouch processing technology (Turtle and Alderson, 1971; Davis et al., 1972).

The exceptions to this increasing process complexity were several studies in India; first using a breathable (cotton plugged) pouch to avoid internal pressure build-up (Kannur et al., 1968) and later the use of steam with overriding air only during cooling (Kannur et al., 1972). The foods processed were presumably conduction heating products with a minimum of free moisture, although heat penetration details were not disclosed.

In view of the increasing interest in, and the present availability of retortable laminates and the obvious utility of the flexible pouch, particularly in circumstances where rigid containers are unavailable or prohibitively expensive, an attempt was made to simplify flexible pouch processing to the point where it could be performed on a pilot-scale without specialized equipment or controls. This report deals with the development of the simplest pouch processing procedure consistent with maintenance of pouch integrity.

EXPERIMENTAL

A POLYESTER-aluminum-nylon laminate (Goldfarb, 1970; 1971) furnished by Reynolds Metals Co. was used to make 17.5 × 20.0 × 2.5 cm pouches with a capacity of about 300g of food. The pouches were filled manually with product at ambient temperature, air spaces were worked out of the filled pouch and the seam area was wiped clean and dry prior to sealing. Side seams and the final seal were made with an M & Q vacuum sealer (used without vacuum) which under about 7 kg pressure for 5 sec delivered a 5 amp impulse for 1.5 sec along a 37 × 0.3 cm sealing wire. A second seal was made adjacent to the first, giving a seam thickness of 0.6 cm. The two food systems selected for studying conduction and convection heating characteristics, respectively, were vegetable soybean paste (70% moisture content) and vegetable soybeans in 2% NaCl brine (bean:brine, 1:1).

A No. 2 Dixie Canning Co. vertical retort was used for the heat processing studies. A horizontal rack with 2.7 cm-spaced 12 gauge stainless steel perforated (2 mm holes on 4 mm centers) shelves was used to hold the pouches in the retort. The pouches were placed on every other shelf to ensure proper circulation of the heating medium.

The retort used is shown schematically in Figure 1. The heat processing procedure observed was as follows:

With valves (1) (2) (5) closed and (6) (8) (9) (10) (14) open the steam (1) was turned on. The bleeder, overflow and drain valves were kept open until all air was purged from the retort, usually 2–3 min, and

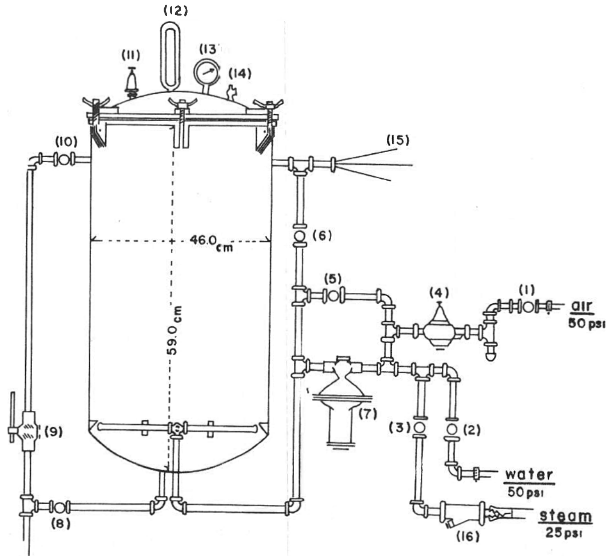

Fig. 1—Experimental retort:

(1) AIR VALVE
(2) WATER VALVE
(3) STEAM VALVE
(4) AIR PRESSURE REGULATOR
(5) STEAM BYPASS VALVE
(6) VALVE
(7) STEAM PRESSURE REGULATOR
(8) DRAIN VALVE
(9) OVERFLOW QUICK OPENING VALVE
(10) OVERFLOW VALVE
(11) SAFETY VALVE 30 PSI
(12) MERCURY THERMOMETER
(13) PRESSURE GAUGE
(14) BLEEDER
(15) THERMOCOUPLE-RECORDER WIRES
(16) FULL-FLOW Y STRAINER

then (9) and (8) were closed. The bleeder was kept half open during the heating cycle. At the end of the process time, the steam was turned off, by-pass opened and the air valve was opened slowly, overriding by 1 or 2 psi the processing pressure (15 or 25 psi). Steam was purged from the retort for about 2 min, while the cooling water valve was slowly opened. The bleeder was closed and the air pressure accurately controlled with the regulator valve. Once the water level reached the overflow, the air valve was closed and override pressure maintained by the quick opening valve. When the temperature at the slowest cooling point in the pouch had dropped below 100°C, the pressure was slowly released with the quick opening valve. The water was shut off, the bleeder fully opened, and the drain valves fully opened.

To obtain the heat penetration data, insulated copper-constantan thermocouples attached to a 4-point Leeds & Northrup Speedomax H recorder were inserted in the geometric center of the pouches through a packing gland. The heat penetration characteristics were determined for each food system studied at both 15 and 25 psi steam (121° and 131°C). An F_0 value (equivalent time at 121°C) of 10 min, representative of many nonacid food products (Turtle and Alderson, 1971; Heid and Joslyn, 1967) was used for the process calculations using the graphical method (NCA, 1968; Stumbo, 1968). Heat penetration data were converted into lethal rates based on the formula:

$$\text{lethal rate} = 1 \,/\, \text{antilog}\,[(250 - ct) \,/\, Z]$$

where: CT = container temp at the geometric center; 250°F = reference temp; and Z = 18F°.

RESULTS & DISCUSSION

A 300g (10.6 oz) PORTION was chosen to check the behavior of a larger pouch and for closer comparison with common can volumes. In other reports, pouch fills ranged from about 80–230g (3–8 oz) with 4.5–6 oz being the most widely cited range; thicknesses varied from 1.0–2.5 cm.

In preliminary experiments, attempting to completely avoid use of compressed air, pouch rupture was extensive, even when smaller pouches containing soy paste were used and retort pressure was reduced to 10 psi (116°C). This rupturing was induced during cooling, since it could be prevented by the impractical step of allowing the retort pressure to drop very slowly without venting or the addition of cooling water.

When the use of overriding air pressure during cooling proved unavoidable, it was necessary to modify the retort only by adding an air pressure control valve (4) and a quick opening valve (9) (Fig. 1). Air pressure served (1) to purge the retort of steam prior to admitting cooling water, thus preventing a sudden pressure drop due to condensation; and (2) to provide a pressure 1–2 psi over the process pressure. This slight overriding air pressure proved adequate, although previous investigators have recommended 5–10 psi (Gould and Geisman, 1962; Pflug et al., 1963; Goldfarb, 1971). The quick opening valve in the overflow line was essential in order to rapidly reduce the pressure surge experienced when the retort filled with water. Once a satisfactory cooling regime was worked out for 15 psi steam pressure it was extended to 25 psi and found to work about as well.

Visual inspection of the pouches immediately after processing showed that the laminate and seal could withstand the treatment if a 1–2 psi override was maintained during cooling. As a general rule, when pressure differential tolerances were accidentally exceeded, pouch leakage invariably occurred at the seal and not on the laminate surface. Seal formation was as critical an operation as pressure control. Seals upon which food particles or brine encroached were defective. Thus, extreme care was required to fill and seal pouches correctly. About 40 sec were required for an experienced operator to fill, seal and inspect each pouch.

The processing method described has the advantages over steam-air mixtures that control of pressure and of media circulation and mixing is unnecessary. The water with superimposed air pressure method has the disadvantage that additional time and energy are required to heat the water at the start of the process and to cool it at the end of the retort cycle. After the retorting procedure had been refined, out of approximately 200 pouches used in this study, plus about another 100 containing a variety of foods, none showed process-induced leakage. However, such low volumes cannot generate the sufficient process reliability data for comparing this process to other prototype systems.

In keeping with the stated goal of maximum process simplicity, the only further retort improvements suggested would be an overhead spray cooling system which should improve cooling efficiency, particularly if the pouches were retained vertically and exposed to a film of cooling water. Futher modifications entailing automatic control devices were deemed outside the scope of this study.

The heat penetration data obtained at 15 and 25 psi (Fig. 2 and 3, respectively) show a faster heat transfer rate for the soybean in brine compared with soybean paste. Process times necessary to achieve an F_0 of 10 min are presented in Table 1. The advantage of being able to process pouches in steam at 25 psi instead of 15 psi is evident from Fig. 4. 1 min at 131°C is about equivalent to 6.4 min at 121°C. Thus, if the contents adjacent to the pouch walls can tolerate the more severe heat treatment to which it is subjected, the center temperature needs to reach only about 125°C prior to the initiation of cooling. The time to steam-off for soybean paste at retort temperature of 131° and 121°C was estimated to be 14 and 25 min, respectively (Table 1). Inspection of the product processed at both temperatures revealed good product color without any overcooked flavor.

Pouch heat penetration characteristics of soybean paste are compared with data obtained for the same product packed in No. 303 cans and processed in the same retort (Fig. 5). The flexible pouch shows a much faster heat penetration rate. The 300g content of a pouch is a slab approximately 10 cm wide, 12 cm long and 2.5 cm thick. A cylindrical can that contains an equivalent weight of product is approximately 6.5 cm in diameter and 8.5 cm high. To further reduce process times, it would be possible to decrease the pouch thickness. Pflug et al. (1963) demonstrated a threefold reduction in process times necessary to achieve an F_0 of 9 min with conduction heating

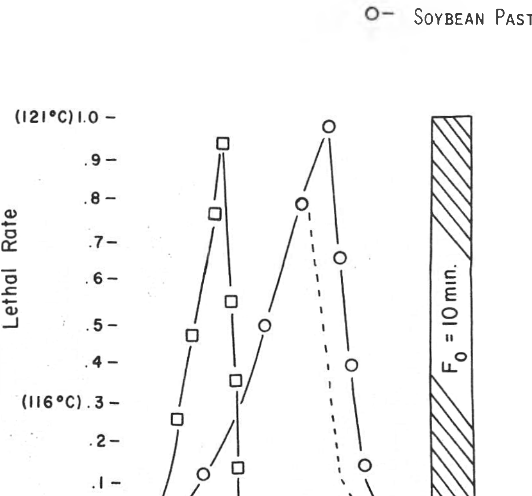

Fig. 2—Lethal rate determinations at 15 psi, retort pressure (121°C).

foods simply by reducing the pouch thickness from 1.00 to 0.25 in. This would, of course, be at the expense of increased handling of a larger number of smaller packages in order to process a given amount of product.

Some experimental work was done on the feasibility of using transparent plastic film pouches made from 3 M Co. "Scotchpack" brand oven film. These were sealed and retorted as described for the laminate pouches. Besides the advantages of the flexible package, the transparent plastic container would contribute eye-appeal (Hu and Nelson, 1955). The pouches withstood the retort treatment at 15 psi well. However, at 25 psi seal failure occurred. The application of microwave processing would be possible with the use of plastic pouches and overriding air pressure. However, no completely satisfactory transparent material has yet been developed, gas permeability being the main probelm (Ayoub et al., 1974).

Preformed polyester-aluminum foil-polyolefin 6 x 7 x 1/2 in, pouches furnished by Continental Can Co. (Davis et al., 1972) were evaluated with respect to the heat resistance of this pouch stock under similar experimental conditions. In limited trials these pouches withstood the process well at 15 psi and 25 psi without signs of delamination or leakage.

It is admittedly more difficult and time consuming to make and fill a pouch, carefully reduce the head space, seal it and go through the retorting procedure described, than to pack the same product in a jar or can and process it routinely. In this country we take for granted the availability of various can and jar sizes for experimental processing. For specialized application, the appropriate container is usually no farther away than a phone call. However, in developing countries cans or jars are frequently difficult to obtain in the proper size or type, if at all, and are invariably quite expensive. A few rolls of retortable pouch laminate could make the difference between being able to readily conduct processing studies and either foregoing such experiments entirely or experiencing lengthy waits for a special shipment of the required container. Sheets of retortable pouch stock about the size and weight of this journal would be

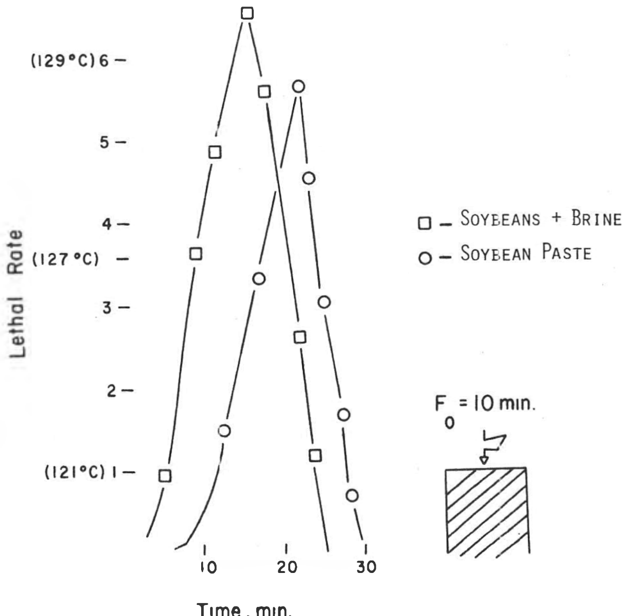

Fig. 3–Lethal rate determinations at 25 psi retort pressure (131°C) 300g pouches.

Table 1–Process time for the food system studied

Food system	Process time[a] at retort temperature of: 121°C (15 psi)	131°C (25 psi)
Soybean paste	25	14
Soybeans in brine	18	7

[a] Min from reaching retort temperature until steam shut off

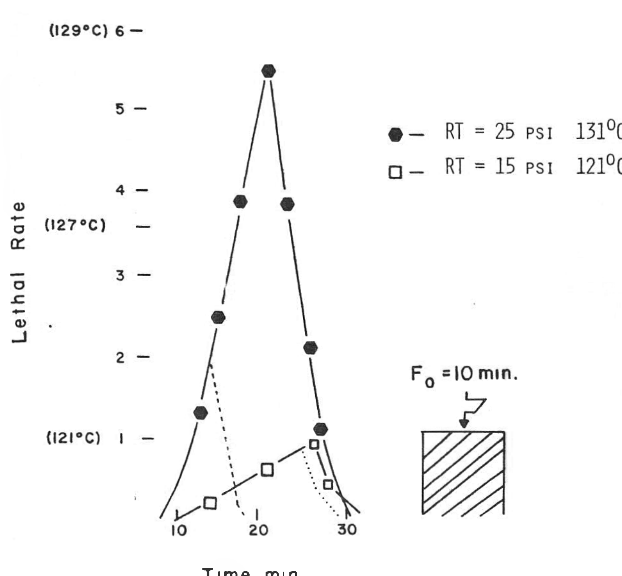

Fig. 4–Lethality comparison at 15 and 25 psi, 300g soybean paste in pouch.

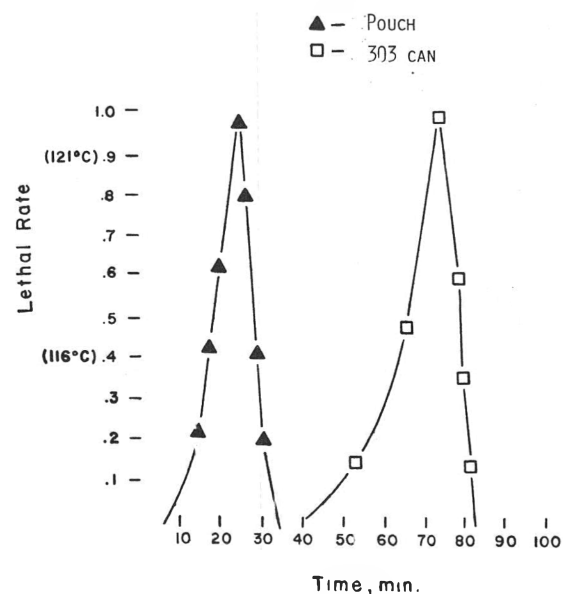

Fig. 5–Comparison between soybean paste in 300g pouch and 303 X 409 can at 15 psi retort temperature.

sufficient to package approximately 100 8 oz packages. In contrast, jars or cans necessary to hold the same quantity would require over 8 cases of 1/2 pt containers with a volume around 2 ft^3.

The versatility, simplicity and eventually lower cost features of pouches make the packaging system a promising alternative to rigid containers in low throughput and laboratory process applications. This processing technique is useful in conducting exploratory studies and preparing samples preliminary to investing in sophisticated prototype or industrial systems.

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AN AUTOMATED CONTINUOUS PROTEIN ANALYZER: MODIFICATION OF THE LOWRY METHOD

INTRODUCTION

IN OUR RESEARCH on increasing the efficiency of automated cleaning and sanitizing systems for food processing plants, aqueous cleaning solutions must be analyzed for changes in concentrations of proteins with time. We needed an automated protein analysis system to provide continuous measurements of the amount of proteins in solutions as a function of time of cleaning. We chose to modify the Lowry method, which was previously automated (Huember and Lee, 1970). The Lowry method is 10–20 times more sensitive than quantitations by absorption at 280 nm and is 100 times more sensitive than the biuret reaction (Lowry et al., 1951). This paper reports our modification and the results of its use in conjunction with logarithmic dilution and logarithmic concentration.

EXPERIMENTAL

Materials

The compositions of the reagents used were as follows: Reagent A contained 10% (percentages are w/v) sodium carbonate in 5.0% sodium hydroxide and distilled water. Reagent B contained 0.5% cupric sulfate in 1.875% sodium or potassium tartrate and distilled water; this reagent was prepared fresh weekly. Reagent C was prepared daily by mixing 48 ml of reagent A with 4 ml of reagent B. Reagent D consisted of one part Folin reagent diluted with two parts distilled water.

Bovine serum albumin (fraction V), casein (Hammersten Quality), egg albumin (2X crystallized), gluten and two soy protein fractions were obtained from commercial sources. Bovine serum albumin was dissolved in distilled water. Egg albumin was dissolved in 0.1M NaCl. Gluten was dissolved in 100 ml of 10.0% NaOH and 5.0% Na_2CO_3, and the mixture was diluted to 500 ml with distilled water. Erroneously high absorbency readings were obtained when gluten was dissolved in only 10.0% NaOH solution. Soy proteins and casein were dissolved in 0.1% aqueous Na_2CO_3. All stock solutions of proteins contained 250 μg/ml of the commercial material. Protein concentrations, as determined by micro-Kjeldahl, were as follows: bovine serum albumin, 92.5%; casein, 90.0%; egg albumin, 93.5%; gluten, 86.1%; soy protein N, 95.0%; and soy protein Supro 610, 95.0%.

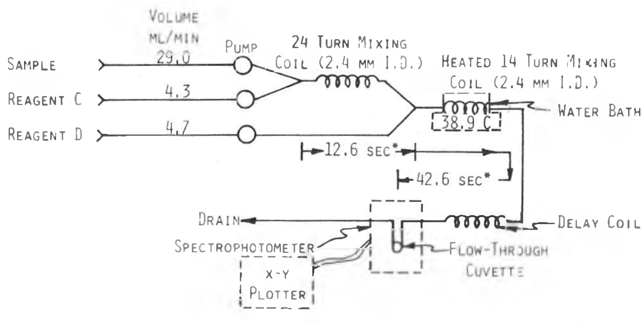

Fig. 1—Schematic diagram of protein analyzer.

Equipment

A schematic diagram and specifications of the protein analyzer are shown in Figure 1. The pumping apparatus consisted of three Masterflex pumps connected to a synchronous motor (30 rpm). The spectrophotometer (Model 20, Bausch and Lomb, Rochester, New York) was equipped with a locally fabricated flow-through cuvette (volume 1.1 ml). The response of the spectrophotometer (mv) was measured with an x-y plotter.

Procedure

The "Logarithmic Dilution" method (MacMullin and Weber, 1935) was used to change the concentration of protein in solutions analyzed by the unit to test its ability to monitor protein continuously. Assuming instantaneous and thorough mixing, the following equation (Yates and Ashton, 1960) describes the decrease in concentration of protein in an aqueous solution when water is added at a constant rate:

$$Ln\frac{X_o}{X_i} = \frac{Q}{V}\,(T) \qquad (1)$$

Where X_o = concentration of protein (μg/ml) in container at time T; X_i = initial concentration of protein (μg/ml) in container; Q = flow rate into or from container, ml/min; V = volume of container, ml; and T = time, minutes.

The following rearrangements of terms permit the determination of time required to reach a specific concentration of protein in solution (logarithmic dilution curve):

$$T = Ln\frac{X_o}{X_i}\cdot\frac{V}{Q} \qquad (2)$$

The following rearrangement of terms permits the determination of tion of protein in an aqueous solution when a solution containing a higher concentration of protein is added at a constant rate (Danckwerts, 1953):

$$X_o = X_F\,(1 - e^{\frac{Q}{V}T}) \qquad (3)$$

Where X_F = concentration of protein (μg/ml) flowing into container.

The time required to reach a specific concentration of protein in a container can be determined after the following rearrangement of terms in equation (3) (logarithmic concentration curve):

$$T = \frac{V}{Q}\,[Ln\,(X_F) - Ln\,(X_F - X_o)] \qquad (4)$$

Standard curves for each purified protein were prepared. Times required to fully dissolve 250 μg/ml of each purified protein were determined. Aliquots of solutions were removed from the freshly prepared sample at varying intervals, and the percentage of transmittance at 748 nm was determined for each. Further experiments were performed only after proteins were completely dissolved as indicated by stabilization of transmittance readings.

Solutions containing 2.5, 12.5, 25, 50, 100, 150, 200 and 250 μg/ml of individual purified proteins were fed into the analyzer, and peak

instrument responses in transmittance at 748 nm were recorded. Heights of these peaks were used to determine the concentration of protein in the container with respect to time. Typical curves for 2.5, 50 and 250 µg/ml of bovine serum albumin are shown in Figure 2. An absorbency vs concentration curve was prepared from the transmittance peaks for each purified protein.

Distilled water was drawn at a constant rate into the flask containing 250 µg/ml of purified protein during each logarithmic dilution test. The volume of solution in the flask remained constant. A concentration of 250 µg/ml of purified protein was drawn into the flask at a constant rate during each logarithmic concentration test. Effluent from the flask was fed into the analyzer, and observed changes in concentration were compared with predicted (theoretical) changes in concentration.

Thorough mixing of reagent C and the protein solutions in the mixing coil was important. Preliminary studies in which a mixing time of about 1 sec was used produced straight curves, but slopes were too steep. Addition of a 24-turn mixing coil corrected this problem. Minimal mixing time for reagent C and protein solutions was about 13 sec.

After the addition of Folin reagent, the pH of the solution had to be 10 or above (Lowry et al., 1951). Below this value, the speed of color development decreased rapidly. The temperature at which the Folin reagent, protein and reagent C were mixed affected absorbency. Color developed best when the temperature of the mixture leaving the heated mixing coil was 33.3°C. Above 33.3°C gas bubbles formed in the tube. The temperature of the solution as it entered the flow-through cuvette was 32.8°C.

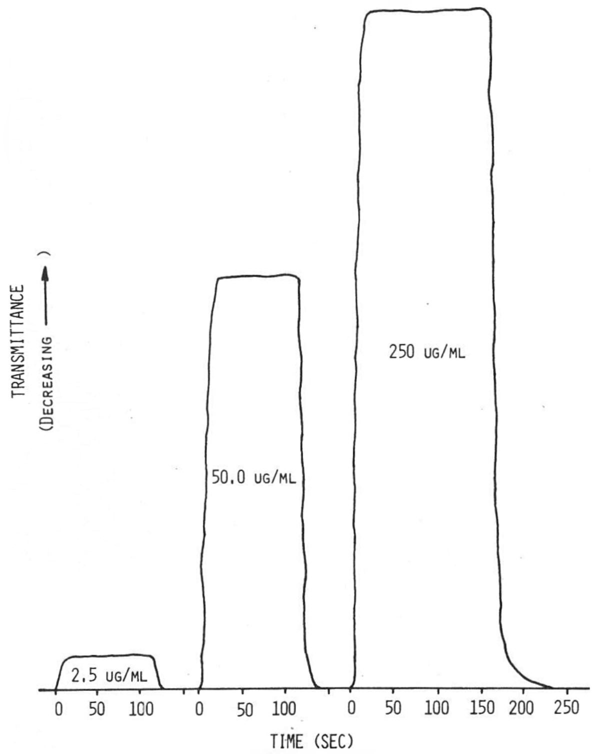

Fig. 2–Typical instrument responses to solutions containing 2.5, 50.0 and 250.0 µg/ml of bovine serum albumin.

RESULTS & DISCUSSION

MEANS and respective standard deviations for times to reach specific concentrations of protein, observed and predicted, during experiments wherein protein concentrations were continuously decreased or increased, are shown in Tables 1 and 2, respectively. Data collected in experiments with casein were plotted (Fig. 3). Curves representing dilution and concentration experiments with other proteins were similar. Data (Tables 1 and 2) indicated some delay in reaching predicted concentrations at given times. Average lag times and standard deviations are given in Table 3. These results suggested that small portions of samples were retained in the tubing of the apparatus or mixed within the cuvette, or probably both. In preliminary experiments, changes from relatively rough to relatively smooth tubing produced more rapid detection of expected concentrations. For maximum efficiency of analysis, retention of traces of sample in the tubing should be minimal. The 1.1 ml capacity of the cuvette and cuvette design, which made flow only semidirectional, would be expected to induce mixing. Furthermore, if each curve were moved toward its origin by the average lag time, the fit between the observed data and the predicted values would be excellent (Fig. 3). It was necessary to design the cuvette with both the inlet and outlet at the top to preclude interruption of the light path by air bubbles that entered the line as analyses were started.

Data in Figure 2 verify these conclusions. Approximate times required to reach 90% of full response after solution started to enter the cuvette were 6, 8 and 10 sec for solutions containing 2.5, 50 and 250 µg/ml of protein, respectively. When the introduction order for the samples was reversed, i.e., 250, 50 and 2.5 µg/ml, times to reach 90% of full response increased to 15, 12 and 10 sec, indicating that small quantities of protein were retained in the system and contaminated incoming solutions.

Absorbency vs concentration curves (Fig. 4) were curvilinear, indicating that the reaction did not follow Beer's law absolutely. The reaction did not go to completion since the

Table 1–Means and standard deviations of times required to reach specific concentrations of proteins during dilution (observed vs theoretical)

	Time (sec)[a] to attain protein concentration of											
	200 µg/ml		100		50		25		12.5		2.5	
Samples	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Theoretical[b]	47.0	–	192.9	–	338.8	–	484.75	–	630.7	–	969.5	–
Casein	51.0	5.5	202.4	7.3	348.2	8.3	488.6	15.1	632.2	17.6	969.5	42.5
Egg albumin	49.0	8.2	205.0	9.4	357.0	13.5	503.0	13.5	654.0	17.8	1005.0	21.5
Bovine serum albumin	39.2	1.1	192.4	2.5	342.0	4.5	485.0	10.0	634.0	11.4	977.0	14.8
Supro 610 modified soy	45.8	4.1	190.2	1.8	334.0	3.8	473.4	5.3	621.2	6.1	955.0	6.1
Soy protein N	44.6	4.6	191.6	7.8	339.6	9.0	480.6	11.5	628.6	14.2	977.6	19.5
Gluten	47.4	7.6	191.5	7.0	335.0	6.3	473.6	12.4	613.0	9.8	937.0	21.5

[a] Based on five observations
[b] Predicted values are for a flow rate of 28.5 ml/min. Actual flow rates for respective proteins were: 28.5–casein, 28.4–egg albumin, 28.9–bovine serum albumin, 29.3–Supro 610, 29.2–Soy protein N and 29.3–gluten.

Table 2–Means and standard deviations of times required to reach specific concentrations of proteins as concentration was increased (observed vs theoretical)

	Time (sec)[a] to attain protein concentration of											
	2.5 μg/ml		12.5		25		50		100		200	
Samples	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Theoretical[b]	2.1	–	10.8	–	22.2	–	47.0	–	107.5	–	338.8	–
Casein	5.4	0.4	16.4	3.1	26.6	3.0	52.2	4.9	112.1	2.0	330.2	8.0
Egg albumin	6.2	1.1	16.2	2.4	28.0	3.1	52.2	2.3	114.6	5.6	340.4	8.8
Bovine serum albumin	6.8	1.5	16.0	1.4	28.4	1.8	53.2	2.4	114.2	1.5	355.0	21.0
Supro 610 modified soy	6.4	3.1	16.0	1.2	27.2	1.9	50.8	1.5	111.0	1.0	330.1	5.8
Soy protein N	6.8	0.8	16.6	0.9	27.6	1.4	51.2	1.8	111.6	3.2	340.2	7.2
Gluten	6.0	1.0	15.0	1.4	26.2	1.5	49.5	1.9	108.0	4.1	318.2	10.9

[a] Based on five observations
[b] Predicted values are for a flow rate of 28.5 ml/min. Actual flow rates for respective proteins were: 28.5–casein, 28.4–egg albumin, 28.9–bovine serum albumin, 29.3–Supro 610, 29.2–Soy protein N and 29.3–gluten.

rate of color development depends on time, and time was limited. However, the same intensity of color developed for any given concentration of a particular protein. Differences in reactivity varied with type of protein. At concentrations of 250 μg/ml, absorbency was greatest for egg albumin and least for gluten. The absorbency vs concentration curve for Supro 610 (not shown in Fig. 4) was essentially the same as that for soy protein N.

Rates of solubilization of four proteins varied considerably (as determined by time required for absorbency to become constant in a mixture of protein and solvent). Casein and egg albumin hydrated completely within 10 min. It took from 15–30 min to hydrate gluten, and absorbency of the solution of soy protein Supro 610 stabilized only after 140 min, indicating that it was the least soluble of the four proteins tested. The data strongly suggest that change in absorbency with time is a good indicator of degree of hydration.

Fig. 3–Predicted and observed concentrations (μg/ml) of casein in solutions during dilution and concentration experiments.

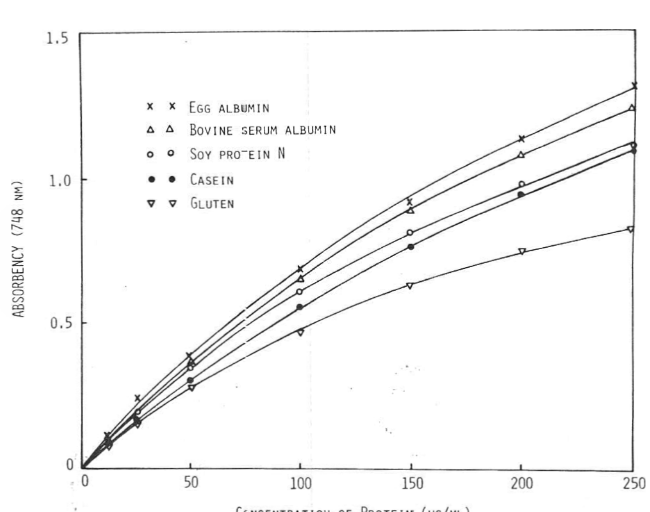

Fig. 4–Absorbency vs concentration for various purified proteins.

Table 3–Mean lag times and standard deviations for continuously diluting and concentrating six purified proteins

	Diluting	Concentrating
	Lag time (sec)[a]	
Protein	Mean S.D.	Mean S.D.
Casein	−7.1 ± 3.9	−5.5 ± 1.8
Egg albumin	−8.7 ± 9.5	−4.8 ± 1.9
Bovine serum albumin	−7.7 ± 6.2	−8.7 ± 5.4
Supro 610	−4.4 + 4.2	−4.5 ± 1.9
Soy protein N	−9.4 ± 8.2	−6.2 ± 1.2
Gluten	−7.7 ± 6.2	−6.8 ± 5.4

[a] Lag time = difference between time concentration was predicted and time it was observed.

We propose to use the modified Lowry method in tests to monitor concentration of protein in cleaning solutions from circulation-type (cleaned-in-place) systems in food plants. Preliminary experiments indicate that it will work well in milk processing plants. Concentrations of protein in cleaning solutions of milk plants are usually within the range of satisfactory operation of our apparatus.

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RUPTURE AND PROTEIN EXTRACTION OF PETROLEUM-GROWN YEAST

INTRODUCTION

ONE APPROACH to the ever-increasing need for protein is the production of single-cell protein (SCP). The term single-cell protein is used to designate food or feed materials produced from microorganisms grown on a wide variety of substrates from crude petroleum to industrial and agricultural wastes. One of the more exciting areas of SCP technology has been the production of food and feed from yeast grown on petroleum hydrocarbons.

British Petroleum (BP) started construction on a 100,000 metric ton/yr plant in 1973 and Amoco Food Co. now produces a yeast product using USP ethanol as a substrate. Today BP and several other large petroleum companies, both in Europe and the United States, are engaged in research and pilot plant production of a yeast biomass produced from petroleum.

The product of fermentation consists of whole yeast cells. The tough wall resists digestion and adds unnecessary bulk to the diet. If the whole cell is ingested, the cell may pass through the digestive tract intact and its protein content may not be available for use. Cell wall disruption is important to the release and solubilization of intracellular proteins Techniques evaluated were pressure release, sonic oscillation, homogenization and others. Pressure release involves the pressurizing of the cell mixture and allowing the continuous phase to penetrate the cell. After the continuous phase has penetrated the cell, the pressure is released instantly and the continuous phase expands as the pressure regains atmospheric pressure with the consequent rupture of the cell wall (Fraser, 1951). Sonic oscillation or sonification is a widely used method for rupturing of bacteria, but has had limited success with yeasts (Nickerson, 1963). Homogenization also has been used to rupture bacteria (Tannenbaum, 1966). One of the objectives of the presented research was to optimize conditions for cell rupture.

Specific problems with the use of SCP as a human food include: residual hydrocarbons; high levels of nucleic acids; and questionable nutritional value. The problem of nutritional value may be approached by separation of the protein by isolation techniques. Alkaline extraction of yeast cells to remove the protein has been accomplished in the past by the use of a 0.05N NaOH solvent (Huang and Rha, 1971; Aries, 1952) or a bicarbonate buffer, pH 9.5–11.0 (Tannenbaum, 1966; Heden, 1971). Objectives of the present research were to optimize the isolation of yeast protein and to determine the amino acid composition of the products produced in the isolation procedure.

METHODS & MATERIALS

Preparation of samples

A large sample of petroleum-grown yeast (Candida Tropicalis) was obtained from the Gulf R&D Company. The yeast was grown at 25°C on N-paraffins and harvested by centrifugation (Leather and Kinsel, 1972). Samples were received in a dry state and stored in a cold room (3–8°C). Proximate analysis of the yeast material appears in Table 1.

Disruption technique

Pressure release. The cell mixture was prepared by adding dried cell material to distilled water in the ratio of 1g per 10 ml of water. The resulting mixture was pH 5.8. The cell mixture was pressurized to 1500 psig. At the end of 30 min the chamber was depressurized, the sample collected and centrifuged at 6000 × G. The liquid was decanted, filtered and protein content was determined.

Sonic oscillation. Each sample was placed under the oscillator probe (Bronwill, Corp.) 12 times for a total sonification time of 3 min. Finally the samples were centrifuged and filtered for protein analysis.

Homogenization. Homogenization was accomplished with the Manton-Gaulin 15M-8TA laboratory homogenizer (Gaulin Corp.). When multiple homogenization was necessary, the sample was placed in an ice-water bath between runs to cool the homogenate to about 35°C.

Other rupture methods. Other methods for cell disruption include: freeze-thaw, stone-milling and manual grinding. Freeze-thaw was accomplished simply by freezing a 10% mixture of cell material in distilled water at −4°C for 24 hr. After freezing, the cells were allowed to stand at ambient temperature until thawed. Cell mixtures were also ground with a Morehouse stonemill (Morehouse-Cowles, Inc.) with a clearance of 0.005 in. between the stones. Dry cells, both 100% cells and 50% cells-50% sand, were ground with a mortar and pestle. After grinding, a known volume of distilled water was added and the contents of the mortar were washed into centrifuge bottles and centrifuged and decanted for protein determination.

Cell integrity determination

Initially, two basic techniques were used to determine the integrity of the yeast cell wall; nitrogen or protein solubility and microscopy. The basis for using the nitrogen or protein solubility is that there will be more soluble protein available when the cell wall has been broken and the solvent can enter the cell and solubilize the protein. Microscopy was used under the assumption that it would be possible to see the effect of a cell disruption technique visually and some sort of subjective measurement might be made. Most of the microscopy was done at 1000×. In later experiments, Gram's stain was used to determine cell rupture.

Table 1—Proximate analysis of petroleum-grown yeast[a] and fractions produced in protein isolation procedure

Fractions	Moisture (%)	Oil (%)	N (%)	Protein (%N X 6.25) (%)	Ash (%)	Crude fiber (%)
Yeast	7.1	4.97	8.6	53.6	8.56	4.89
Fraction 1 (cell residue)	4.54	0.26	5.7	35.6	4.99	12.59
Fraction 2 (alkaline extract)	4.72	0.44	8.6	53.8	28.06	0.09
Fraction 3 (protein isolate)	7.83	10.83	11.2	70.0	4.3	0.22
Fraction 4 (supernatant)	b	b	3.8	23.8	48.3	b

[a] Candida tropicalis Gulf R&D Co., Pittsburgh, Pa.
[b] Not determined

Solubility characteristics

In the solubility studies, both solubility of nitrogen and protein were determined, usually at 5–10 points over the pH scale. Solubility studies were conducted in the following manner:
(a) A 10% (w/v) mixture of cells was prepared.
(b) Sample preparation or treatment, if any.
(c) The sample was divided accurately into the appropriate number of centrifuge bottles and adjusted to the proper pH with 0.1N HCl or 0.1N NaOH.
(d) The samples were stirred with a magnetic stirrer for 30 min at ambient temperature.
(e) The pH was readjusted, if necessary, and the samples were centrifuged at 6000 × G for 10 min.
(f) The liquid was decanted, filtered and nitrogen and/or protein determinations were made.

Nitrogen and protein analysis

All nitrogen determinations on solid samples were accomplished by the Kjeldahl method. The percent protein was calculated by multiplying the percent nitrogen by 6.25. Liquid samples were often quantified by Lowry's modification of the Folin-Ciocalteau method for protein analysis (Lowry et al., 1951) and in some cases both methods were used. Since the Kjeldahl procedure measures nitrogen and the Lowry procedure measures protein, there is a basic difference in their measurements and all solubility studies herein are marked "% soluble nitrogen" or "% soluble protein" to indicate use of the Kjeldahl and Lowry method, respectively.

Protein isolation and nitrogen distribution

Protein isolation was accomplished by alkaline extraction and subsequent isoelectric precipitation of the protein fraction. Protein recovery was determined in this report by quantitative evaluation of the distribution of the nitrogen throughout the isolation technique. The procedure used was as follows:
(a) 500g of yeast material was used to form a 10% (w/v) mixture.
(b) The cell mixture was homogenized three times at 10,000 psig, adjusted to pH 10 and centrifuged at 6,000 × G.
(c) To the residue in the centrifuge bottle (Fraction 1), was added enough 0.1N NaOH to fill the bottle which was shaken, stirred and centrifuged again. This extraction was repeated five times.
(d) Except for a small aliquot for analysis, the extract (Fraction 2) was adjusted to pH 3.8, centrifuged and the precipitate (Fraction 3) freeze dried as was an aliquot of the supernatant (Fraction 4).

Table 2—Effect of multiple passes through the homogenizer at 10,000 psig

No. of passes through homogenizer	% Soluble protein
0	6.3
1	13.2
2	16.1
3	19.7
4	21.0
5	15.7

Table 3—Effects of other cell rupture techniques

Treatment	% Soluble Protein
None	5.0
Grinding (mortar and pestle)	5.8
Grinding with sand (mortar and pestle)	5.5
Freeze-thaw	6.5
Stone-milling only[a]	6.4
Freeze-thaw + stone-milling[a]	7.6

[a] 0.005 in clearance

Amino acid analysis

Amino acid analyses were performed with a Beckman Model 120°C analyzer. Samples were hydrolyzed in constant-boiling 6N HCl for 24 hr under nitrogen. The amino acid analyses were not corrected for losses due to hydrolysis. All four fractions from the protein isolation procedure were desalted after hydrolysis due to a high ash content. A desalting Ion Retardation Resin, AG 11A8 (Bio-Rad Laboratory) in a small (1 × 24 cm) column was used to desalt the hydrolyzed samples. The sample was eluted through a UV monitor to a fraction collector. Citrate buffer (pH 2.2) was added to the dried sample and a small sample (0.1 ml) was submitted for amino acid analysis. The remainder of the sample was utilized for nitrogen analysis.

RESULTS & DISCUSSION

THE INDIGESTIBLE CELL WALL must be ruptured to achieve maximum protein extraction. Rapid decompression using nitrogen gas increases the soluble protein by 29% when a cell mixture is decompressed from 1500 psig. Because of concern over corrosion of the equipment, when the yeast material was mixed with distilled water, the pH of the resulting mixture (pH 5.8) was not changed. It is important to remember that the protein should have a limited solubility at this pH since its isoelectric point is pH 3.8 and an increase of 29% in soluble protein is considered to be substantial.

Using sonic oscillation at the maximum intensity, there was a 61% increase in soluble protein over the cells which received no treatment. All methods of rupture were arbitrarily considered for their feasibility in pilot plant scale or even full scale industrial application and since sonic oscillation, even in a continuous process, imposes severe limitations on the capacity, it thusly seems poorly suited to any large scale production of ruptured cells.

Since homogenization can conceivably be scaled-up, it appears at the outset to be more feasible than other techniques evaluated in this report. The laboratory scale homogenizer used in these experiments had a capacity of 57 liters/hr. A homogenization pressure of 10,000 psig was selected as a maximum pressure for this work. Table 2 indicates the effect of homogenization at 10,000 psig on a 10% cell mixture. Although the maximum extraction of protein is possible after four passes through the homogenizer, each pass heats the sample. Extensive heating was avoided by cooling the samples between passes. Since protein solubility of the samples homogenized three times was high (310% higher than untreated cells) and further homogenization resulted in smaller particles and increased processing times, three passes through the homogenizer were used exclusively in later experimentation.

Effects of other rupture methods can be found in Table 3. Except for homogenization and stone-milling, all techniques are more or less restricted to laboratory scale use. The stone-mill can be operated continuously but is less effective than homogenization.

Cell integrity

When the yeast material is mixed with distilled water it has a tendency to agglomerate or clump. Clumps varied widely in size and shape, even in the same sample, and only a small percent of the cells appeared to exist in a free or individual state. The exact reason for this clumping effect is unknown. Evidence exists that the cells grown on hydrocarbon substrates retain a small amount of the substrate in the cell wall (Ludvik et al., 1968). This seems reasonable since, presumably, the method for introduction of the hydrocarbon substrate is through the cell wall and cell membrane and finally into the cytoplasm where it undergoes a modified form of fatty acid oxidation.

So far as is known, all intact yeast cells are intensely Gram-positive while cells even slightly ruptured are Gram-negative (Nickerson, 1963; Northcote and Horne, 1952). Gram's staining of all the yeast samples indicates that the individual cells (cells not in the clumps) are Gram-negative. Clumps are

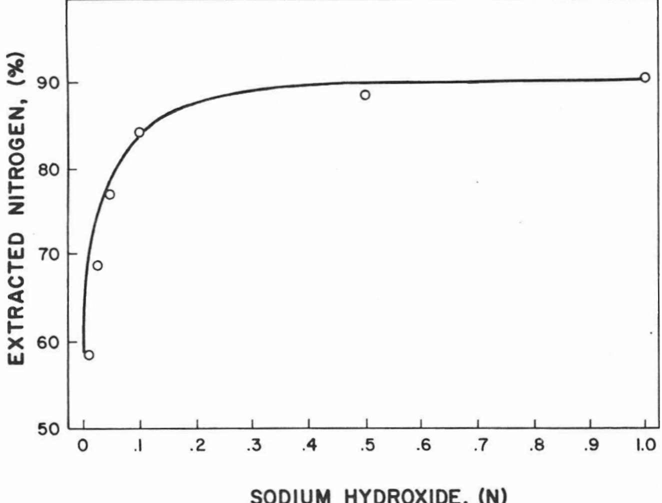

Fig. 1–Effect of NaOH concentration on nitrogen extraction of homogenized yeast.

Fig. 2–Solubility profile of protein isolate (Fraction 3).

Gram-positive presumably because the iodine dces not wash from the large masses of cells with the alcohol wash. Samples of untreated yeast cells show Gram-negative individual cells and the periphery of the clump appears Gram-negative but the inside may be too dense to be effectively washed so it appears to be Gram-positive. However, if some treatment is effected to physically break-up the clumps, then all cells are Gram-negative. Thus the yeast material is Gram-negative, in other words, at least slightly ruptured, even prior to any treatment in this laboratory. If the cells are already ruptured, then why do methods used increase the extraction of protein? This might possibly be explained by presence of residual hydrocarbons in the cell wall and their tendency toward hydrophobic bonding which results in clumping. The untreated cells have a limited protein solubility but when the cell mixture is homogenized or some other treatment is applied, the clumps are broken-up and the area of cell material available to the water solvent substantially increases. Approximately 70–90% of the cells in an untreated mixture exist in clumps while this clumping effect is completely absent in a mixture which has been homogenized three times. Thus, it appears that it is the break-up of the clumps of cells, not the cell itself, which increases the extraction of protein.

Protein isolation

NaOH was selected for use in this report, and Figure 1 indicates the effect of NaOH concentration on percent of nitrogen extracted. The nitrogen extracted with any given concentration of NaOH was calculated by the difference in the nitrogen content in the initial sample and the nitrogen content in the exhaustively extracted residue (Fraction 1). The supernatant from all the extractions was combined and adjusted to pH 3.8 after collection, centrifuged and the precipitate freeze dried. The various samples were compared on the basis of nitrogen content, color and weight. A NaOH normality of 0.1 was selected for further work because of the tan color of the protein concentrate and its nitrogen extraction capacity. Stronger NaOH concentrations produced darkly colored materials with no significant advantage in extraction.

Production of a protein isolate from the yeast material was performed. A 10% cell mixture was exhaustively extracted with 0.1N NaOH and fractions 1, 2, 3 and 4 were dried, weighed and analyzed. Results are given in Table 1. In previous work, protein isolation has been less than 25% effective in removing the protein from the cells. As Table 4 indicates, at least 41.5% of the nitrogen was recovered in the protein isolate (Fraction 3). Of the starting material, approximately 32% remains as the cell wall residue (Fraction 2) and 34% of the material is in the form of a protein isolate. This procedure thus produces a cell wall residue fraction (34.8% protein) and a protein isolate (70.1% protein) in approximately equal amounts.

A solubility profile of the protein isolate was used to determine the effect of pH on protein solubility. Results are presented in Figure 2. Since solubility of the protein is one of the prime functional properties and Fraction 3 does exhibit appreciable solubility in the pH 7–8 range, it seems reasonable to suggest further work on the functionality of this protein isolate.

Amino acid analyses

The original Gulf material and all four fractions of the protien isolation procedure were analyzed for amino acid composition. Calculation at this laboratory for amino acid content is

Table 4–Distribution of nitrogen through protein isolation procedure[a]

Fraction	Material recovered (g)	Nitrogen recovered (g)	Total nitrogen (%)	Recovered nitrogen[b] (%)	Material recovered (%)
Fraction 1 (cell residue)	155.79	9.25	22.0	26.6	31.9
Fraction 2 (alkaline extract)	311.17	26.26	62.5	–	–
Fraction 3 (protein isolate)	163.92	17.42	41.5	50.2	33.6
Fraction 4 (supernatant)	167.96	8.1	21.2	25.2	24.4

[a] Contribution of 0.1N NaOH to solids was not calculated.
[b] From the initial sample of 500g only 462.5g was recovered.

Table 5—Amino acid analyses of yeast material and fractions produced in protein isolation procedure[a]

	Gulf	Fractions			
Correction	original	1	2	3	4
factor	1.0601	1.0596	1.4124	1.0472	1.6156
Amino acids	Grams amino acid/16g N				
Lysine	7.7	7.2	6.9	7.4	4.7
Histidine	2.0	2.1	1.7	1.9	1.3
Arginine	3.5	5.0	4.1	4.7	2.7
Aspartate	9.8	11.0	12.0	11.7	7.8
Threonine	5.3	7.0	6.0	5.6	4.4
Serine	4.5	5.2	5.3	,5.2	3.5
Glutamate	13.8	12.2	18.5	12.8	23.2
Proline	3.5	3.9	4.2	4.1	2.8
Glycine	4.4	4.4	5.8	4.9	5.3
Alanine	6.3	6.0	8.4	6.5	9.5
Valine	5.5	6.5	8.3	6.5	3.8
Methionine	1.2	1.3	1.1	1.5	0.4
Isoleucine	4.5	6.0	5.9	6.3	2.7
Leucine	7.5	8.9	9.3	9.9	4.3
Tyrosine	3.5	4.5	4.4	4.7	1.8
Phenylalanine	5.3	5.6	5.3	5.5	2.6

based on the amount of sample applied to the ion-exchange column of the amino acid analyzer. Since it was suspected that a significant amount of protein would be lost in desalting (resulting from less than 100% transfer from one flask to another and loss in elution), samples to be placed on the amino acid analyzer were analyzed for nitrogen. By measuring the nitrogen which was actually applied to the column and dividing this into the quantity of nitrogen assumed to go onto the column, it is possible to produce a correction factor for more accurate interpretation of the results. This calculation indicates that approximately 95% of the protein is recovered in the undesalted samples and 62–72% of the protein was recovered from the desalted samples. Application of the correction factor implies 100% recovery of the nitrogen and results are presented in Table 5.

Since Fraction 3 is intended here as a protein isolate, it is desirable to concentrate as much of the essential amino acids as possible into this fraction. Fraction 2 which is the precursor of both Fraction 3 and Fraction 4 appears to donate the greater share of most of the amino acids to the protein isolate with the exception of alanine, glycine and glutamate. The protein isolate has a desirable quantity of lysine but lacks methionine. Analysis of cystine and tryptophan was not performed.

If petroleum yeasts are to be used as human food the problems of toxicity, and nutritional value must be solved. Protein isolation techniques may effect a separation of the protein fraction from the undesirable cell wall and conceivably commercial plants producing the cells could bypass the drying step with part of the cell material and direct it into a continuous process of homogenization, extraction and protein precipitation to produce a protein isolate. The cell wall fraction retains about 35% of the protein and is quite possibly an acceptable animal feed. Since the isolate is 70% protein, and contains a high lysine content it could make a welcome addition to the world's protein resources.

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THE FUNCTIONALITY OF BINDERS IN MEAT EMULSIONS

INTRODUCTION

FRANKFURTERS enjoy wide acceptance as a processed food. Their composition is closely regulated by Federal Law (Bailey, 1970) and they can only be called "frankfurters" if they conform to standards of identity of the U.S. Department of Agriculture. Until recently, an "imitation" label was required if these standards of identity were not met. New regulations (Mulhern, 1973) now permit marketing of imitation frankfurters meeting certain standards for nutrition and composition without an "imitation" label. The same regulations prohibit their designation as frankfurters.

Imitation frankfurters produced under the new regulations will require similar nutritional and functional characteristics as conventional frankfurters. Reduced costs may be necessary for economic success. These objectives can be most logically met by using a class of materials known as sausage binders or extenders.

Binders are permitted in conventional frankfurters at a level of 3.5%; in nonstandard products there is no restriction. Acceptable binders include such materials as starches and cereals as well as products containing significant amounts of protein such as soy flour, soy isolate (permitted at a 2% level), soy concentrate or nonfat dried milk (Bailey, 1970).

While percent moisture content is restricted by regulations to 4 times % protein plus 10%, only meat protein and not binder proteins can be considered in this equation. Meat ingredients vary in their activity as fat stabilizers (Carpenter and Saffle, 1964). Therefore, an active binder can be used to compensate for reduced functionality when a nutritionally equivalent meat source of lower cost and reduced functionality is used. This is the function of binders in standard frankfurters.

To be successful under the new regulations for imitation frankfurters, a binder should be capable of replacing some of the functions of the meat in an all-meat frankfurter, the most critical of which is to stabilize the fat during smoking and cooking. Recently binders have been criticized (Smith et al., 1973; Swift, C.E., 1973, Private communication; Sulzbacher, 1973) as not performing this function.

The research reported here had a broad objective of determining whether binders in general, and whey products in particular, function as active sausage binders. The specific objective was to determine if binders could be used as partial replacements for meat in production of imitation frankfurters with resulting reduced cost.

EXPERIMENTAL

CALCULATIONS to achieve least-cost emulsion formulations were performed using the IBM linear program LP-MOSS (Anonymous, 1967) and an IBM 1130 computer. The methods used with meat emulsions were somewhat modified versions of those reported by Saffle (1966). Linear programming is a standard technique which will not be discussed in detail in this paper. It should be recognized, however, that each least-cost formulation presented is unique for the composition of the ingredients, the assumed costs of the ingredients and the constraints imposed for frankfurter composition and quality.

The water content was controlled to meet regulations for conventional frankfurters. However, the binder protein was considered on an equal basis with meat protein. That is, maximum water was restricted to 4 times % of meat and binder protein plus 10%. The binder level was not restricted. A minimum of 10% of protein was chosen as a limit for the finished frankfurter. Requirements for dry ingredients, fat content and the cost of ingredients are included with the data reported later in this paper.

Saffle (1966) described and controlled both fat binding and color intensity in frankfurters by using the data obtained from his studies with meat ingredients. Both binding and color are functions of the type and level of meat protein.

Fat binding in meats is a function primarily of the salt-soluble protein fraction. The salt-soluble protein from each meat source was first characterized for its ability to emulsify fat by titrating liquid fat into a saline extract of meat proteins. The data for different sources were then compared on an equal protein basis (Carpenter and Saffle, 1964). Since the amount of salt-soluble protein also varies for different meats, the fraction of the total protein which was salt-soluble was also determined (Saffle and Galbreath, 1964).

Multiplication of fat binding per unit weight of salt-soluble protein by the fraction of total protein that was salt-soluble resulted in a product called the constant bind value. This value characterized a unit weight of total protein from each meat source for fat-binding activity. For each meat ingredient, the fraction of total protein in the meat was multiplied by the appropriate constant bind value. The new product is the bind value per unit weight of the meat ingredient used in the formulation. When this bind value is multiplied by the amount of the meat ingredient used and is summed for all the meat ingredients, the result is the total bind value for the formulation. In this investigation, total bind values were held above a minimum level during formulation and are reported in the data and the text. A similar approach was used to estimate and control the color of the finished product (Saffle, 1966).

Meat ingredients

The compositions of the various meat ingredients used in the laboratory and pilot plant experiments are reported in Table 1. The analyses were performed using standard methods of the AOAC (1970). All samples were ground, frozen and thawed before use. The constant bind values reported by Saffle (1966) were reduced by 10% to allow for loss in functionality caused by freezing (Saffle, R.L., 1973, Private communication). The bind value for each individual meat source is not reported here but can be easily calculated.

Emulsion preparation and evaluation

Meat emulsions were prepared in the laboratory in 100g batches with or without addition of the 10% of water which is used to replace water lost during cooking of products such as frankfurters. Room tem-

Table 1—Composition of meat ingredients used in imitation frankfurter formulations

	Protein (%)[a]	Fat (%)	Water (%)
Laboratory			
Beef, chuck	18.6	14.0	66.9
Pork, picnics	17.8	23.0	60.3
Fat pork	0.9	88.0	11.8
Pilot plant			
Beef, chuck, cow	20.67	6.44	73.42
Pork, picnics	17.50	16.95	65.34
Fat pork	1.91	90.00	7.06

[a] TKN X 6.25

perature water was first placed in the metal cup of a Servall® Omni-Mixer followed by the refrigerated meat ingredients and dry ingredients (salt, sugar and sodium nitrite curing salt) in that order.

Binders were either added as dry ingredients or, more often, were pre-dispersed in the water. The cup was attached to the Omni-Mixer and immersed, unless otherwise specified, in ice water. The mixture was chopped at full power, with intermediate scraping, for consecutive periods of 5, 10, 5 and 5 sec. Since all-meat controls were most difficult to emulsify, chopping was continued until the control was finished as judged by its appearance. In some experiments, one less or one more final chop-period of 5 sec was needed with the all-meat control. The same chop schedule was then used with the binder formulations. Final emulsion temperatures were recorded and were similar provided that the protein to moisture ratios were similar. An increase in water content caused lower final emulsion temperatures.

25-g samples of the emulsions were stuffed into 40 ml glass centrifuge tubes and were cooked by immersing in a water bath at 82°C for 30 min. These experiments were carried out both with and without prior refrigeration in 250 ml metal beakers covered with Saran wrap. The cooking schedule resulted in an internal emulsion temperature of about 79°C when the emulsions were at refrigerator temperature (ca. 4–5°C) before cooking. The free liquid that formed after cooking was pressed with a rubber plunger into a graduated cylinder, and the volumes of free fat and water were recorded as a % of the uncooked emulsion weight.

Pilot plant production and subsequent evaluation of frankfurters followed the methods of Townsend et al. (1971) with some modifications. The emulsions were chopped in a 25 liter Schnell Kutter meat chopper at a speed of 3600 rpm. The blade tip speed was equivalent to that of a standard silent cutter. The emulsions were chopped to a final temperature of 15.6°C as measured by a thermistor in the bowl. The binders to be tested were pre-dissolved in the water. The frankfurters were smoked to a final temperature of 71°C. Toughness was estimated with a Warner-Bratzler shear (Bratzler, 1932). Press juice was determined by a method (Ackerman, S.A., 1973, Private communication) which is reported to correlate with juiciness scores in taste panels. Juiciness is regarded as desirable in frankfurters.

A taste panel, composed of untrained panelists was conducted. The frankfurters were heated in hot water and members were asked to record their preferences by ranking them from most to least preferred.

Binder identification and evaluation

ENR-EX® and **ENRPRO® 50**—partially delactosed whey and whey protein concentrate, respectively, are products of Stauffer Chemical Company, Westport, Conn., produced by a commercial gel filtration process.

Lactalbumin—a modified whey product obtained from Crest Foods, Inc., Ashton, Ill.

Nonfat dried milk (NFDM)—a low heat type obtained from Land O'Lakes, Inc., Minneapolis, Minn.

Whey—dried sweet whey supplied as KRAFEN® by Kraftco, Corp., Chicago, Ill.

PROMINE® D—a soy protein isolate supplied by the Central Soya Co., Chicago, Ill.

The fat bind value of a selected product, ENR-EX, was estimated by two methods. One was the model system method used by Carpenter and Saffle (1964). An alternate method was to arbitrarily assign a series of bind values per unit weight of binder and then to calculate least-cost formulas for each case. These formulations were then prepared and evaluated in the laboratory with the results shown in Table 2.

The performance of various binders in least-cost formulas was determined first by laboratory methods. The formulations and their performance appear in Table 3. The assumptions made before calculation of these formulas are explained under Results and Discussion. Similar formulations were evaluated with pilot plant methods and materials and are described in Tables 4 and 5.

Statistical methods

Laboratory data were analyzed by the methods of step-wise multi-

Table 2—Estimation of fat binding by ENR-EX in sausage emulsions

	Control	Assumed ENR-EX bind values: 1.07	1.964	2.86	3.7	5.6	6.5	Limits
Ingredients (%)								
Beef ($1.39/lb)	52.6	46.9	37.6	34.2	34.2	34.2	34.2	
Pork ($1.58/lb)	15	15	15	15	15	15	15	≥15
Fat pork ($0.22/lb)	19.5	20.4	21.9	22.5	22.5	22.5	22.5	
ENR-EX ($0.27/lb)	None	12.6	17.9	15.1	11.6	7.7	7.4	
Added water	8.9	1.1	3.6	9.3	12.7	16.6	16.9	
Salt and dextrose (1:1)	4	4	4	4	4	4	4	=4
Predicted properties of final products								
Cost $/100 lb of product	101	97	86	81	80	78.5	78.5	Minimum
Water[a]	4.9	−10	−10	−2.3	3.2	9.6	10	−10 to 10
Fat (%)	28	28	28	28	28	28	28	28 to 31
Bind, total	155	155	155	155	155	155	160	≥155
Protein (%)	12.6	13.6	12.6	11.5	11.0	10.4	10.4	≥10
Color	218	197	162	150	150	150	150	≥150
Per cent fat loss from cooked emulsions[b]								
ENR-EX added dry								
	2.0–2.8	24.4–	26.0–	23.2–	13.0–11.8		1.2–	
	1.6–1.2	24.4	22.8	20.0	7.2–8.8		1.6	
ENR-EX predispersed in water								
	2.4–		25.6–		8.7–	0.8–	0.8–0.8	
	1.6		24.4		8.5	1.2	0.8–0.8	

[a] The total predicted water content (%) of the final frankfurter equals 4 times percent protein plus the number shown.

[b] The two estimates of fat loss were made after aging the fresh emulsion overnight in the refrigerator and loading 25g into glass centrifuge tubes. The first figure was obtained by cooking on the same day and the second figure after holding the loaded tube overnight in the refrigerator.

Table 3—Least-cost formulations and performance of sausage emulsions with and without various meat binders

		Binders					
	Control	ENR-EX	KRAFEN	PROMINE D	ENRPRO 50	NFDM	Lactalbumin
A. Ingredients (%)							
Beef ($1.00/lb)	52.6	34.2	34.2	34.2	34.2	34.2	34.2
Pork ($1.14/lb)	15	15	15	15	15	15	15
Fat pork ($0.16/lb)	19.5	22.5	22.5	22.5	25.9	22.5	22.5
Salt and dextrose (1:1)	4	4	4	4	4	4	4
Added water	8.9	16.9	16.4	21.8	18.0	19.4	21.5
Binder level	None	7.43	7.88	2.56	2.94	4.89	2.84
B. Predicted cost and composition of final product							
Cost $/100 lb of product	73.6	57.69	56.63	56.81	57.99	57.69	57.59
Water	4.9	10	10	10	10	10	10
Fat (%)	28	28	28	28	31	28	28
Protein (%)	12.63	10.37	10.25	11.59	10.74	11.0	11.51
C. Characteristics and performance of binders							
Binder protein content (%)		15.2	12.9	91.8	50.0	36.0	80.0
Assumed binder cost ($/lb)		0.27	0.12	0.44	0.60	0.41	0.67
Fat loss (% of cooked emulsion)	4.4–	3.6– 4.8	3.2– 0.8	1.2– 0.4	0.8– 1.2	0.6– 0.8	1.2– 0.6
Water loss (% of cooked emulsion)	12.0–	12.0– 18.0	16.0– 5.2	4.8– 4.8	4.8– 4.8	5.2– 5.2	11.2– 6.0

[a] For explanation of data see the note and footnote attached to Table 2.

ple regression (Efroymson, 1966) using a second-order model. Error estimates were obtained and were used to eliminate occasional outliers. The predictive equations derived by statistical methods were examined and any trends of interest, such as the effects of final emulsion temperatures, were tested in separate experiments.

Table 4—Typical least-cost formulas used in frankfurter production in the pilot plant

Composition %	Control	ENR-EX	ENRPRO 50
Beef	35	25.55	25.55
Pork	20	20	20
Pork fat	27.1	25.52	25.52
Salt	2	1.4[a]	2
Sugar	1.6	1.6	1.6
Water	14.3+	18.05+	21.4+
	10	10	10
Binder		7.89	3.96

[a] Salt was reduced when ENR-EX was used to allow for the salt naturally present in the material.

Table 5—Replacement of beef by binders in least-cost formulations

	Source parameters			Formulation parameters	
Protein source	Protein (%)	Parts protein	Parts used	Water added	Binder plus water
Beef replaced	20.57	1.95	9.45		
KRAFEN	12.9	1.01	7.84	17.5	25.34
ENR-EX	15.2	1.20	7.89	18.1	25.99
NFDM	36.0	1.75	4.87	20.5	25.37
PROMINE D	91.8	2.33	2.54	22.8	25.34

RESULTS & DISCUSSION

THE LABORATORY EXPERIMENTS reported in Tables 2 and 3 involved making emulsions without adding the 10% of water normally added to frankfurter emulsions to compensate for losses during cooking (shrink). Processed meats of the loaf type are prepared and cooked in metal cans by placing them in hot water. The laboratory method was therefore more comparable to production of loaf products rather than frankfurters, particularly if the added shrink water was omitted.

The approximate fat-binding potential of ENR-EX was estimated by the experiment described in Table 2. A series of bind values for ENR-EX was assumed ranging from 1.07 to 6.5, and least-cost formulas were calculated for each case. When the assumed bind values per unit weight of ENR-EX were below 5.6, the requirement to meet the minimum total bind value of 155 caused drastic changes in the formulations. Water was removed from the formulation and replaced by ENR-EX in order to meet the bind value constraint at minimum cost. A negative value in the water estimate means that there was less water in the emulsion than in meat of equivalent protein content. In extreme cases, lack of water resulted in overall loss of fat-binding capacity by the emulsion. Such failures occurred when the assumed bind value per unit weight of ENR-EX was 3.7 or lower.

At an assumed bind value for ENR-EX of 6.5, the total emulsion bind value became greater than the minimum of 155. This means that further increases in assumed bind value of ENR-EX would result in no change in the least-cost formulas. This places an upper limit on the bind value range that can be estimated by this method. If the assumed bind value is above such a limit for any binder, the least-cost formula will be determined by considerations of cost and protein content rather than fat-binding capacity.

Evaluation of the data for fat loss (Table 2) revealed that, as the assumed bind values for ENR-EX were increased, three things occurred:

1. Fat stability increased as water content increased.

Table 6–Performance of selected binders in frankfurters produced in the pilot plant[a]

	Emulsion preparation				Finished product evaluation			
	Binder (%)	Chopping time (sec)	Emulsion "viscosity" number	"Viscosity" std dev	Fat caps (%)	Shrink during smoking (%)	Shear (lb)	Press juice (%)
Control	None	180	57.6	3.1	23	11.8	3.3	29.5
ENR-EX	7.89	226	39.2	1.2	25	9.8	2.6	37.9
NFDM	4.87	300	46.2	1.3	37	11.0	2.3	34.0
Lactalbumin	2.83	225	49.1	3.7	41	12.7	2.8	38.2
PROMINE D	2.54	250	47.9	1.6	39	9.3	2.4	34.1
ENRPRO 50	3.96	295	36.7	1.3	35	9.4	2.4	35.0
KRAFEN (Dried Sweet Whey)	7.84	270	41.9	2.4	29	9.6	2.8	31.0

[a] An estimated difference of about 4% in percent Fat Caps is required to indicate a significant difference. The error (standard deviation) for viscosity estimates was based on nine determinations.

2. Emulsions made with pre-dissolved ENR-EX were more stable than those made with ENR-EX added in dry form.
3. At an assumed bind value of 6.5, the ENR-EX emulsion retained more fat than the control. This suggests that in this system the bind value of ENR-EX was so high that it had no effect on formulation for least-cost.

Sulzbacher (1973) recommends model systems to judge the effects of binders. Such a system yielded a bind value of 1.07 for ENR-EX while an experiment with meat emulsions indicated a value of 6 and above. Although the model system method of Carpenter and Saffle (1964) apparently failed with ENR-EX, it is also true that their methods have been very successful in the study and control of the emulsifying properties of salt-soluble meat proteins. The bind value of 1.07 for ENR-EX in a model system suggests that part of the action of ENR-EX is similar to that of meat protein. More of the functionality of ENR-EX in meats may result from an enhancement of the fat-binding capacity of the meat protein system. Pyrophosphates, for example, increase fat binding in meats with no demonstrable effect on fat emulsification when meat is absent. ENR-EX may exert much of its effect on the protein of meat and therefore be more similar in its mode of action to pyrophosphate than to a fat-binding protein system.

A comparison was made of the fat-binding capacities of various commercial protein-containing binders. The bind value of each was assumed to be high enough so that least-cost formulations were, therefore, dependent on protein content and costs. This resulted in binders being added on an approximately equal protein basis. Meat ingredients and the sum of binder plus added water were held constant as the protein content of the binder varied (see Table 5 as discussed below). These formulations, along with the performance of each binder, appear in Table 3. A comparison of performance (Table 3,C) of various binders with the all-meat control suggests that protein-containing binders, as a class, can replace meat protein and that they may be much better than meat in binding fat. The bind-value per unit weight of protein was estimated in this research as being about 2.5 times as high for ENR-EX as for the most effective meat source known. One explanation may be the reported low salt-solubility of meat protein (Saffle and Galbreath, 1964). Another explanation may be the somewhat low availability of water in the all-meat control formula (Table 3,B).

Frankfurter types of emulsions were also prepared in the laboratory by simply adding the 10% of water which is normally cooked out during smoking of frankfurters. Considerable data were collected but are not presented because all the binders were so effective that no significant differences between them could be detected. The formulations with binders seemed to outperform the all-meat controls, however.

The binders were next evaluated in pilot plant experiments. Based on the composition of pilot plant meat ingredients (Table 1), least-cost formulas were calculated with a lower total bind value constraint of 137. Typical formulas appear in Table

Table 7–First preferences in a taste panel evaluation of imitation frankfurters

Preference	Smokers	Nonsmokers	Total
Control	5	2	7
ENR-EX	3	8	11
KRAFEN (Dried sweet whey)	1	4	5

Table 8–Laboratory evaluation of pilot plant formulations for percent loss of fat during cooking of emulsions

	Fat loss (%)		
	Time, days after preparation		
	0	1	2
ENR-EX	1.0	0.8	1.0
PROMINE D	>1.6	0.8	0.6
ENRPRO 50	1.8	1.4	0.8

Table 9–Effects of finished emulsion temperature and aging in the refrigerator on fat loss by cooked meat emulsions[a]

Approximate emulsion temp (°C)	Fat loss (%)			
	Immediate cook		After refrigeration	
	Control	ENR-EX	Control	ENR-EX
14	2.4	3.0	1.8	1.2
25	7.2	1.0	2.8	0.6
28		8.0		1.4
30	18.8		13.2	

[a] Meat ingredients and formulas were the same as in Table 4 including the 10% added water normally added to replace losses during cooking.

4. Sodium ascorbate was used to increase the rate of development of cured color since the frankfurter emulsions were cooked on the same day that they were prepared. All formulas allowed for the maximum water content of 4 times % total meat and binder protein plus 10%.

The results of basing formulations on least-cost are clarified by the data in Table 5. All the binders replaced 9.45 parts of beef. The indicated quantities of dry binders varied both in the amount that was used and in the protein which they contributed. A variable quantity of water was added with each binder. The weight of binder plus water was constant in each case as was the quantity of meat and dry ingredients.

The results of the pilot plant evaluation appear in Table 6. All the frankfurters were of good quality but did differ in several aspects. The most critical and meaningful data are those for fat caps appearing on frankfurters after smoking and cooling (Townsend et al., 1971). The data of Table 6 indicate that only ENR-EX equalled the control in fat stability and was completely successful in replacing beef. Whey was significantly less effective but was still a reasonably good replacement for beef. Whey produced (1) emulsions which were slower to chop to emulsion temperature and (2) frankfurters which were tougher and less juicy than those made with ENR-EX.

All the whey products except denatured lactalbumin produced thin emulsions. Emulsion viscosity seemed unrelated to final frankfurter quality although thin emulsions have been traditionally associated with poor performance.

In frankfurters, the tendency for soy isolate and ENRPRO 50 to bind more water, as indicated by reduced shrink, is of little importance since less water could then be used in preparing the emulsions and the water content would then be the same for all finished frankfurters.

The only frankfurters with a definite off-flavor were those made with soy isolate. Since dried whey frankfurters were the only products that were functionally comparable to ENR-EX frankfurters, they were compared with the all-meat controls by a taste panel with the results summarized in Table 7. Only first preferences are reported in the data. The majority of the panelists preferred ENR-EX frankfurters although smokers seemed to prefer the controls. All the samples contained spices.

The formulations and ingredients used in the pilot plant were also tested in the laboratory with the results presented in Table 8. The relative differences between binders with respect to fat stability which were observed in the pilot plant (Table 6) were confirmed in the laboratory when cooking was done on the same day. When the emulsions were refrigerated for 1 or 2 days before cooking, the differences between binders disappeared and the results were very similar to data obtained with laboratory methods and meat ingredients.

The laboratory and pilot plant meat ingredients and formulations were used to study the effect of final emulsion temperature on emulsion performance. Step-wise multiple regression of laboratory data indicated that all-meat controls should perform best at 14°C while ENR-EX should perform best at room temperature. The data of Table 9 were obtained when final emulsion temperature was controlled in the laboratory by changing the temperature of the water bath around the Omni-Mixer cup. It appears that ENR-EX will perform quite well at typically low chop temperatures for high water frankfurter emulsions. As the chop temperature was increased, the performance of ENR-EX improved and reached an optimum between 20°C and 25°C. This improvement persisted even after overnight refrigeration to remove cooking temperature variations. These results were obtained in the laboratory with meat ingredients and formulas used in the pilot plant studies. Similar results, although less pronounced, were obtained with laboratory meat formulations having higher predicted bind values (⩾ 155 rather than ⩾ 137).

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EFFECT OF PORK HEARTS, ADDITIVES AND pH ADJUSTMENT ON PROPERTIES OF MEAT LOAVES

INTRODUCTION

HIGH-PROTEIN plant seed products such as soy protein concentrate and isolated soy protein along with nonfat dry milk are most commonly used in sausage formulation. Reducing production costs is the primary purpose for using these substances although several research laboratories claim that these additives can increase the binding capacity of comminuted meat mixtures. Results reported to date do not agree with each other (Smith et al., 1973; Van Eerd, 1971; Cook et al., 1969; Rongey and Bratzler, 1966; Pearson et al., 1965; Inklaar and Fortuin, 1969 and Glabe et al., 1956).

According to Hamm (1960), changes in charges and structure of muscle proteins significantly influence water-holding capacity of meat. He stated (Hamm, 1960) that the pH at which water-holding capacity is minimal corresponds approximately to the isoelectric point of actomyosin (pH 5.0). Although moving the pH of a sausage mixture toward neutrality may improve water-binding capacity, Wilson (1960) and Fox (1966) reported that less nitrous acid is available at pH 7.0; thus, development of cured meat color may be impaired.

Zirkel (1965) ranked the water-holding capacity of 17 types of sausage meats commonly used in industry. She reported that pork hearts ranked as a medium binding meat with a binding capacity lower than that of skeletal muscle, but higher than those of smooth muscle and muscle with higher fat content.

The objectives of this research were to determine the effects of formulation with pork hearts and various additives as well as the adjustment of pH of the sausage batters on shrinkage, texture, firmness and overall acceptability of meat loaves.

MATERIALS & METHODS

EXTRA-LEAN pork trimmings (80% lean), boneless picnics (75.1% lean) and regular pork trimmings (56% lean) were purchased from Gold Kist, Talmo, Ga. Lean cow meat (95% lean) was obtained from a local meat processor while pork fat and pork hearts were supplied by the Meat Lab., Food Science Dept., University of Georgia. The meats were separately ground twice through a 0.31 cm plate, packed into 2.2 kg batches and stored in a freezer (−20°C) for not more than 2 wk. The frozen meats were removed from the freezer and stored in a 5°C cooler for 24–36 hr prior to manufacturing.

Nonfat dry milk (NFDM) was purchased from a local store. Isolated soy protein (ISP, Promine D) was supplied by Central Soya Chemurgy, Chicago, Ill. with soy protein concentrate (SPC) GL-301 supplied by Griffith Lab., Inc., Chicago, Ill. Peanut grits (PG) and peanut flour (PF) were supplied by Gold Kist Lab., Lithonia, Ga.

Batches were formulated to approximately 30–33% fat which would be less than commercial loaf production and would not exceed the fat-binding capability of the meat mixtures. Pork hearts were added at three levels: 10, 20 and 30% of the total formula. Additives were included at levels of 0, 3.5 and 7.0% to measure response over a wide range of usage. Formulations for these experiments are shown in Table 1. Formula 1 was used in the experiments evaluating additives and pork hearts while Formula 2 was used in the experiments evaluating effects of additives and pH adjustment.

Manufacture of meat loaves

Batches for processing were selected randomly to minimize the effect of time and/or order of preparation. Meat for each batch was chopped in a Hobart chopper (equipped with four blades) along with 2.5% salt, seasoning, additives (when used), ascorbic acid, sodium nitrite and water (20%) until the temperature of the batter reached 15°C. The meat batter was removed from the chopper and packed into two aluminum loaf pans (6.25 × 10 × 25 cm), each containing approximately 500g of batter. The prepared raw batter was covered and stored in a cooler (5°C) until all batches were accumulated.

All batches were cooked in an electric oven with the temperature set at 65°C for the first 30 min then increased to 150°C until the internal temperature of the loaf reached 74°C. Internal temperature was recorded by a copper-constantan thermocouple (Type T) attached to a recorder. After cooking, the loaves were drained of all fluids and returned to a 5°C cooler for chilling. The fluid released by cooking was measured and the amounts of fat and water released were recorded. To determine the effects of hydrogen ion concentration, the pH of the batter was adjusted by adding citric acid or sodium carbonate to the mixture during comminution and was determined by placing the electrode of a pH meter (Corning Model 10) directly into the meat mass at two different locations.

Table 1—Formulations of meat, additives, seasonings and water used in manufacture of meat loaves

	Cow meat (9.75%) (g)	Pork fat (81.00%) (g)	Pork heart (10.00%) (g)	Additive (g)	Fat content
Formula 1 (29.9% fat)[a]					
	618	282	100	0	29.87%
	579	286	100	35	29.91%
	538	292	100	70	29.89%
	518	282	200	0	29.89%
	451	286	200	35	29.56%
	438	292	200	70	29.90%
	418	282	300	0	29.92%
	379	286	300	35	29.86%
	339	292	300	70	29.96%

	Boneless picnics (24.90%) (g)	Pork trim (44.50%) (g)	Additive	Fat content
Formula 2 (33.5% fat)				
Control	554	446	0	33.64%
w/additive	489	476	35	33.41%

Salt, seasonings and water (used with Formulas 1 and 2).

Salt	25.0 grams
Seasoning	5.5
$NaNO_2$	0.2
Na ascorbate	0.2
Water	240.0

[a] Figure in parenthesis is fat percentage of raw material.

In order to avoid the difficulty of adjusting the pH during the comminution, the following experiment was also conducted. A meat batter using Formula 2 was prepared, then divided into four parts and the pH of each part was adjusted to 5.0 ± 0.1, 6.0 ± 0.1, 7.0 ± 0.1 and 8.0 ± 0.1 with 0.5N HCl or 0.5M NaOH. Various properties including cooking, shrinkage, amount of water-soluble and salt-soluble proteins and amount of acetone-nitrosoheme complex (hematin) were determined.

Determination of cooking shrinkage

After cooking the loaves were removed from the oven and the fluid was poured into a graduated cylinder. Upon standing the fluid separated into two layers, fat and water. The amount of water and fat separated was expressed as ml of liquid/100g of meat batter. The weight of meat loaf with pan after removal of the separated fluid was recorded and the difference between the weight before and after cooking was expressed as cooking shrinkage.

Cooking loss was determined by the following procedures of Townsend et al. (1968) with slight modifications. 30g of raw batter were placed into a 500 ml flask, the flask was stoppered, cooked in a 70°C water bath for 30 min and the fluid immediately decanted into a graduated cylinder. Cooking loss was expressed as weight of liquid lost divided by total weight of raw batter.

Determination of meat loaf texture

The texture of the meat loaf was measured with a Universal Instron testing machine (Table Model 1130, Instron Corporation, Canton, Mass.) with recorder. Samples from each batch were sliced to uniform thickness and then cut into a final size of 6.25 × 2.50 × 1.25 (cm). The sample was placed into the cell (Allo-Kramer multi-blade press cell, C-266) and the force required to shear the sample was automatically plotted on the recorder chart. The speed of the shaft was 10 in./min. Data were collected from the reading of each peak with the full scale calibrated to 220 kg and reported as the average of three replications.

Evaluation of loaf by taste panel

Samples were prepared for organoleptic evaluation by selecting from batches at random. Uniform slices were served to a trained panel of 12 members who evaluated the samples for texture (firmness) and preference. Samples were rated for preference on a scale of one through five with five being the most and one the least desirable. The same scales were used for texture (firmness) with five being tough and unacceptable, three being most desirable, and one being soft and unacceptable. Results of panel scores were subjected to statistical analysis.

Extraction of water-soluble and salt-soluble proteins

Water-soluble and salt-soluble proteins were extracted following the procedures of Carpenter and Saffle (1965). 5g of raw batter were mixed with 15 ml of the extraction medium at different pH levels. Water-soluble proteins were extracted with a solution of 0.1M potassium phosphate in distilled water and salt-soluble proteins were extracted with a solution of 0.1M potassium phosphate in distilled water and salt-soluble proteins were extracted with a solution of 0.1M potassium phosphate in 0.6M NaCl. Protein concentration of the extract was determined by the Biuret procedure (Gornall et al., 1949).

Determination of acetone-soluble nitroso pigment in the meat loaves

The procedure of Hornsey (1956) was used to determine the color of the meat loaves. 10g of minced sample was mixed with 50 ml of acetone:water (40:4 in volume) solution for 5 min. The slurry was then filtered through two layers of Whatman #2 filter paper and the filtrate collected in a wrapped cylinder. Light was excluded from the extraction by wrapping the glassware (funnel and cylinder, etc.) with aluminum foil. The light absorption was measured at a wavelength of 540 nm in a double-beam spectrophotometer (Perkins-Elmer double-beam, Coleman 124) using a 1 cm cell containing an 80:20 solution of acetone:water as a blank. The values obtained were used as a comparative measure of the soluble nitroso-pigment concentration. The concentration of nitroso-pigment in 10g of meat is equal to the absorption of the acetone-nitroso-heme at 540 nm in a 1 cm cell multiplied by a factor of 290 and is expressed as ppm of hematin.

Statistical analysis

Data collected from experiments were subjected to an analysis of variance and Duncan's multiple range test according to the procedures of Steel and Torrie (1960).

RESULTS & DISCUSSION

THE RESULTS of experiments using Formula 1 show that the heart content of the formula had significant effects on all responses: shrinkage, texture measured mechanically and subjective measurements for firmness and preference (Table 2). Increasing the level of pork heart in the loaf formula increased the shrinkage and decreased the sensory panel scores for firmness and preference of the products. The type of additive had no effect on shrinkage or texture measured mechanically; however, the panel rated samples containing SPC as being firmer than those with either NFDM or PF. Products containing NFDM produced significantly higher preference ratings than those containing either SPC or PF. No significant differences were observed between the latter two additives. The level of additive did not affect shrinkage, but texture (firmness) increased linearly as the level of the additive increased. The panel did indicate a preference (significant at 5% level) for loaves made with 3.5% of NFDM over loaves made with the other additives or levels of additives. No significant interactions among treatment means were observed.

Table 3 shows the effects of using high-protein additives (NFDM, SPC, ISP and PG) at the 3.5% level along with no additive in the manufacture of loaves from an all-pork formula (Formula 2). Loaves containing NFDM (3.5%) exhibited significantly ($P < 0.05$) less shrinkage than loaves made with any other additive; however, the NFDM loaves were not significantly different from the control loaves. These data are in agreement with the result of the experiment using Formula 1. The control sample (Table 3) was not significantly different from PG and SPC, but all samples were significantly different from ISP. Products with additives exhibited a more rigid structure than the control as with the first experiment. Panel scores indicated a higher preference for loaves made without additives and made with NFDM than for loaves made with SPC, ISP and PG with no difference among the latter treatments. The panel members were unable to detect any differences in texture (firmness) among the products made with additives and that made without additives (control), except when ISP was used.

Table 2–Treatment means showing the effects of heart content, additive type and additive level on shrinkage, texture (mechanical shear), firmness (panel evaluation) and panel preference of meat loaves[a]

	Heart			Additive Type			Additive Level		
	10	20	30	NFDM	SPC	PF	0	3.5	7.0
Shrinkage (%)	15.86a	17.56b	19.64c	16.99a	18.32a	17.75a	18.44a	17.51a	17.11a
Texture-Instron kg/g	2.43a	2.23b	1.98c	2.09a	2.26a	2.29a	1.90a	2.25b	2.50c
Firmness-panel score	3.17a	2.76b	2.50c	2.62a	3.06b	2.75a	2.19a	2.88b	3.36c
Preference-panel score	3.35a	2.97b	2.69c	3.55a	2.72b	2.74b	2.94a	3.16b	2.91a

[a] Numbers with different letters are significantly (5%) different from other numbers in the same row.

Figure 1 illustrates the effects of pH adjustment of the raw batter on shrinkage, mechanical texture and on panel scores for texture (firmness) and preference of the cooked products. Adjusting the pH toward neutrality reduced the shrinkage and

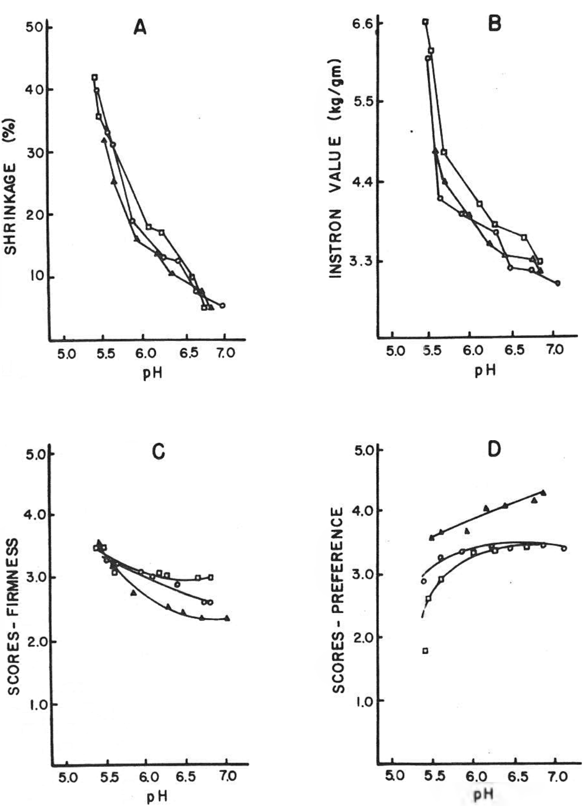

Fig. 1—The effect of pH of the sausage batter on: (A) cooking shrinkage, (B) texture measured mechanically, (C) panel scores for firmness, and (D) panel scores for preference of cooked meat loaves. (o__o Control; ▲__▲ NFDM; □__□ ISP.) Panel scores for firmness: 1.0 = soft, unacceptable; 3.0 = most desirable; 5.0 = tough, unacceptable. Panel scores for firmness: 1.0 = least desirable; 5.0 = most desirable.

Table 3—Effect of high-protein additives on the cooking shrinkage, Kramer shear values and panel scores for preference and texture (firmness) of meat loaves

Treatment	Shrinkage[a,f] (%)	Kramer Shear Value[b,f] (kg/gram)	Panel Scores[c,g] Preference[d]	Firmness[e]
Control	18.64bc	1.62c	3.83a	2.92b
With NFDM	13.33c	2.55b	4.08a	3.33ab
With PG	21.04b	2.38b	2.92b	3.00b
With ISP	33.69a	2.88a	2.00b	3.67a
With SPC	21.00b	2.55b	2.52b	3.58ab

[a] Values are means of two determinations.
[b] Values are means of three determinations.
[c] Values are means of 12 determinations (1.0 = least desirable, 5.0 = most desirable)
[d] 1.0 = least desirable, 5.0 = most desirable
[e] 1.0 = soft, unacceptable; 3.0 = most desirable; 5.0 = tough, unacceptable
[f] Values with different superscript in the same column are significantly different at 1% level.
[g] Values with different superscript in the same column are significantly different at 5% level.

texture, measured mechanically, for all treatments; however, there were no significant differences in responses to pH adjustment among the treatments. There seemed to be a difference in panel scores for products made with NFDM and those made with ISP and no additives for both responses. Panel members rated products containing NFDM as being softer, and they indicated a greater preference for these products over products made without additives or with ISP. Panel scores for texture (firmness) decreased and preference scores increased as the pH was adjusted toward 7.0.

The adjustment of pH from 5.0 to 8.0 in the raw batter produced a significant effect on cooking shrinkage, extractable salt-soluble proteins and acetone-nitroso-heme pigment (hematin) in loaves made without additives and with 3.5% NFDM or ISP (Table 4). The amount of extractable water-soluble protein was not influenced by pH adjustment or by use of additives.

The use of pork hearts in loaves is self-limiting due to shrinkage and organoleptic properties. Incorporation of pork hearts at higher percentages (30%, for example) into the formula produced higher cooking shrinkages and lower panel scores for texture (firmness) and preference when compared with products containing lower percentages of pork hearts.

Additives used in this study did not improve the water-holding capacity of meat batter when substituted for an equal part of meat in the formula. The high protein content of these materials causes them to be over-rated in their ability to hold water. In every case, these additives do hold water at the first stage of loaf preparation, i.e., during chopping; but, the absorbed water is lost during the heating process causing high cooking loss. Glabe et al. (1956) studied Gelsoy, a spray-dried aqueous extract of ethanol-washed soy meal, with NSI as high as 90%. They reported that products with 1% Gelsoy in the formation released 29 ml liquid (17 ml water, 12 ml fat) compared to a control with 35 ml liquid separation (20 ml water and 15 ml fat) and concluded that Gelsoy used at a 1% level exerted a binding effect on fat and water in meat loaves. No further data were given to support whether the two values (29 ml and 35 ml) were significantly different. Pearson et al. (1965) found sodium soy proteinate (Promine D, Central Soya Co., Inc., Ill.) to be a poor emulsifier in the usual pH range of meat and suggested that Promine D does not serve any major function in emulsifying the fat when added to sausage formulation. Inklaar and Fortuin (1969) used the same technique and found that additives with higher Nitrogen Solubility Indices (NSI) significantly decreased fat separation. The NSI of additives in the latter work were generally low (ISP, 11%; PG, 28%; PF, 26%; SPC, 42%) with the exception of NFDM (95%). Inklaar and Fortuin (1969) also reported that in actual emulsion systems, those containing 2% soy protein exhibited 0.4% separable fat; whereas, 8.2% fat separated from the all-meat products. Cook et al. (1969), using an experimental luncheon loaf formulation of beef chuck and rendered lard (total protein, 14%; fat, 35% and moisture, 48%) processed at a high temperature and humidity to reduce cooking time, reported that the all-meat samples, shrank in excess of 25%, whereas those samples in which 2% and 4% of the meat proteins were replaced with soy protein, shrank 2% and 4%, respectively. The authors did not present the experimental data to support these statements nor did they disclose the NSI of the soy protein. The results of this work cannot confirm their description; however, functionality comparable to that mentioned by Raymond (1965) for NFDM, Inklaar and Fortuin (1969) for soy proteins and Toledo (1973) for animal protein concentrates was obtained in the present study.

Satterlee et al. (1973a) showed that hydrolyzates of beef or pork skin, when used to replace NFDM in a sausage formulation, produced a sausage with a slightly higher water and fat binding capacity than the control. Satterlee et al. (1973b) found that tissue powders prepared from pork and beef heart

Table 4–Effect of pH and additives on the cooking shrinkage, salt-soluble protein extractability, water-soluble protein extractability and hematin content (all values are means of two determinations)[a,b]

	No Additive				NFDM				ISP			
	pH				pH				pH			
	5.0	6.0	7.0	8.0	5.0	6.0	7.0	8.0	5.0	6.0	7.0	8.0
Cooking shrinkage (%)	37.22	21.59	16.28	13.67	33.72	21.08	14.00	10.37	36.14	25.82	18.91	14.94
Salt-soluble protein (mg/gm)	0.90	3.80	6.10	9.10	1.30	4.00	6.60	9.60	1.10	3.60	6.40	9.30
Water-soluble protein (mg/gm)	0.98	1.18	1.18	1.57	1.18	1.47	1.57	1.76	1.18	1.57	1.57	1.66
Acetone-nitroso-heme complex (ppm hamatin)	113	98	63	41	103	74	57	38	98	88	54	33

[a] Values in the same row within treatment blocks are significantly different at 5% level.
[b] Difference of treatments among types of additives are not significant at 5% level.

had poor emulsifying properties, while tissue powders from blood, lung and stomach could be incorporated into a sausage formulation emulsifying properties comparable to NFDM. Their data (Satterlee et al., 1973b) indicated that emulsifying capacities for blood, lung and stomach powders are comparable to those of lean beef. No explanation was given concerning this difference; presumably the proteins in heart tissue, when used as meat, are not efficient in binding water and fat.

Toledo (1973) reported that low temperature (30°C) extracted animal protein concentrate increased the fat and water binding capacity in comminuted meat products. The oil-binding capacity of protein could be altered by the extraction procedure. Extraction temperature of the process plays a role in the preservation of the functionality of the protein concentrate. The amount of salt-soluble protein decreased considerably at high extraction temperature. These results suggested that protein origin and the preparation procedure for the protein concentrate contributed to their functional properties. The results of the present experiments showed that binding between water and the protein additives used in this study is much weaker than that of water and the proteins of meats, with the exception of formulas using NFDM. In a similar study, Smith et al. (1973) reported that muscle proteins were excellent emulsion stabilizers and that they exhibited superior emulsion stabilizer characteristics when compared with most of the vegetable proteins presently offered to the trade.

Adjustment of pH is an effective way to prepare meat loaf with lower cooking loss. Increasing pH toward neutrality (pH 7.0) reduces cooking shrinkage. Presumably at this pH, gel formation between proteins, mainly myosin, and water is promoted. Meat with pH of below 5.5 (normal pH of carcass) is less effective in binding water possibly due to the tight structure of meat protein molecules at this pH. Hamm (1960) showed that the portion of water present in meats that can be affected by changing protein structure and/or protein charges is that portion that can be immobilized by capillary condensation, that is, fluid existing in the capillary space of the myofibrillar structure of muscles. This portion amounts to 90% of the total moisture content of muscle; thus, the effect of pH on water-binding capacity can be very significant.

Although increasing the pH of meat batter has numerous advantages such as reducing cooking shrinkage and increasing salt-soluble protein extractability, these could be overshadowed by poor color development in the product due to lack of nitrous acid (Wilson, 1960). Thus, prior to incorporating this procedure into a commercial practice, it will be necessary to determine the optimal pH that will produce products with low cooking loss and at the same time induce good color development.

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QUALITY CHARACTERISTICS OF BROILED AND ROASTED BEEF STEAKS

INTRODUCTION

BROILING is a popular method for cooking meat that is difficult to standardize whereas roasting is easy to standardize because there are fewer variables. In broiling, the heat source is primarily radiant heat; air convection and conduction from the rack or pan supporting the meat contribute minor amounts of heat (Rogers, 1969; Paul and Palmer, 1972). Broiling temperature is related to the distance of the meat from the broiling unit. However, radiation intensity varies among broiler units, even those of the same brand. In preliminary experiments, the temperature at a distance of 4 in. from the broiler unit ranged from 150–204°C among a group of individual electric ovens of the same make.

In roasting, heat is transferred by air convection with radiant heat having a minor effect. A thermostat determines the roasting temperature in an oven and maintains a preset temperature by repeatedly switching the oven's heating units on and off. The method called "oven broiling" in some publications by Cover and "oven cooking" in others (Cover and Hostetler, 1960) is actually roasting because the meat is heated in hot air.

Many studies have compared the characteristics of beef cooked by roasting or broiling and braising procedures (Dawson et al., 1959; Cover and Hostetler, 1960). Roasted and broiled meat qualities have not been compared directly, however. To determine which method, broiling or roasting, resulted in the better product, the quality characteristics of beef round steaks broiled at 176°C (the temperature at the top surface of the meat) were compared with those roasted in air in a 176°C oven. Comparisons were made on meat cooked to internal temperatures of 60 and 71°C.

MATERIALS & METHODS

SIX WHOLE TOP ROUNDS (Semimembranosus muscle) from U.S. Choice carcasses were obtained from the retail market and frozen at −23°C. Five steaks, 3.8 cm thick, were cut from each frozen round, wrapped in heavy duty aluminum foil, and held in freezer storage at −23°C until used (within 3-1/2 wk). Analyses were done on one raw and four cooked steaks from each round.

For each of the 12 taste panel sessions, two adjacent steaks from a round were thawed in the refrigerator (30 ± 1°C) for about 20 hr. The two thawed steaks were roasted or broiled at 176°C in separate preheated wall-type electric ovens, each equipped with a two-element watthour meter, to an internal temperature of either 60 or 71°C (the temperatures often associated with rare and medium done beef). In broiling, the unit was set on "Broil," the broiling temperature was determined at the top surface of the meat, and the oven door was opened 7.6 cm during the broiling period. The top surface of the meat was about 10 cm from the broiling unit. Temperatures of the oven and the meat were measured and recorded with thermocouples on a recording potentiometer. Broiled steaks were turned when the internal temperature of the steak reached 38°C. All steaks were cooked on open wire racks placed 2.5 cm above the enameled broiling pans.

Time required for each steak to reach a given internal temperature was recorded as well as the total cooking losses, drippings and evaporation.

Six people were chosen for the taste panel on the basis of consistency in selecting the odd sample in a series of triangle tests. (In the triangle tests, panel members evaluated round steaks cooked to different internal temperatures or by different methods and which varied in juiciness, tenderness, or mealiness. Like samples were taken from the same slices of steak; odd samples were taken from another steak cooked in a different way.) Panel members scored 3.8 × 5 × 0.6 cm center slices of meat for juiciness, tenderness, mealiness and flavor on a 9-point rating scale, and for degree of doneness on a 5-point scale. The highest scale values indicated the most juicy, most tender, most mealy, very full, characteristic flavor and most well done meat. Each panel member rated two slices from each of the two steaks; the four slices were served one at a time. To eliminate bias, order of serving followed a predetermined schedule.

In addition to the panel evaluations, juiciness and tenderness were determined objectively. Sanderson and Vail (1963) described the method adapted for the press fluid determination, a measure for juiciness, using 0.5-g samples of meat and the Carver press. Shear force, as a measure of tenderness, was determined on 20-g cubes of cooked steak with the Kramer shear press (Batcher and Dawson, 1960). The instrument charted the pounds of force required to shear across the grain of a piece of meat. Both maximum (peak) force and average force were calculated as pounds force required to shear 1g of meat.

Total solids contents of raw and cooked steaks were determined by drying 5-g samples of ground lean meat in a vacuum oven at 70°C for 24 hr. The lipid fraction from the ground lean meat was extracted with chloroform:methanol by using the procedure described by Bligh and Dyer (1959) wherein an aliquot of the extract was evaporated to dryness, and the lipid residue was weighed. Each measurement was done in triplicate on each raw or cooked steak.

A balanced incomplete block design (Cochran and Cox, 1957) was used in the study; sources of variation included both animals and panel members. Statistical differences among means were tested for significance by using Duncan's multiple range test (1955). Interrelationships among the various chemical, objective, and panel measurements were determined by correlation methods outlined by Snedecor and Cochran (1967).

RESULTS & DISCUSSION

COOKING TIME for roasted steaks was about twice as long as that for broiled steaks to reach the desired internal temperature, but less than half the kilowatt-hours were needed (Table 1). The electrical current cycled "on" and "off" during roasting as is required to maintain a desired oven temperature. The current was never off during broiling because the door was ajar. In today's conservation and cost conscious society, savings in the use of electrical energy are important.

Cooking losses, evaporation and drippings, were much lower in roasted steaks than in broiled steaks. As expected, steaks roasted or broiled to 71°C required more time and had greater cooking losses than steaks cooked to 60°C by either roasting or broiling procedures. Of course, the lower the cooking losses, the greater the amount of edible cooked meat. Others have also reported increased cooking losses with an increase in internal temperature of beef (Cover and Hostetler, 1960; Paul, 1962).

When cooked to either 60 or 71°C, roasted steaks were scored more juicy and tender, and less mealy than steaks broiled to the same internal temperature. Mealiness is characterized by tiny, dry and hard fragments of meat which cling to the surfaces of the mouth (Cover et al., 1962). Therefore, mealiness usually varies inversely with juiciness and is most prevalent in very well done and braised meat.

Except for degree of doneness, no significant differences were observed in the palatability characteristics of broiled steaks cooked to the two internal temperatures. However, panel members rated steaks roasted to 60°C more juicy, tender, and flavorful and less mealy than steaks roasted to 71°C. Steaks roasted to 60°C were scored less well done than all other steaks, even those broiled to the same internal temperature. Panel members scored steaks broiled to 60°C as medium in doneness and those roasted to 60°C as rare, the degree of doneness usually associated with 60°C. Paul and Palmer (1972) also noted that the stage of doneness at a given internal temperature will vary with the time required to reach that temperature. Panel members were unanimous in their evaluation of the effects of cooking method on one characteristic–degree of doneness. Each of the panel members was quite consistent in his evaluation of all palatability characteristics of two samples from the same steak.

More press fluid was obtained from steaks roasted to 60°C than from steaks broiled or roasted to 71°C which supported the panel members' evaluations of juiciness. The percentage of press fluid from the cooked steak was positively correlated with the mean panel scores for juiciness (Table 2).

Neither cooking method nor internal temperature had a significant effect on either peak or average shear force values. However, panel members did find differences in tenderness, and both peak and average shear force values were negatively correlated with tenderness scores. More force was required to shear samples that were scored low in tenderness.

Total solids contents were higher in cooked than in raw steaks, as expected (Table 1). Broiled steaks contained more solids (less moisture) than roasted steaks. Steaks with the highest total solids content or highest cooking losses were scored the most mealy and least juicy (Table 2).

The percentage of total lipids was significantly lower in raw steaks than in broiled steaks. However, the percentage of total lipids in raw and roasted steaks did not differ significantly (Table 1). Differences in the percentages of total lipids in roasted and broiled steaks were not significant on either a wet or dry basis. The percentage of total lipids in the steaks was not correlated with either the objective measures or panel scores for juiciness, tenderness, mealiness, or flavor.

Besides producing a more juicy, more tender and less mealy product, roasting had additional advantages over broiling. Less spattering occurred when meat was roasted than when it was oven broiled. Roasting required a minimum of the chef's attention during cooking of the meat. Many other foods can be cooked simultaneously in the oven with the meat thus conserving energy.

Table 1–Mean values and scores for selected palatability, physical and chemical characteristics of beef round steaks broiled or roasted to an internal meat temperature of 60 or 71°C

Characteristics	No. of detm in mean	Raw	Broiled at 176°C to an internal meat temp of 60°C	Broiled 71°C	Roasted at 176°C to an internal meat temp of 60°C	Roasted 71°C
Cooking time[a]						
Total minutes	6	–	37a	41a	70b	90c
Minutes per kg	6	–	41a	46b	78c	100d
Electrical energy (kwh)	6	–	253b	331c	115a	135a
Cooking losses						
Total, %	6	–	33.3c	40.5d	13.4a	26.2b
Drippings, %	6	–	11.0ab	11.9b	7.7a	9.3ab
Evaporation, %	6	–	22.3c	28.6d	10.7a	16.9b
Palatability scores[b]						
Juiciness	72	–	6.3bc	5.1c	8.3a	6.6b
Tenderness	72	–	5.0c	4.8c	6.7a	5.9b
Mealiness	72	–	4.5ab	4.8a	2.8c	4.0b
Flavor	72	–	6.8ab	6.1b	7.4a	6.6b
Doneness	72	–	3.2b	4.1a	1.6c	3.7ab
Press fluid, %	18	–	51.6ab	46.8b	55.7a	50.3b
Shear force						
Peak lb force per gram	18	–	15.2a	16.7a	14.8a	15.6a
Average lb force per gram	18	–	9.0a	10.6a	8.3a	8.6a
Total solids, %	18	26.1a	42.6c	44.7d	35.0b	37.6b
Total lipids, %	18	4.2a	9.0b	9.4b	8.7ab	6.4ab

[a] Means with same letter on a line are not significantly different at the 5% level of probability based on Duncan's multiple range test.
[b] Except for doneness, scales ranged from 1–9, with the higher values indicating greater quantity of the characteristics–most juicy, most tender, most mealy, and very full, characteristic flavor. Scale for degree of doneness is rare = 1, medium = 3, well-done = 5.

Table 2–Correlation coefficients between taste panel scores and objective measurements of quality characteristics of meat

Panel score	Press fluid	Total solids	Shear force Peak	Shear force Average
Juiciness	0.822**	–0.491*		
Mealiness		0.618**		
Tenderness		–0.351ns	–0.609**	–0.719**

* $P < 0.05$
** $P < 0.01$
ns not significant

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CHARACTERISTICS OF CONVENTIONALLY AND HOT-BONED BOVINE MUSCLE EXCISED AT VARIOUS CONDITIONING PERIODS

INTRODUCTION

THE EFFECTS of hot boning on the quality of bovine muscle has been studied in initial investigations by Schmidt and Gilbert (1970). They found that bovine muscles removed within 2 hr after exsanguination, vacuum packaged, and held at 15°C for 24 hr were usually as tender as the same muscles excised after a 24 hr chill at 9°C. When the conditioning period for the muscles, held at 15°C, was extended to 48 hr postmortem, the mean shear force values for the biceps femoris, anterior longissimus dorsi, and posterior longissimus dorsi muscles were significantly ($P < 0.05$) less than the controls. However, the same authors observed that aging the pre-rigor excised semimembranosus muscles for 24 hr at 15°C and the semitendinosus muscles for 24 and 48 hr postmortem, at the same temperature, gave mean shear force responses that were greater than the controls. Taste panel assessments of tenderness, juiciness, texture and general acceptability of the pre-rigor excised muscles, that were conditioned for 24 or 48 hr, indicated that they were generally as acceptable as the controls and in some instances superior. Microbial spoilage was satisfactorily controlled in these samples that were held in vacuum packages at 15°C for 24 or 48 hr postmortem. These same authors concluded that pre-rigor excision of prime cuts of beef can yield organoleptically acceptable meat of satisfactory microbiological standards.

Kastner et al. (1973) excised bovine muscles at 2, 5 and 8 hr postmortem (hot boning) during which times the holding temperature was 16°C. The corresponding half of each carcass was held at 2°C until 48 hr postmortem prior to muscle excision (cold boning). The hot-boned muscles were placed in cryovac bags immediately post-excision and stored at 2°C until 48 hr postmortem. When the hot- and cold-boned muscle (for the 8 hr holding period) responses for shrink (percent loss), shear force, color value notation, flavor, cooking loss, water-binding capacity, percent moisture and fat were compared statistically, the data indicated that the hot-boned treatment was equal or superior to its cold-boned counterpart. The mean shear force responses for muscles excised at 2 and 5 hr postmortem were statistically ($P < 0.05$ and $P < 0.10$) different and larger than the corresponding cold-boned samples. However, these authors pointed out that the differences may not be economically important. Considering the parameters evaluated and the methodology used in this preliminary study, Kastner et al. (1973) concluded that muscle excision at 8 hr postmortem could produce a product of acceptable quality and yield.

In a more recent study by Schmidt and Keman (1974), yield, shear force, fiber diameter and various palatability characteristics were evaluated for hot-boned, vacuum packaged bovine muscles. The right half of each carcass was hot boned within 1 hr postmortem and the corresponding halves were held at 1°C for 8 days prior to muscle excision (cold boning). Hot-boned muscles were held at 7°C for 4 hr post-excision, chilled overnight at 1°C, vacuum packaged and stored at 1°C for 7 days. These authors observed no statistical difference ($P > 0.05$) between the shear force means for hot- and cold-boned steaks and roasts. In addition, the taste panel observed no statistical differences ($P > 0.05$) between hot- and cold-boned steaks and roasts when flavor, juiciness, tenderness and overall acceptability were evaluated. It appeared that difficulty in trimming the fat cover of hot-boned cuts caused a statistically greater ($P < 0.05$) mean retail yield value for the hot-boned product. Differences between the hot- and cold-boning means for fiber diameter were statistically significant ($P < 0.01$) and larger for the hot-boned psoas major, gluteus medius and semitendinosus muscles; however, these differences were not supported by shear force and taste panel responses. These authors concluded that a very acceptable product can be produced by hot boning.

The purpose of this study was to evaluate the yield (percent loss), tenderness, flavor and color of bovine muscles held at 16°C and excised at 6, 8 or 10 hr postmortem (hot boning) as compared to muscles held at 2°C and excised at 48 hr postmortem (cold boning).

MATERIALS & METHODS

15 CHOICE AND GOOD grade heifers, ranging in weight from 367–501 kg, were assigned to one of three postmortem holding periods. One-half of each carcass was held for 6, 8 or 10 hr at 16°C prior to muscle excision. The corresponding half of each carcass was held 48 hr at 2°C before the muscles were excised. The animals were held off feed for 24 hr prior to slaughter. Upon exsanguination, the time was recorded and all postmortem holding periods were based on this zero time. Each carcass was divided in the conventional manner, weighed, washed and the halves randomly assigned to either hot or cold boning. Within 2 hr postmortem, one-half was placed in a cooler at 2°C and the corresponding half in a 16°C holding room.

pH and temperature determinations

In order to follow postmortem pH decline, samples (10g) were excised from both halves at hourly intervals beginning at 2 hr postmortem and continuing until fabrication of the hot-boned half. Samples were taken from corresponding locations on each psoas major muscle. The samples were minced and placed in 50 ml of distilled, deionized water. The pH was measured, and used as an index of the extent and rate of postmortem glycolysis (Khan and Lentz, 1973). In addition, 24- and 48-hr postmortem samples were taken in the same manner.

The extent of temperature decline on each half was measured by inserting a thermometer such that it passed through the obturator foramen into the round. The thermometer location was standardized for each round. Internal round temperatures for both treatments were recorded immediately prior to fabrication of each carcass half to be hot boned.

Hot and cold boning

After the appropriate postmortem holding period (6, 8 or 10 hr), the halves to be hot boned were fabricated into lean trim, fat trim, bone and intact muscles and muscle systems (clod, supraspinatus, inside chuck, longissimus dorsi, gluteus medius, quadriceps femoris, semimembranosus and adductor, biceps femoris and semitendinosus). Each component was placed into individual Cryovac bags (B-620) immediately post-excision. Upon complete fabrication (approximately 1.5 hr), the

resulting components were placed in the 2°C cooler along with the control (cold-boned) half.

At 48 hr postmortem, the cold-boned half was fabricated in the same manner as the hot-boned half. Fabrication was completed within 2.5 hr of initiation. Upon complete fabrication, the components of each side were weighed and totaled and the yield, expressed as percent loss, was calculated based on the inital weights of the carcass halves.

Five test muscles were selected from each half (semimembranosus, semitendinosus, biceps femoris, longissimus dorsi and supraspinatus). Steaks were cut from corresponding locations on each set of paired muscles for further quality evaluation. The maximum length of each muscle was measured and divided by two; thus, the location of the midline of each muscle was determined. Two steaks for each parameter measurement were taken from alternating sides of this midline. On the anterior or origin side of the midline, samples for taste panel, color panel, shear force and color by reflectance were cut 2.5, 2.5, 5.0 and 2.5 cm thick respectively, beginning at the midline. On the posterior or insertion side of the midline, again beginning at the midline and continuing consecutively, color by reflectance, shear force, color panel and taste panel steaks were cut 2.5, 5.0, 2.5 and 2.5 cm thick.

Color difference assessment

Two 2.5 cm steaks from each muscle were allowed to oxygenate under atmospheric conditions for 2 hr at 2°C before Photovolt Reflection Meter (Model 670) readings were taken. The search unit was equipped with a green filter, and the meter was adjusted to 100% reflectance using a smooth magnesium oxide surface as a standard. The reflectance readings were then converted to Munsell color value scores (Judd and Wyszecki, 1952).

Another set of samples, as those taken for Photovolt reflectance measurements, were frozen at –40°C, thawed at 2°C, and exposed to atmospheric conditions for the same period of time. The Hunterlab Digital Color Difference Meter (Model D25D) equipped with the D 25 optical head was used to record L, a and b values for corresponding steaks derived from the hot- and cold-boned test muscles. The meter was standardized using the pink Hunterlab standard (No. W824). The differences between the sample L, a and b values and the same readings for the pink standard were recorded as ΔL, Δa and Δb. Using these values, ΔE as defined by $\Delta E = [(L_1 - L_2)^2 + (a_1 - a_2)^2 + (b_1 - b_2)^2]^{1/2}$ was calculated (Francis and Clydesdale, 1969; Clydesdale and Francis, 1971). The a/b ratios were also calculated for the hot- and cold-boned samples.

Organoleptic evaluation

The same samples used for Hunterlab Color Difference Meter measurements were initially evaluated by a color difference panel. Each panelist was requested to assume a standard position from the samples which were displayed on a black background utilizing a Macbeth light as the only source of illumination. Corresponding steaks from each muscle for both the hot- and cold-boning treatments were displayed side by side and the panelists were asked if the two samples were the same or different in color.

The same panel members were presented three samples (standard size) of longissimus dorsi muscle that was cooked to an internal temperature of 66°C. Two samples were from either the hot- or cold-boning treatment and the other from the corresponding treatment. The panelists were requested to select the odd sample. The taste panel room was equipped with red fluorescent lights in order to standardize product appearance.

Statistical significance for the paired comparison and triangle tests was evaluated as presented by Kramer and Twigg (1966).

Shear force

Two 5.0 cm steaks were cut from each muscle and frozen at –40°C for later analysis. Samples were thawed at 2°C, oven roasted at 163°C to an internal temperature of 72°C, and chilled at 2°C for 24 hr. If sample size permitted, three 2.5 cm diameter cores were taken from each steak, and each core was sheared three times with a Warner-Bratzler shear apparatus (Kastner and Henrickson, 1969).

The sources of variation in the analysis for a muscle and holding period were treatments, animals and animal × treatment so that means for percent loss and shear force had five observations (animals). Pooled error terms across all holding periods were used to test mean differences for percent loss and shear force data. Color measurement mean differences were tested using the animal × treatment mean square with 4 degrees of freedom.

Table 1–Percent loss means for hot versus cold boning by postmortem holding periods

Holding periods (hr)	Hot (%)		Cold[b] (%)
6[a]	1.11		1.23
$s_{\bar{x}}$		0.078	
8[a]	1.27		1.39
$s_{\bar{x}}$		0.063	
10[a]	1.15[c]		1.42
$s_{\bar{x}}$		0.120	

[a] Postmortem holding periods 6, 8 or 10 hr for hot-boned halves
[b] Cold-boned halves held 48 hr postmortem for all holding periods
[c] ($P < 0.10$). Significant difference between hot and cold boning

Table 2–Flavor and color panel results for hot- versus cold-boned samples by postmortem holding periods

	Flavor		Color	
Holding periods (hr)	Total triangle comparisons	No. identifying odd sample	Total no. of paired comparisons	No. detecting a difference
6[a]	30	10[b]	300	107[b]
8[a]	30	11[b]	300	70[b]
10[a]	30	13[b]	300	84[b]

[a] Postmortem holding periods 6, 8 or 10 hr for hot-boned halves
[b] ($P > 0.05$). Statistically nonsignificant

RESULTS & DISCUSSION

Percent loss

The hot-boned halves were consistently lower in percent loss than the cold-boned halves. The differences between the mean percent loss values for hot- and cold-boned halves were statistically nonsignificant ($P > 0.10$) for the 6- and 8-hr holding periods (Table 1). However, the difference between the hot- and cold-boning treatments were statistically significant ($P < 0.10$) for the 10-hr holding period. These data agree with previous work by Kastner et al. (1973) even though the magnitude of the differences were not as great in this study.

Flavor and color panel

Flavor panel evaluation of the hot- and cold-boned samples indicated that no statistical difference ($P > 0.05$) existed between these treatments for each postmortem holding period (Table 2).

When samples from each treatment were visually evaluated for color, no statistically detectable difference ($P > 0.05$) was observed for any of the postmortem holding periods (Table 2). Regardless of the postmortem holding period, the flavor and color panelists found the hot-boned samples to be comparable to the cold-boning treatment.

Color by reflectance

Differences in the color measurement parameters between hot and cold boning were statistically nonsignificant ($P > 0.10$) for the 6-hr holding period (Table 3). Statistical differences were observed between hot- and cold-boning means for ΔL, Δb, ΔE and color value for both the 8- and 10-hr holding periods. The ΔL and ΔE values for the hot-boning treatment in the 8- and 10-hr holding periods were statistically different [8 hr ($P < 0.10$); 10 hr ($P < 0.01$)] and smaller than the corresponding mean values for the cold-boning treatment. On the other hand, the color value means for the hot-boned sam-

Table 3—Color measurement means for hot versus cold boning by postmortem holding periods

Holding periods (hr)	ΔL		Δa		Δb		a/b		ΔE		Color value	
	Hot	Cold[b]	Hot	Cold[b]	Hot	Cold[b]	Hot	Cold[b]	Hot	Cold[b]	Hot	Cold[b]
6[a]	46.48	46.82	2.30	2.31	2.63	2.54	1.57	1.59	46.62	46.97	3.52	3.54
$s_{\bar{x}}$	0.124		0.121		0.031		0.011		0.129		0.019	
8[a]	45.69[c]	46.48	1.39	1.36	2.37[e]	2.16	1.51	1.55	45.80[c]	46.58	3.64[d]	3.52
$s_{\bar{x}}$	0.238		0.124		0.025		0.012		0.240		0.029	
10[a]	44.65[e]	45.55	1.75	1.62	2.71[d]	2.44	1.58	1.52	44.80[e]	45.69	3.63[c]	3.54
$s_{\bar{x}}$	0.082		0.098		0.051		0.062		0.083		0.027	

[a] Postmortem holding periods 6, 8 or 10 hr for hot-boned halves
[b] Cold boned halves held 48 hr postmortem for all holding periods
[c] ($P < 0.10$)
[d] ($P < 0.05$)
[e] ($P < 0.01$) Significant difference between hot and cold boning

ples were statistically different [8 hr ($P < 0.05$); 10 hr ($P < 0.10$)] and larger than the cold-boning means for these two postmortem holding periods. The Δb values for the hot-boned samples were statistically different ($P < 0.01$) and ($P < 0.05$) and larger for the 8- and 10-hr holding periods respectively when compared to the cold-boned samples (Table 3). Even though these statistical differences existed between the treatment means for the various parameters these differences appeared to be visually subtile as they were not detected by the color panel (Table 2).

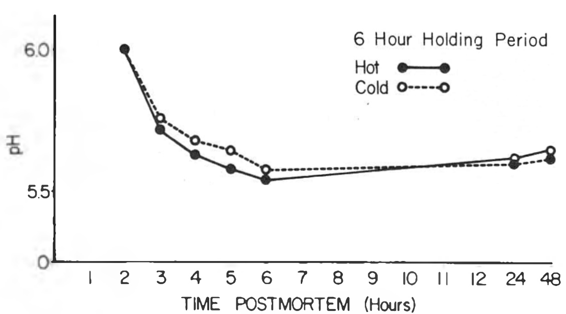
Shear force

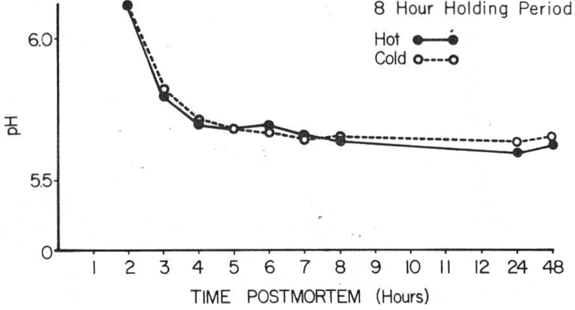
When each postmortem holding period was evaluated, considering all test muscles, the differences between corresponding treatment means were statistically significant ($P < 0.05$) for the 6-hr holding period and nonsignificant ($P > 0.10$) for the 8- and 10-hr holding periods (Table 4). When the shear force responses for each test muscle were analyzed, it was observed that there was no statistically significant difference ($P > 0.10$) between corresponding means for each of the test muscles in the 10-hr holding period (Table 4). Shear force means for the hot- and cold-boned semitendinosus, biceps femoris and longissimus dorsi muscles were statistically different ($P < 0.10$) in the 8-hr holding period (Table 4). The hot-boning treatment (8-hr holding period) gave larger mean shear force values than the corresponding cold-boning means for the semitendinosus and biceps femoris muscles; however, the reverse was true for the longissimus dorsi muscle (Table 4). For the 6-hr holding period, the mean shear force values for the hot-boned semitendinosus and supraspinatus muscles were statistically different ($P < 0.05$) and larger than their cold-boned counterparts (Table 4). Differences in shear force that exist between hot and cold boning in the 8-hr holding period may not be of economic importance as these differences are generally quite small. Considering an overall view of all test muscles, tenderness, as defined by shear force, appears to be comparable between hot and cold boning if the hot-boned carcass halves are held intact until 8 hr postmortem.

Postmortem pH and temperature decline

The average ultimate pH value for the hot-boned halves assigned to the 6-hr holding period was achieved at approximately 6 hr postmortem (Fig. 1), and the initiation of fabrication of these hot-boned halves closely corresponded to the attainment of this ultimate pH. At 6 hr postmortem, the average internal round temperature was 33.9°C for the hot-boned halves and 32.2°C for the cold-boned halves assigned to the 6-hr holding period.

The average ultimate pH was attained prior to fabrication of the hot-boned halves assigned to the 8-hr holding period (Fig. 1). At 8 hr postmortem the hot-boned halves had an average internal round temperature of 32.2°C whereas the cold-boned halves had an average internal temperature of 28.9°C.





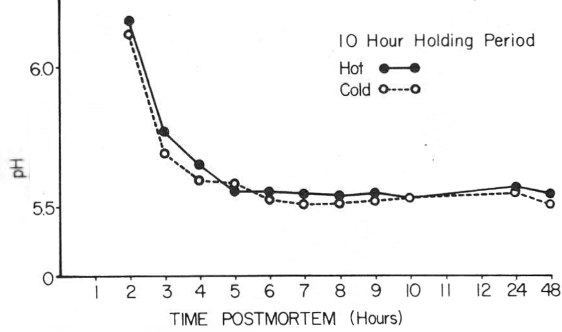


Fig. 1—Postmortem pH decline for hot- and cold-boned psoas major muscles for 6, 8 and 10-hr holding periods.

Table 4–Shear force means (kg) for hot versus cold boning by muscle and postmortem holding periods and by postmortem holding periods for all test muscles

Holding periods (hr)	Muscles											
	Semimembranosus		Semitendinosus		Biceps femoris		Longissimus dorsi		Supraspinatus		All muscles	
	Hot	Cold[b]	Hot	Cold[b]	Hot	Cold[b]	Hot	Cold[b]	Hot	Cold[b]	Hot	Cold[b]
6[a]	13.1	12.4	11.0[d]	10.4	12.3	11.7	9.0	9.4	12.6[d]	10.8	11.6[d]	10.9
$s_{\bar{x}}$	0.38		0.16		0.44		0.37		0.54		0.18	
8[a]	14.1	14.9	11.9[c]	11.3	13.7[c]	12.6	9.8[c]	11.5	12.6	12.0	12.4	12.7
$s_{\bar{x}}$	0.16		0.24		0.22		0.84		0.41		0.17	
10[a]	12.6	12.4	11.0	10.7	13.5	12.5	7.7	8.8	12.3	11.3	11.4	11.1
$s_{\bar{x}}$	0.41		0.22		0.48		0.30		0.52		0.11	

[a] Postmortem holding periods 6, 8 or 10 hr for hot-boned halves
[b] Cold-boned halves held 48 hr postmortem for all holding periods
[c] ($P < 0.10$)
[d] ($P < 0.05$) Significant difference between hot and cold boning

For the 10-hr holding period, the ultimate pH was reached approximately 4 hr prior to fabrication of the hot-boned halves (Fig. 1), and the average internal round temperatures were 30.0 and 26.1°C for the hot- and cold-boned halves, respectively.

When the ultimate pH for the psoas major muscle was reached prior to muscle excision, there were small overall differences between the shear force means for the hot- and cold-boning treatments. Refer to the 8- and 10-hr holding periods (Table 4 and Fig. 1). In addition, any differences in internal round temperatures did not appear to significantly affect the rate and extent of postmortem pH decline. Any differences in shear force for these two holding periods were generally small and in most cases statistically nonsignificant. In addition, when the shear force values for each postmortem holding period were evaluated, over all test muscles, the differences between corresponding treatment means were statistically nonsignificant ($P > 0.10$) for the 8- and 10-hr holding periods (Table 4).

For each holding period, the pH decline for the hot- and cold-boned halves was essentially the same. The average ultimate pH values were comparable between hot- and cold-boned halves in each postmortem holding period and among holding periods (Fig. 1).

Considering an overall analysis of all parameters (percent loss, flavor, color and shear force), if the halves to be hot boned were held until 8-hr postmortem the hot boning method was comparable to the conventional method. These results agree with the findings of Kastner et al. (1973).

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THE REMEDIAL AND PREVENTATIVE EFFECT OF DIETARY α-TOCOPHEROL ON THE DEVELOPMENT OF FISHY FLAVOR IN TURKEY MEAT

INTRODUCTION

IT IS WELL ESTABLISHED that the development of fishy flavor in turkeys fed fish oil is related to the linolenate (ω-3 fatty acids) content of the oil. Crawford and workers reasoned that the flavor probably resulted from in vivo and/or postmortem oxidation of the linolenates especially the long chain homologues such as C20:5, C22:5, and C22:6. Alpha tocopherol may reduce fishiness in vivo by significantly preventing the oxidation and breakdown of linolenates and/or may react with the oxidative products thus changing their flavor characteristics. If the latter hypothesis is correct, then α-tocopherol may be used therapeutically toward the end of the feeding of fish oil either by incorporation into the diet a few weeks before slaughter or by injection a few days before slaughter.

Crawford et al. (1974) verified the findings of Dreosti (1970) and Opstvedt et al. (1971), that α-tocopherol acetate does retard the development of fishy flavor in poultry when the α-tocopherol acetate is fed concomitantly with fish oil. The recent work of Webb et al. (1973) further confirms this finding. This preventative effect of α-tocopherol acetate may have commercial significance, depending on the degree of effectiveness and the economics.

Feeding fish or other polyunsaturated oils to turkeys significantly increases the unsaturated fatty acid content of the carcass, and carcass storage stability is related to the degree of unsaturation and tocopherol content (Mecchi et al., 1956a, b; R.W. Webb et al., 1972; J.E. Webb et al., 1972). This effect should also be considered when appraisal is made of the cost: benefit ratio of the use of α-tocopherol, especially for further processed products like turkey rolls, precooked dinners, etc.

This paper reports on: (1) the level of effectiveness of dietary α-tocopherol acetate in preventing the development of fishy flavor in turkeys fed fish oil; (2) the effect of dietary α-tocopherol acetate on the flavor of turkeys when included in the diet after the feeding and withdrawal of fish oil; and (3) the effect of α-tocopherol injection (a few days before slaughter) on the flavor of turkeys fed fish oil.

EXPERIMENTAL

Oils

Freshly rendered beef fat and polished (twice centrifuged) tuna oil were obtained unstabilized. Ethoxyquin was added to each oil and fat to give 125 ppm after the oil or fat was mixed into the diet.

Feed

The following basal diet was used in the feeding of all turkeys with modifications as indicated:

Basal diet	g/100g
Soybean oil meal (50%)	50.00
Mineral mix	2.58
Vitamin mix (in corn starch)	1.00
$CaHPO_4 2H_2O$	1.70
$CaCO_3$	1.90
Choline Cl (50%)	0.20
Dl methionine	0.40
Ground corn	38.22

Diets contained 10 mg Vitamin E (dl α-tocopherol acetate) per kilo and 0.66 ppm sodium selenite. Diets were mixed with the stabilized oils every 1 or 2 wk prior to use and stored in a refrigerator. Fresh feed was added to the bottom of the feed tray so that feed remaining from the previous day would be consumed first.

Diets and feeding

Experiment 1, the preventative effects of α-tocopherol. 80 White Broad Breast poults were fed 1/2 starter (6.75% fishmeal) and 1/2 basal diet for 2 days. On day 3, they were divided into 8 groups of 10 turkeys each, and fed the basal diet plus the following oil supplement until they were 9 wk old:

Group	Oil supplement to basal diet[a]
1	4% BF
2	3% BF + 1% TO
3	3% BF + 1% TO + 100 mg Vit. E/kilo
4	3% BF + 1% TO + 200 mg Vit. E/kilo
5	2% BF + 2% TO + 100 mg Vit. E/kilo
6	2% BF + 2% TO + 200 mg Vit. E/kilo
7	2% BF + 2% TO + 500 mg Vit. E/kilo
8	2% BF + 2% TO

[a] BF = Beef fat; TO = Tuna oil; Vit. E = dl α-tocopherol acetate

Experiment 2, the remedial effect of tocopherol after withdrawal of dietary fish oil. 50 White Broad Breast turkey poults were treated as follows: Chick starter (6.75% fishmeal) was fed to 3 wk of age. They were then fed a 50:50 mixture of chick starter and the 50% soybean meal basal diet for a few days followed by the 50% soybean basal diet supplemented with 2% soybean oil and 2% beef fat to 8 wk of age. At 8 wk of age the soybean meal content of the diet was reduced to 30% and the methionine to 0.3% with a corresponding increase in corn. The turkeys were then divided into 5 groups of 10 each and the following fat supplements (replacing the previous one) were fed from 8–14 wk of age:

Group	Oil supplement to basal diet[a]
1	4% BF
2	2% BF + 2% TO
3	2% BF + 2% TO
4	2% BF + 2% TO
5	2% BF + 2% TO

[a] See oil supplement, Experiment 1

At 14 wk of age, the above groups of turkeys were fed a soybean meal basal diet and oil supplement to 16 wk of age as follows:

Group	Oil supplement to basal diet[a]
1 Keep on	4% BF
2 Change to	4% BF
3 Change to	4% BF + 100 mg Vit. E/kilo
4 Change to	4% BF + 200 mg Vit. E/kilo
5 Keep on	2% BF + 2% TO

[a] See oil supplement, Experiment 1

Experiment 3, the effect of tocopherol injection. 50 White Broad Breast poults were obtained and handled as in Experiment 1 through day 2. On day 3, they were fed a basal diet plus 4% beef fat to 9 wk of age. At 9 wk of age to the end of the experiment, the fat supplement remained 4% beef fat but the soybean oil meal content of the diet was

Table 1—Duncan's multiple range test of mean[a] taste panel scores[b] for breast and thigh meat and skin of turkeys fed to 9 wk of age diets containing varying levels of tuna oil and beef fat with and without α-tocopherol acetate supplementation

Treatment[c]	Breast meat	Treatment[c]	Thigh meat	Treatment[c]	Skin
8 2% TO	3.74	8 2% TO	2.81	8 2% TO	2.49
5 2% TO + 100E	3.24	5 2% TO + 100E	2.47	5 2% TO + 100E	2.22
2 1% TO	2.85	2 1% TO	2.25	2 1% TO	2.11
3 1% TO + 100E	2.07	6 2% TO + 200E	1.86	7 2% TO + 500E	1.98
6 2% TO + 200E	1.99	3 1% TO + 100E	1.59	6 2% TO + 200E	1.79
1 4% BF	1.96	7 2% TO + 500E	1.57	3 1% TO + 100E	1.32
4 1% TO + 200E	1.69	1 4% BF	1.51	4 1% TO + 200E	1.32
7 2% TO + 500E	1.59	4 1% TO + 200E	1.31	1 4% BF	1.12

[a] Mean taste panel scores connected by a common line are not significantly different at the 0.05 probability level.
[b] Taste panel scoring, 1 = no fishy flavor, 5 = very fishy flavor
[c] TO = tuna oil, BF = beef fat, E = mg α-tocopherol acetate/kilo diet. All diets containing tuna oil were made isocaloric to 4% with beef fat.

reduced to 30% and the methionine to 0.3% with corresponding increases in corn. From 14–16 wk of age, they were divided into 5 groups of 10 turkeys each and fed as follows:

Group	Oil supplement to basal diet[a]
1	4% BF
2	2% BF + 2% TO
3	2% BF + 2% TO (+ inject 170 mg of α-tocopherol into thigh 72, 48, 24 hr before sacrifice)
4	2% BF + 2% TO + 100 mg Vit. E/kilo
5	2% BF + 2% TO + 500 mg Vit. E/kilo

[a] See oil supplement, Experiment 1

Sampling and analysis

Sampling, determination of oil content, fatty acid distribution by GLC and organoleptic analysis using a balance incomplete block design were previously described by Crawford et al., 1974. Duncan's multiple range test (α = 0.05) was used to compare the adjusted mean scores of 7 trained panelists. The scoring was, 1 = no fishy flavor, 5 = very fishy flavor.

RESULTS & DISCUSSION

Experiment 1, the preventative effects of α-tocopherol

The turkeys had a mean dressed weight of 1817g, with no significant differences between groups. Growth was normal and uneventful.

The authors have learned by experience that breast meat reflects more consistently the organoleptic character of fish oil fed turkeys. This is probably because of the relative blandness of the breast meat as compared to the thigh and skin. Therefore, more reliance is placed on the taste panel results for the breast meat.

Table 2—Methyl ester fatty acid composition of tuna oil and rendered beef fat.

Fatty acid	% Distribution	
	Rendered beef fat	Tuna oil
C14	3.4	2.9
C14-1	1.0	0.2
C15	0.6	0.8
ISO C16	0.2	0.2
C16	24.7	16.7
C16-1	3.6	4.4
C17	1.5	1.5
ISO C18	1.2	0.8
C18	17.8	4.7
C18-1	43.2	15.0
C18-2	1.7	4.0
C18-3 ω 3	0.3	1.0
C20-4	0	1.6
C20-5 ω 3	0	9.2
C22-5 ω 3	0	1.6
C22-6 ω 3	0	28.2
Others	0.8	7.2

Table 3—Methyl ester fatty acid composition of lipids extracted from the breast meat of turkeys fed to 9 wk of age, diets containing varying levels of beef fat and tuna oil with and without α-tocopherol acetate supplementation

	% Distribution in extracted oil							
Fatty acids Diets[a]	1 4% BF	2 1% TO	3 1% TO 100 mg E	4 1% TO 200 mg E	5 2% TO 100 mg E	6 2% TO 200 mg E	7 2% TO 500 mg E	8 2% TO
C14	0.7	0.6	0.8	0.9	0.6	0.6	0.6	0.4
C14-1	0.2	0.1	0.2	0.2	0.1	0.1	0.1	0.1
C15	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
ISO C16	3.8	4.4	3.7	3.3	4.3	4.1	4.2	4.9
C16	16.1	16.7	17.0	17.8	16.7	16.6	17.1	16.1
C16-1	1.4	1.2	1.5	1.6	1.3	1.4	1.4	1.1
C17	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6
ISO C18	2.1	2.1	1.8	1.6	2.1	1.8	1.8	2.2
C18	14.6	13.7	13.1	13.0	13.3	13.2	12.8	12.4
C18-1	20.4	16.6	18.6	20.0	16.1	15.6	15.8	15.4
C18-2	23.7	17.4	18.8	18.4	15.8	17.1	17.0	16.1
C18-3 ω 3	0.6	0.4	0.5	0.8	0.4	0.5	0.6	0.4
C20-4	8.2	6.3	5.7	4.8	5.5	5.8	5.4	6.4
C20-5 ω 3	0.6	3.2	2.6	2.6	4.4	4.4	4.2	4.3
C22-5 ω 3	1.2	1.7	1.6	1.5	1.6	1.6	1.8	1.6
C22-6 ω 3	1.4	12.8	11.1	10.6	15.0	14.4	14.6	15.2
Others	4.2	2.0	2.2	2.1	2.0	2.0	1.8	1.6
g Lipid/100g tissue	0.90	0.86	0.96	0.85	0.81	0.83	0.89	0.82

[a] BF = beef fat; TO = tuna oil; mg E = mg/kilo dl α-tocopherol acetate.

Table 4—Duncan's multiple range test of mean[a] taste panel scores[b] for breast and thigh meat and skin of turkeys fed from 8–14 wk of age a diet with 2% tuna oil plus 2% beef fat and fed 14–16 wk a diet with no tuna oil but 4% beef fat with and without α-tocopherol acetate supplementation

Treatment[c]	Breast meat	Treatment[c]	Thigh meat	Treatment[c]	Skin
5 2% TO	3.14	5 2% TO	3.41	5 2% TO	2.28
2 4% BF[d]	2.43	2 4% BF[d]	1.70	3 4% BF + 100E	1.81
3 4% BF + 100E	1.31	1 4% BF	1.50	4 4% BF + 200E	1.50
1 4% BF	1.29	4 4% BF + 200E	1.32	2 4% BF[d]	1.46
4 4% BF + 200E	0.99	3 4% BF + 100E	1.30	1 4% BF	0.98

[a,b] See footnote to Table 1
[c] See Experimental, Diets and Feeding.
[d] Diet 2 was changed from a fat content of 2% BF and 2% tuna oil when turkeys were 14 wk of age to 4% beef fat from age 14 wk to 16 wk.

Table 1 reports the mean scores of taste panel evaluation by balanced incomplete block design (t = 8, r = 7). Fishiness tended to increase with the amount of fish oil in the diets and to decrease as the amount of α-tocopherol in the diets increased. Duncan's multiple range test showed that Diet 8 (2% fish oil + 2% beef fat) produced the more fishy turkey meat and skin. In those diets that contained 2% tuna oil, it was noted that for the breast meat, 200 mg/kilo of α-tocopherol acetate reduced fishiness significantly more than 100 mg/kilo but that 500 mg/kilo was not much more effective than 200 mg/kilo. In fact, 200 mg and 500 mg in the diets that contained fish oil invariably resulted in taste panel scores for thigh and breast meat that were about equal to or lower than the control although the differences were not statistically significant. Considering economics and efficient utilization, it would then appear that about 200 mg/kilo of α-tocopherol acetate exerts optimum protection against the development of fishy flavored turkey when 2% tuna oil is contained in the diet. However, it should also be noted that the fish oil (tuna) used in this experiment has, by far, the highest level of long chain linolenate content (Table 2) of oils in commonly used fishmeals. Since fishiness is related to the linolenate content of dietary oils, it is reasonable that oils containing lower levels of linolenates would require lower levels of α-tocopherol to impede the development of fishy flavor. Webb et al. (1973) reported some reduction of fishiness with 22 mg/kilo of α-tocopherol acetate.

Table 3 reports the results of GLC analysis of the fatty acid distribution of extracted lipids from the breast meat of turkeys for all treatments. As expected, the level of C20:5 and C22:6 increased as the amount of fish oil in the diet increased, and α-tocopherol acetate had no effect on the uptake of these fatty acids. These linolenates seem to increase at the expense of oleic, linoleic and arachidonic acids.

Experiment 2, the remedial effect of α-tocopherol after withdrawal of dietary fish oil

The mean average dressed weight of the turkeys in this group was 3637g. Growth to time of slaughter was uneventful and all birds appeared to be in good nutritional health.

Table 4 demonstrates the remedial effect of α-tocopherol acetate on fishy flavor. Duncan's multiple range test showed that the more fishy flavored breast meat were from those turkeys fed fish oil to the time of slaughter (Diet 5) and the ones switched from fish oil to 4% beef fat (Diet 2) for 2 wk before slaughter. However, the addition of α-tocopherol acetate to the beef fat diet significantly reduced the development of fishy flavor in the breast meat (Diets 3 and 4). The optimum level appeared to be between 100 mg and 200 mg/kilo. This level effected taste panel scores which were lower or equal to the control for breast and thigh meat but not statistically significant. Had these turkeys been fed tuna oil for longer periods of time (thus increasing the amount of carcass linolenates), about 200 mg probably would have been required. The fatty acid distribution of lipids extracted from the breast meat are given in Table 5. As before, the carcass fatty acid distribution reflected that of the dietary oil and after withdrawal of the fish oil, the level of long chain linolenates was reduced. It is of interest that the control turkeys (Diet 1, 4% beef fat) have higher amounts of C22:6 than expected. This amount may be a reflection of the fishmeal (starter diet). It would appear that this amount cannot be removed from the tissue since these turkeys were fed a basal diet plus 4% BF and no fish oil for

Table 5—Methyl ester fatty acid composition of lipids extracted from the breast meat of turkeys fed from 8–14 wk of age a diet with 2% tuna oil plus 2% beef fat and fed from 14–16 wk a diet with no tuna oil but 4% beef fat with and without α-tocopherol acetate supplementation

	% Distribution in extracted oil				
Fatty acids Diets[a]	1	5	2	3	4
C14	1.0	0.7	0.8	0.7	0.5
C14-1	0.2	0.1	0.2	0.1	0.1
C15	0.2	0.2	0.2	0.2	0.2
ISO C16	3.5	3.6	3.2	3.8	3.9
C16	17.3	15.3	15.3	14.8	14.5
C16-1	1.6	1.5	1.5	1.6	1.0
C17	0.6	0.6	0.6	0.5	0.5
ISO C18	1.8	1.8	1.7	1.9	2.2
C18	13.1	13.2	13.7	12.9	14.3
C18-1	23.6	17.4	20.7	19.6	18.2
C18-2	21.0	18.5	19.9	19.1	18.8
C18-3 ω 3	0.8	0.6	0.7	0.6	0.4
C20-4	6.1	6.3	5.9	6.6	6.3
C20-5 ω 3	0.6	3.0	1.9	2.0	2.0
C22-5 ω 3	0.8	1.3	1.2	1.2	3.2
C22-6 ω 3	5.1	14.5	10.9	12.8	11.9
Others	2.7	1.4	1.7	1.6	2.0
g Lipid/100g tissue	1.0	1.0	0.9	0.9	0.9

[a] All groups (except Group 1, the control which was maintained on diet with 4% beef fat for all 16 wk) were fed a basal diet with 2% tuna oil plus 2% beef fat from 8–14 wk of age and from 14–16 wk of age, they were fed a basal diet with: Group 1 = 4% beef fat; Group 2 = change to 4% beef fat; Group 3 = change to 4% beef fat + 100 mg/kilo dl α-tocopherol acetate; Group 4 = change to 4% beef fat + 200 mg/kilo dl α-tocopherol acetate; Group 5 = keep on 2% tuna oil + 2% beef fat.

Table 6–Duncan's multiple range test of mean[a] taste panel scores[b] for breast and thigh meat and skin of turkeys fed from 14–16 wk of age diets containing 2% tuna oil plus 2% beef fat with and without α-tocopherol acetate supplement or α-tocopherol injection

Treatment[c]	Breast meat	Treatment[c]	Thigh meat	Treatment[c]	Skin
2 2% TO	2.23	2 2% TO	2.82	2 2% TO	3.11
5 2% TO + 500E	2.18	5 2% TO + 500E	1.95	5 2% TO + 500E	1.87
4 2% TO + 100E	1.86	3 2% TO + In[d]	1.70	3 2% TO + In[d]	1.66
3 2% TO + In[d]	1.32	4 2% TO + 100E	1.64	4 2% TO + 100E	1.65
1 4% BF	1.19	1 4% BF	1.31	1 4% BF	1.00

[a,b,c] See footnotes to Table 1.
[d] In = α-tocopherol injected into the thigh at 72, 48 and 24 hr before slaughter (170 mg per injection).

several weeks following the feeding of the starter diet. Although this level is higher than expected, it has not proven to be high enough to cause fishiness or off flavor.

Experiment 3, the effect of tocopherol injection

All turkeys were of apparent good nutritional health. They had a mean dressed weight of 3924g.

The breast and thigh meat from turkeys fed 2% fish oil (Diet 5) do not have much off flavor. This in turn makes difficult the task of evaluating the relative merits of injecting α-tocopherol. However, Duncan's multiple range test of the mean taste panel scores in Table 6 showed Diet 2 (2% fish oil) and Diet 5 (2% fish oil and 500 mg/kilo tocopherol acetate) tended to result in the more fishy turkeys and there were no statistical differences between any of the treatments for breast meat. Within the limits of interpretation, injection α-tocopherol was at least as effective as feeding it. Statistical analysis does show some benefit for thigh meat and skin when tocopherol is injected or added to the diet. It was somewhat puzzling that 500 mg in the diet did not give better results than 100 mg. It is possible that 500 mg is not as efficiently absorbed from the diet by the turkey as 100 mg (no level of 200 mg was tested). Therefore, this relatively short time feeding study would not reflect the advantage (if any) of the higher level of tocopherol acetate. Tappel (1973) reported that intestinal absorption and tissue deposition of Vitamin E show a logarithmic relationship with respect to dietary concentration such that levels exceeding the RDA are increasingly less efficiently utilized. He points out that about 10 times the RDA would be required to double the tissue concentration of Vitamin E.

The fatty acid distribution of the lipids extracted from breast meat again reflected the composition of the dietary oil supplement but the levels of long chain linolenates are somewhat lower than those of the preceding experiments (Table 7). This could be expected, since the turkeys in this experiment were fed fish oil for only 2 wk.

SUMMARY

THREE EXPERIMENTS were conducted to evaluate the preventative and remedial effect of α-tocopherol on the development of fishy flavor in turkeys fed fish oils. It was found that about 200 mg/kilo α-tocopherol acetate afforded optimum

Table 7–Methyl ester fatty acid composition of lipids extracted from the breast meat of turkeys fed beef fat and tuna oil with and without α-tocopherol acetate supplementation

Fatty acids	Diets[a] 1 4% BF	2 2% TO	3 2% TO + In	4 2% TO + 100E	5 2% TO + 500E
	% Distribution in extracted oil				
C14	0.6	0.9	0.6	0.7	0.7
C14-1	0.2	0.2	0.1	0.1	0.2
C15	0.2	0.2	0.2	0.2	0.2
ISO C16	3.8	4.1	4.4	3.5	3.7
C16	14.8	15.8	15.7	16.5	14.9
C16-1	1.8	1.9	1.3	1.8	1.6
C17	0.5	0.6	0.5	0.5	0.6
ISO C18	1.9	1.7	1.9	1.8	2.0
C18	13.9	12.2	16.2	12.9	12.8
C18-1	22.4	19.9	17.6	20.0	19.3
C18-2	23.0	20.0	18.5	21.2	20.7
C18-3 ω 3	0.7	0.9	0.5	0.7	0.7
C20-4	9.1	7.4	8.8	8.3	8.6
C20-5 ω 3	0.4	1.8	1.4	1.5	1.9
C22-5 ω 3	1.4	1.7	1.7	1.2	1.2
C22-6 ω 3	1.7	7.5	7.4	6.6	8.4
Others	3.6	3.2	3.2	2.5	2.5
g Lipid/100g tissue	1.0	1.1	1.0	1.1	1.0

[a] BF = beef fat; TO = tuna oil; E = mg/kilo dl α-tocopherol acetate; In = inject 170 mg α-tocopherol at 72, 48 and 24 hr before sacrifice.

prevention of fishy flavor in turkeys fed as much as 2% tuna oil in their rations. Withdrawal of fish oil and beef fat substitution for 2 wk before slaughter, caused some decline in fishiness. The addition of α-tocopherol to the beef fat diet significantly accelerated the decline of fishiness in breast meat. It was also noted that the oil (tuna) used in these experiments contain many fold higher levels of long chain linolenates (ω-3 fatty acids) than other fish meals (oils) used to feed poultry. There, it is more than a specious argument to assume that less α-tocopherol acetate would be required to achieve the same effect with such oils since fishiness is related to linolenate content.

Tocopherol injection 72, 48 and 24 hr before slaughter was as effective as feeding it concomitantly with fish oil in the reduction of fishiness in the thigh meat and skin. The same trend was noted for breast meat although not statistically significant. Further work is needed to more accurately assess the value of injection.

Fatty acid distribution analyses were performed on the extracted lipids from the breast meat of turkeys from all treatments. In general, the results were as expected.

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

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DISCOLORATION IN PROCESSED CRABMEAT. A Review

INTRODUCTION

DESPITE almost a century of study, general agreement has not been reached on the causes of or cures for discoloration of processed crabmeat. The problem is compounded by several factors. There are different types of discoloration which may appear similar, and these occur in many different species of crabmeat. Processing methods vary widely, and processors sometimes find discoloration to occur sporadically and unpredictably. Even under controlled laboratory conditions, workers have reported different results in crabmeat discoloration studies.

The various explanations of and cures for crabmeat discoloration are presented here, for the most part, without interpretation. The literature references were readily organized into five categories of discoloration. The ease of organization implies but does not guarantee that the five categories are discrete and comprehensive. The discoloration categories are blue, evidently from a crab blood reaction; brown, due to the Maillard reaction; black, from the formation of iron sulfide; discoloration from oxidation in frozen crabs; and discoloration from diffusion of pigments.

BLUE DISCOLORATION

A COMMON PROBLEM with processed crabmeat is a discoloration usually called blueing although the actual color may range from light blue to blue-gray to black. This form of discoloration is generally associated with heat processing, the probability of discoloration increasing with higher processing temperatures and longer cooks (Howe, 1933; Waters, 1970). Thus, blue discoloration occurs in heat sterilized crabmeat more often than in pasteurized. Blue discoloration also occurs in frozen King crabmeat, *Paralithodes camtschatica* (Dassow, 1950; Groninger and Dassow, 1964), frozen cooked carcasses of Dungeness crabs, *Cancer magister* (Babbitt et al., 1973a), and, rarely, in the meat of crab carcasses several hours after preliminary heat treatment (Tanikawa and Doha, 1965). Blueing has even been reported in live crabs (Oshima, 1931).

Blue discoloration is a greater problem in meat of some species than in others. Jarvis (1944) reports that *Callinectes sapidus* meat is more subject to blue discoloration than other species. According to Tanikawa and Doha (1965), blueing appears in canned meat of the Horse Hair crab *Erimacrus isenbeckii* more often than in King crab. In pasteurized *C. sapidus* crabmeat blueing may occur during the heat treatment and intensify during storage (Waters, 1970) or, in canned Dungeness crabmeat, it may not develop until after several months storage (Harvey and Mann, 1948).

Discoloration that takes place after processing depends on storage temperature. Harvey and Mann (1948) recommend incubation of canned crabmeat for 2 days to 2 wk at 37°C to accelerate discoloration development in order to assess the extent of discoloration that will develop later in cans held at room temperature.

The cause of blue discoloration

Blue discoloration is believed to be caused by some constituent in crab blood. Discoloration is reduced by bleeding crabs before processing. Other decapod crustacea with, of course, similar blood, are also subject to a similar, if not the same form of discoloration: lobster (Harrison and Hood, 1923; Reed and MacLeod, 1924; Nielsen, 1955), and shrimp and prawns (Landgraf, 1956; Hansen, 1967; Nandakumaran et al., 1969, 1970).

There is disagreement as to the specific blood constituents and reaction mechanism involved in the formation of the blue pigment. Specific proposed mechanisms fall roughly into five categories which are not necessarily mutually exclusive: formation of (1) iron compounds, (2) copper compounds, (3) melanin, (4) copper proteins or biuret complexes, and (5) hemocyanin compounds. Oshima (1931) suggested iron sulfide as a possible cause of discoloration, but considered a copper sulfide compound more probable. Waters (1971) added ferric and ferrous salts to canned *C. sapidus* crabmeat and found blue discoloration after processing. Added cupric and cuprous salts caused less discoloration than iron, while controls were not discolored. Babbitt et al. (1973b) found ferrous and ferric iron and cuprous and cupric copper equally effective in causing blueing when added to canned Dungeness crabmeat. Inoue and Motohiro (1970a) processed King crabmeat without additives, separated the blue and normal meat, and found the iron content to be the same.

Many investigators agree that copper is related to blue discoloration. A correlation between copper concentration and discoloration intensity was found in Dungeness crab by Elliott and Harvey (1951) and Babbitt et al. (1973b) and in King crab by Inoue and Motohiro (1970a). Copper is implicated further because the discoloration reaction is inhibited by chemicals which react with copper, such as phenylthiourea (Babbitt et al., 1973a) and by cyanide (Groninger and Dassow, 1964). Three inorganic copper compounds have been suggested as causes of discoloration: compounds of copper sulfide (Oshima, 1931), copper sulfate (Spears, 1973), and the copper-ammonia complex (Fellers, 1936; Harris, 1939).

Another suggested mechanism for the blueing in crabmeat is the oxidation of phenolic compounds, such as tyrosine, to form a group of dark pigments called melanin. This reaction is sometimes accelerated by enzymes, such as tyrosinase. Fellers and Parks (1926) state that fresh crabmeat contains tyrosinase which causes gray, then brown discoloration. Oshima (1931) considered melanin as a cause of blue discoloration but rejected the idea after adding tyrosinase to meat of the King crab, and finding a dark discoloration "much different from the color of blue meat." Babbitt et al. (1973a) found tyrosinase in Dungeness crab and believe the discoloration in crabmeat may be similar to the phenol enzyme system in shrimp (Bailey et al., 1960a, b) and lobster (Kakimoto and Kanazawa, 1956). Florkin (1960) maintains that tyrosinase is a characteristic constituent of crustacean blood, but Groninger and Dassow (1964) found neither tyrosinase nor polyphenols in King crab blood. Bailey et al. (1960b) did not find tyrosinase in *C. sapidus* blood, although they did find that hemocyanin acts as an enzyme to oxidize the phenol, pyrocatechol.

These enzymes may remain active to cause discoloration after heat processing. Although a tyrosinase system studied by Pinhey (1930) was destroyed at 60°C, Babbitt et al. (1973a) found only a slight loss of phenol enzyme activity in Dungeness crab blood after 2 min at 85°C. Some enzyme systems of the red crab, *Geryon quinquedens*, survived 30 min at 100°C

(Smith, 1970). In any event, Babbitt et al. (1973a, b) proposed nonenzymatic as well as enzymatic pathways for the oxidation of phenols.

Babbitt et al. (1973a, b) removed phenols from Dungeness crabmeat by extraction and dialysis. This meat did not turn blue after heat processing if copper or iron ions alone were added, but did turn blue if a phenol (dopa) was also added. They separated copper from the blue pigment by dialysis against deionized water, demonstrating that copper may be a catalyst rather than a constituent of the pigment. This separation also demonstrates a similarity of the blue pigment to hemocyanin, because the copper of hemocyanin may be removed by dialysis (Inoue and Motohiro, 1971a). Copper is a required catalyst for the activity of phenol enzymes (Bailey et al., 1960a).

According to Fellers and Parks (1926), blue discoloration in crabmeat is caused by the reaction of biuret and copper, both found in crab blood, in the presence of ammonia. Oshima (1931) induced discoloration via the biuret reaction by adding sodium hydroxide to King crabmeat. The discoloration was similar to that of blue meat, but Oshima believed the biuret reaction to be an improbable cause due to the high pH required. However, Groninger and Dassow (1964) found the blueing reaction to proceed between pH 5 and 9 in King crabmeat. The reaction required oxygen and involved oxidation and reduction. Groninger and Dassow (1964) suspect that the biuret reaction is the cause of blue discoloration.

Hemocyanin or its derivatives have been suspected as the cause of discoloration for some time. However, the mechanism of hemocyanin blueing is debatable. Hemocyanin contains copper, it is a protein, and a phenol, containing tyrosine (Goodwin, 1960). As mentioned above, hemocyanin may also act as a phenol enzyme (Bailey et al., 1960b). Therefore, hemocyanin may be the source of copper for discoloration said to be from copper compounds. It may participate in the biuret reaction (Schulman and Wald, 1951), it may be oxidized to melanin, or it may catalyze that reaction. Inoue and Motohiro (1970b) found hemocyanin in the region of blue discoloration of King crabmeat but not in normal meat. They (1970c) produced a blue-green substance by exposing hemocyanin of King crab to sulfide and heating. The colored substance was not formed by exposing ground crab leg meat to hydrogen sulfide, indicating that hemocyanin contains a constituent of the blue substance, but the muscle does not. Inoue and Motohiro analyzed the blue green substance and their results (1970e, f) suggest a hemocyanin-sulfide complex. They found linear equations relating hemocyanin-sulfide complex content with color intensity (1970d).

Prevention of blue discoloration with acid

Addition of acid to prevent blue discoloration by lowering the pH of the crabmeat is an idea consistent with some of the proposed mechanisms. Fellers (1936) recommends addition of 0.03–0.1% weak organic acid to prevent formation of ammonia and therefore prevent the formation of the blue copper ammonia complex in canned "blue, rock, and sand" crabs. Webb (1945) suggests lowering the pH to 4.7–5.0 with a weak organic acid to prevent discoloration by sulfide formation. Groninger and Dassow (1964) found that the blueing reaction will not occur below pH 4 which is consistent with their theory that the blueing agent is a biuret complex.

Different authors recommend different acids at various concentrations. Jones (1968) observed less discoloration in canned King crab with 0.25–0.35% sodium acid pyrophosphate added at pH 6.0 and 6.2. Gordievskaya (1964) observed a threefold reduction in discoloration with the addition of 1–2% lactic acid. For canned Dungeness crabmeat, Harvey and Mann (1948) recommend a citric acid dip to give the product a pH of 6.3–6.5, and Lantz (1951) reports that phosphoric, malic, acetic, and tartaric acids are also used. Jarvis (1948) advises home canners to add lemon juice, citric acid, or vinegar to Atlantic and Gulf coast crabs.

Some authors attribute the benefits of added acid to effects other than the lowering of pH. In fact, acid additions may lower the pH only temporarily. Webb et al. (1969) observed an initial pH drop after adding citric acid to *C. sapidus* meat before pasteurization. However, depending on the method of packaging, the pH in some cases rose during storage to equal that of controls. After 9 months storage, the pH of canned *Scylla serrata* meat dipped in citric acid rose from 5.8 to 6.3 and with added citric acid the pH rose from 6.1 to 7.3 (Gangal and Magar, 1967). Possible explanations for the pH rise in canned crabmeat are given in the section on blackening. Contrary findings are reported by Varga et al. (1969) who observed no significant change in pH of *Chionoecetes opilio* crabmeat during 6 months storage after canning with citric acid added.

According to Howe (1933), rinsing crabmeat in organic acid solutions prevents discoloration by extracting color-forming constituents, particularly iron and sulfur. Farber (1953) presumed that the negative ions of weak organic acids prevent discoloration by forming weakly or nonionizing complexes with copper. He recommends washing eviscerated crab sections in 1% sodium citrate-5% sodium chloride and adding the same solution to the pack. Farber found this solution effective in preventing discoloration in Dungeness crabmeat. But, using the same solution as a dip, Strasser et al. (1969) observed little or no improvement in pasteurized or sterilized *C. sapidus* meat. Groninger and Dassow (1964) found ascorbic acid to inhibit the blueing reaction in King crab, probably by acting as an antioxidant to prevent formation of the biuret complex. Babbitt et al. (1973b) suggest that the blueing reaction in Dungeness crabmeat is inhibited by ascorbic or citric acid because of their respective antioxidative and metal chelating properties. Ascorbic acid may prevent the oxidation reaction of phenols to melanin while citric acid may inhibit the reaction by chelating catalysts such as iron or copper. The antioxidative power of ascorbic acid as well as phosphoric acids may also be due to chelation of metals (Stuckey, 1968).

Not all authors agree that crabmeat is improved by acid treatment. Gangal and Magar (1967) prevented blueish-gray discoloration in canned meat of *S. serrata* by keeping the pH below 7.4 with citric acid, but they report that many additives have an adverse effect on flavor. They found that lower concentrations of citric acid affect crabmeat flavor least. Acid addition to Dungeness crabmeat has the disadvantage of increasing shrinkage and degrading the texture and flavor (Farber, 1953). Citric acid added to meat of *C. opilio* in concentrations of 0.8% of the can contents prevents blueing entirely but concentrations greater than 0.1% have an adverse effect on quality (Varga et al., 1969). A 2% sodium acid pyrophosphate dip improved the appearance of pasteurized *C. sapidus* meat but seemed to have a negative effect on storage stability (Strasser et al., 1969). Dassow (1950) found no evidence that treatment with citric or acetic acid reduced discoloration in canned King crabmeat. Waters (1971) induced discoloration in *C. sapidus* by adding iron salts. Adding ascorbic or lactic acid did not prevent the induced blueing, and tartaric acid was of limited benefit. Added citric acid in concentrations high enough to prevent blueing imparted an unpleasant flavor to the meat.

Prevention of blue discoloration without acid

Meade and Gray (1973) had poor results when they tried a 1% citric acid dip to prevent darkening around the membranes attached to the shoulders of uncooked, iced Red crab sections, *G. quinquedens*. Discoloration was prevented, however, when they dipped the crab sections in solutions of 0.5 and 1% sodium bisulfite with and without citric acid. They assumed that the dark pigment was a melanin and chose a dip of sodium

bisulfite for its specific inhibition of phenol enzyme reactions. This inhibition is due to the antioxidant properties of sodium bisulfite, at least in shrimp (Bailey et al., 1960b).

Most authors agree that iron and/or copper take part in the blue discoloration reaction, either as direct participants, or as catalysts. The use of ethylenediaminetetraacetic acid (EDTA) as an additive in crabmeat and other seafood products is patented by Fellers (1954), who claims that EDTA chelates copper and iron, thereby preventing discoloration. EDTA chelates the copper of hemocyanin (Inoue and Motohiro, 1970f). Ladenburg (1959) and Fellers (1961) hold patents on the production of EDTA tablets to be added to canned seafood. The Food and Drug Administration approves use of up to 275 ppm EDTA in canned crab (Anonymous, 1961). EDTA does not inhibit blueing in King crab (Groninger and Dassow, 1964), or in *C. sapidus* (Waters, 1971). Ganucheau (1948) holds a patent on the addition of buffered disodium phosphate which, he claims, binds iron, copper and other metals and retains the original color of canned crabmeat. Black discoloration in boiled and frozen Kegani crabs, probably due to tyrosinase activity, was prevented by soaking the boiled crabs in a phosphate-brine solution before freezing (Ichisugi and Inokawa, 1969). Spears (1973) holds a patent on packing a disc of aluminum foil with crabmeat. Copper and iron are reduced to the metallic state by the aluminum and discoloration is prevented.

There are several suggestions for the prevention of blue discoloration in canned crabmeat by washing or rinsing to remove the causative agent. Oshima (1931) advises a thorough wash to remove blood from crabmeat before canning. Fellers (1936) claims that washing "blue, rock and sand" crabmeat in 2–3% brine before canning tends to whiten the meat. Harvey and Mann (1948) advise a water wash to reduce the probability of discoloration in canned Dungeness crabmeat. The customary acid bath treatment used before canning King crabmeat can be omitted if the meat is thoroughly washed in brine (Miyahara, 1954). Motohiro et al. (1972) lessened blue and grayish brown discoloration caused by iron and copper sulfides in *C. opilio* meat by washing with running water to remove the offending ions. According to Gangal and Magar (1967), a brine rinse before canning meat of *S. serrata* may prevent discoloration but causes extensive leaching of flavoring agents. Howe (1933) recommends a brine rinse to flavor crabmeat but claims its effect in reducing coloration in canned meat is practically nil.

Butchering methods which quickly separate meat and visceral material help prevent discoloration in Alaskan King crabmeat (Pottinger, 1956b) and in Dungeness crabmeat (Harvey and Mann, 1948). Some Pacific coast processors use such a butchering method for Dungeness crabs but Elliott and Harvey (1951) consider this method insufficient. They bled the crabs before butchering and found less copper and less discoloration in the meat after canning. The same results are reported by Tanikawa (1943) for King crab and Farber (1953) for Dungeness crab. Bleeding Dungeness crabs is not commercially practiced on the West Coast (Babbitt, 1973). Jarvis and Puncochar (1946) recommend evisceration before cooking for home canners to prevent discoloration in *C. sapidus* and Dungeness crabmeat. Lobster and other crustacea may be eviscerated with vacuum and water pressure methods patented by Trelease et al. (1973).

Harris patented (1939) a preliminary heat treatment for processing Atlantic crabs to reduce blueing in the canned product. According to Harris, discoloration is caused by copper which is primarily concentrated in inedible portions of live crabs. Harris' method subjects live crabs to a mild heat treatment which allows separation of the meat from parts of the crab with high copper concentration. Then, when heat processing of the separated meat is completed, copper contamination and discoloration are reduced. A similar preliminary heat treatment was proposed for *E. isenbeckii* (1957) and patented (1961) for all forms of crustaceans by Osakabe. Osakabe specifically provided for the separation of meat and hemocyanin with a cook above 50°C to coagulate meat but below 70°C so that blood is not coagulated. Babbitt et al. (1973b) used Osakabe's preliminary heat treatment with Dungeness crab and confirmed reduction in blueing, although they believe the method works by reducing the phenol concentration. Some King crab processors believe that a single fast cook prevents discoloration by coagulating all the protein and that less flavor is lost than with a preliminary heat treatment (Anonymous, 1965a).

Discoloration in Dungeness crab is less likely if the precook is with live steam rather than boiling water (Harvey and Mann, 1948). Undercooking increases blueing in Dungeness crab (Babbitt et al., 1973a). Although many crab processors agree that undercooking causes discoloration in King crab, an increased cook does not necessarily solve the problem according to the observations of Groninger and Dassow (1964).

If the crustaceans are not fresh when processing begins, the probability of discoloration increases. This is true for King crab (Dassow, 1950), Dungeness crab (Harvey and Mann, 1948; Babbitt et al., 1973a, b), *E. isenbeckii* (Tanikawa et al., 1953), and lobster (Reed and MacLeod, 1924), but not true for *C. sapidus* (Waters, 1971). Dassow (1950) suggests that although the crabs are kept alive and wet with sea water, the accumulation of toxic products from the body wastes tends to affect adversely the quality of the meat, noticeable after processing and storage. The "toxic products" may be an accumulation of clotted blood cells in the muscle of the crab. Inoue and Motohiro (1971b) observed clotting of *E. isenbeckii* blood. Cell membranes ruptured during clotting leaving a gelatinous substance containing hemocyanin in the muscle fibers of the crab. This gelatinous substance is difficult to remove by washing with water. Inoue and Motohiro had previously observed (1970b) hemocyanin in blue crabmeat, but not in normal colored meat. They conclude (1971b) that discoloration may result from processing crabs which are not fresh because of hemocyanin from the clotted blood. Another interpretation of the observations of Inoue and Motohiro is suggested by the work of Pinhey (1930). She found tyrosine in the blood plasma of the spider crab, *Maia squinado*, and tyrosinase in the cells. Darkening occurred when the cells ruptured, allowing contact between tyrosine and tyrosinase to form melanin.

If processing is delayed, the concentration of volatile nitrogen and sulfur compounds increases in meat of King crab (Sekine and Kakizaki, 1926) and Dungeness crab (Fellers and Parks, 1926). Tanikawa et al. (1953) measured increases in volatile nitrogen compounds in raw *E. isenbeckii* meat with delayed processing. They observed more blueing in canned meat which had been held longer before processing and thus contained more volatile nitrogen compounds. Babbitt et al. (1973b) observed increases in both pH and phenol content in live Dungeness crabs held at 1–3°C. However, Waters (1971) found no increase in discoloration when he processed *C. sapidus* which were dead or moribund from exposure to the sun. Also, the aging of precooked, picked *C. sapidus* meat for a few days at 3°C before pasteurization reduced shalf life but did not cause discoloration (Waters, 1970).

Clough (Anonymous, 1948) observed that blue discoloration was related to the level of vacuum in canned Pacific crab, with higher vacuum increasing the tendency for discoloration development. Vacuum did not cause discoloration in canned *C. sapidus* (Waters, 1971). Howe (1933) prevented discoloration in crabmeat by adding sugars or oils or various oxidizing agents. A North Carolina firm (Anonymous, 1965b) proposed use of 250 parts per million or less sodium nitrite as a color fixative in canned crabmeat. A 1% sodium nitrite dip improved appearance of canned *C. sapidus* according to Strasser et al. (1969). Fellers and Harris (1940) describe a patented (Fellers,

1936) method to prevent discoloration by adding 50 to 500 parts per million of aluminum, zinc, and/or tin salts.

BROWN DISCOLORATION

A DETAILED STUDY of browning in canned King crabmeat was done by Nagasawa (1958; 1959a, b, c, d; 1960). He demonstrated that the brown pigment is the product of the Maillard reaction, a reaction between reducing sugars and amino acids. Nagasawa found that browning is accelerated by copper in crab blood and iron in crabmeat. If the meat is alkaline when packed, the probability of browning increases. More browning occurs at higher processing temperatures and longer cooks and when the meat is not fresh when processed. Nagasawa proposes three methods to prevent browning: keep crabs fresh until processing, improve boiling methods, and discard meat which fluoresces under ultraviolet light, indicating precursors of the brown pigment. Nagasawa's 1960 paper contains a summary of his work. Safronova and Shchigoleva (1973) found browning proportional to the hexosamine content of crabmeat, again implicating the Maillard reaction.

The Maillard reaction may be the cause of discoloration reported by other authors who do not specify the mechanism. Puncochar and Pottinger (1954) observed scorching in *C. sapidus* meat at higher sterilization temperatures. According to Fellers (1936), scorching is reduced by addition of a brine solution which facilitates heat transfer within the can. Dickerson and Berry (1974) observed considerable difference between heat received in the center and inside surfaces of cans of *C. sapidus* meat during pasteurization. They suggest use of smaller cans. Byrd (Anonymous, 1952) and Tatro (1970) mention dark and gray discolorations, respectively, in pasteurized *C. sapidus* meat due to excessive processing time or temperature. Brown discoloration, or scorch, in canned Dungeness crabmeat may be caused by air cooling after retorting, rather than water cooling (Farber, 1953), especially after retorting at higher temperatures (Dewberry, 1955). Russian processors rapidly cool canned crabmeat to prevent discoloration (Carlson, 1945). Cahn (1948) reports that the Japanese prevent discoloration by immersing crabs, which had just been boiled, in cold (8°C) sea water for 15 min.

BLACK DISCOLORATION

BLACKENING may occur in canned crabmeat from the combination of volatile sulfide compounds, such as hydrogen sulfide, evolved from the meat during processing, and ferrous ions dissolved from the can to form black ferrous sulfide (Okuda and Matsui, 1916; Fellers and Parks, 1926; Oshima, 1931). Black crabmeat contains more sulfur and ammonia than normal meat (Okuda and Matsui, 1916), and the black substance may also contain organic iron compounds (Yamada and Tanaka, 1959). Although this form of discoloration and blue discoloration may appear very much alike, the cause of blue meat originates in the meat itself, whereas blackening is caused by contact of the meat with the metal of the can (Oshima, 1931). At first, blackening is noticed only where meat touches the can (Puncochar and Pottinger, 1954) but, if liquid is present, blackening may spread throughout the can (Fellers and Parks, 1926). Iron contamination may also come from processing equipment, so stainless steel, aluminum, or glass should be used (Howe, 1933).

Blackening occurs in alkaline crabmeat, and canned crabmeat becomes increasingly alkaline with time in meat of a Japanese crab (Okuda and Matsui, 1916), in Dungeness crabmeat (Dewberry, 1955), and *S. serrata* (Gangal and Magar, 1967), but not in King or Horse Hair crabmeat. Okuda and Katai (1936) report a constant pH in canned King crabmeat during 5 years storage. Motohiro and Inoue (1970) followed the pH in canned King and Horse Hair meat for 2 months and found it constant. Possible explanations of the rise in pH are autolysis (Okuda and Matsui, 1916), the liberation of volatile bases (Legendre, 1926) such as nitrogen compounds (Dewberry, 1955), and the liberation of carbon dioxide from malic acid in a reaction involving an enzyme stable to heat (Morrison, 1956).

Blackening does not occur below pH 7.5 (Oshima, 1931), because less hydrogen sulfide is evolved during the heating process at low pH (Johnson and Vickery, 1964). The lower pH suppresses the ionization of hydrogen sulfide, making it less likely that the solubility product of the metallic sulfide is exceeded (Johnson and Frost, 1951). However, at high sulfide concentrations, a low pH may drive hydrogen sulfide gas into the headspace, causing discoloration there. This may explain the observation of Kimura and Akiba (1971) that headspace blackening is accelerated by low pH in canned extracts of King crabmeat with added cystine. Nevertheless, blackening may be controlled by lowering the pH (Okuda and Matsui, 1916). Patents are held by Oshima (1927, 1928) for addition of a buffer solution such as sodium acetate and acetic acid, and by Webb (1945) for a dip treatment with, for example, 4% sodium chloride, 0.5% citric acid, and an optional 0.5% magnesium sulfate. Jones (1968) found less sulfide blackening in King crab with added sodium acid pyrophosphate. Cahn (1948) reports that the Japanese prevented alkaline conditions and consequent iron sulfide formation by washing the meat to remove blood. Since hemocyanin contains sulfur (Goodwin, 1960), this reduces potential sulfide concentrations. Blood removal also reduces copper concentrations which were found to accelerate blackening in canned King crabmeat extracts with added cystine (Kimura and Akiba, 1971). Other metals, including iron, catalyze the release of sulfide from sulfur-containing amino acids (Gruenwedel and Patnaik, 1971).

Iron sulfide discoloration may be reduced by the use of C-enamel cans. Hydrogen sulfide preferentially reacts with zinc in the enamel forming white zinc sulfide rather than black iron sulfide. Scratches in the enamel cause discoloration (Waters, 1970). Harrison and Hood (1923) reduced discoloration in canned lobster by using a lining of parchment paper, especially one that had been soaked in a zinc salt solution. C-enamel eliminates the need for parchment liners in canned Pacific crabs (Harvey and Mann, 1948; Dewberry, 1959). However, USDI (no date) states that parchment liners and organic acids may still be needed to some extent with crabmeat in C-enamel cans. Aluminum cans and aluminized lacquers reduce discoloration according to Dewberry (1970) and Finch (1966), respectively. Flynn and Tatro (1966) found the color of pasteurized crabmeat was better in plastic containers, which are approved for use by the Maryland State Department of Health (Semling, 1965).

Legendre (1926) reviewed the early literature on darkening of canned crustacea, especially blackening of canned lobster meat.

Idler and MacLeod (1953) observed a different form of black discoloration on the ventral surface of crabs from the Nass River, British Columbia, caused by oxides of manganese. The discoloration was removed by dipping the whole crab in a solution of 3% acetic acid and 3% hydrogen peroxide. This treatment dissolved the oxides of magnesium and did not harm the meat. The cause of accumulation of the oxides is unknown.

DISCOLORATION OF FROZEN CRABS

ACCORDING to Pottinger (1956a), crabmeat has a very slight susceptibility to oxidative changes in comparison with fish. However, frozen precooked crabmeat is subject to color changes. The cause is generally considered to be oxidation, which may be reduced with packaging which excludes oxygen, lower temperatures which decelerate oxidation, or with antioxidants, which have had variable success. Raw crabs and crab-

meat are not suitable for freezing because of rapid deterioration, including discoloration (Dassow, 1950; Tanikawa et al., 1964; Babbitt et al., 1973a). Frozen crabmeat discussed below is precooked. It is liable to blue discoloration, discussed in that section. Blueing in frozen King crabmeat is reduced with a blanket of carbon dioxide (Groninger and Dassow, 1964).

Color stability of frozen crabmeat differs with species. Gangal and Magar (1963) observed blackening in *S. serrata* and yellowing in *Neptunus pelagicus* crabmeat under identical freezing and storage conditions. They found that discoloration increases with frozen storage time, as did Barnett et al. (1967) with Dungeness crabmeat. The meat of some crabs is pigmented, and oxidation may cause this pigment to fade during frozen storage. Ravesi (1969) identified the pigment in King crabmeat as astaxanthin and its derivatives. Ravesi observed less fading at lower storage temperatures, with dips of ascorbic acid and other antioxidants, and with a short, high temperature cook in fresh water. Fading of color was somewhat less in meat frozen in the shell. Ravesi observed little improvement with cans packed with nitrogen or vacuum in comparison with cans sealed with air. However, Heerdt (1947) reduced fading in Dungeness crabmeat during storage with vacuum packing as well as by packing the meat with water or brine. This form of discoloration may be similar to that observed in frozen meat of lobster (Bligh et al., 1957) and shrimp (Faulkner and Watts, 1955).

Gangal and Magar (1963) reduced browning in frozen meat of the above mentioned crabs by a citric acid dip. Discoloration may be prevented in frozen Alaskan King crabmeat by proper washing and exclusion of air spaces according to Pottinger (1956b) and Dassow (1950). Impure grades of salt used during processing may cause yellowing after long storage periods (Dassow, 1950). Strasser et al. (1969; 1971a, b) investigated preservation methods, especially freezing, for *C. sapidus* meat. They found that quality, including appearance, was improved by rapid freezing, low temperature storage, and vacuum or sealed packaging or glaze. No significant improvement was observed with dips of sodium nitrite, ascorbic acid, sodium acid pyrophosphate, brine with sodium citrate or trypolyphosphate. A malic acid dip improved the appearance of frozen *C. sapidus* meat according to Webb et al. (1974).

RED DISCOLORATION

IF PIGMENTED LEG or claw meat is canned with white body meat, the body meat may become discolored pink or red by the pigments. Separate packing of different colored meats may avoid this problem. Discoloration is reduced in King crabmeat by rapid cooling of the retorted cans (Anonymous, 1942). According to Motohiro and Inoue (1970), this red discoloration occurs at pH above 7.4 in Horse Hair and King crabmeat. They suggest using a small proportion of meat from paper shell crabs, because the processed meat has a higher pH than canned hard shell crabmeat.

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IDENTIFICATION OF THE MAJOR VOLATILE COMPONENTS OF BLUEBERRY

INTRODUCTION

THE BLUEBERRY is a plant native to North America which is grown commercially for its edible fruit. The flavor components of blueberries have received little attention; Nursten and Williams (1967) omitted them from their review of fruit flavor components. Hall et al. (1970) reported the fruit of the low-bush blueberry (*Vaccinium angustifolium*) contains acetaldehyde, methyl acetate, ethyl acetate, ethanol and ethylene. The volatiles of other members of the genus *Vaccinium* have been studied. For example, von Sydow and Anjou (1969, 1970) investigated the bilberry (*Vaccinium myrtillus*), a fruit native to Europe. They identified a large number of compounds and concluded that trans-2-hexenal, ethyl-3-methyl butyrate and ethyl-2-methyl butyrate are of the major importance to bilberry aroma.

The volatiles of the high-bush blueberry (*Vaccinium corymbosum*), grown commercially in the mid-Eastern United States, have not been previously reported. The purpose of this study was to identify the major compounds of commercial high-bush blueberries and to establish the relative importance of these compounds to the characteristic blueberry aroma.

MATERIALS & METHODS

Blueberry concentrate

900g of high-bush blueberries (*Vaccinium corymbosum*) were obtained from commercial sources. These were placed in a commercial Waring Blendor with 300 ml water, the container flushed with nitrogen for 3 min, sealed, and the system was macerated for 30 sec. The resultant slurry was vacuum distilled at 25 in. Hg (54.4°C) for 1 hr and the volatiles were trapped in a dry ice-acetone bath. This yielded 525 g of volatile material which was saturated with sodium chloride and extracted with three 200-ml portions of diethyl ether. The ether was washed with a small quantity of 5% sodium carbonate to remove the free acids. The ethereal solution was then dried and concentrated by slow distillation to a volume of about 1 ml.

Separation and identification

Separation of the mixture was accomplished by gas-liquid chromatography (GLC) in a Perkin-Elmer Model 990 gas chromatograph using 8 ft × 1/8 in. o.d. column containing 10% SP 1000, a modified Carbowax 20M, on 80–100 mesh Supelcoport. The column was temperature programmed from 60°C to 275°C at 6°C per min. Mass spectra were obtained using tandem gas chromatography-mass spectrometry. The column effluent was passed through a glass jet separator maintained at 275°C into the ion source of a DuPont Model 21-491 mass spectrometer. Mass spectra were obtained at 70eV and a source temperature of 250°C.

Sample identification was accomplished by comparison of mass spectra and gas chromatographic retention times of the isolated materials to that of known standards. Where mass spectral identification was not unequivocal, as in the case of the trans-2-hexenal and trans-2-hexenol, infrared spectra were prepared. The individual components were trapped in a dry ice-cooled melting point tube and the condensed components were transferred to a pair of sodium chloride plates. The spectra were run as capillary films in a Perkin-Elmer Model 467 infrared spectrophotometer.

Sample quantitation was performed by triangulation of the GC peaks and the data are reported as area percent.

RESULTS & DISCUSSION

IN ANY MEANINGFUL ANALYSIS of natural products it is important that the concentrate which is analyzed represents accurately the composition of the starting material. In the

Table 1—Components identified in blueberry essence

Peak no.	Identity	Rel. R_t^a	Conc. (%)
1	Ethyl acetate	0.18	0.29
2	Ethanol	0.24	5.74
3	Ethyl isovalerate	0.56	0.13
4	1-Hexanal	0.59	4.86
5	1-Penten-3-ol	0.77	0.30
8	Limonene	0.96	0.97
9	trans-2-Hexenal	1.00	71.13
10	2-Penten-1-ol	1.28	0.24
11	1-Hexanol	1.40	2.0
12	cis-3-Hexenol	1.50	0.41
13	trans-2-Hexenol	1.56	12.39
14	1-Heptanol	1.72	0.01
15	2-Ethyl-1-hexanol	1.81	0.04
16	Linalool	2.00	0.67
18	1-Nonanol	2.35	0.02
19	α-Terpineol	2.45	0.03
20	Nerol	2.75	0.04
21	Geraniol	2.85	0.61

[a] Relative to trans-2-hexenal = 1.00

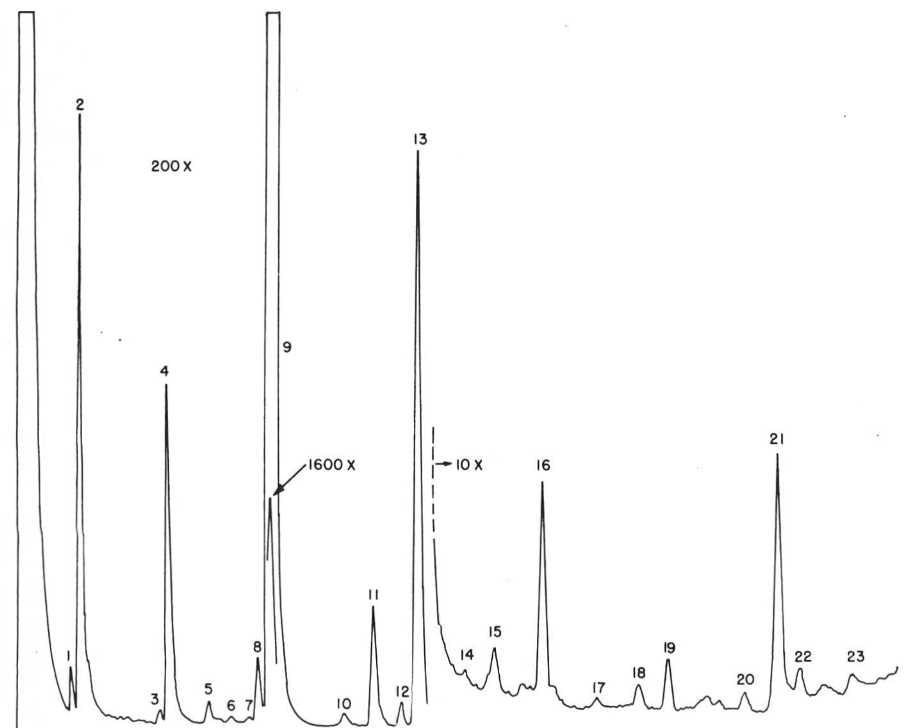

Fig. 1–Gas chromatogram of the neutral components of blueberry. 8 ft × 1/8 in. SP 1000 column programmed from 60°C to 275°C at 6°C per min with a helium flow rate of 25 ml per min.

present case, the aroma of the aqueous distillate was found to be characteristic of fresh blueberries. The ethereal essence retained this character although the green note seemed disproportionately high.

Identification of the major neutral components of blueberries is given in Table 1. The GC curve is presented as Figure 1. The composition of the essence is given in the table in terms of area percent, calculated on a solvent free basis. The compounds which we have identified represent over 99% of the total volatiles of the essence.

It is worth noting that a large percentage of the volatile components identified in this study are composed of six carbon compounds. Taken together these hexanols, hexenols, hexanals and hexenals comprise over 91% of the volatiles. These compounds were all judged to contribute to the pleasant, fruity, fresh green character of blueberries. Organoleptic evaluation demonstrated that linalool also makes a major contribution to the characteristic flavor. Arctander (1969) states that linalool is used in imitation blueberry flavors and that it possesses a floral-woody odor with a faint citrusy note. Although the level of linalool in this essence was less than 1%, in other samples it was found to be as high as 6%. To demonstrate the organoleptic importance of these compounds, a 3:1:1 mixture of trans-2-hexenal, trans-2-hexenol, and linalool was evaluated in a sucrose-citric acid base. The consensus of an informal bench-top evaluation of this system was that it possesses the character impact of fresh blueberries.

SUMMARY & CONCLUSIONS

VOLATILE COMPONENTS of high-bush blueberries (*Vaccinium corymbosum*) were separated from the fruit by vacuum steam distillation, extracted with ether and concentrated by distillation. The essence was fractionated by gas chromatography. Eighteen individual components were identified by mass spectrometry, infrared analysis and gas chromatographic retention times. The concentrations of the components in the essence were determined by peak area measurement. The major component is trans-2-hexenal; and the next largest is trans-2-hexenol. Other compounds present at greater than 1% levels are ethanol, hexanal, hexanol and limonene. Minor compounds identified include ethyl acetate, ethyl isovalerate, 1-penten-3-ol, 2-pentenol, cis-3-hexenol, 2-ethyl-1-hexanol, linalool, nonanol, α-terpineol, nerol and geraniol.

Recombination of the identified compounds demonstrates the importance of trans-2-hexenal, trans-2-hexenol and linalool to the characteristic blueberry flavor.

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THERMOBACTERIOLOGY OF CANNED WHOLE PEELED TOMATOES

INTRODUCTION

UNDERPROCESSING of whole peeled tomatoes usually results in spoilage by butyric acid anaerobes such as *C. pasteurianum* or *C. butyricum* (NCA, 1968). Spoilage outbreaks caused by *B. coagulans*, or flat souring, are fewer in number but of no less importance. At present, the recommended sterilizing value of a process for canned whole peeled tomatoes in juice against spores of butyric acid anaerobes is $F_{200}^{15} = 10$ min when the pH is above 4.3 or $F_{200}^{15} = 5$ min when the pH is less than 4.3, and against spores of *B. coagulans* it is $F_{250}^{18} = 0.7$ min at pH 4.5 (NCA, 1968). These recommendations, however, are not sufficient in cases of excessive contamination, warned NCA (1968) basing process derivation on measuring can-center temperatures.

A method of evaluating the sterilizing effect of a process by determining the reduction of viable spores was explored and published by Yawger (1967). Leonard et al. (1975) successfully used *B. coagulans* spores in the count reduction system of process lethality evaluation, and related the method to classical procedures, using both flame and rotary pressure cooker systems of food preservation.

Increasing consumer awareness of nutrition, dependence on canned foods, and energy problems have caused a reexamination of food processing practices. They should not only be safe, but should rate high in the retention of all quality attributes as well. A step in this direction has been made with whole peeled tomatoes by defining the criteria for microbial stability of the canned product.

EXPERIMENTAL

Sample preparation for determination of microflora initially present in canned whole peeled tomatoes

Microbiological testing was conducted on both commercial and laboratory samples. The samples of commercially prepared peeled whole tomatoes packed in juice in 303 × 406 cans were randomly taken at the closing machine, after sealing, in three different canneries. In addition, at one of the canneries, five cans of whole peeled tomatoes without covering juice and five cans of the hot covering juice itself were also secured so that the types and number of microorganisms in each could be evaluated separately.

The commercially prepared tomatoes were lye peeled and handled in the regular cannery manner (Good Manufacturing Practice) through the sealing of the cans. Tomatoes which were canned experimentally in the laboratory were freeze-heat peeled using the method of Leonard and Winter (1974). The 303 × 406 cans were filled with 10.5 oz freeze-heat peeled tomatoes, and 5.5 oz single strength (97% extraction) tomato juice. Both commercial and laboratory samples were frozen immediately and stored at −10°F (−23.3°C) until examined.

The frozen cans were thawed in cold tap water. The use of warm or hot water was avoided to prevent any possible heat shocking or growth of organisms in the samples. As soon as a sample thawed, it was mixed by shaking the can vigorously. The can was opened aseptically and subsampled. Serial dilutions were made in sterile water. The samples for spore counts were heated at 176°F (80°C) for 5 min to inactivate vegetative cells before plating. When possible, plates showing standard plate counts (30–300 colonies) were counted and recorded.

Total bacteria count

The total numbers of microorganisms and spores were determined by pour plating duplicate samples at different dilutions with glucose-tryptone-yeast extract (GTY) agar, pH 7.0. The plates were incubated 48 hr at 98.6°F (37°C). Colonies were counted for both heated and unheated samples, and reported as the initial total number of spores and total bacterial count, respectively.

Total acid-tolerant bacteria count

Heated and unheated samples were plated in duplicate in GTY agar, pH 4.6. Plates were incubated 48 hr at 98.6°F (37°C). Colonies were counted, and the results were reported as above.

Flat sour spore count

Heat-treated samples were pour plated in duplicate with dextrose-tryptone agar containing 0.04% bromcresol purple. The plates were incubated 48 hr at 98.6°F (37°C). Typical colonies producing the yellow halo characteristic of flat sour organisms were counted and are reported as number of flat sour spores initially present.

Spores of butyric acid anaerobes

Samples heated at 149°F (65°C) for 15 min were used to inoculate exhausted fluid thioglycollate medium which was then sealed with sterile vaspar (equal volumes of petroleum jelly and paraffin) and incubated 48 hr at 98.6°F (37°C). The presence of turbidity and/or the presence of gas were considered positive indicators of growth. Positive samples were then used to inoculate skim milk media. The tubes were sealed with sterile vaspar and incubated 48–72 hr at 98.6°F (37°C). Stormy fermentation of the skim milk medium and/or the production of gas indicated by the rising of the vaspar plug were considered as positive evidence that butyric acid anaerobes were present in the sample. No quantitative determination of the initial number of spores of the butyric acid anaerobes was made.

Lactic acid bacteria count

Unheated samples were plated in duplicate in liver infusion sorbate (0.1% sorbic acid) agar. The plates were incubated for 48 hr at 98.6°F (37°C).

Cultivation and determination of thermal resistance of the test organism

Spores of *B. coagulans* ATCC No. 8038 were chosen as test organisms in this work because they are more heat resistant than spores of butyric acid anaerobes (NCA, 1968), and the metabolism of the organism expedites identification during the experiments.

Spores were cultivated on tomato juice nutrient agar, pH 4.5–5.0, from stock cultures of *B. coagulans* ATCC No. 8038 grown on glucose-tryptone-yeast slants at pH 7.0. Harvesting was done when sporulation reached 90–95%. The spores were washed twice and stored in sterile isotonic saline solution at 37.4°F (3°C) until used, usually, within 1 wk of harvesting. The concentration of spores was maintained at about 1×10^9 spores per ml of inoculum. Sterile glass beads were used to minimize clumping. Prior to inoculation, the spore suspension to be used was heated 5 min at 176°F (80°C) to eliminate vegetative cells of *B. coagulans.*

The thermal resistance of *B. coagulans* ATCC No. 8038 spores was determined and monitored in tomato juice at pH 4.1 to 4.2 using the flask method with either water or oil as the heating medium. Continuous stirring was achieved with a magnetic stirrer arrangement. Survivors were obtained after 24 hr incubation at 98.6°F (37°C) on dextrose-tryptone agar containing 0.04% bromcresol purple. Thermal death rates were determined for at least five temperatures ranging from 176–218°F (80–103.3°C).

The temperature working range was chosen at 176–218°F for two reasons. First, temperature was relatively easy to control and remained constant. Second, at these lower temperatures the thermal death rates were lower thus the time factor could be measured more accurately.

The data obtained were used to calculate the "phantom" death time curve and to define the "z" value for the test organism. The curve is as follows:

$$\log_{10} D_T = 7.565 - 0.037T$$

where T is sterilization temperature (°F) and the correlation coefficient r = −0.980 for n = 7.

The "z" value was found to be 27°F. The thermal resistance of the test organism was tested periodically and remained in close agreement with the initial death rate data throughout the experiments.

Inoculated packs

Groups of 30 cans (303 × 406) of whole peeled tomatoes packed in juice inoculated with 1 ml of approximately 1×10^9 spores of *B. coagulans* ATCC No. 8038 were given varied thermal treatments (Leonard et al., 1975), and placed in a 98.6°F (37°C) incubator. For each group, the Integrated Sterilizing value ($I.S._{212}^{27}$) of the thermal treatment given, the number of surviving spores, and pH were determined at the end of a process by destructively sampling five cans. At monthly intervals, five additional cans were opened and the pH measured to detect flat souring. After 4 months of incubation, in addition to pH measurements, each can of whole peeled tomatoes was analyzed to determine the number of viable spores or cells of *B. coagulans*. Wet mounts of the samples were examined microscopically.

Determination of process lethality

Process lethality was determined in terms of Integrated Sterilizing values ($I.S._{212}^{27}$) using spores of *B. coagulans* (ATCC No. 8038) as described by Leonard et al. (1975):

$$I.S._{212}^{27} = D_{212} (\log a - \log b)$$

where a = initial number of spores, and b = surviving number of spores.

Influence of pH on germination and growth of *B. coagulans* spores in the tomato product

Seven lots of tomato juice were prepared in a range of pH from 4–5 by adjusting with HCl or NaOH. The juice at each pH level was divided into 10 capped culture tubes and autoclaved 15 min at 250°F (121.1°C). When cooled, the first tube in each pH group was inoculated to approximately 1×10^6 spores per ml of juice with *B. coagulans* (ATCC No. 8038). Five additional serial dilutions were made from the first set of tomato juice samples to give approximate spore concentrations of 1×10^1 to 1×10^6 per ml. The extra tubes were incubated as controls. The culture tubes were not airtight.

Dilutions in each group were plated with dextrose tryptone agar to determine the actual initial concentration of spores. The initial pH of each group was measured using a control juice which was autoclaved but not inoculated.

The tomato juices were aseptically subsampled and plated at regular intervals to monitor growth. The experiment was terminated after 6 wk when the number of viable microorganisms began to decline. The final pH of the juices was measured at the conclusion of the experiment.

Influence of pH and heat on germination and growth of *B. coagulans* spores in the tomato product

Three additional sets of tomato juice samples, with adjusted pH values ranging from 4.00–4.81 were prepared. Each tube (25 × 155 mm) at each pH level was inoculated to approximately 1×10^8 spores of *B. coagulans* (ATCC No. 8038) per ml tomato juice. The first set of tubes with different pH values was heated 10 min without agitation at 194°F (90°C); the second and third sets were heated in the same manner for 20 and 30 min, respectively. The initial number of surviving, viable organisms was determined as previously described. Counts were then made weekly, or bi-weekly near the end of the experiment. The pH measurements were taken on controls which were heated but not inoculated.

RESULTS & DISCUSSION

Initial number and types of microorganisms

The initial numbers of microorganisms present in unprocessed whole peeled tomatoes packed in juice are presented in Table 1. The microorganisms varied in kind and number by canneries and method of peeling used in the preparation of the samples.

The counts in positive tests for lactic acid bacteria, and for acid tolerant bacteria were low. The data were not considered significant enough to be presented in detail. Of the 91 samples tested, 45 were positive for lactic acid bacteria, and 35 for acid-tolerant bacteria.

Spores of butyric acid anaerobes were found in 25 of the 34 lye-peeled samples prepared commercially and in 26 of the 57 freeze-heat peeled samples prepared experimentally.

The results suggest that under good commercial practice the total microbial count does not exceed 1×10^4 per ml in cans of whole peeled tomatoes in juice prior to processing.

The microbial analysis of the lots of tomatoes taken as they emerged from the lye peeler, samples of juice used for filling the cans, and cans as they emerged from the filling machine are reported in Table 2. These samples were acquired to indicate the source of the contaminants.

The data indicate that while the tomatoes following lye peeling had about 3×10^3 bacteria per ml, they had low flat sour spore counts and were negative for butyric acid anaerobes. Although the covering juice had lower total bacterial counts it tested positive for butyric acid anaerobes. Thus, the use of high temperature-short time pretreatment of the covering juice should be recommended as a good precautionary practice (Troy and Schenck, 1960).

As the tomatoes and juice are combined in canning, there is a lowering of the total bacteria count caused in part by the sterilizing effect of the hot juice on heat sensitive microorganisms and by an averaging of the initial numbers and types found in each. The lower pH value of these combined samples reflects the effect of the citric acid present in the salt tablet added as customary practice.

Thermal resistance of *B. coagulans* spores

Up-to-date research, in general, tested the thermal resistance of *B. coagulans* spores in terms of thermal death times in tomato juice, citing F values which were admittedly ex-

Table 1—Initial numbers of microorganisms per milliliter of unprocessed canned whole peeled tomatoes packed in juice in 303 X 406 cans

	Total bacteria no./ml sample		Total spores no./ml sample		Flat sour spores no./ml sample		No. of samples
	Range	Median	Range	Median	Range	Median	
			Lye peeled, commercial samples				
Cannery A	140–5,800	590	35–2,350	122	1–330	40	18
Cannery B	150–8,500	740	40–1,555	400	1–245	55	12
Cannery C	470–1,255	578	30–70	35	1–25	18	4
			Freeze-heat peeled, experimental samples				
	5–60,000	1,400	5–14,000	480	5–4,050	270	57

trapolated. In tomato juice, when heated without agitation, we found that the heat transfer by convection in the first phase of heating usually changes to a conduction like heating as the temperature of the juice approaches a level which would have any lethal effect on the organism. Thermal death time determination methods are usually nonagitating, in-container processes. A difference in methodology is well exemplified when comparing the following thermal death rates for spores of *B. coagulans* ATCC No. 8038 at 194°F, in tomato juice at pH 4.2. With continuous stirring D_{194} = 2.6 min, and without stirring D_{194} = 13 min. Although the thermal resistance of ATCC No. 8038 determined in this work cannot be related directly to the findings of other investigators (Troy and Schenck, 1960; NCA, 1968), we can extrapolate and reduce some data from Troy and Schenck for the purpose of comparison. The heat resistance of 10,000 spores/ml tomato juice was found to be less than 0.7 min at 250°F, from 5–37 min at 200°F and 1–10 min at 212°F, based on product incubation. The organism was isolated in having caused an outbreak of flat sour spoilage of tomato juice. Using the high and low values at 200°F and 212°F, the F value at 194° estimates at 11.5 min low and 70 min high. The "z" values for the organism are estimated 17°F and 22°F respectively, and both are lower than z = 27°F for ATCC No. 8038. Assuming that the F value of Troy and Schenck (1960) represents five decimal reductions, i.e., from 10,000 spores/ml to < 1 spore/ml, the maximum D_{194} value for their organisms is estimated to be 14 min.

Considering the errors introduced in extrapolating the small amount of data available, we may conclude that *B. coagulans* ATCC No. 8038 spores are as heat resistant as those isolated from the outbreak of flat sour spoilage, and that the use of this test organism in determining a process for commercial sterilization of canned whole peeled tomatoes in 303 X 406 cans is adequate. Although we limited our work to strain ATCC No. 8038 using standardized procedures, it should be recognized that the heat resistance of *B. coagulans* spores can vary greatly depending on age, conditioning, and individual strains of bacteria. These variables deserve thorough investigation but are too extensive to be covered in this work.

Table 2—Microbial analysis and pH values of the covering juice, lye-peeled tomatoes and peeled tomatoes packed in juice encountered in a typical commercial operation

pH	Total initial no. microorganisms/ml sample	Total initial no. spores/ml sample	Total flat sour spores/ml sample	Test for butyric acid anaerobes
		Covering juice		
4.25	95	25	10	N.A.[a]
4.28	70	5	1	positive
4.30	25	10	1	positive
4.30	95	1	5	positive
4.28	105	10	1	positive
		Lye peeled tomatoes		
4.35	985	5	1	negative
4.38	1495	1	1	negative
4.42	1835	280	100	negative
4.50	2280	1	1	negative
4.50	2790	1	15	negative
		Peeled whole tomatoes packed in juice		
4.25	660	30	10	positive
4.20	1255	70	25	positive
4.18	470	30	1	positive
4.12	495	40	25	positive

[a] Information not available

Inoculated packs

The data on the experimental packs inoculated with *B. coagulans* are presented in Table 3. The heat treatments for each pack are given in terms of the Integrated Sterilizing values based upon the actual recorded reduction in microorganisms. The pH values reported are those determined after processing.

With time in storage the data indicate a consistent decrease in the number of viable organisms for all lots. Their longevity was found to be dependent upon the initial heat treatment received at the recorded level of pH values in both the Stériflamme (registered trademark of Filper Corp., San Ramon, Calif.) and Steritort (Rotary Sterilizer, Canning Machinery Div., FMC Corp., San Jose, Calif.) processing procedures.

All thermal treatments indicated in Table 3 are apparently sufficient to control flat-sour spoilage in peeled whole tomatoes at the recorded pH values. Cans receiving lesser heat treatment swelled after a few days of incubation. Spoilage resulted from growth of lactic acid bacteria; the highest count recorded being 1.44 X 10^5 per ml of spoiled macerated tissue. Tests for butyric acid anaerobes were positive but the drop in pH value that accompanies flat-sour spoilage had not yet occurred.

Table 3—Effect of the thermal treatment and incubator storage time on the viability and longevity of B. coagulans spores (ATCC No. 8038) in canned whole peeled tomatoes

$I.S._{212}^{27}$ value of process (min)	Initial pH	Initial no. of survivors/ml	Number of survivors/ml after storage at 37°C		
			90 days	120 days	150 days
			Stériflamme		
0.69	4.20	3.3 X 10^5	2.4 X 10^2	3.2 X 10^4	1.0 X 10^3
0.98	4.10	2.9 X 10^5	1.5 X 10^3	6.8 X 10^2	1.3 X 10^2
1.07	4.10	7.6 X 10^4	2.4 X 10^2	6.5 X 10^1	N.A.[a]
1.30	4.16	3.7 X 10^4	None	None	None
			Steritort		
0.27	4.12	2.7 X 10^5	6.8 X 10^4	2.6 X 10^4	1.1 X 10^4
0.72	4.00	3.7 X 10^4	2.6 X 10^2	3.3 X 10^2	2.6 X 10^2
0.99	4.18	6.9 X 10^3	N.A.[a]	1.0 X 10^1	N.A.[a]
1.52	4.16	1.6 X 10^4	None	None	None

[a] Data not available

Influence of pH

Rice and Pederson (1954a) showed that pH is a significant factor in inhibiting the germination and growth of *B. coagulans* spores in tomato products. Tomato juice, inoculated with 65 million spores/ml of NCA strain No. 710 which were heated at 203°F (95°C) for 5 min, did not spoil at pH 4.18. They also found that the pH tolerance of spores was directly influenced by heating at 200°F (93.3°C) before subculturing into tomato juice. As the heat treatment increased, the minimum pH at which the subcultures were able to grow increased. In a study using other strains of *B. coagulans* spores, Rice and Pederson (1954b) found that a million spores did cause spoilage in 25 to 33 percent of the tomato juice samples with natural pH 4.18, but no spoilage was observed at pH 4.10 or less.

In our studies when the pH level of the juice was adjusted to be 4.46 or less, growth of spores occurred at all but the very lowest levels of inoculation without reflecting significant changes in pH values (Table 4). Samples adjusted to pH 4.71 showed a significant increase both in the counts of viable organisms and in pH values, at all levels of inoculation. Stanier et al. (1963) describe the metabolism of microorganisms which can utilize specific organic acids, ordinarily considered metabolic wastes, when fermentable sugars of low concentration are depleted in the growth medium. However, when fermentable sugars are abundant enough, the organism utilizes the available sugars at a much faster rate than the intermediate organic acids which are excreted as wastes to a concentration which inhibits any further growth. The first situation would be marked by a rise in the pH value of the medium which Becker and Pederson (1950) also experienced, whereas the second is marked by a significant drop in the pH value which would be considered as flat sour spoilage. Since the volume of samples at pH level of 4.71 in Table 4 was adequate to titrate with 0.1 NaOH to pH 8.0 end point, we found that titratable acidity in the spoiled samples had decreased by as much as 26% compared to the uninoculated controls.

The data are too limited, however, to permit a conclusive explanation of the findings. Strains of *B. coagulans* are known to vary widely in both their physiological characteristics and their response to outside influences. It would appear from these tests that pH alone is not a completely reliable indicator of spoilage by this microorganism. Also, *B. coagulans* spores (ATCC No. 8038) are capable of germination and growth in tomato juice at a low pH value of 3.85 under aerobic conditions when no additional heat treatment was given to the juice after inoculation.

Influence of pH and heating

The influence of hydrogen-ion concentration on the germination and growth of *B. coagulans* spores in tomato juice after heating for 30 min at 194°F (90°C) is shown in Figure 1. At pH 4.81, germination and growth were definite as indicated by total viable cell count. Growth at the other pH levels was questionable, but microscopic examination confirmed growth in the presence of both vegetative cells and germinating spores at pH 4.81, 4.56 and 4.45. At pH values of 4.31 or lower, only spores were observed which were either maintaining or losing their refractility. In view of the decreasing number of viable microorganisms, loss of refractility would seem to indicate death rather than germination of spores at and below pH 4.31. Samples which received only 10 or 20 min of heating at 194°F (90°C) after inoculation behaved in a similar manner. Heat applied in conjunction with hydrogen-ion concentration appear to determine whether the spores of *B. coagulans* ATCC No. 8038 will germinate and grow in tomato juice.

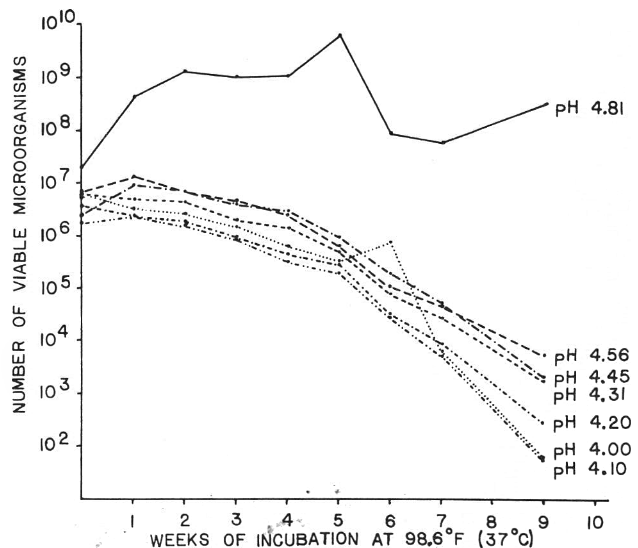

Fig. 1—Influence of pH on germination and growth of B. coagulans spores (ATCC No. 8038) in tomato juice after heating at 194°F (90°C) for 30 min.

Table 4—The effect of initial spore concentration on the minimum pH value at which spores of B. coagulans, ATCC No. 8038 germinated and grew in tomato juice

pH of uninoculated tomato juice		Average concentration of inoculated spores per ml tomato juice											
		6 X 10^0		6.4 X 10^1		6.4 X 10^2		6.4 X 10^3		6.4 X 10^4		6.4 X 10^5	
		Final pH and number of viable cells per ml tomato juice											
Before incubation	After incubation	pH	no. of cells	pH	no. of cells	pH	no. of cells	pH	no. of cells	pH	no. of cells	pH	no. of cells
4.71	4.76	4.81	∿1 X 10^9	4.81	∿1 X 10^9	4.89	∿1 X 10^9	4.87	∿1 X 10^9	4.87	∿1 X 10^{10}	4.93	∿1 X 10^{10}
4.46	4.47	4.43	<5	4.44	20	4.45	4.7 X 10^3	4.47	4.4 X 10^5	4.46	4.4 X 10^6	4.55	3.5 X 10^9
4.31	4.33	4.34	25	4.34	45	4.33	9.5 X 10^3	4.34	6.5 X 10^5	4.34	1.1 X 10^7	4.33	2.1 X 10^9
4.18	4.21	4.21	5	4.22	115	4.20	8.1 X 10^3	4.21	2.7 X 10^5	4.20	2.2 X 10^8	4.20	1.9 X 10^9
4.07	4.10	4.11	10	4.12	140	4.10	2.2 X 10^4	4.10	7.5 X 10^5	4.10	4.4 X 10^7	4.10	2.6 X 10^9
3.97	4.02	4.04	<5	4.04	145	4.02	4.6 X 10^4	4.02	1.5 X 10^6	4.02	5.2 X 10^7	4.02	2.0 X 10^9
3.81	3.85	3.86	20	3.86	83	4.06	3.8 X 10^5	3.85	1.7 X 10^6	3.86	6.4 X 10^7	3.85	2.3 X 10^9

The behavior of *B. coagulans* spores given different thermal treatments is compared in Figures 2, 3 and 4 at pH levels of 4.45, 4.31 and 4.20, respectively. Spores which were losing refractility and vegetative cells were observed for all samples at pH 4.45. At pH 4.31 and 4.20 vegetative cells were not found in any of the samples. The data reported in Figures 3 and 4 further substantiate that a heat treatment of greater severity than that usually used for heat shocking the spores, combined with pH of 4.31, or less, would be effective in retarding and, with time, destroying the exposed spores of *B. coagulans* (ATCC No. 8038). The behavior observed in this experiment seems to have also occurred in the experimental inoculated packs (Table 3).

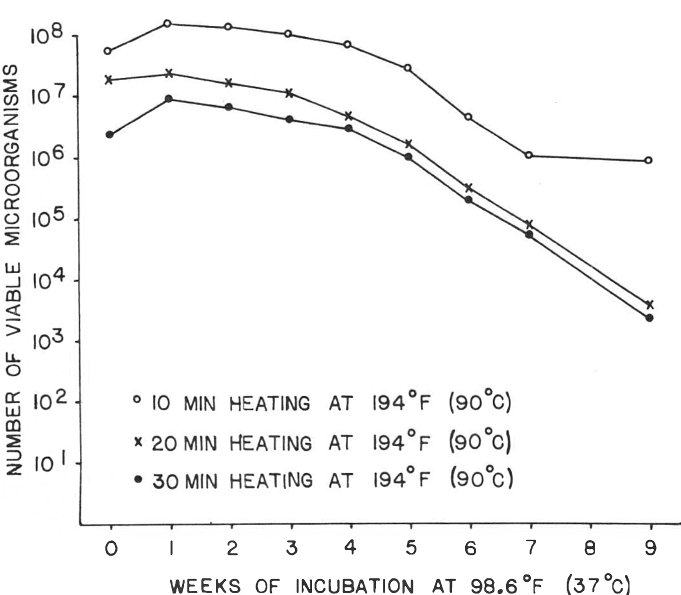

Fig. 2–Influence of thermal treatment on germination and growth of B. coagulans spores (ATCC No. 8038) in tomato juice at pH 4.45.

Process calculation

The variable heat resistance of flat-sour spores and variable pH in tomatoes has caused problems regarding the establishment of suitable processes for tomato products. Troy and Schenck (1960) and NCA (1968) investigating the heat resistance of organisms that caused outbreaks of flat sour spoilage in tomato juice, proposed a thermal process which provides an F_{250}^{18} = 0.7 min for such tomato products. Since this lethality requirement came into practice, the acidification of canned whole peeled tomatoes in juice has been legalized. Investigations revealed and confirmed the influence of pH on the thermal resistance of *B. coagulans* spores and their capability of causing spoilage in tomato products. Application of this information resulted in some reduction of processing times and thereby improved the quality of canned tomatoes without jeopardizing safety.

Leonard et al. (1975) showed the relationship of classical single point F values to Integrated Sterilizing (I.S.) values (Yawger, 1967) for canned whole peeled tomatoes. The F_{212}^{27} values determined by general method process calculations were found to show a logarithmic relationship to actual microbial destruction represented by the I.S.$_{212}^{27}$ value of a thermal treatment. A margin of safety was realized by the fact that in the F determination of process lethality, the data are taken at the slowest heating point in the can, whereas the I.S. value of a process accounts only for organisms that were completely destroyed throughout the can without consideration for heat damaged organisms capable of growth when subcultured, but not able to grow in the product. The magnitude and meaning of the margin of safety in each method merits separate investigation employing the various methods and principles of thermal processing currently available. The work conducted to date indicates that such information is essential for defining a process which will achieve the microbial and biochemical stability desired with minimum impairment of nutritional and aesthetic value of canned tomatoes, or canned foods in general.

The method used for determining the appropriate I.S. value for peeled whole tomatoes in 303 X 406 cans is as follows: The average number of flat sour spores found in commercial samples of unprocessed canned tomatoes was 55 spores/ml.

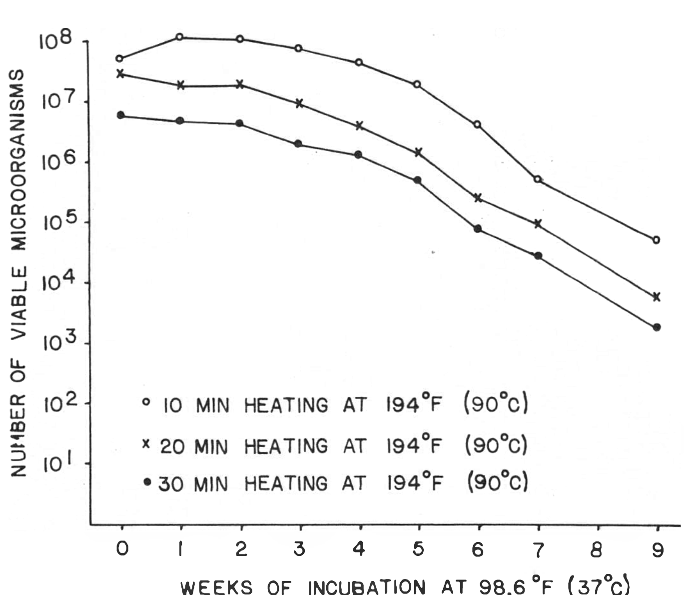

Fig. 3–Influence of thermal treatment on germination and growth of B. coagulans spores (ATCC No. 8038) in tomato juice at pH 4.31.

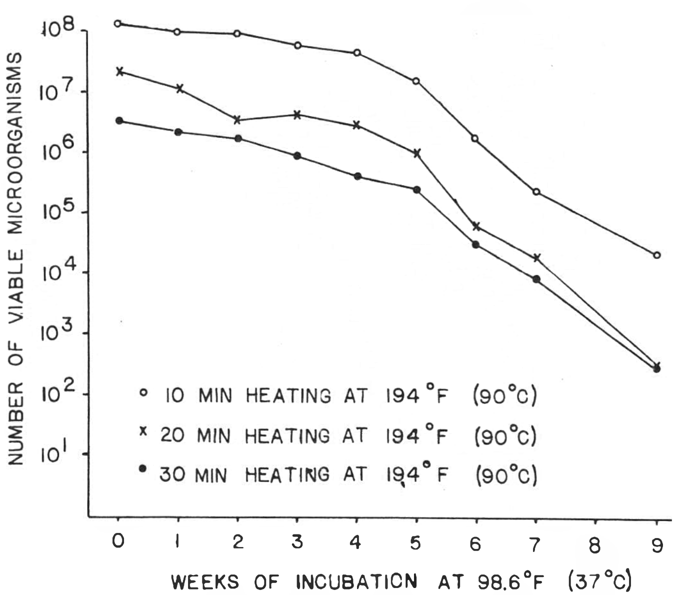

Fig. 4–Influence of thermal treatment on germination and growth of B. coagulans spores (ATCC No. 8038) in tomato juice at pH 4.20.

The allowable final concentration of surviving spores can be approximated from the fact that with pH level 4.46 or less, 640 spores/ml tomato product definitely germinated and grew when the spores were given a heat shock treatment but not heated in the tomato product prior to incubation. Assuming a tolerance level of 0.01% spoilage in any commercial pack of canned whole peeled tomatoes, and a D_{212} value of 0.53 min the thermal process should provide the following lethality in terms of $I.S._{212}^{27}$ value with spores of *B. coagulans* (ATCC No. 8038).

Avg initial spore conc X can vol (ml) 55 X 454 = 24,970; a = avg initial number of spores in 10,000 cans = 249,700,000; b = conc of spores to cause spoilage X can vol (ml) = 640 X 454 = 290,560

D_{212} = 0.53 min

$$I.S._{212}^{27} = D_{212}\,(\log_{10} a - \log_{10} b)$$
$$= 0.53 \text{ min } (8.40 - 5.46)$$
$$I.S._{212}^{27} = 1.56 \text{ min}$$

The $I.S._{212}^{27}$ = 1.56 min is equivalent to F_{212}^{27} = 1.25 min in the rotary pressure cooker process or an F_{212}^{27} = 15.4 min in the Stériflamme system of sterilization (Leonard et al., 1975). Converted to experimental times and temperature these F values for the retort would be 28 min at reel speed of 1.67 rpm at 215°F, and for the Stériflamme, 4 min in the preheating steam chamber, 2 min on the riser, 4 min in the holding section at 240°F, and 4 min in the cooling section. The probability of 290,560 spores surviving within one single can, after the given process is small. The margin of safety is therefore large.

It was also found that one million spores of *B. coagulans*/ml of tomato product did not grow at pH 4.31 or less after receiving any lethal heat treatment ($I.S._{212}^{27}$ of 0.06–0.84 min). The rate at which the number of viable organisms decreased was increased by both lower pH and the increasing severity of the heat treatment. The reported research findings clearly indicate that a process lethality of $I.S._{212}^{27}$ = 1.56 min is safe from a spoilage standpoint for canned peeled whole tomatoes acidified to pH 4.3 or less. In addition, there is the evidence of a season's commercial pack we monitored (over 100,000 cases), prepared in good manufacturing practice, and laboratory-prepared packs with comparable process lethality which failed to produce any spoilage related to underprocessing, even when excessive numbers of lactic acid bacteria were experimentally introduced into the product. Cooling water contamination and double seam failures are of separate concern.

CONCLUSION

EARLY INVESTIGATORS found that spores of *B. coagulans* were easily rendered harmless as spoilage causing organisms in tomato products with pH 4.2 or less when given only a marginal heat treatment. In the present work it was shown that although a minimal thermal treatment did not completely destroy many of the spores, since they recovered in subsampling, it damaged them to a degree where they were not only incapable of germinating but also unable to sustain life in the tomato product at pH ⩽ 4.31. As heat resistant as the spores of *B. coagulans* ATCC No. 8038 were, we were unable to induce flat sour spoilage experimentally when the spores received any lethal heat treatment in the acidified tomato product. At pH ⩽ 4.31, with a lethal treatment equivalent to $I.S._{212}^{27}$ = 1.56 min, neither organoleptic, nor microbiological spoilage by lactics, butyric acid anaerobes, flat sour organisms or *B. coagulans* was experienced, either commercially or in the laboratory.

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IMPORTANCE OF ENZYME INACTIVATION PRIOR TO EXTRACTION OF SUGARS FROM PAPAYA

INTRODUCTION

THE COMPOSITION of sugars in papayas (*Carica papaya*) has been reported by numerous workers. The reported values for sucrose conflict and vary considerably. King et al. (1951) and Pratt and Del Rosario (1913) reported that sucrose comprised 1.2–1.3% of the total sugars. Pope (1930) and Thompson (1914) reported sucrose was 0–13.2% of the total sugars. Stahl (1935) reported sucrose was from 0–4.4% while Jones and Kubota (1940) reported sucrose as 18% of the total sugars. Chen (1963) using a hot alcohol extraction method reported that 60% of the sugars were sucrose. Most recently Dollar et al. (1969) reported that sucrose was not present at any time during postharvest ripening. Varietal differences do not provide a plausible explanation for the discrepancies since Chen (1963), Jones and Kubota (1940), King et al. (1951) and Dollar et al. (1969) reported sucrose values for the Solo variety. The discrepancy in the reported values may be caused by the existence of an invertase enzyme in papaya. Reported herein are the results of a study showing the existence of an invertase enzyme in papayas and its influence on papaya sugar composition.

MATERIALS & METHODS

Preparation of fruit

Papayas (*Carica papaya*) Solo variety were purchased from a commercial source and allowed to ripen at room temperature. Two lots of three or more fruits were treated in the following manner: papayas were cut longitudinally into quarters and deseeded. A segment from each papaya was heated with microwaves for 2 min. The skin was removed from the flesh. While still hot, 90–95°C, 100g of flesh was blended with 300 ml of 80% ethanol for 5 min in a Waring Blendor. The mixture was then extracted for sugars. Another segment of unheated papaya was treated in the following manner to determine if any invertase was present. The flesh was scooped out in large chunks from the skin and blended for 1 min. The pureed papaya was then subdivided into 100-g portions. At 5, 10, 15 and 20 min, a 100-g sample was immediately blended with 300 ml of 80% ethanol for 5 min and further treated for the extraction of sugars. Puree was made from the flesh portion of the remaining quarter segments of papayas by blending for 5 min and without further treatments frozen at −17°C. After 2 wk the puree was extracted for sugar analysis by TLC.

Extraction and silylation of sugars

The ethanolic mixtures of papayas were further treated for the extraction and isolation, and silylation of sugars by the method of Kline et al. (1970).

Gas and Thin-Layer Chromatography

The silylated sugars were gas chromatographed as previously described (Chan and Kwok, 1975).

The sugar extracts in ethanol (2 μl) were applied directly to precoated cellulose sheets (Polygram Cellulose MN 300, Brinkmann Instruments, Inc.). The sheets were developed and the spots visualized as described previously (Chan and Kwok, 1975).

Extraction of papaya invertase

Acetone powders were prepared from papaya. Fresh, ripe papaya flesh, 100g, was blended with 300 ml of cold acetone, −18°C, in a Waring Blendor for 1 min and filtered in vacuo through a Whatman No. 1 filter paper. The procedure was repeated three or more times on the residue, filtering to dryness on the last extraction. The powder was stored at −18°C. The yield of acetone powder from 100g of papaya was 5.0g.

Enzyme extracts were prepared by mixing 5g of acetone powder with 100 ml of 0.01M acetate buffer (pH 6.8) for 5 min at 4°C. The slurry was squeezed through a Nitex nylon cloth (Tobler, Ernest, Trobler, Inc.) with 37 micron orifices. The extract was dialyzed in a Spectrapor membrane tubing No. 1 (Spectrum Medical Ind.) for 24 hr at 4°C. The protein content in the dialyzed extract, determined by the method of Lowry et al. (1951), was 394 μg/ml.

Assay of invertase activity

Invertase activity was measured by the rate of glucose formation. The enzymatic reaction mixture consisted of 0.1 ml of 0.1M acetate buffer pH 5, 0.05 ml of 0.5M sucrose and 0.05 ml papaya enzyme dialysate diluted to 39.4 μg protein/ml. A series of test tubes containing the above mixture was incubated at 30°C. At 1, 3, 5 and 10 min a tube was removed, 1.8 ml 0.1M phosphate buffer (pH 7.0) was added, and the tube placed in boiling water for 3 min to inactivate the enzyme. After the tubes were cooled under running tap water 2 ml of Glucostat Special reagent (Worthington Biochemical Corp.) were added, incubated for 10 min at 30°C, and the reaction stopped by adding 6 ml of 6N HCl. After standing 5 min at room temperature the absorbance was measured at 530 nm in a Bausch Lomb Spectronic 20. A solution consisting of 0.05 ml heat-inactivated papaya enzyme dialysate, and the same quantities of the remaining reagents used in the reaction mixture was used as a blank. One unit of invertase was defined as "the amount of enzyme which catalyzes the production of 1 μmole of glucose/min."

RESULTS & DISCUSSION

SUGAR EXTRACTS from fresh, microwave-heated papaya when chromatographed in cellulose showed the presence of three sugars. The three sugars had Rg values corresponding to those for fructose, glucose and sucrose (1.22, 1.00 and 0.76, respectively).

TLC of papaya that was pureed and frozen without further treatment showed only the presence of two sugars with Rg values corresponding to fructose and glucose. The identity of each of the sugars was further confirmed by their characteristic color development with diphenylamine-p-anisidine spray.

The absence of sucrose in the unheated puree and its presence in the microwave-heated puree provided the initial evidence of invertase in the papayas.

The sugars in the microwave-heated papaya was quantitated and their identities further confirmed by GLC.

Papaya TMS sugars chromatographed in the SE-30 column at 170°C had peaks with relative retention times corresponding to those of TMS fructose, α-glucose and β-glucose (0.32, 0.43 and 0.66, respectively). Papaya TMS sugars chromatographed on SE-30 at 225°C showed a peak with a relative retention corresponding to TMS sucrose, 5.67. When the TMS sugars were chromatographed on 30% OV-17 at 190°C the relative retentions of their peaks matched those of fructose, α-glucose, and β-glucose (0.36, 0.56 and 0.77, respectively). TMS sugars chromatographed on OV-17 at 250°C had a peak with a relative retention of 4.75 which corresponded to TMS sucrose.

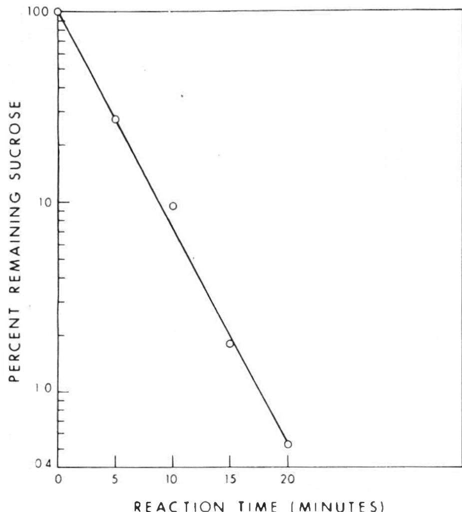

Fig. 1–Sucrose loss in papaya puree.

Table 1–Quantitative determination of sugars in heat-inactivated and untreated papaya after 20 min of invertase action

% Sugar	Heat-inactivated papaya	Untreated papaya
% Fructose	3.30	7.09
% D-glucose	4.50	8.47
% Sucrose	7.29	0.06
% Total	15.09	15.62

The quantitative determination data obtained by GLC are shown in Table 1. Glucose was expressed as the total of the α- and β-anomers. In the heat-inactivated papaya sucrose was found to be the predominant sugar, constituting about 48.3% of the sugars followed by glucose 29.8%, and fructose 21.9%. Contrastingly, in the untreated papaya puree which was allowed to incubate at room temperature for 20 min, only traces of sucrose were found with large increases in both fructose and glucose. In fact, the changes were stoichiometric; a decrease of 21.5 mmoles of sucrose resulted in an increase of 22 mmoles of glucose and 21 mmoles of fructose which clearly establishes the loss of sucrose as due to inversion.

The sucrose concentration in unheated papaya puree was determined by GLC at 5-min intervals after tissue disruption. The results, when plotted semilogarithmically as shown in Figure 1, indicate that the loss of sucrose in papaya puree follows first-order kinetics. The first-order rate constant, was calculated to be 0.261 min^{-1} and the half-life of sucrose in this particular instance was determined to be 2.66 min.

To further demonstrate that the inversion of sucrose is due to the presence of an invertase enzyme in papayas, enzyme extracts from papaya acetone powder were added to model systems of sucrose in buffer. Invertase action was then followed by the determining of the rate of glucose production. The papaya enzyme extract was found to have a specific activity of 0.821 invertase units/mg protein.

The conflicting and low values for sucrose reported by previous workers (King et al., 1951; Pratt and Del Rosario, 1913; Pope, 1930; Thompson, 1914; Jones and Kubota, 1940; Stahl, 1935; Dollar et al., 1969) may be explained by the high invertase activity in papayas. If invertase is not inactivated prior to or immediately after tissue disruption low levels of sucrose will be found.

In summary, this work reports positive evidence for the presence of high concentrations of both sucrose and invertase in papayas. This work also shows that if invertase is not inactivated prior to extraction of the sugars 50% of the sucrose will be lost within 2.66 min.

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SUGAR COMPOSITION AND INVERTASE ACTIVITY IN LYCHEE

INTRODUCTION

THE LYCHEE (*Litchi chinensis* Sonn) a native of southern China is grown in many areas throughout the tropics. Its small ovate fruit, about 1-1/2 in. in diameter, is eaten fresh, canned in syrup, or dried to produce "lychee nuts" (Purseglove, 1968). Horticultural (Yee, 1972) and processing studies (Chan and Cavaletto, 1973; Ross et al., 1969; Wu, 1970) have been reported recently. Information on composition includes analysis of the fruit's nutrients (Wenkam and Miller, 1965), its nonvolatile acids (Chan and Kwok, 1974), and a qualitative study of its sugars by Mathew and Pushpa (1964). These latter workers, using paper chromatography, estimated that glucose was the main sugar and that there were smaller amounts of fructose and sucrose.

Reported here is a study of the sugar composition plus evidence of an invertase enzyme in lychee.

MATERIALS & METHODS

LYCHEE var. Brewster were harvested from the Hawaii Agricultural Experiment Station farms, frozen and stored at −17.8°C. 100g of peeled and pitted fruit were blended with 300 ml of 80% ethanol for 5 min in a Waring Blendor. The mixture was then extracted for sugars, To determine if an invertase enzyme was present, another batch of peeled and pitted fruits was blended for 5 min and without further treatment frozen at −17°C to allow for invertase action. After 2 wk the puree was extracted with 80% ethanol for sugar analysis as stated above.

Extraction and silylation of sugars

The ethanolic mixtures of lychee were further treated for the extraction, isolation and silylation of sugars by the method of Kline et al. (1970). The alcoholic mixture was transferred quantitatively to a 500-ml flask and diluted to volume with 80% ethanol. After standing for 1 hr or more a portion of the mixture was filtered. A 10-ml aliquot of the filtrate was transferred to a centrifuge tube to which was added 0.5 ml of saturated lead acetate solution. The tube was capped, shaken thoroughly, and allowed to stand 15 min. The mixture was centrifuged at 2000 × G for 10 min. An aliquot, 1.05 ml, of clear supernatant was pipetted into a 7-ml screw-cap vial (Pierce Chemical Co.) containing ca. 0.1g of Celite (Johns Manville Co.). The solution was evaporated to dryness in a vacuum oven at 30°C. To each vial a weighed amount of myo-inositol (Calbiochem. Co.), 4–6 mg, was added as an internal standard along with ca. 0.1g of Drierite. The sugars were silylated with 2 ml of TriSil (Pierce Chemical Co.), sealed with a teflon-lined screw-cap (Pierce Chemical Co.), shaken and let stand at 45°C for 1/2 hr. After the solids were allowed to settle, ca. 0.5–1.0 μl of the clear solution was injected into the gas chromatograph.

Gas chromatography

A Varian Aerograph Model 200 gas chromatograph with a flame ionization detector was used. The carrier gas (nitrogen) flow rate was 25 cm³ per min and the hydrogen gas flow rate was 44 cm³ per min. The column was a 7 ft × 0.093 in. i.d. stainless steel column, packed with 3% OV-17 on Chromosorb G A/W HMDS 60/80. Two temperatures were used to facilitate the GLC analysis since sucrose elutes considerably slower than inositol. The column was operated isothermally at both 170°C and 250°C with detector temperature at 270°C and injector temperature at 260°C.

Quantitative data were calculated from the ratio of the peak areas with reference to the internal standard in six or more replications. Ratio of peak areas to internal standard was then converted to ratio of weights, sugar/internal standard, through a linear regression equation. The regression equation was established for each sugar from a standard curve of ratio of weight, sugar/internal standard, versus their corresponding peak areas obtained through GLC. The quantity of each sugar is determined by multiplying its weight ratio by the amount of internal standard (McNair and Bonelli, 1966).

Thin-layer chromatography

The sugar extracts in ethanol (2 μl) were applied directly to precoated cellulose sheets (Polygram Cellulose MN 300, Brinkmann Instruments, Inc.). The sheets were developed in an ascending direction with FMBW (formic acid-methyl ethyl ketone-tert-butanol-water) 15:30: 40:15 (v/v) (Vomhof and Tucker, 1965), dried, and developed again in the same direction with the same solvent. After development, the spots were visualized with diphenylamine-p-anisidine spray reagent (Bailey, 1962). Rg values as well as the characteristic colors (fructose, yellow; glucose, bluish-gray; sucrose, grayish-yellow) were used to identify the sugars.

Extraction of lychee invertase

Acetone powders were prepared from lychee. Frozen, peeled and pitted lychee, 100g, was blended with 300 ml of cold acetone, −18°C, in a Waring Blendor for 1 min and filtered in vacuo through a Whatman No. 1 filter paper. The procedure was repeated three or more times on the residue, and was filtered to dryness on the last extraction. The powder was stored at −18°C. The yield of acetone powder from 100g of lychee was 3.0g.

Enzyme extracts were prepared by mixing 3g of acetone powder with 100 ml of 0.01M posassium phosphate buffer (pH 7.4) for 4 min at 4°C. The slurry was centrifuged at 12,000 × G for 10 min. The supernatant was dialyzed in a Spectrapor membrane tubing No. 1 (Spectrum Medical Ind.) for 24 hr at 4°C. The protein content in the dialyzed extract as determined by the Lowry et al. (1951) method was found to be 520 μg/ml.

Assay of invertase activity

Invertase activity was measured by the rate of glucose formation. The enzymatic reaction mixture consisted of 0.1 ml of 0.1M glycine-HCl buffer pH 2.6, 0.05 ml of 0.5M sucrose, and 0.05 ml lychee enzyme dialysate diluted to 173 μg protein/ml. The mixture in a test tube was incubated at the enzyme's optimal temperature, 55°C. After 10 min 1.8 ml 0.1M phosphate buffer (pH 7.0) was added, and the tube placed in boiling water for 3 min to inactivate the enzyme. After the tube was cooled in ice water 2 ml of Glucostat Special reagent (Worthington Biochem. Corp.) was added, incubated for 10 min at 30°C, and the reaction stopped by adding 6 ml of 6N HCl. After the mixture was allowed to stand for 5 min at room temperature the absorbance was measured at 530 nm with a Bausch & Lomb Spectronic 20. A solution consisting of 0.05 ml heat-inactivated lychee enzyme dialysate, and the same quantities of the remaining reagents used in the reaction mixture were used as a blank. One unit of invertase was defined as the amount of enzyme which catalyzes the production of 1 μmole of glucose/min under the above conditions.

pH Optimum of invertase

The pH optimum for lychee invertase activity was determined by measuring invertase activity in Sorenson's glycine-HCl 0.1M buffer from pH 1.25–3.65 and in 0.1M acetate buffer from pH 3.65–5.75. Solutions of heat-inactivated lychee invertase in their corresponding

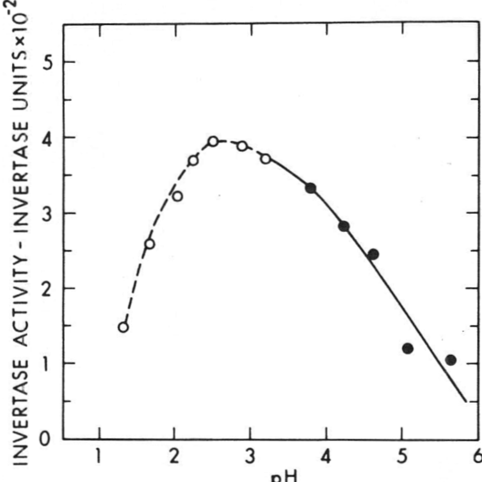

Fig. 1–pH Optimum of lychee invertase in (○) 0.1M Sorenson's glycine-HCl buffer and (●) 0.1M acetate buffer at 55°C.

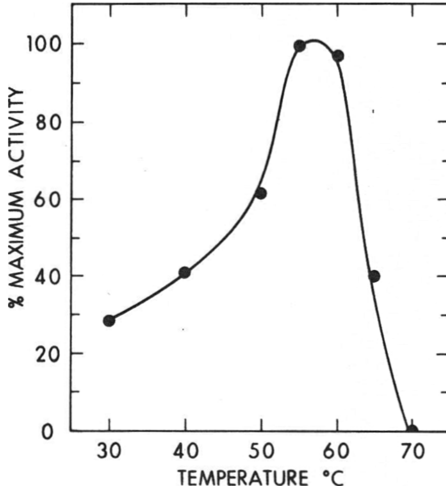

Fig. 2–Temperature optimum of lychee invertase.

buffer solutions were used to correct for any inversion due to nonenzymatic hydrolysis.

RESULTS & DISCUSSION

WHEN THE LYCHEE TMS sugars were chromatographed on 3.0% OV-17 at 170°C, the relative retentions of their peaks matched those of fructose, α-glucose and β-glucose (0.36, 0.56 and 0.77, respectively). TMS sugars chromatographed on OV-17 at 250°C had a peak with a relative retention of 4.75 which corresponded to TMS sucrose.

The quantitative determination data obtained by GLC showed that in whole frozen lychee sucrose was the predominant sugar, constituting ca. 51.1% of the sugars, followed by glucose 30.1% and fructose 18.8%. Total sugars comprised 16.75% of the fruit.

The identity of the sugars was further confirmed by TLC. Sugar extracts from frozen lychee when chromatographed on cellulose and developed twice in the same direction with FMBW solvent showed the presence of three sugars. The three sugars had Rg values corresponding to those for fructose, glucose and sucrose (1.22, 1.00, and 0.74, respectively).

TLC of lychee that was pureed and frozen without further treatment showed only the presence of two sugars with Rg values corresponding to fructose and glucose. The identity of each of the sugars was further confirmed by their characteristic color development with diphenylamine-p-anisidine spray.

The absence of sucrose in the puree and its presence in the whole frozen fruit provided the initial evidence of the possibility of invertase being present in lychee.

To further demonstrate that the inversion of sucrose is due to the presence of an invertase enzyme in lychee, enzyme extracts from lychee acetone powder were added to model systems of sucrose in buffer. Invertase action was then followed by the determining of the rate of glucose production. The lychee enzyme extract was found to have a specific activity of 0.188 invertase unit/mg protein. The optimum pH for lychee invertase was found to be 2.6 as shown in Figure 1. This is somewhat higher than the optimum pH 2.0 reported for grape invertase by Arnold (1965). However, it is lower than pH optima reported for other invertase systems: yeast, pH 4.0–5.5 (Myrbäck, 1957); tomato, pH 4.5 (Nakagawa et al., 1971); dates, pH 4.5 (Hasegawa and Smolensky, 1970); sweet potatoes, pH 4.6 (Matsushita and Uritani, 1974), and it is somewhat lower than the pH of the lychee fruit, which is pH 4.6.

Table 1–Quantitative determination of lychee sugars

Sugar	g/100g fruit	Std dev
Fructose	3.154	0.41
D-Glucose[a]	5.036	0.06
Sucrose	8.562	0.63
Total	16.752	

[a] Expressed as total glucose

The temperature optimum for lychee invertase was found to be 55°C which is similar to that of yeast invertase (Myrbäck, 1957) but somewhat higher than tomato invertase which was described by Nakagawa et al. (1971) as unstable above 30°C.

The relative abundance of each of the three sugars reported in this work differs from those reported by Mathew and Pushpa (1964) who reported sucrose to be present in lesser amounts with glucose being the predominant sugar. The differences in the reported values may be explained by the invertase activity in lychees. If invertase is not inactivated prior to or immediately after tissue disruption, low levels of sucrose will be found.

In summary, this work reports positive evidence of the presence of invertase activity and high concentrations of sucrose in lychee. This work also reports that lychee invertase has an unusally low pH optimum and a high temperature optimum. This enzyme therefore may have potential applications where processing conditions of low pH and high temperatures prevail.

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CHARACTERIZATION OF ANTHOCYANINS IN FIG (*Ficus carica* L.) FRUITS

INTRODUCTION

STRIKING CHANGES in pigmentation occur during the ripening of Mission fig fruits. In only a few days, color of the cell layers comprising the fruit skin changes from deep green to intense bluish-black. In an early survey of anthocyanins, Robinson and Robinson (1932) reported the occurrence of cyanidin 3-monoglucoside in *Ficus carica* L. fruits. However, it is unclear which of the many cultivars of this species was examined. The present study was undertaken to characterize anthocyanins in fruits of the Mission fig, one of the four leading commercial cultivars in Califronia.

MATERIALS & METHODS

THE MISSION FIG FRUITS used were from the Wolfskill Experimental Orchards, Winters, Calif., as were the fruits of Kadota and Calimyrna, which were used for comparative purposes.

Extraction and preparation of crude pigments

75g of skin were peeled from well-colored, fully ripened figs, and immediately immersed in chilled 1% methanolic HCl (methanol:HCl 00:1 v/v) to minimize contact with air. The pigments were extracted by maceration for 5 min in a Waring Blendor containing 1% methanolic HCl. The slurry was centrifuged at 10,000 × G and the pigment-containing supernatant fluid was decanted. The pellet was remacerated several times in 0.1% methanolic HCl and re-centrifuged, until the color of the supernatant fluid indicated that very little additional pigment was being extracted. The supernatant fluids from the several extractions were combined and evaporated in vacuo to dryness at 25°C. The residual pigment was resuspended in 0.1% methanolic HCl and divided into 10 parts, each equivalent to approximately 7.5g of skin. The individual portions were evaporated to dryness and stored in the dark under a nitrogen atmosphere at about −18°C.

Paper chromatography

All paper chromatography was of the descending type, using the various solvent systems listed in Table 1. Chromogenic visualization reagents were as follows:

DSA Prepared fresh as needed by mixing stock solutions of 0.9% sulfanilic acid and 5% sodium nitrite in a 1:5 (v/v) ratio (Ames and Mitchell, 1952). Alkaline degradation products of fig anthocyanidins and standard benzoic acid derivatives were located by spraying developed chromatograms with DSA.

AHP Prepared by dissolving 2.5g of aniline hydrogen phthalate in 100 ml of 95% ethanol. Sugar chromatograms were sprayed with freshly prepared AHP and heated at 80°C until the brown spots became visible (Partridge, 1949).

PdB Prepared fresh as needed by mixing stock solutions of 0.1M periodic acid and acetone in a 1:19 (v/v) ratio. Chromatograms were dipped in the periodic acid-acetone solution and allowed to dry 3–4 min. When just dry, the papers were dipped in 0.01M benzidene reagent and then dried thoroughly. Sugars and sugar alcohols were detected as distinct cream-colored spots on a blue background (Gordon et al., 1956).

Densitometry

The various anthocyanins present in crude skin extract were resolved by chromatography on separate Whatman 1 papers, using BAW and HAc-HCl. Strips from developed chromatograms were exposed for several minutes to fumes of concentrated HCl to maximize color of the separated pigments. Color density was measured on a Spinco Analytrol Model RB Densitometer (Beckman Instruments, Inc., Palo Alto, Calif.) fitted with 500-nm interference filters and an automatic integrator.

Partial acid hydrolysis

Prior to hydrolysis, the various anthocyanins were purified by repeated paper chromatography. The glycosidic nature of the individual purified pigments was determined using essentially the techniques described by Abe and Hayashi (1956) and Harborne (1958a). In each instance, about 30 mg of pigment material were dissolved in a few drops of methanol; and, after addition of 3 ml of 4N HCl, the mixture was immersed in a boiling brine bath. Samples were withdrawn every 5 min for a total of 1 hr, and were spotted on Whatman No. 1 paper. The papers were developed in BAW, HAc-HCl, Bu-H, propionic and formic solvent systems.

Identification of sugars

Sugars were purified by washing the full hydrolyzed pigment solution with an equal volume of n-amyl alcohol. The mixture was cooled rapidly by immersion in an acetone-dry ice bath. The aglycone was extracted into the organic phase, and free sugars were retained in the aqueous phase. The sugar solution was rewashed several times with

Table 1—Solvent systems used for paper chromatography

Abbreviations	Composition	Proportions (v/v)	Phase used	Solvent used for	Time (hr)
BAW	n-butanol:acetic acid:H_2O	4:1:5	Upper	Anthocyanins, Sugars, Phenolics	18, 20, 20
BuHCl	n-butanol:2N HCl	1:1	Upper	Anthocyanins	20
1% HCl	H_2O:concn HCL	97:3	Miscible	Anthocyanins	4
HAc-HCl	Acetic acid:H_2O:concn HCl	15:82:3	Miscible	Anthocyanins, Anthocyanidins	6
2% AW	Acetic acid:H_2O	2:98	Miscible	Anthocyanins	7
Propionic	Propionic acid:formic acid:concn HCl:H_2O	2:5:1:6	Miscible	Anthocyanins, Anthocyanidins	10
Bu-H	n-butanol:concn HCl:H_2O	7:2:5	Upper	Anthocyanins, Anthocyanidins	20
Bu-Pyr	n-butanol:pyridine:H_2O	6:3:1	Miscible	Sugars	18, 36
EtAc-Ac	Ethyl acetate:acetic acid:H_2O	9:2:2	Miscible	Phenolics	18
Bu-EtOH	n-butanol:abs ethanol:H_2O	5:1:4	Upper	Sugars	16
Formic	Formic acid:4N HCl	2:1	Miscible	Anthocyanins	7.5
Iso-ProH	Iso-propyl alcohol:5% HCl	55:45	Miscible	Anthocyanins, Anthocyanidins	20

n-amyl alcohol to remove any remaining aglycone, and was then evaporated in vacuo to dryness. The sugar residue was neutralized by resuspension in redistilled pyridine followed by evaporation, until no traces of HCl were detectable. For paper chromatographic analysis, sugar samples were dissolved in 0.5 ml of redistilled pyridine to which 3 drops of distilled water were added. Samples (50–100 μl) of each sugar solution were spotted on Whatman No. 1 paper along with standards of glucose, rhamnose, xylose, galactose, fructose and arabinose. The papers were developed in Bu-Pyr, BAW or Bu-EtoH, and the sugars were located with AHP and PdB respectively.

Gas chromatography

The neutralized sugars were taken up in anhydrous, redistilled pyridine, and their trimethylsilyl derivatives were formed by addition of hexamethyldisilizane and trimethylchlorosilane in a 2:1 v/v ratio (Sweeley et al., 1963). After 2 hr at room temperature, 0.05–.01 μl volumes of the silylated sugars were injected for analysis. Authentic standards were silylated and injected for comparison. A Varian Aerograph Series 2100–20 gas chromatograph equipped with a hydrogen flame ionization detector and a linear temperature programmer was used for separation of the silylated sugars. Comparison of retention times with that of authentic standards allowed sugar characterization.

Alkaline degradation

Phloroglucinol and benzoic acid derivatives are produced by alkaline hydrolysis of the aglycone (Smith and Luh, 1965). About 15 mg of purified anthocyanidin were combined with 3 ml of hot 15% barium hydroxide solution. The reaction tube was connected to a nitrogen stream and then immersed in a boiling brine solution for 60 min. The hydrolysate was acidified with HCl and then washed with four 1-ml portions of diethyl ether. Samples of the ether extract were spotted on Whatman No. 1 paper along with standards of gallic acid, p-OH benzoic, protocatechuic acid and phloroglucinol. Separate papers were developed in BAW and EtAc-Ac.

Spectrophotometry

A Beckman DK2A (Beckman Instruments, Inc., Fullerton, Calif.) ratio-recording spectrophotometer was used for spectral characterization of individual anthocyanins and their aglycones. Spectra of purified pigments in methanolic 0.01% HCl were obtained over the range 250–750 nm. Spectral shifts in the presence of aluminum chloride were determined by addition of 3 drops of a 5% w/v solution of the anhydrous salt in absolute ethanol.

Authentic pigments

Pelargonidin 3,5-diglucoside, perlargonidin chloride and cyanidin chloride were purchased from Fluka A.G., Buchs, Switzerland. Cyanidin 3-monoglucoside, isolated from the 'Tokay' cultivar of *Vitas vinifera* grapes, was kindly provided by M. Akiyoshi (Dept. of Viticulture & Enology, University of California at Davis). Pelargonidin 3-rhamnoglucoside and cyanadin 3-rhamnoglucoside were isolated from orange-yellow (Harborne, 1958b), and crimson cyanidin 3,5-diglucoside was isolated from *Rosa gallica*, 'Chrysler Imperial,' (Mayer and Cook, 1943).

RESULTS & DISCUSSION

Pigment isolation

The crude 1% methanolic HCl extract from 'Mission' fig skin was resolved on chromatograms developed in BAW into 3 distinct anthocyanin bands. The pigments were designated A, B and C, commencing with the slowest moving band. After elution of the pigments, and their respective rechromatography in 2% AW, an additional anthocyanin was found associated with each. The pigments separated by rechromatography were designated a, b and c, according to the major pigment from which they were separated. Rechromatography of the 6 anthocyanin bands in HAc-HCl, Iso-PrOH, BuHCl, and BAW failed to separate any additional pigments.

Sugar moieties

To ensure removal of free sugars originating from the skin of ripe fruits, strips from preparative chromatograms were monitored for the movement of sugars and sugar alcohols by dipping them in the periodic acid and benzidine visualization reagent. The purification routine of chromatography in BAW followed by 2% AW was effective in removing free sugars from anthocyanin pigments present in the skin. The sugars present in detectable amounts with AHP were glucose and rhamnose in bands a, B and C, and glucose in bands A, b and c (Table 2).

An improved distinction between glucose and galactose was obtained by gas chromatography of their trimethylsilyl derivatives. The retention times of silylated sugars and of authentic standards are listed in Table 3. The retention characteristics of α-glucose and α-galactose were sufficiently unlike to permit exclusion of galactose as a possible glycoside from any of the pigment bands examined. Results from gas chromatography supported paper chromatographic data and eliminated other sugars from consideration (Table 2). Also, rhamnose was confirmed as the additional sugar glycosidating bands a, B and C.

Table 2–R_f and R_g values of sugars hydrolyzed from fig anthocyanins and those of authentic standards

Pigment source	Bu-Pyr (R_f)	Bu-Pyr[a] (R_g)	BAW (R_g)	Bu-EtOH (R_f)	Identification
Band A	0.14	1.00	0.18	0.14	Glucose
Band a	0.14	0.97	0.18	0.14	Glucose
	0.45	2.17	0.39	0.15	Rhamnose
Band B	0.10	1.00	0.18	0.33	Glucose
	0.45	2.11	0.38	0.12	Rhamnose
Band b	0.11	1.00	0.18	0.13	Glucose
Band C	0.15	0.98	0.17	0.32	Glucose
	0.43	2.24	0.40	0.14	Rhamnose
Band c	0.17	0.98	0.18	0.15	Glucose
Standard sugars					
Glucose	0.18	1.00	0.18	0.14	
Rhamnose	0.46	2.23	0.39	0.34	
Xylose	0.31	1.59	0.26	0.24	
Galactose	0.16	0.94	0.18	0.11	
Fructose	0.24	1.36	0.20	0.16	
Arabinose	0.22	1.47	0.22	0.19	

[a] Papers were allowed to develop for 36 hr.

Table 3–Gas chromatography of sugars hydrolyzed from fig anthocyanins[a]

Pigment source	Retention temp (°C)[b] α	Retention temp (°C)[b] β	Retention time (min) α	Retention time (min) β	Identification
Band A	203	212	25.7	28.0	Glucose
Band a	204	212	26.0	28.0	Glucose
	174	181	18.5	20.2	Rhamnose
Band B	204	212	26.0	28.0	Glucose
	174	181	18.5	20.2	Rhamnose
Band b	203	212	25.7	28.0	Glucose
Band C	204	212	26.0	28.0	Glucose
	175	182	18.7	20.5	Rhamnose
Band c	203	212	25.7	28.0	Glucose
Standard sugars					
Glucose	204	212	26.0	28.0	
Rhamnose	175	182	18.7	20.5	
Galactose	213	215	28.2	28.75	
Xylose	184	190	21.0	22.5	
Arabinose	174.5	177.5	18.6	19.4	

[a] Linear temperature programmed analysis 100–250°C at 4°C/min
[b] Varian Aerograph Series 2100–20, 3% SE-30 coiled 5 ft X 0.125 inch o.d.

Minor pigments

During the course of the investigation it became apparent that bands a and c, which were present in low concentrations, were chromatographic artifacts produced, respectively, by overloading and a high sugar content retained near the origin of BAW chromatograms. Band a was produced by streaking of band B, and was identical to it. Band c was identical to band b, and resulted from insufficient separation of band b from band C on preparative chromatograms.

Partial acid hydrolysis

This technique was used to determine the position of glycosidation and the number of sugar residues attached to each anthocyanin molecule. The results are presented in Figure 1. The sugar residues were completely removed from each pigment within 40–60 min. Band A fluoresced under UV illumination, indicating that the 5-position was substituted (Harborne, 1958a, b). Its hydrolytic products were separated into 4 spots after 15 min, confirming it as a 3,5-di-monoside. Hydrolysis of bands B and C yielded 3 spots for each band after 10 min. As there was no fluorescence observed in either case, the compounds were both identified as 3-biosides. Band b lacked fluorescence and yielded 2 spots after 10 min, indicating it was a 3-monoside.

Spectral analysis

Spectral measurements in the range 240–600 nm are used in anthocyanin characterization to indicate (a) the nature of the aglycone, (b) the position of glycosidation and (c) the possible acylation of the pigment by a hydroxy aromatic acid. Examination of the spectral data in Table 4 reveals that the anthocyanins corresponding to bands A, B, a, b and c have very similar absorption maxima (λmax 530 nm). This, together with ratios of the optical density of their absorption spectra (E_{440}/E_{max}), indicated they were glycosides of either cyanidin or peonidin. Since peonidin does not undergo a bathochromic shift in the presence of anhydrous $AlCl_3$, it was immediately excluded as a possibility. Thus, based on its absorption maxima, E_{440}/E_{max} values, and free o-dihydroxy groups, the aglycone of bands A, B, a, b and c was identified as cyanidin. The E_{440}/E_{max} values, absorption maximum, and lack of reaction with $AlCl_3$ clearly established band C as a glycoside of pelargonidin.

Absorption spectra of pigments substituted in the 5-position have a pronounced shoulder in the 410–450 nm region, causing decreased absorbance at 440 nm and a lowering of the E_{440}/E_{max} values. Substitution in the 5-position lowers E_{440}/E_{max} values, for pelargonidin glycosides 17%, and those for cyanidin glycosides 9%. Based on their E_{440}/E_{max} values, bands B, C, a, b and c fall into the 3-substituted pigment category, and band A into a 3,5-substituted category.

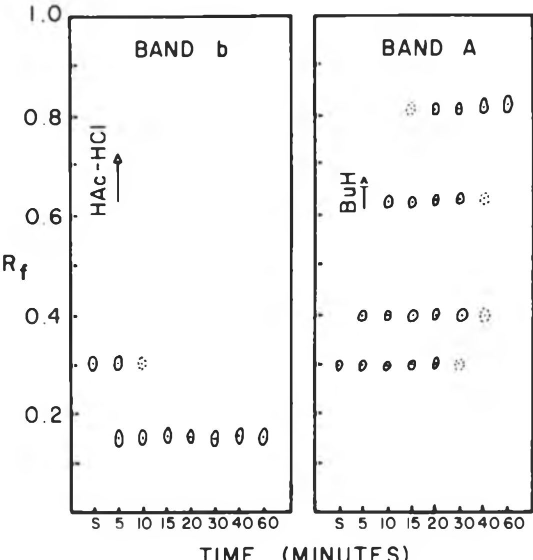
Acylated pigments have 2 distinct peaks, an anthocyanin peak in the region of 280 nm, and an acyl peak at 310 nm. Spectral absorption of the fig anthocyanins had a single peak at 280 nm, indicating that the pigments were not acylated.

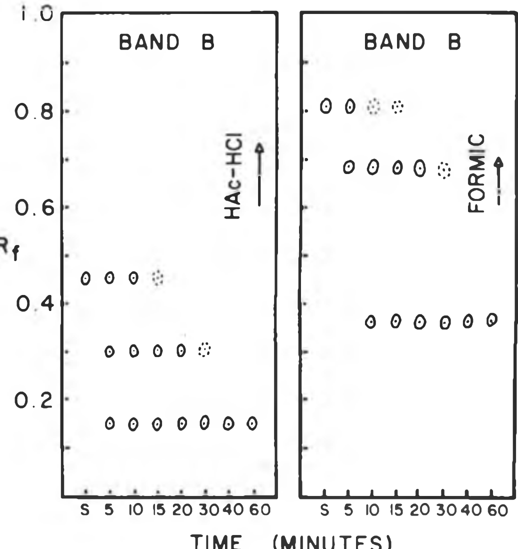
Comparison of spectral data with that from authentic standards indicated that the fig anthocyanins were as follows: band A, cyanidin 3,5-diglucoside; band B, cyanidin 3-rhamnoglucoside; band b, cyanidin 3-monoglucoside; and band C, pelargonidin 3-rhamnoglucoside. Band a, cyanidin 3-rhamnoglucoside, was confirmed identical to band B; and band c, cyanidin 3-monoglucoside, was confirmed identical to band b.

The anthocyanin around the drupelets ("seeds") of each of the 'Mission,' 'Kadota' and 'Calimyrna' cultivars had spectral (Table 4) and chromatographic properties similar to those of band B. Thus, it was tentatively identified as cyanidin 3-rhamnoglucoside pending analysis of the sugar moieties.

Alkaline hydrolysis

Further evidence concerning the structure of the pigment aglycone can be obtained by its hydrolytic cleavage in the





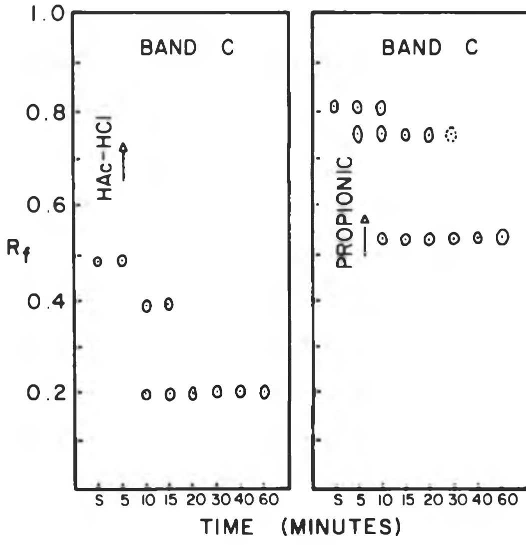


Fig. 1—Degradation by partial acid hydrolysis of bands A, b, B and C isolated from preparative chromatograms of Mission fig anthocyanins.

Table 4–Spectral absorption characteristics of anthocyanins isolated from fig fruits as compared to characteristics of these pigments obtained from other sources

Pigment origin	Band	λmax (nm) Pigment	λmax (nm) Aglycone	E_{440}/E_{max} %	$AlCl_3$ shift	Identification
Mission skin	A	525	540	15	+	Cyanidin 3,5-diglucoside
	a	530	540	22	+	Cyanidin 3-rhamnoglucoside
	B	530	540	23	+	Cyanidin 3-rhamnoglucoside
	b	529	540	25	+	Cyanidin 3-monoglucoside
	C	510	522	41	–	Pelargonidin 3-rhamnoglucoside
	c	529	540	25	+	Cyanidin 3-monoglucoside
Mission drupelets		530	540	25	+	Cyanidin 3-rhamnoglucoside
Calimyrna drupelets		530	540	25	+	Cyanidin 3-rhamnoglucoside
Kadota drupelets		530	540	23	+	Cyanadin 3-rhamnoglucoside
Standard pigments						
Chrysler Imperial rose		526	540	13	+	Cyanidin 3,5-diglucoside
Antirrhinum majus		530	540	24	+	Cyanidin 3-rhamnoglucoside
Tokay grape		529	540	23	+	Cyanidin 3-monoglucoside
Antirrhinum majus		510	522	42	–	Pelargonidin 3-rhamnoglucoside
Fluka A.G.		508	–	21	–	Pelargonidin 3,5-diglucoside
Fluka A.G.		–	521	39	–	Pelargonidin chloride
Fluka A.G.		–	540	22	+	Cyanidin chloride

Table 5–R_f values and color characteristics of alkaline degradation products from fig anthocyanins

Compound	BAW	EtAc-Ac	DSA color	Identification
Unknown A	0.67	0.75	Yellowish	Phloroglucinol
Unknown B	0.82	0.86	Yellowish orange	Protocatechuic acid
Unknown C	0.85	0.96	Yellow	p-OH benzoic acid
Phloroglucinol	0.64	0.75	Yellowish	
Protocatechuic acid	0.82	0.88	Yellowish orange	
p-OH benzoic acid	0.90	0.98	Yellow	
Vanillic acid	0.86	0.97	Orange	
Gallic acid	0.61	0.74	Greyish	

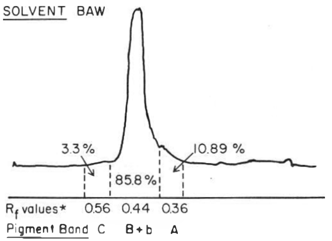
presence of strong alkali. The A ring yields phloroglucinol and, depending on its hydroxylation or methylation, the B ring yields various benzoic acid derivatives. Since, due to the absence of a bathochromic shift, malvidin and peonidin had been eliminated as possible aglycones for bands A, B and b, it was necessary to eliminate the only other possibility, delphinidin. The R_f values, and the colors obtained with DSA (Table 5), indicated that the degradation products of aglycones from bands A, B and b were phloroglucinol and protocatechuic acid. As these can only arise from cyanidin, it was confirmed as the aglycone of those bands. The production of p-OH benzoic acid from degradation of the band C aglycone substantiated its identification as pelargonidin. The yields from alkaline hydrolysis are low and typically around 10%. Since the concentrations of bands a and c were low, and sufficient evidence had already been accumulated to indicate they were chromatographic artifacts, they were not subjected to alkaline degradation.

Chromatographic analysis

R_f values have been used extensively for identification purposes, and factors affecting them have been reviewed by Smith (1969), Lederer and Lederer (1957), and others. The relationship between R_f values and anthocyanin structure has been described by Bate-Smith (1950), Abe and Hayashi (1956) and Harborne (1958a). The R_f values obtained for fig pigments (Table 6) were similar to those of the authentic standards. As there was no pigment hydrolysis observed in the acidic HAc-HCl and 1% HCl solvents, it was confirmed that none of the pigments was acylated.

Table 6–R_f values of Mission fig and of authentic anthocyanins

Pigment	Identification	R_f values BAW	HAc-HCl	1% HCl
A	Cyanidin 3,5-diglucoside	0.12	0.42	0.16
B	Cyanidin 3-rhamnoglucoside	0.41	0.41	0.16
C	Pelargonidin 3-rhamnoglucoside	0.34	0.44	0.21
a	Cyanidin 3-rhamnoglucoside	0.41	0.41	0.16
b	Cyanidin 3-monoglucoside	0.41	0.26	0.07
c	Cyanidin 3-rhamnoglucoside	0.41	0.41	0.16
Authentic standards				
Cyanidin 3,5-diglucoside		0.13	0.42	0.16
Cyanidin 3-rhamnoglucoside		0.41	0.41	0.15
Pelargonidin 3-rhamnoglucoside		0.34	0.44	0.23
Cyanidin 3-monoglucoside		0.41	0.26	0.07



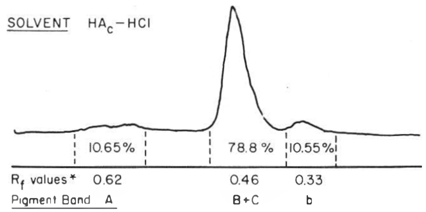


Fig. 2—A densitometric measurement of the relative concentration of various anthocyanins in skin of ripe Mission figs. (R_f values vary due to presence of a high sugar concentration and other pigment-contaminating substances in the original skin extract.*

Densitometry

Since none of the individual solvents used for anthocyanin chromatography in this study distinctly resolved all the fig pigments on a single chromatogram, it was necessary to use two solvents for measurement of the relative pigment concentration. The major pigment, comprising about 75% of the total anthocyanin present, was cyanidin 3-rhamnoglucoside (Fig. 2). Cyanidin 3,5-diglucoside and cyanidin 3-monoglucoside were present in equivalent amounts, and together accounted for about 22% of the total pigment. It was not established whether cyanidin 3-monoglucoside was derived from partial hydrolysis of either cyanidin 3-4-diglucoside or cyanidin 3-rhamnoglucoside. Pelargonidin 3-rhamnoglucoside accounted for about 3% of the pigment content in the skin.

CONCLUSION

THE DATA obtained provide strong evidence, indicating the presence of specific anthocyanins in 'Mission' figs. There is good general agreement between the values obtained and those reported for these pigments in the literature. While Robinsons' earlier finding of cyanidin 3-mono-glucoside in *Ficus carica* is confirmed here, this is the first report of cyanidin 3-rhamnoglucoside, cyanidin 3,5-diglucoside and pelargonidin 3-rhamnoglucoside occurring in fruits of *Ficus carica* 'Mission.' It appears that the predominant pigment cyanidin 3-rhamnoglucoside found in the skin of ripe 'Mission' figs also occurs in its drupelets and the drupelets of two other cultivars, 'Kadota' and 'Calimyrna,' which do not have anthocyanin pigments in their skins.

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TASTE THRESHOLDS OF FATTY ACID ESTERS IN RAISINS AND RAISIN PASTE

INTRODUCTION

THE HISTORY of the use of fatty acid esters and other compound-treatment combinations to facilitate drying of grapes into raisins was outlined by Ponting and McBean (1970). These authors also conducted experiments which indicated that ethyl oleate was convenient and effective in increasing the drying rate of Thompson Seedless grapes. Petrucci et al. (1974) pointed out the problems involved in the time honored natural sun-drying process, and the advantages derived by using mechanical harvesting and fatty acid esters to speed on-the-vine or tunnel drying. In connection with the use of different fatty acid ester mixtures and drying procedures, formal sensory evaluation of the finished raisins indicated large variations in flavor. Various types of off-flavors were observed in some of the samples but not in others. At that time, the raisins were not analyzed for fatty acid ester residue; hence, it was not possible to relate off-flavor with fatty acid ester content. Subsequently, Stafford et al. (1974) developed a procedure for fatty acid ester analysis in processed raisins. Since analysis of the raisins showed substantial variation in fatty acid ester content, it appeared essential to determine the thresholds or minimum amounts of the different esters that could be detected in the raisins. This information is needed to design and control processing treatments that will prevent the occurrence of fatty ester off-flavor in the finished raisins.

MATERIALS & METHODS

THE RAISINS USED in these studies were obtained from California State University, Fresno; detailed procedures for harvesting and processing the grapes (Thompson Seedless) are described by Petrucci et al. (1974). Briefly, the fruit was mechanically harvested, yielding individual berries which were dipped in plain water and in 0.5–10.0% emulsions of the various fatty acid ester mixtures prior to drying in a two-tunnel dehydration system. The raisins were dried to 14–16% moisture and held at 1.1°C until used for the sensory experiments. The analytical procedures for fatty acid ester analysis and composition of the dipping emulsions used in these experiments are described by Stafford et al. (1974). Most of the compounds or mixtures were obtained from various commercial sources and were variable in their ester composition depending on the raw material from which they were made. Thus the thresholds obtained from these sources represent mixture thresholds rather than the threshold of the principal constituent of the mixture. High purity methyl and ethyl oleate were prepared from a common sample of oleic acid. The acid was prepared by saponification of high oleic safflower oil followed by fractional crystallization of the urea complex of the acid (Parker et al., 1955). The oleic acid was then converted to methyl and ethyl esters which were distilled under vacuum. Purity of the esters by GLC analysis was 96–98%. Principal impurities were traces of the corresponding stearate and linoleate esters. Methyl and ethyl linoleate were similarly prepared, except that the source of linoleic acid was ordinary safflower oil.

Sensory evaluation

Thresholds in the dipped raisins and raisin paste were determined by the same procedures and in the same facility described by Guadagni et al. (1973). For the whole raisins, samples dipped in various concentrations of the fatty acid esters were paired with a water-dipped sample from the same lot and presented to a panel of 20 judges trained to detect oleate off-flavors in raisins. The task was to determine the sample with the off-flavor. Each concentration pair was replicated at least three times giving 60 judgments per concentration. Thresholds were determined by plotting the results from four or five concentrations on log-probability paper and determining the concentration where 50% of the judgments detected an off-flavor.

Thresholds were determined in raisin paste to eliminate the large variability in ester concentration among individual berries. Water-dipped, tunnel-dried raisins were ground and extruded through a power-driven food chopper into a uniform paste (three passes). Representative samples of the paste were weighed to 0.5g and thoroughly mixed with varying amounts of each individual compound or mixture to give a series of increasing concentrations in the paste. Each of the concentrations was then paired with the same paste containing an equivalent amount of distilled water and presented to the sensory panel for threshold determinations as described above for whole raisins.

All samples were coded with two-digit numbers, and order of presentation was completely randomized. Because of the persistency of fatty acid ester flavors, only one pair was presented per session, held in mid-morning and mid-afternoon. The thresholds and 95% confidence intervals derived from the sensory data are equivalent to the familiar LD_{50} used in dose-response experiments (Litchfield and Wilcoxon, 1949). In addition to the threshold measurements, flavor differences among samples treated with various commercial mixtures were determined by duo-trio test (ASTM, 1968).

RESULTS & DISCUSSION

Flavor variability in whole raisins

Preliminary sensory results on raisins prepared from grapes dipped in emulsions of commercial preparations of methyl and ethyl oleate gave inconsistent results. Some samples of grapes dipped in 2% emulsions of the oleates were undistinguishable from water-dipped samples, but others had strong off-flavors. Since there was some question as to possible differences in the grapes representing the different samples, the experiments were repeated with a reasonably uniform lot of grapes. The first five comparisons shown in Table 1 were between duplicates of the same treatment. A significant difference ($P \geqslant$

Table 1—Flavor variability of raisins from grapes dipped in oleate emulsions

Comparison[a]	N	No. correct	Preference for[b] control
Ethyl oleate 1 vs ethyl oleate 2	37	27**	25
Methyl oleate 1 vs methyl oleate 2	41	25	15
Methyl oleate 3 vs methyl oleate 4	41	24	12
Sun-dried 1 vs sun-dried 2	59	24	13
Soda-dipped 1 vs soda-dipped 2	39	25	14
Sun-dried vs ethyl oleate	37	23	20
Sun-dried vs soda-dipped	39	20	10
Sun-dried vs methyl oleate 5	34	26**	26
Sun-dried vs methyl oleate 6	38	20	12

[a] Comparisons were by duo-trio test. Oleate-treated raisins were dipped in 2% emulsions in water.
[b] Of the correct judgments
** $P \leqslant 0.01$

0.01) was found between samples dipped in 2% emulsions of ethyl oleate, but no significant differences were found between duplicates of the other treatments. In the last four comparisons in Table 1, sun-dried controls were compared against different treatments. No differences were observed for ethyl oleate or soda dip, but one of the methyl oleate samples was significantly different ($P \geqslant 0.01$) and all of the correct judgments preferred the control, indicating an off-flavor in the oleate-treated sample. Since none of the nonoleate-treated samples showed significant differences among themselves or against the natural sun-dried raisins, it appeared that the flavor variation among oleate-treated samples was due to variations in the amounts of oleate retained on finished raisins.

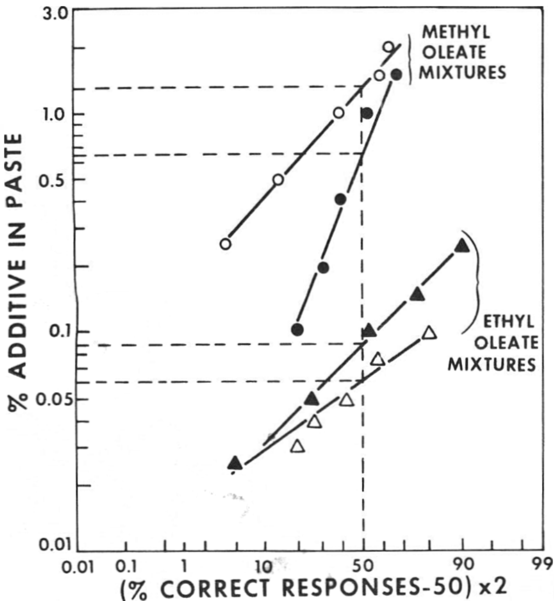

Fig. 1—Relationship between oleate concentration in raisin paste and off-flavor detection for commercial ethyl and methyl oleate mixtures. [Ethyl oleate mixture A (▲); ethyl oleate mixture B (△); methyl oleate mixture E (○); methyl oleate mixture F (●). Methyl oleate mixtures E and F correspond to methyl oleate dipping oils A and B respectively in Table 1 of Stafford et al., 1974.]

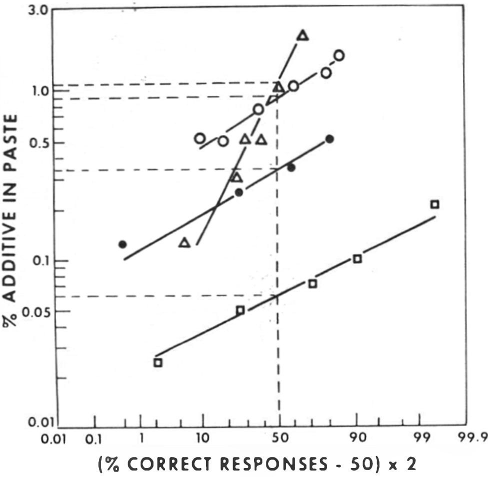

Fig. 2—Relationship between oleate concentration in raisin paste and off-flavor detection for high purity laboratory prepared oleates. [□—ethyl oleate; ●—ethyl linoleate; △—methyl oleate; ○—methyl linoleate.]

Oleate thresholds in raisin paste

The first experiments with raisin paste prepared from water-dipped raisins were done with the commercial mixtures used in dipping fresh grapes prior to drying. The sensory results obtained with these mixtures are shown in Figure 1. Since the paired comparison results corrected for chance [(% correct responses − 50) x 2] plot as a reasonably good straight line on log probability paper, the threshold is taken at a value of 50 which corresponds to LD_{50} in dose-response experiments. From these data it is clear that the methyl oleate mixtures have much higher thresholds than the ethyl oleate mixtures. The slopes of the lines for methyl oleate are also higher than those for ethyl oleate, indicating that these panelists were less sensitive to increasing amounts of methyl oleate than of ethyl oleate. This indicates that if the drying efficiency of methyl oleate were similar to that of ethyl oleate the former would be the preferred additive since approximately 10 times more methyl than ethyl oleate was required for 50% detection. Unfortunately, the composition of the mixtures (Stafford et al., 1974) indicates the presence of other fatty acid esters and unknown components, so that the data in Figure 1 represent the effect of a composite mixture rather than a pure compound. Therefore, some of the other components may have contributed a substantial amount to the off-flavor imparted to raisins by the composite mixture.

Thresholds of high purity compounds

Thresholds of highly purified laboratory preparations of ethyl and methyl oleate and methyl and ethyl linoleate were determined in raisin paste prepared from the same lot of raisins. The data from these tests are shown in Figure 2. The slopes of the lines for ethyl and methyl linoleate and ethyl oleate are very similar, indicating about the same panel sensitivity to increasing amounts of these compounds. Again, the slope for the pure methyl oleate was much higher, indicating less sensitivity to this material. The main significance of these data is that pure methyl oleate has a threshold about 16 times higher than that of pure ethyl oleate prepared from the same oleic acid source. Thus, it is clear that much more methyl than ethyl oleate could be used on raisins without causing significant off-flavors. This would provide a much greater latitude in the application and control of these materials on grapes prior to drying. The relationship between additive concentration and off-flavor detection for the other fatty acid esters found in the commercial dipping mixtures is shown in Figure 3. These compounds and an additional sample of ethyl oleate were all obtained from the same reputable commercial source. It is clear that the low threshold of ethyl oleate dipping mixtures was not due to the other fatty acid ester impurities in the mixture since their thresholds were several times higher than that of pure ethyl oleate. The thresholds and 95% confidence intervals for all the compounds tested in raisin paste are shown in Table 2. Even though there was a large variation in ethyl oleate threshold, depending on its source, it was consistently below that of all the other compounds tested. The threshold of methyl oleate was about 6.5 to 100 times higher than ethyl oleate, depending on which samples were compared. Regardless of the variation among samples, however, it is clear from the thresholds of the other fatty acid esters that these compounds are not responsible for the low thresholds of the mixtures.

Contribution of individual compounds to mixture thresholds

Since the thresholds of the known individual components of the mixtures and of the total mixtures themselves were determined in the same raisin paste, it is possible to estimate the probable contributions of the individual components as-

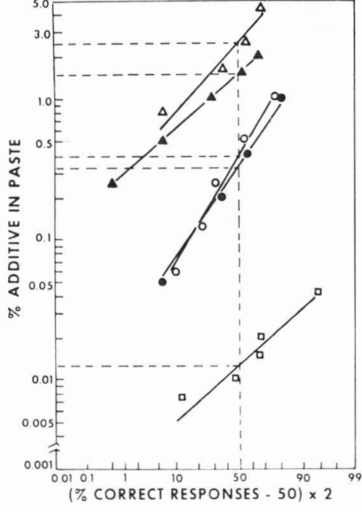

Fig. 3–Relationship between oleate concentration in raisin paste and off-flavor detection for individual fatty acid esters. [□–ethyl oleate; ●–methyl stearate; ○–methyl palmitate; △–ethyl palmitate; ▲–ethyl stearate.]

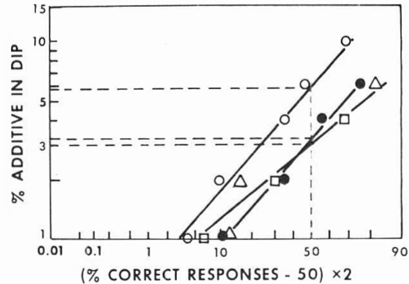

Fig. 4–Relationship between dip concentration of oleate mixtures and off-flavor detection in whole raisins. [○–mixture A vs water dip raisins; ●–mixture E vs sun dried raisins; △–mixture E vs water dipped raisins; □–mixture E vs soda dipped raisins.]

suming they behave additively at subthreshold concentrations (Guadagni et al., 1963). At threshold of the mixture in raisin paste, the concentration of mixture (C_m) is equal to the threshold concentration (T_m) and C_m/T_m is equal to 1. If the subthreshold concentrations of the various components behave additively, $C_1/T_1 + C_2/T_2 + C_3/T_3 + \text{---} C_n/T_n = C_m/T_m = 1$ at threshold of the mixture, and the contribution of each component is estimated by dividing its concentration in the mixture at threshold of the mixture by its individual threshold. Using this relationship as a model, the contributions of the different components in the four different mixtures used in raisin paste were calculated and are shown in Table 3. In mixtures B, E and F the sum of the contributions from the individual compounds in the mixture is within ± 20% of the theoretical value of 1 assuming additivity. This deviation from unity is considered well within the accuracy attainable with the materials and sensory procedures employed; therefore, the mixture thresholds are reasonably well accounted for by the individual components except for mixture A. In mixtures B, E and F this suggests that unknown impurities had little or no effect in contributing to the threshold of the mixture and that the flavor perceived at threshold was due to a combination of the known components in the mixture. The threshold evidence for these three mixtures also indicates that the subthreshold interaction of the components was additive as assumed in the model and not synergistic or suppressive. In mixture A, the known components accounted for less than 50% of the mixture threshold. Since this mixture also contained the greatest amount of unknown impurities, it is probable that these unknowns also contribute to the threshold if the relationship is additive as in the other three mixtures. Another possibility is that the unknown impurities behave synergistically with some or all of the components rather than additively. Either possibility or a combination of both could explain the results obtained in mixture A.

Oleate thresholds in whole raisins

Thresholds of commercial mixtures A (ethyl oleate) and E (methyl oleate) on whole raisins are shown in Figure 4. The

Table 2–Taste thresholds of different fatty acid esters in raisin paste

Material[a]	Threshold (%)	95% confidence limits
Mixture A, ethyl oleate emulsion	0.088	0.07–0.11
Mixture B, ethyl oleate emulsion	0.060	0.05–0.07
Mixture C, ethyl oleate emulsion	0.108	0.09–0.12
Ethyl oleate, commercial	0.013	0.01–0.015
Ethyl oleate, high purity	0.061	0.056–0.066
Mixture E, methyl oleate emulsion	1.30	1.06–1.58
Mixture F, methyl oleate emulsion	0.65	0.42–1.01
Methyl oleate, high purity	1.05	0.74–1.50
Methyl palmitate, commercial	0.40	0.30–0.55
Methyl stearate, commercial	0.34	0.26–0.44
Ethyl palmitate, commercial	2.45	2.0–3.0
Ethyl stearate, commercial	1.50	1.3–1.8
Methyl linoleate, high purity	0.88	0.70–1.10
Ethyl linoleate, high purity	0.34	0.30–0.38

[a] High purity samples were synthesized in the laboratory; commercial samples were all obtained from the same reputable manufacturer. The mixtures contained other fatty acid esters and unknown impurities in addition to methyl or ethyl oleate.

Table 3–Contribution of individual fatty acid esters to the threshold of commercial mixtures of these components in raisin paste

Compound		C/T[a]	
		Mixture E	Mixture F
Methyl oleate		0.77	0.33
Methyl linoleate		0.17	0.07
Methyl palmitate		0.16	0.05
Methyl stearate		0.07	0.45
	Sums----------	1.17	0.90
		Mixture A	Mixture B
Ethyl oleate		0.42	0.85
Ethyl stearate		0.01	—
Ethyl palmitate		0.005	0.001
	Sums----------	0.435	0.851

[a] Concentration of compound in paste at threshold of mixture divided by threshold of pure compound

threshold of mixture E was determined against three different lots of control raisins—water dipped, soda-dipped and sundried. It is apparent that the different controls did not cause any significant differences in oleate threshold. Fatty acid ester content of the whole raisins corresponding to these thresholds indicated that only 20–30% of the pure ethyl oleate required for detection in the paste was found on the whole raisins (120–200 ppm ethyl oleate). One of the main reasons for a lower threshold in whole raisins compared to paste is probably associated with the fact that most of the oleates are concentrated on the surface area of the whole raisins in contrast to the uniform mixture in the paste. When the raisins are placed in the mouth, the surface area comes in contact with the taste buds before chewing and mastication makes a reasonably uniform mixture, and hence initial perception is based on surface concentration rather than the concentration based on total weight of the raisins. In addition, the lower the purity of the oleate mixture, the greater the probability that nonvolatile or unknown constituents contribute to the detectability of flavors foreign to natural raisins.

Individual thresholds

On the basis of individual thresholds, it was found that the most sensitive 10% of the panel could detect 40 ppm, 50% of the panel detected 200 ppm or less, and the least sensitive 10% required 1300 ppm of ethyl oleate for significant detection. This represents over a 30-fold change in concentration from the least to most sensitive 10% of the panel. While this panel may not be representative of average consumers, it is probable that a similar range of sensitivities would be encountered among the consuming public. Thus, a substantial proportion of the consumers would detect 100 ppm of ethyl oleate on treated raisins, and some of these would probably object to the oleate flavor. In view of the large differences in thresholds of the various fatty acid ester mixtures, and large differences in individual sensitivity to these materials, it is important that the application of these drying aids be accompanied by adequate quality control procedures designed to control oleate content of treated raisins at acceptable levels.

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

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STRUCTURAL FUNCTIONS OF TASTE IN THE SUGAR SERIES: GUSTATORY PROPERTIES OF ANHYDRO SUGARS

INTRODUCTION

STUDIES of the stereochemical basis of sweetness in sugar molecules are limited by the availability of suitable model substances, and by their intrinsic stability, ring size and shape. Thus most sugars other than sucrose (cane sugar) and trehalose (mushroom sugar), which are encountered in foods, possess reducing properties due to their free anomeric centers, and are generally unsuitable models.

Previous reports in this field (Shallenberger, 1966; Shallenberger and Acree, 1967; Shallenberger et al., 1969; Hodge et al., 1972; Hodge and Inglett, 1973; Kier, 1972) have educed molecular patterns, which elicit sweetness, and which are related to hydrophilic or hydrophobic bonding ability. Chemical modification of sugar molecules at selected sites will alter these bonding properties and allow conclusions to be drawn about the stereospecific moieties within a sugar ring which elicit the sweet response. Such an approach might, for example, lead to the location of Shallenberger's AH,B system (Shallenberger, 1963, 1964). When the lipophilicity of sugar molecules is increased, either by increasing the size of an aglycone (Birch and Lindley, 1973a) or selectively removing oxygen atoms from the ring or glycol groups (Birch and Lindley, 1973b; Birch and Lee, 1974), the gustatory properties of the molecule alters although its overall shape does not. Most sugars, so far studied, exist in the favored $^4C_1$ conformation and their gustatory properties are therefore geometrically analogous. A notable exception, however, is β-D-fructopyranose, which exists in the alternative $_4C^1$ conformation, and which is the sweetest simple sugar known. Molecular models of this latter type can be obtained as stable substances when water is intramolecularly eliminated from sugar molecules under suitable conditions. Anhydro sugars are in fact formed under conditions of pyrolysis, when foods are subjected to conditions of high temperature, pressure and acidity, and it is therefore of general fundamental interest that their sensory properties should be evaluated. Accordingly this paper describes some structural functions of taste in a number of anhydro derivatives.

EXPERIMENTAL

THE FOLLOWING 1,6-anhydro-β-D-hexopyranoses were obtained as gifts from Professor P.A. Seib,[1] Kansas State University, Kansas, USA; Dr. A.C. Richardson,[2] Queen Elizabeth College, London; Dr. P. Köll,[3] University of Hamburg, Germany; and Dr. J. Pacak,[4] Charles University, Prague, Czechoslovakia:

1,6-anhydro-β-D-galactopyranose[1,3]
1,6-anhydro-β-D-mannopyranose[3]
1,6-anhydro-β-D-allopyranose[3,4]
1,6-anhydro-β-D-gulopyranose[3]
1,6-anhydro-β-D-talopyranose[3,4]
1,6-anhydro-β-D-idopyranose (deuteriated at 3 position)[3]
1,6-anhydro-2-O-methyl-β-D-glucopyranose[1]
1,6-anhydro-2-deoxy-β-D-xylo-hexopyranose[1]
1,6-anhydro-2-deoxy-β-D-ribo-hexopyranose[1]
1,6-anhydro-2-deoxy-β-D-arabino-hexopyranose[1]
1,6-anhydro-2-deoxy-β-D-lyxo-hexopyranose[1]
1,6-anhydro-3-dimethyl-3-deoxy-β-D-glucopyranose[2]
1,6-anhydro-3-dimethyl-3-deoxy-β-D-altropyranose[2]
1,6-anhydro-3-acetamido-3-deoxy-β-D-glucopyranose[2]
1,6-anhydro-3-acetamido-3-deoxy-β-D-gulopyranose[2]
1,6-anhydro-3-nitro-3-deoxy-β-D-idopyranose[2]
1,6-anhydro-3-amino-3-deoxy-β-D-gulopyranose[2]
Sedoheptulosan[2,3]

Levoglucosan (1,6-anhydro-β-D-glucopyranose) and 1,6-anhydro-β-D-altropyranose were obtained from British Drug Houses, Chemicals, Poole, Dorset, England, and 1,4:3,6-dianhydro sorbitol from Graesser Salicylates Ltd., Deeside, Hintshire. All these compounds were purified by repeated crystallizations. All the 3,6-anhydro and 3,6:3′,6′-dianhydro analogues of methyl-α-D-glucopyranoside, α,α-trehalose and sucrose were prepared by classical or novel carbohydrate techniques (Lee, 1973; Lee and Lindley, 1972; Khan et al., 1972; Buchanan et al., 1972). Known compounds synthesized in this way agreed in melting points and optical rotations with literature reports. The structure of all compounds prepared was confirmed by nuclear magnetic resonance spectroscopy (Lee, 1973).

Each sample was treated in the following way before serving to the panelists: 50–100 mg of the purified sugar was dissolved in about 10 ml of distilled water and evaporated under vacuum at 40–50°C using a rotary evaporator to give an amorphous solid or a glass. This was repeated two to three times to ensure complete removal of solvent(s) used for recrystallizing the compound. Such treatment ensures uniformity of the samples to be tasted, thus eliminating or minimizing errors due to rate of dissolution of the sugars (Birch et al., 1970). If the sugars had been tasted as solutions rather than crystals we would anticipate (as in previous studies) no qualitative differences, due to the intrinsic stability of the glycoside structures (1,6-anhydrides are internal glycosides and as such are nonreducing in alkaline medium).

Panelists were selected and trained from College personnel according to a previous publication (Birch et al., 1972) and were asked to place a few milligrams of each substance (prepared as described above) on the tongue and to comment whether they were trace sweet (tr. S), sweet (S) or intensely sweet (SS) or trace bitter (tr. B), bitter (B) or intensely bitter (BB). Tastelessness is designated '0'. The decisions listed in the tables are those obtained in at least 70% of total judgements, each panelist carrying out duplicate tasting sessions. The total number of panelists was ten. Each panelist tasted all substances listed in the tables once at each of the two tasting sessions, rinsing with distilled water between substances, and pausing 1 min before passing on to the following substances. The two sessions were conducted with one week intervening. Swallowing was permitted but none of the substances tasted had any tactile or aroma characteristics which might influence the results obtained.

RESULTS & DISCUSSION

SUGAR MOLECULES tend to adopt the energetically preferred $^4C_1$ conformation. A change in conformation (to the $_4C^1$ conformation) can be achieved by a bridging reaction to form the anhydro derivative (Fig. 1).

1,6-Anhydro-β-D-hexopyranoses have been widely studied by proton magnetic resonance spectroscopy and the $_4C^1$ conformation confirmed (Hall and Hough, 1962; Hall et al., 1967; Horton and Jewel, 1967; Seib, 1969). No NMR studies of 3,6-anhydro hexopyranosides have however, been described previous to the current work but our studies (Birch, Lee and Richardson, 1971a, b; 1974) have confirmed the $_4C^1$ conformation of this fused ring system.

It is quite possible that changes in the ring size and conformation may, by affecting the molecular geometry, also cause changes in the taste of the sugar. Because of the different conformation, the sensory properties of anhydro derivatives cannot be strictly compared with those discussed in our previous publications (Birch and Lindley, 1973a, b; Birch and Lee, 1974). However, all the 1,6-anhydro-β-D-hexopyranoses tasted (Table 1) are sweet and have in common a free hydroxyl group at C-4, thus indicating the importance of this grouping in the $_4C^1$ conformation. From the results in Table 1 it is clear that the C-2 and C-3 hydroxy groups and the ring oxygen atom could function with the C-4 hydroxy group as AH,B system(s). However, which particular combination of moieties constitute the AH,B system(s) is not apparent.

Shallenberger (1966) stated that vicinal hydroxyl groups in the anti-clinal conformation cannot elicit the sweet taste.

Fig. 1—$^4C_1$—$_4C^1$ conformational change.

Table 1—Sensory properties of 1,6-anhydro-β-D-hexopyranoses

Substances	Sweetness	Bitterness
1,6-Anhydro-β-D-glucopyranose	S	B
1,6-Anhydro-β-D-galactopyranose	S	B
1,6-Anhydro-β-D-mannopyranose	S	B
1,6-Anhydro-β-D-altropyranose	S	B
1,6-Anhydro-β-D-allopyranose	S	B
1,6-Anhydro-β-D-gulopyranose	S	B
1,6-Anhydro-β-D-talopyranose	S	B
1,6-Anhydro-β-D-idopyranose (C_3—D)	S	B
1,6-Anhydro-2-O-methyl-β-D-glucopyranose	Tr	B
1,6-Anhydro-2-deoxy-β-D-ribo-hexopyranose	Tr	BB
1,6-Anhydro-2-deoxy-β-D-arabino-hexopyranose	Tr	BB
1,6-Anhydro-2-deoxy-β-D-xylo-hexopyranose	Tr	BB
1,6-Anhydro-2-deoxy-β-D-lyxo-hexopyranose	Tr	BB
1,6-Anhydro-3-dimethylamino-3-deoxy-β-D-glucopyranose	Tr	?
1,6-Anhydro-3-dimethylamino-3-deoxy-β-D-altropyranose	Tr	?
1,6-Anhydro-3-acetamido-3-deoxy-β-D-glucopyranose	Tr	?
1,6-Anhydro-3-acetamido-3-deoxy-β-D-gulopyranose	Tr	B
1,6-Anhydro-3-amino-3-deoxy-β-D-gulopyranose	Tr	B
1,6-Anhydro-3-nitro-3-deoxy-β-D-idopyranose	0[a]	BB

[a] Some panelists reported a trace of sweetness.

Hodge, however, recently stated (Hodge, 1973) that this conformation does induce sweetness since trans-diaxial 1,2-cyclopentanediol, threitan and 1,6-anhydro-β-D-glucopyranose are all sweet. However, one cannot necessarily equate this with the reactive conformation. The gustatory properties of many of these compounds have been substantiated by us but we are more inclined to explain the sweet taste of these compounds as being due to the participation of the ring oxygen with one of the hydroxyl groups (center of oxygen orbitals separated by 2.8–4.0 Å). We have previously stated that the trans-diaxial hydroxyl arrangement in D-protoquercitol is the factor that causes steric hindrance to binding to the taste bud receptor site, thus causing tastelessness (Birch and Lee, 1971). The importance of the ring oxygen in inducing sweetness is probably one of the main reasons for the lower intensity of sweetness of the cyclitols (as a general rule) compared to sugars (Lee and Lindley, 1972).

The C-3 hydroxyl group has been shown to be of unique importance in the taste of sugars in the $^4C_1$ conformation (Hodge et al., 1972; Birch and Lee, 1974). This does not seem to be the case in the $_4C^1$ conformation. In 1,6-anhydro-β-D-talopyranose, 1,6-anhydro-β-D-galactopyranose, 1,6-anhydro-β-D-mannopyranose, 1,6-anhydro-β-D-glucopyranose, its 2-O-methyl ether, and the 2-deoxy 1,6-anhydro-β-D-arabino and lyxo-hexopyranoses, the C-3 hydroxyl group is ca. 2.6 Å (Table 4) away from the anhydro-ring oxygen atom, which is well within hydrogen bonding distance. Thus the C-3 hydroxyl group would not be expected to elicit sweet taste. The sweet taste of C-3 substituted derivatives (Table 1) substantiates this.

X-Ray crystallographic studies of 1,6-anhydro-β-D-glucopyranose (Park et al., 1971) have shown that the oxygen-oxygen spacing between C-2 and C-4 hydroxyl groups is 3.3 Å. It may be that in 1,6-anhydro-β-D-glucopyranose and its C-3 substituted derivatives, these same hydroxyl groups could also constitute the AH,B system. Although Shallenberger (1966) predicted that α-glycol groupings having the eclipsed conformation cannot elicit the sweet taste due to intramolecular hydrogen bonding, this is not so with cis-diaxial 1,3-diols, as it has been reported that cis-1,3-cyclohexanediol is sweet (Lindemann and Baumann, 1929; Hodge, 1973); the observed conformation of cis-1,3-cyclohexanediol is not the chair in which the hydroxyl groups are in the cis-diequatorial relation as might be inferred from simple considerations of instability factors, but rather that in which they are cis-diaxial and in which they are hydrogen bonded intramolecularly (Vinogradov and Linnell, 1971). This has also been observed in methyl-2-deoxy-α-D-ribopyranoside (Lemieux and Levine, 1963).

An analogous system exists in anhydropyranosides. However, with 3,6-anhydro hexopyranosides (Table 2), due to the distortion at C-3 caused in the formation of the anhydro bridge (Birch, Lee and Richardson, 1971a, b; 1974), the O–2....O–4 distance is shortened to about 2.4 Å (Table 5); a strong intramolecular hydrogen bonding would be expected between such hydroxyl groups, and the lack of sweet taste in these compounds is therefore not surprising. It is, however, interesting to note that while the dianhydro trehalose derivative is tasteless, the monoanhydro trehalose is sweet. Similar observations are made with the anhydro derivatives of sucrose (Table 3). While 3,6:3′,6′-dianhydro and 3,6:1′,4′:3′,6′-trianhydro sucrose were virtually without any sweet taste, 3,6-anhydro and 1′,4′:3′,6′-dianhydro-sucrose were distinctly sweet. These examples certainly provide further proof of the truth of Shallenberger's hypothesis and also of our suggestion (Birch et al., 1970; Birch and Lee, 1974) that only one-half of a disaccharide molecule binds to the receptor molecule. Further studies of asymmetrically substituted analogues would be valuable in this context. The results in Table 1 and Table 2 also show that all the 2-deoxy 1,6-anhydro derivatives have a bitter taste. This is in accordance with our earlier proposal

(Lee, 1973; Birch and Lee, 1974) that the creation of lipophilic site(s) (CH_2 groups) in the sugar ring causes the molecule to align itself differently on the receptor site from the parent sugar, thus eliciting the bitter response. The chlorodeoxy derivatives (Table 2) also have a bitter taste. This is also shown by all the halodeoxy derivatives in the $^4C_1$ conformation we have tasted (Lee, 1973), but unlike the deoxy derivatives they do not possess any sweet taste, even with one halogen atom. This can be rationalised in terms of the combined effect of an increased negative charge and smaller atomic radius of the halogens (compared to oxygen).

Shallenberger (1964, 1966, 1969) suggested that for sweetness, the AH,B separation should be 2.5–4.0 Å. Our previous (Birch et al., 1970, 1974; Birch and Shallenberger, 1973) and present results clearly support this, though we favor an optimum distance of 2.8–2.9 Å. Hodge (1973), however, suggested that the figure should be widened to 3.5–5.0 Å, corresponding to an oxygen to proton spacing of ca. 3–5 Å.

The sweet taste of the anhydro furano derivatives (Table 3) (Birch and Lee, 1971) is especially interesting as they do not contain any α-glycol groupings involving AH,B systems of the usual interatomic A-B distance of 2.8–4.0 Å. However, the center of the oxygen orbital attached to C-5 is about 2.6–2.8 Å from the center of the oxygen orbital in the furanogluco-ring, assuming ring distortion takes place, and could therefore operate in this way as an AH,B system. The gustatory properties of these anhydro furano derivatives and the 1,4-anhydro alditols, particularly erythritan, threitan and xylitan, show that the ring oxygen can function as B with a hydroxyl group as AH, if the A-B spacing is between 2.8 to 4.0 Å. Indeed, by elimination, an AH,B system involving the ring oxygen in the 1,6-anhydro-β-D-hexopyranoses appears to be the only possible explanation of their sweetness (the C-2 and C-4 hydroxyl groups in all compounds other then 1,6-anhydro-β-D-glucopyranose and allopyranose have the centers of their oxygen orbitals separated by 4.3–4.7 Å). It therefore appears that the ring oxygen functions in an analogous manner in both sets of compounds since both the 1,6-anhydro compounds and 3,6-anhydro compounds always exists in the $_4C^1$ conformation according to the NMR data which are at our disposal (Hall and Hough, 1962; Hall et al., 1967; Birch et al., 1971a).

The sweetness of sugars is generally rather low when compared with artificial sweeteners. This seems odd, particularly when it is considered that these compounds possess a number of glycol groupings, each satisfying Shallenberger's requirement of a geometrically suitable AH,B system, whereas only one such unit is available in sweeteners like saccharin and cyclamate. We have previously suggested (Birch and Lindley, 1973a, b; Lee, 1973) that, although a polar moiety is likely

Table 2—Sensory properties of 3,6-anhydro-α-D-hexopyranosides

Substances	Sweetness	Bitterness
Methyl-3,6-anhydro-α-D-glucopyranoside	0	0[a]
Methyl-3,6-anhydro-4-chloro-4-deoxy-α-D-galactopyranoside	0	B
Methyl-3,6-anhydro-4-deoxy-α-D-xylo-hexopyranoside	0	B
3,6-Anhydro-α-D-glucopyranosyl 3,6-anhydro-α-D-glucopyranoside	0	0
3,6-Anhydro-α-D-glucopyranosyl α-D-glucopyranoside	S	0
3,6-Anhydro-4-chloro-4-deoxy-α-D-galactopyranosyl 3,6-Anhydro-4-chloro-4-deoxy-α-D-galactopyranoside	0	B
3,6-Anhydro-4-deoxy-α-D-xylo-hexopyranosyl 3,6-anhydro-4-deoxy-α-D-xylo-hexopyranoside	0	B

[a] Some panelists detected a trace of bitterness. This is probably due to residual ethyl acetate used to recrystallize the sugar.

Table 3—Sensory properties of 3,6-anhydro-furanosides

Substances	Sweetness	Bitterness
Methyl-3,6-anhydro-α-D-glucofuranoside	S	Tr
3,6-Anhydro-α-D-glucofuranosyl 3,6-anhydro-α-D-glucofuranoside	S	Tr
1,4:3,6-Dianhydro sorbitol	S	Tr
1,4:3,6-Dianhydro mannitol	S	Tr
α-D-Glucopyranosyl 3,6-anhydro-β-D-fructofuranoside	S	Tr
α-D-Glucopyranosyl 1,4:3,6-dianhydro-α-D-fructofuranoside	S	Tr
3,6-Anhydro-α-D-glucopyranosyl 3,6-anhydro-β-D-fructofuranoside	0[a]	Tr–B
3,6-Anhydro-α-D-glucopyranosyl 1,4:3,6-dianhydro-β-D-fructofuranoside	0	Tr–B

[a] Some panelists reported a trace of sweetness.

Table 4—Interatomic oxygen-oxygen distances in 1,6-anhydro-β-D-hexopyranoses, measured using Dreiding stereo models

	Gluco	Allo	Altro	Manno	Galacto	Talo	Gulo	Ido
0–2....0.3	3.6	3.6	3.6	2.6	3.6	3.6	2.6	2.9
0–2....0.4	3.3[a]	3.3	4.3	4.3	4.3	4.7	4.3	4.7
0–3....0–4	3.6	2.6	2.8	3.6	2.7	2.9	2.9	2.9
0–2....Sugar ring-0	2.9	2.9	3.6	3.6	2.8	3.6	2.8	3.6
0–3....Sugar ring-0	3.6	3.9	4.3	3.6	3.6	3.6	4.2	4.2
0–4....Sugar ring-0	2.9	2.9	2.9	2.9	3.6	3.6	3.6	3.0
0–2....Anhydro ring-0	3.6	3.6	2.8	2.8	3.6	3.0	3.6	3.0
0–3....Anhydro ring-0	2.6	4.3	4.2	2.6	2.6	2.6	4.2	4.2
0–4....Anhydro ring-0	4.3	4.3	4.3	4.3	3.9	3.9	3.9	3.9

[a] X-ray crystallographic data of Park et al., 1971

Table 5—Interatomic oxygen-oxygen distances in 3,6-anhydro-sugars measured using Dreiding stereo models

0–2....0–4	2.4	–	–
0–2....Sugar ring-0	2.8	3.4	3.3
0–4....Sugar ring-0	2.7	–	–
0–5....Sugar ring-0	–	2.8	2.8
0–2....Anhydro ring-0	3.6	3.4	2.6
0–4....Anhydro ring-0	3.5	–	–

needed to cause the sweet taste, the preferable structural feature may be a five or six-membered ring with a polar substituent including an AH,B system outside the ring. Sedoheptulosan and the 1,4-anhydro hexitols (Lee, 1973) are for example quite sweet, and possess this structural feature. Also, it is significant that β-D-fructopyranose, the sweetest of the simple sugars, has such a structure.

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COMPUTER DERIVED PERCEPTUAL MAPS OF FLAVORS

INTRODUCTION

RECENTLY, psychologists interested in sensory measurement have focused attention upon a set of procedures known collectively as multidimensional scaling. These procedures attempt to develop a geometrical representation of stimuli (e.g., odorants, flavors), whereby the properties of perceptually similar stimuli are placed close to each other in the space, whereas perceptually dissimilar stimuli are placed far away from each other. Nearness thus reflects subjective similarity. Multidimensional scaling has been used previously to represent odor quality of simple odorants (Moskowitz and Gerbers, 1974; Woskow, 1968; Yoshida, 1964), the taste quality of different compounds (Schiffman and Erickson, 1971), and for the compatibility of different foods (Moskowitz et al., 1974). These procedures provide adjunct analyses of sensory judgments from panelists.

Most multidimensional scaling methods require that the panelist directly estimate how subjectively dissimilar two objects appear to be (Shepard, 1962), or the opposite—how similar they appear to be. For n stimuli (e.g., 20) the method of paired comparisons that is used to determine subjective dissimilarity can produce the tedious number of 20(19)/2 = 190 comparisons. The requisite number of comparisons grows as the square of n. A variation of the scaling procedure can reduce the effort considerably, and as will be shown below, may illustrate various aspects of sensory profiles. Basically, the procedure is to consider the matrix of flavor stimulus × descriptor as a matrix of similarities. For example, if a panelist rates 10 flavor stimuli (e.g., juices) on each of 30 attributes, then the judgments yield a matrix of 10 × 30, containing 300 numbers. The higher an entry is in the matrix the 'closer' is that juice to the attribute. Therefore, the profile matrix may be interpreted as a matrix of the closeness of juices (stimuli) to attributes or descriptor terms (concepts).

The analyses of such profiling data (and in fact all such interstimulus distance data) are relatively straightforward. A simple analogy will illustrate the logic. If pairwise distances between 100 major cities in the U.S. are known, but the locations of the cities are not, then these 100(99)/2 = 4950 distances are constraints. The final placement of the cities will not be random, but must obey the constraints set up by the interpoint distances. In fact, as the number of such distances increases the location of each city becomes increasingly fixed. New York could not lie next to Chicago, nor to Los Angeles in the map that is developed from these distances. That unusual placement would violate many of the other constraints (e.g., the distance between New York and Miami, or New York and Minneapolis); similarly for distances developed by subjective estimation. If these distances are known then one can develop a 'map' in which the stimuli (e.g., flavors, descriptors) are points, so that the distances between the points reflects judged dissimilarity (or similarity).

In the present study the scaling procedure concerned the analysis of profiles by two procedures:

(a) A large-scale analysis of a 32(descriptor) × 18(juice flavor) matrix, in which average data from a group of six expert panelists were used to generate a map comprising both juice flavors and descriptors;

(b) A smaller-scale analysis in which matrices of individuals × descriptors were analyzed to determine whether (1) individuals used descriptors in the same way for a single juice flavor; (2) whether individuals used descriptors in the same way across days (i.e., the problems of concensus and the problems of reliability, respectively). Multidimensional scaling is particularly useful to analyze these two aspects of profiling, since it presents a map of attributes as well as the idiosyncratic 'points of view' of different panelists whose data generate this map.

EXPERIMENTAL

FULL DETAILS of the procedure have been published elsewhere (Von Sydow et al., 1974). Briefly, the experiment comprised two parts. In the first part panelists and the experimenters decided upon a list of appropriate descriptor words to reflect the flavor nuances of the four test juices (blueberry, cranberry, apple and grape). The resulting list of descriptors were then presented to the panelists, along with any necessary explanations, when appropriate, and along with typical chemical stimuli that reflected the attribute. During this initial phase the panelists also evaluated the apple and grape juices, and the cranberry and blueberry juices (with 1 level of sucrose added to cranberry juice). This initial phase served to familiarize a large panel of people with the proce-

Table 1—Fruit juices and descriptors

Flavors	Aroma Descriptors	Taste Descriptors
Apple	Total odor strength	Total taste strength
Grape	Apple	Astringent
Cranberry +	Musty, mouldy	Biting
8% sucrose	Sweet odor	Bitter
12% sucrose	Spicy	Metallic
18% sucrose	Fermented, winelike	Puckery
28% sucrose	Blueberrylike	Sour
Blueberry +	Estery, hard candy	Sweet
0% sucrose	Aromatic	Taste pleasantness
6% sucrose	Sharp, pungent	Total pleasantness
18% sucrose	Cranberrylike	
	Woody, sawdust	
	Floral	
	Grape	
	Resinous	
	Fruity, berrylike	
	Green, cutgrass	
	Fragrant	
	Earthy	
	Vinegar	
	Etherish	
	Odor pleasantness	

dure. The panelists were instructed to sample the juices, either by smelling the headspace over a cup of juice, or by sipping the juice. They scored the juice on all of the 22 aroma descriptors with a 10-point category scale (0 = not present, 9 = extremely strong). When sipping the juice the panelists also evaluated the juices on an additional 10 descriptors.

In phase 2 the six most consistent panelists were selected from the group, and tested with cranberry and blueberry juices + varying amounts of sucrose, as well as with apple and grape juices at sucrose concentrations specified by the manufacturer. For the analysis of grape juice from the expanded population of 10 observers, 16 replicate judgments were taken for each panelist. These 16 replicate judgments were subsequently averaged in 4-day segments to yield 4 such segments.

RESULTS

Analysis I

Profile representation in geometrical coordinates. The juices are listed in Table 1, along with the descriptors used for both aroma and taste. Note that the panelists evaluated each juice both by aroma alone, and by aroma + taste (i.e., by nose alone vs by mouth, respectively). Details of the experiment have been previously published (Von Sydow et al., 1974). The present paper concerns the reanalysis of these data by multidimensional scaling procedures.

Table 2 presents the matrix of 32 descriptors X 18 juices on the 10-point scale. These data were obtained from the replicate judgments (at least 3) of a panel of six expert individuals. Entries that are blank correspond to inappropriate descriptors (viz. taste descriptors for evaluations by nose alone). In the development of geometrical maps that embed both juice flavors and descriptors these blank entries were ignored, and did not act as constraints.

A multidimensional scaling program, MDSCAL 5M (Kruskal and Carmone, 1969) was used to obtain the geometrical representation for Table 2. The unfolding option (specifically designed for profiling data such as that obtained here) was used and the computer program was instructed to place the flavors and descriptors in geometrical spaces of 3, 2 and 1 dimension, respectively. As the dimensionality increases the points have more leeway to move around, and can more easily satisfy the constraints posed by the profile entries. However, offsetting the goodness-of-fit afforded by high dimensional spaces are the problems of interpreting these dimensions.

Figure 1 shows the two dimensional geometry developed with the method. Note that there are several clusters of different descriptors. One cluster, earthy, etherish, woody and green seem not to apply very well to these juices. Other descriptors, such as sweet odor, estery, sweet, spicy, etc., apply more to the juices, and probably more to the flavor induced by blueberry juice than to the flavor induced by cranberry juice. Tastes themselves are also placed close to these juice flavors, and indicate the great importance taste qualities for the evaluation of juice flavor.

Table 2—Descriptor beverage matrix beverage treatment, sampling method[a]

	B	B	B	B	B	B	C	C	C	C	C	C	C	C	A	A	G	G
	0	6	18	0	6	18	4	12	18	4	12	18	28	28				
Descriptor	N	N	N	M	M	M	N	N	N	M	M	M	M	N	N	M	N	M
1 Total odor strength	5.00	4.53	4.33	3.46	4.06	4.30	4.30	4.57	4.47	3.15	3.97	4.21	4.71	4.40	5.40	4.72	5.93	5.30
2 Apple-like	0.94	1.17	0.85	0.79	0.94	1.25	1.67	1.60	1.47	0.52	1.09	1.39	1.71	1.51	5.79	5.18	1.05	1.37
3 Musty, moldy	1.66	1.53	1.44	1.27	1.00	1.13	1.06	0.94	0.87	0.67	1.06	1.15	1.03	1.24	2.13	1.76	1.66	1.64
4 Sweet odor	4.82	4.72	4.26	2.09	4.00	4.71	2.94	3.26	2.32	0.94	2.03	2.72	2.94	3.51	3.94	3.53	4.39	4.57
5 Spicy	1.79	1.33	1.67	1.42	1.85	1.79	1.79	1.86	1.79	1.21	1.80	1.95	1.85	2.12	1.87	1.76	1.89	1.91
6 Fermented, wine-like	1.88	1.79	1.78	2.03	1.88	1.89	1.70	1.63	1.66	1.12	1.36	1.87	1.79	1.68	2.05	1.94	2.71	2.50
7 Blueberry-like	5.49	5.33	5.03	3.46	4.98	5.03	0.01	0.01	0.03	0.01	0.01	0.03	0.01	0.01	0.13	0.21	1.53	1.62
8 Estery (hard candy)	1.90	1.33	1.63	1.00	1.32	2.14	1.61	1.71	1.55	1.00	1.60	2.05	1.79	2.24	2.03	2.01	2.24	2.45
9 Aromatic	4.06	3.72	3.51	2.76	3.88	4.00	2.79	2.89	2.74	2.06	2.91	3.26	2.32	3.39	3.75	3.43	4.14	3.80
10 Sharp, pungent	1.88	1.69	1.44	2.54	1.68	1.07	3.39	3.49	3.37	4.36	4.00	3.67	3.53	3.58	2.58	2.21	2.18	2.03
11 Cranberry-like	0.03	0.00	0.04	0.46	0.53	0.32	5.06	5.37	5.00	3.67	4.30	4.87	5.29	5.39	0.32	0.42	0.26	0.37
12 Woody, sawdust-like	0.82	0.75	0.70	0.97	0.94	0.64	0.73	0.77	0.79	0.76	1.00	1.00	0.88	0.88	1.02	0.00	0.32	0.74
13 Floral	2.73	2.72	2.70	1.35	2.95	3.03	1.32	1.77	1.68	1.03	1.69	2.26	1.88	2.52	2.83	2.52	3.09	2.35
14 Grape-like	1.76	1.69	1.33	0.97	1.88	1.95	0.79	1.66	0.71	0.21	0.63	0.85	0.62	0.91	0.53	0.69	6.35	5.76
15 Resinous	1.21	0.97	0.93	1.52	1.19	0.93	0.91	1.09	0.90	1.64	1.31	1.10	1.15	1.24	1.24	1.17	1.39	1.39
16 Fruity, berry-like	3.76	3.56	3.38	2.54	3.76	4.04	2.79	2.80	2.66	1.79	2.54	3.10	2.68	3.42	2.20	2.09	3.40	3.46
17 Green, cut grass	0.21	0.19	0.22	0.64	0.29	0.25	0.88	0.83	0.74	1.06	0.06	0.94	0.88	0.88	0.93	0.73	0.48	0.48
18 Fragrant	3.68	4.00	3.56	2.15	3.79	4.04	2.79	2.80	2.66	1.33	2.51	3.03	2.79	3.42	3.71	3.32	4.40	4.00
19 Earthy	1.00	0.81	0.74	1.03	0.91	0.93	0.79	0.87	0.79	0.73	0.91	0.87	0.82	0.82	0.97	1.02	0.67	0.73
20 Vinegar-like	1.00	0.92	0.93	2.33	1.21	0.61	1.68	1.74	1.68	2.85	2.71	1.80	1.77	1.49	1.93	1.86	1.15	1.18
21 Etherish, anesthetic	0.79	0.86	0.78	0.76	0.89	0.64	0.91	0.94	0.74	0.76	1.00	0.90	0.82	0.88	1.02	0.83	0.77	0.77
22 Odor pleasantness	5.91	5.94	5.56	3.76	5.47	5.54	5.03	4.97	5.08	2.91	4.54	5.12	5.06	5.70	5.42	5.41	6.62	6.32
23 Total taste strength				4.70	4.53	5.29			6.33	5.69	5.69		5.72			5.32		5.86
24 Astringent				4.00	2.97	2.11			5.97	5.20	4.49		3.88			2.84		3.08
25 Biting				2.12	1.53	0.96			4.94	4.34	3.49		3.03			1.92		1.87
26 Bitter				1.61	0.85	0.57			4.27	3.69	2.59		2.09			0.94		0.96
27 Metallic				1.51	1.00	0.86			3.76	3.51	2.77		2.24			1.43		1.29
28 Puckery				4.09	3.03	1.93			5.81	5.34	4.31		3.91			2.68		3.03
29 Sour				4.88	3.11	1.86			7.27	3.46	5.41		4.49			3.66		3.19
30 Sweet				2.03	4.53	6.82			1.33	2.66	3.85		5.21			4.47		5.81
31 Taste pleasantness				3.58	5.18	5.68			2.27	3.34	5.26		5.45			5.45		6.19
32 Total pleasantness				3.64	5.24	5.57			2.36	3.54	5.18		5.64			5.67		6.32

[a] Number in middle represents the percent of added sucrose. (A = apple juice; B = blueberry juice; C = cranberry juice; G = grape juice; M = by mouth; N = by nose)

One of the important aspects of Figure 1 is that it represents relations among descriptors. Appropriate descriptors for each juice flavor are located near that flavor. The descriptors metallic and bitter are more closely associated with the concept of cranberry than with blueberry, grape or apple. The descriptors musty, aromatic and sweet odor seem to apply more appropriately to grape and blueberry juice.

The addition of varying amounts of sucrose affects the flavor characteristics of cranberry juice far more than it does the flavor of blueberry juice. By consulting the profiles in Table 2 one may see greater variation among the aroma and taste profiles for cranberry juice in comparison to the same profiles for blueberry juice. Figure 1 shows this comparison immediately. The cranberry juice variants spread far apart, indicating that their profiles differ from each other, and that some descriptors are emphasized, whereas others are de-emphasized. Low levels of sucrose in cranberry juice produce a tart drink that makes the cranberry juice perceptually unique, and moves it away from the center space. In contrast different levels of sucrose in blueberry juice do not exert as much of an effect perceptually.

Quite often the aim of multidimensional scaling is to reveal the underlying aspects or attributes along which a panelist may make his judgments of qualitative similarity or dissimilarity. That is, if the panelist is presented only with pairs of juice flavors without descriptors and asked to judge perceived dissimilarity, then one may assume that the panelist uses certain dimensions. Through multidimensional scaling the experimenter can embed the juices on a geometrical space (e.g., 2 dimensions) and also name those dimensions. Here the use of descriptors makes the attempt to name dimensions less important. Rather, the aim is to show a picture of the data matrix and illustrate relations among elements of the matrix. The picture shows the food technologists many relevant relations in the data matrix.

Finally, the geometrical map can be considered as a matrix of qualitative similarities or dissimilarities. A fixed distance represents a specific degree of similarity. If qualitative dissimilarities of a fixed degree are desired then one need only locate a point and swing out a circle of fixed radius. All points (e.g., descriptors, stimuli) meeting on that circumference of the circle are equally dissimilar to the center point. For iso-dissimilarity contours with pleasantness as the nodal point one need only swing out the circle from the central point 'odor pleasantness.' All descriptors and juices that intersect the circle are equally dissimilar to 'odor pleasantness.' Thus the map becomes a tool for uncovering iso-similarity or iso-dissimilarity contours, whether this be for pleasantness (a hedonic attribute) or for qualities (e.g., sweetness, floral, etc.). Note, however, that the contours are qualities, not quantities.

Analysis II

Individual differences and temporal consistencies. One of the more important and recurring problems in sensory analysis of flavors, textures, etc., is to ascertain that all panelists participating in an experiment are using the same language to describe flavors, and are perceiving the stimuli in the same way. The former is the problem of calibrating the panelists so that they use descriptors in the same manner, whereas the latter is the problem of eliminating those panelists with specific sensory losses or weaknesses (e.g., altered threshold for some tastes and odors).

In the present experiment individual profile ratings (averaged over at least 16 replicate judgments) were available for 10 panelists, for the evaluation of the flavor of grape juice by mouth (i.e., aroma and taste considered together). The matrix of panelists X attribute was subjected to a computer program known as INDSCAL (Individual Differences Multidimensional Scaling, Carroll and Chang, 1971). INDSCAL attempted to

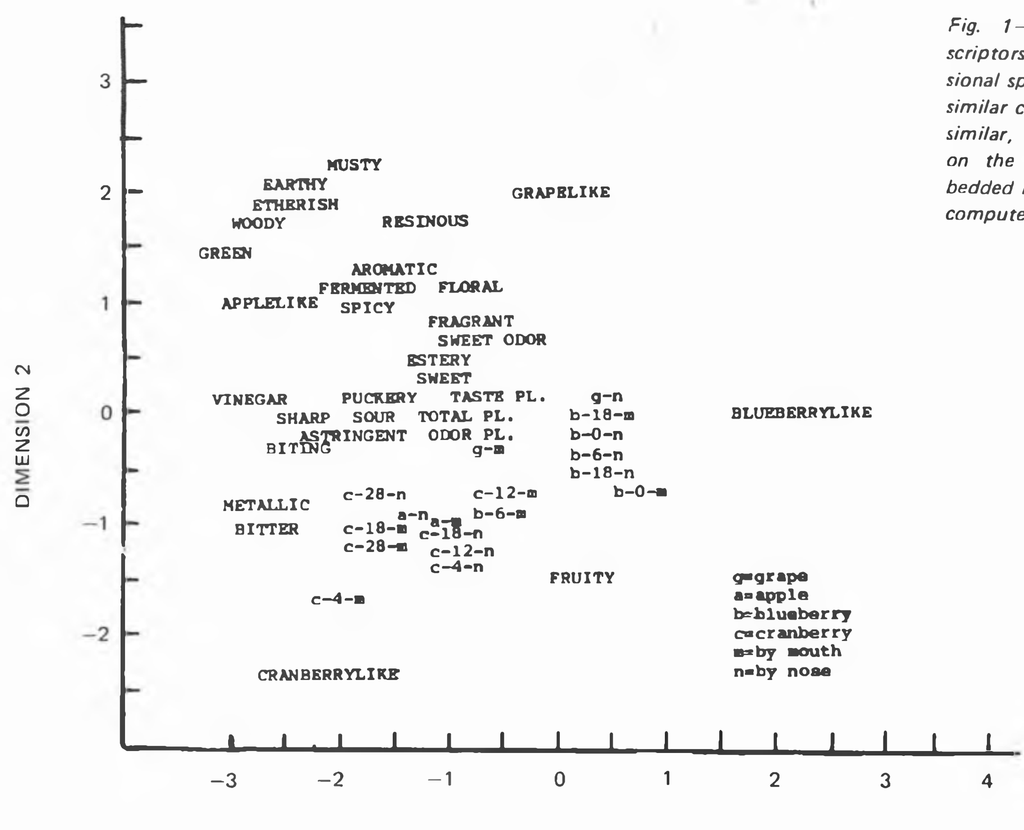

Fig. 1—Relative location of profile descriptors and fruit juices in a two-dimensional space. Points close together represent similar concepts, juices that are qualitatively similar, or a high profile rating of the juice on the descriptor. The points were embedded in the two-dimensional space by the computer program MDSCAL 5M.

decompose the profile matrix into two sections: (a) A geometrical space in which are embedded all of the descriptors. This matrix is common to all of the panelists; (b) A separate set of weighting factors, one factor for each panelist for each dimension of the geometrical space. That is, INDSCAL resolves the judgment of many panelists to a common geometry of descriptors, and idiosyncratic ways of using those descriptors. Some individuals may perceive large differences among descriptors—their weighting factors would be positive and high, so that they stretch distances between descriptors, and thus accentuate dissimilarities among descriptors. Other panelists may not perceive very much difference among descriptors—they share the same basic geometrical space for descriptors, but they have low weights, and compress the distances between descriptors.

Finally, some panelists may invert the dimensions entirely, and use descriptors in ways opposite to the rest of the population. Their weighting factors are negative. If they expand the differences among descriptors their weighting factors are high and negative whereas if they contract the differences then their weighting factors are low and negative.

Table 3 lists the order of descriptors for grape juice on two dimensions, as uncovered by INDSCAL. The matrix of panelist X descriptor could not be represented by a one-dimensional space, but the descriptors seemed to have two major aspects—one that may be labelled (a) bad-good (or hedonic) and (b) relevance-vs-nonrelevance for grape juice. The first dimension (or axis of the descriptor space) is relatively easy to label. The second dimension is harder, but the descriptors at the top are probably those that first are used to describe grape, whereas those at the bottom are probably not very salient to the panelist. Panelists agree both on the ordering of the first dimensions (no panelist inverted the order, nor perceived odor pleasantness to be a hedonically negative attribute), and on the weighting of the dimensions. The panelists were thus homogeneous on the first dimension, that of hedonics. On the second dimension five of the panelists perceived the dimension to lie in the order shown, whereas five others inverted the order. Apparently for this dimension panelists disagree among each other as to what attributes constitute 'insistent' or 'immediately applicable' (salient) descriptors. In addition, even within a group of panelists showing the same ordering the expansion or shrinkage factors differed quite a bit, so that panelists did not concur about the relative differences in relevance for given pairs of descriptor terms.

A second INDSCAL analysis was run, this time on the matrix of profile ratings for apple juice evaluated over 4 blocks of 4 days, by nose (i.e., aroma) alone. The matrix of descriptor X day showed one dimension and there were no differences across days. Both the sign (order of the dimension) and the weighting factors were virtually identical from one block of four days to the next. This finding means that panelists used the descriptors in the same way from one day to the next, and did not change their criteria during the course of the experiment.

Table 3—Rank order of attributes on two dimensions (evaluation of 10 panelists of grape juice, by taste and smell)

Dimension 1	Dimension 2
Cranberrylike	Grape
Green, cut grass	Total pleasantness
Etherish	Floral
Woody, sawdust	Odor pleasantness
Applelike	Taste pleasantness
Bitter	Sweet
Metallic	Bitter
Vinegar	Sharp, pungent
Resinous	Musty, mouldy
Earthy	Puckery
Musty, mouldy	Fragrant
Spicy	Astringent
Blueberrylike	Metallic
Biting	Earthy
Sharp, pungent	Green, cut grass
Estery, hard candy	Sweet odor
Fermented, winelike	Resinous
Puckery	Biting
Floral	Cranberrylike
Astringent	Woody, sawdust
Sour	Sour
Fruity, berrylike	Spicy
Aromatic	Etherish
Fragrant	Applelike
Sweet odor	Vinegar
Sweet	Fruity
Grape	Fermented, winelike
Taste pleasantness	Aromatic
Total pleasantness	Estery, hard candy
Odor pleasantness	Blueberrylike
(Bad – Good)	(Tentatively 'insistence of the attributes to the perceiver,' but there is substantial variability among panelists in the way they use this dimension.)
All panelists concur on the relative ordering of these attributes from bad to good.	Panelists 2, 7, 8, 9 and 10 perceive the attributes in the above order.
	Panelists 1, 3, 4, 5 and 6 invert the order relative to Dimension 1.

DISCUSSION & CONCLUSIONS

THE PRESENT PAPER illustrates the potential usefulness of new psychometric procedures, called multidimensional scaling, for the analysis of sensory profile data. The data discussed here are meant solely for illustrative purposes—a full analysis has been published elsewhere, detailing the ratings and relations among attributes so that their use here provides an example of how sensory data can be more completely analyzed and new information obtained (see Von Sydow et al., 1974).

At least two major advantages accrue to the flavor scientist who employs multidimensional scaling as an adjunct to the standard statistical procedures.

(a) The scaling provides a visual map of the data, in which both stimuli (e.g., compounds isolated from foods, or foods themselves) and descriptors are simultaneously represented. Hence, one may immediately obtain an idea of the relations among descriptors (viz. which descriptors are redundant, which are highly dissimilar to each other), as well as relations among foods, and relations between food and descriptors. In this way a general picture of flavor can be readily seen and appreciated. On the line connecting two descriptors there lie other descriptors whose meaning is somewhat intermediate (e.g., between floral and estery lies the term 'sweet odor'), or stimuli that possess some of each attribute. Standard multidimensional scaling, of the type afforded by MDSCAL 5M (Kruskal and Carmone, 1969) provides this mapping.

(b) The use of individual differences scaling (INDSCAL) allows the flavor scientist to consider individual differences in the profiling of foods and flavors. Here such individual differences scaling illustrated that some panelists possessed conceptions of attributes slightly different than the group's, but that

the profiling behavior of individuals along a stretch of 16 days was essentially unchanged. The conformity of different panelists to an overall criterion, and the discovery of systematic individual differences provide a powerful insight into individual's responses to the manifold aspects that flavor comprises.

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EFFECTS OF AMYLOLYTIC ENZYMES ON "MOISTNESS" AND CARBOHYDRATE CHANGES OF BAKED SWEET POTATO CULTIVARS

INTRODUCTION

SWEET POTATOES have been grouped by cultivar into two major classes on the basis of the textural properties of the baked root. The "yam" type has a soft, syrupy texture and is a "moist" type; at the other extreme is the firm, mealy textured, "dry" type (Hayword, 1938). The terms "moist" and "dry" as used here designate organoleptic characteristics of the baked sweet potato and are not related to water content. There is no clear line of separation between "moist" and "dry" cultivars, so it is probably more realistic to consider a continuum of textural properties. All cultivars are "drier" at harvest than after curing and storage, thus a "moist" cultivar may have a wide range of textural properties depending upon the history of the roots.

A number of workers have studied "moist" and "dry" roots with the idea of determining their basic differences. Ali and Jones (1967) found no significant correlation between softness of baked roots and reducing sugar, maltose or dextrins in contradiction to at least two other groups. Hammett and Barrentine (1961) found that the cured Porto Rico, a "moist" type, had much higher amylopectin, dextrin and reducing sugar values than the intermediate type, Allgold. These differences became apparent only after baking. Swingle (1965), however, found no relationship between "moistness" and amylopectin content of cured baked roots and concluded that "moistness" did seem to be associated with starch content and insoluble pectins.

Sweet potatoes contain large amounts of starch which is largely converted during baking into maltose and dextrins (Ali and Jones, 1967). Gore (1920, 1923) demonstrated the presence of an active diastase in sweet potato reporting that slow cooking of the roots through a range of 60–100°C converted large amounts of starch into soluble carbohydrates. Maltose has been shown to be the sole sugar produced by cooking sweet potatoes (Sistrunk, 1954). Balls et al. (1948) crystallized the diastatic enzymes from sweet potatoes and observed that the amylolytic activity was almost entirely due to β-amylase. The presence of β-amylase in sweet potatoes and the production of maltose during baking indicates that starch conversion is due in part to this amylolytic enzyme.

The other major product of starch conversion, dextrins, are polysaccharides having a broad spectrum of molecular size formed by the action of α-amylase on starch. Ikemiya and Deobald (1966) isolated sweet potato α-amylase which had an optimum activity temperature of 70–75°C at pH 6.0. Hoover (1967) showed that in sweet potato puree, starch was not converted into maltose and dextrins until the temperature was above that for starch gelation. The object of this study was to determine the relationships among amylolytic enzyme activities, carbohydrate changes and differences in organoleptic properties in sweet potato cultivars.

MATERIALS & METHODS

FOR 1971 AND 1972, Centennial, Jewel, Porto Rico, Nuggett, Australian Canner and Pelican Processor sweet potatoes were obtained at the North Carolina Agricultural Experiment Station at Clayton, N.C. A representative sample of each cultivar was analyzed at harvest. The remainder were cured at 29–32°C and 85% relative humidity for 7 days, then stored at 16°C until used.

Random samples of about 2.5 kg were taken from each cultivar for analytical and baking tests. Only sound roots, about 1-7/8–3-3/8 in. in diameter and 3–9 in. in length, were used. About 75% of the sample was baked for organoleptic tests and the remainder used for enzyme assays. For 1972, carbohydrate analyses were also performed on raw and baked samples of Centennial, Jewel, Pelican Processor and Porto Rico cultivars.

Enzyme assays

Replicate samples of two or three roots were selected for each analysis. Roots were hand-peeled, grated and juice equivalent to 20–25% of the weight was pressed from the grated material in a hydraulic press. Enzyme assays of the juices were run in duplicate at 60°C. Substrates were prepared at the 2% level using as solvent 0.02M phosphate buffer (pH 6.0) containing 0.3% NaCl. Controls which contained heat-denatured samples were run with each set of samples.

α-Amylase

α-Amylase activity was determined by the chromogenic starch method (Walter and Purcell, 1973). Amylopectin Azure (Calbiochem.) served as substrate. Enzyme activity is expressed as APA amylase units per ml of sweet potato juice. The data were handled by analysis of variance (Snedecor, 1950).

β-Amylase

Activity of β-amylase was determined on the 1972 crop only using Lintner starch as the substrate. A 1.0 ml sample of juice or dilution was added to 15.0 ml of substrate. The reaction was continued for 5 min, then stopped with 0.2N NaOH. The amount of reducing sugar produced was determined as maltose (Hodge, 1964), and expressed as mg of maltose per ml of juice per min.

Organoleptic evaluations

For each taste panel, at least four roots of each cultivar were washed, punctured to prevent bursting and baked in a pre-heated, 191°C oven for 70 min. After baking and cooling the flesh was removed and presented to the panel on a white plate scored into six equal coded wedges. Deep red filters were used in taste panel booths to prevent the influence of color (Nelson, 1973). Panelists were instructed to rank samples in increasing intensity of moist mouthfeel with six most moist. The scores were ranked and analyzed statistically as described by Kramer and Twigg (1966).

Carbohydrate analysis

At each sampling period in 1972, several raw roots of Centennial, Jewel, Porto Rico and Pelican Processor cultivars were hand-peeled, grated and homogenized with sufficient boiling ethanol to give an 80:20 ethanol-water ratio. The slurry was heated in a boiling water bath for 1 hr, cooled and filtered. Those baked roots used for taste panel evaluations were treated in the same manner. The resulting filter cake was extracted two additional times by heating with 80% ethanol for 1 hr followed by filtration. Filtrates were combined and assayed for total carbohydrate (Dubois et al., 1956) and for reducing sugars (Hodge, 1964). The filter cake containing the alcohol insoluble substances (AIS) was retained for starch and dextrin analyses. Dry matter content was determined on a separate portion of each sample by drying weighed samples at 110°C for 24 hr.

Dextrins. Dextrins were extracted with water-ethanol (9:1) from weighed samples of AIS by shaking on a rotary shaker for about 4 hr at 200 rpm. The pellet was dehydrated by washing successively with

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ethanol, ether and hexane. The dextrin-free material was air-dried and retained for measurement of starch content.

The supernatant, which contained the total dextrin extract (TDE), was diluted and carbohydrate content was measured by the phenol-sulfuric acid procedure (Dubois et al., 1956). Dextrin content was quantitatively measured on alcohol-free aliquots of the TDE solution with the amyloglucosidase-glucose oxidase procedure of Dekker and Richards (1971).

If viscosity measurements were to be made, the TDE was freeze dried on a Freeze-Mobile apparatus (Virtus Co., Gardiner, N.Y.). Where pure dextrins were needed, the TDE solution was treated with excess saturated lead acetate solution to remove co-extracted contaminants (e.g., pectins) and excess lead removed by shaking with IRC 50 (Na^+) ion exchange resin. The dextrin solution was freeze dried, extracted with ethanol to remove sodium acetate and finally solvent dehydrated and dried in vacuo. Viscosity measurements were obtained on solutions of this material.

Starch. Starch content of the dextrin-free material was measured by the amyloglucosidase hydrolysis method of Dekker and Richards (1971) except that the glucose formed from the starch was quantitated using the alkaline copper reagent (Hodge, 1964). The glucose concentration was multiplied by appropriate factors to give the amount of starch in the original sample.

Viscosity measurements. Intrinsic viscosity determinations were made with a #50 semi-micro Cannon Ubbelohde dilution viscometer (Cannon Instruments, State College, Pa.). The sample was dispersed in 0.1M potassium chloride, filtered, and adjusted to about 2% concentration. A 1.0-ml portion was charged into a calibrated viscometer, in a water bath at 25.1 ± 0.05°C, and equilibrated. Flow time was measured in triplicate. A measured amount of solvent was added, mixed, temperature equilibrated, and flow time again measured in triplicate. This procedure was continued until a fourfold dilution was obtained. The diluted sample was removed and the carbohydrate content measured using the phenol-sulfuric acid reagent (Dubois, 1957). Using the flow times and substrate concentrations, the intrinsic viscosity $[\eta]$ was obtained (Greenwood, 1964). Viscosities for all samples were measured on solutions of 0.65%–2.2% carbohydrate.

RESULTS & DISCUSSION

Amylase activities and moistness scores (Table 1)

For both years, Pelican Processor ranked lowest in "moistness," while Centennial and Porto Rico consistently ranked highest. Jewel ranked high in moistness early in the tests but declined in rank as the seasons progressed. Australian Canner ranked low in "moistness" early in the storage period and increased in rank toward the end of storage. In all cultivars and both years, α-amylase activities increased with storage time. For both years, roots of Pelican Processor had lowest, while Centennial and Porto Rico had highest α-amylase activity. In Australian Canner, enzyme activity increased late in storage corresponding to the shift in taste panel rankings. Alpha-amylase activity in Nugget and Jewel increased steadily, but was always less than in other varieties except Pelican Processor.

Beta-amylase activities (Table 1) of the cultivars changed erratically as the season progressed. Centennial, Porto Rico and Pelican Processor were highest in β-amylase. Jewel was intermediate and Nugget and Australian Canner had the lowest activity.

Correlation coefficients between β-amylase and taste panel scores were very low and are not reported. Although α-amylase activities and "moistness" scores were not significantly correlated in the early part of the season, there was a trend toward significance as the season progressed (Table 2). In 1971, α-amylase activities were significantly correlated with "moistness" scores at 45 ($P < 0.01$) but not at 20 days. In 1972, the correlation increased between 31 and 48 days and was significant ($P < 0.05$) at 71 days. For two testing dates for 1971 and three for 1972, correlations were significant at the 0.01 and 0.05 levels, respectively. Values "at harvest" were not included. These correlations suggested that α-amylase influenced the textural properties of baked sweet potatoes.

Amylase activities and carbohydrate changes

Numerous workers have reported the conversion of starch into maltose and dextrins during baking of sweet potatoes (Southern Cooperative Series, 1970). In general, our data are similar to those reported for other cultivars; the content of sugars in raw and baked roots increased with storage. Sugar concentration in baked roots was similar for all cultivars within any sampling period, although increases with time of storage tended to be erratic. Sugar in raw roots increased during storage from about 2.0g per 100g fresh weight at harvest to about 6.0 per 100g after 71 days.

Starch and starch transformation products are shown in Table 3. Even in freshly harvested roots large amounts of starch were converted into maltose during baking. For Centennial, Jewel and Porto Rico, ranges of conversion were 63–69% at harvest and 91–95% at 71 days. Pelican Processor, a typical "dry" variety, converted only 63% of its starch when the 71-day sample was baked.

Beta-amylase levels did not seem to directly affect the amount of maltose produced during baking. For example, Jewel has significantly less activity than any of the other varieties and yet baking produced very similar amounts of maltose in all varieties. We concluded, therefore, that factors other than juice β-amylase levels control conversion of starch into maltose.

Dextrins, as well as maltose, are produced from starch during baking of sweet potatoes. The dextrin content of raw sweet potatoes is less than 0.1g per 100g fresh weight. All varieties have similar amounts and no increases are noted during storage. In all varieties, the percent of starch converted to dextrins by baking (Table 3), was lowest at harvest, and for Centennial, Jewel and Porto Rico, increased sharply during storage, and was about 27% at 71 days. For Pelican Processor, conversion into dextrins was only about 9% at 71 days.

The relationship between α-amylase activity (Table 1) and dextrin formation (Table 3) appears to be complex. Within each variety, dextrins produced by baking and raw juice α-amylase increased simultaneously with storage time of the raw roots. In Pelican Processor, enzyme levels show very little change until after 48 days of storage. Dextrin formation follows an identical path. Centennial, Jewel and Porto Rico were alike in amounts and rate of increase in formation of dextrin during baking but differ in their levels of α-amylase. Alpha-amylase was related to starch conversion but not in a simple linear fashion.

Alpha-amylase activity is also characterized by the range of dextrin molecular sizes produced. Increasing enzyme activity causes reduction in the average molecular size. Changes in molecular size can be estimated by comparing intrinsic viscosities (INV). Table 4 indicates that INV of the TDE decreases steadily with length of storage before baking, indicating decreases in molecular size. This steady decrease in the INV from stored roots occurs concurrently with increases in α-amylase activity. However, the enzyme levels are not linearly related to changes in molecular size.

The INV values for purified dextrins are inconsistent with the 48-day values for all varieties being greater than those for the 31 days. This apparent anomaly might have been caused by the procedure used to isolate the purified dextrins. We have found, in agreement with others (Diemair and Koelbel, 1963), that 10–30% of the dextrins could have been removed by co-precipitation during removal of contaminants with lead acetate. Moreover our study shows that the dextrins of highest molecular size are selectively removed. Thus a leveling effect would occur with observed INV for purified dextrins being shifted to lower values and differences between cultivars becoming smaller.

Apparently, "moistness" scores are significantly and negatively related to INV of the total dextrin extract (Fig. 1). The

Table 1—Taste panel scores and amylase activities for sweet potato varieties after baking

	1971 Crop			1972 Crop			
Variety	Harvest[a]	20-Day	45-Day	Harvest[a]	31-Day	48-Day	71-Day
			Panel "moistness" scores[b]				
Centennial	5.7t	5.3t	4.4v	5.0t	4.4t	4.8t	4.9t
Porto Rico[c]	4.3u	4.9t	5.5t	4.3u	3.4u	3.7u	4.5t
Jewel	4.5u	4.3u	2.9w	4.0u	4.5t	3.6u	3.3u
Nugget	2.2v	2.7v	2.2x	3.9u	2.6v	4.0u	3.1u
Australian Canner	2.5v	2.9v	4.9u	2.9u	4.0u	4.0u	4.3t
Pelican Processor	1.3w	1.0w	2.3y	1.0v	1.0w	1.0v	1.0v
			Alpha-amylase activity[d]				
Centennial	2.8	13.6	35.3	1.5	27.7	45.9	61.8
Porto Rico[c]	4.9	26.6	46.3	1.9	27.5	24.2	35.6
Jewel	1.2	1.9	5.6	0.6	6.5	10.9	22.7
Nugget	2.8	9.3	11.4	2.5	9.7	14.0	18.5
Australian Canner	1.9	6.6	31.3	1.5	11.8	29.2	53.1
Pelican Processor	0.7	2.9	1.5	0.8	3.3	3.1	10.3
LSD at 0.05 confidence level	1.4	9.2	5.6	1.5	3.2	4.5	12.1
			Beta-amylase activity[e]				
Centennial				32.1	28.9	32.4	22.6
Porto Rico				30.5	35.3	27.2	23.3
Jewel				9.6	11.9	11.2	10.6
Nugget				4.2	5.1	3.3	4.3
Australian Canner				8.0	8.8	6.8	7.1
Pelican Processor				19.7	22.4	16.9	21.3

[a] Not cured
[b] High score means large degree of moist "mouthfeel." Scores in same column not followed by a common letter are different at the 0.05 confidence level.
[c] Porto Rico Mutant used in 1972 crop
[d] Activity in APA units per ml of sweet potato juice at 60°C during a 15 min reaction period
[e] Activity in milligrams of maltose per ml of sweet potato juice per min at 60°C X 100

Table 2—Correlation coefficients between taste panel scores and α-amylase in baked sweet potato varieties

	Simple correlation	N
1971 Study		
20 Day	0.570	6
45 Day	0.949**	6
Both testing dates	0.714**	12
1972 Study		
31 Day	0.476	6
48 Day	0.775	6
71 Day	0.861*	6
All testing dates	0.663*	18

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

Table 3—Changes in starch and starch conversion products during baking of cultivars of sweet potatoes[a]

			Maltose produced[b]		Dextrins produced[d]	
		% of Total starch converted	Amount[c]	% of Converted starch	Amount[c]	% of Converted starch
Centennial	At Harvest	68.5	14.6	99.0	0.2	1.0
	31 Day	75.1	11.4	79.8	2.9	20.2
	48 Day	82.7	9.8	73.8	3.5	26.2
	71 Day	95.4	10.8	73.5	3.9	26.5
Jewel	At Harvest	62.9	14.2	99.2	0.1	0.8
	31 Day	84.8	13.6	80.8	3.2	19.2
	48 Day	81.0	9.3	71.5	3.7	28.5
	71 Day	92.0	9.9	71.9	3.9	28.1
Porto Rico Mutant	At Harvest	65.4	13.8	99.1	0.1	0.9
	31 Day	62.6	9.8	85.6	1.7	14.4
	48 Day	84.8	10.4	73.8	3.7	26.2
	71 Day	91.4	11.8	72.4	4.5	27.6
Pelican Processor	At Harvest	53.8	15.5	99.0	0.2	1.0
	31 Day	49.5	13.3	98.6	0.2	1.4
	48 Day	42.0	9.9	97.3	0.3	2.7
	71 Day	63.4	13.6	90.7	1.4	9.3

[a] In 100g of raw sample
[b] Sugar in baked root less sugar in raw root
[c] Grams in 100g of a raw sample
[d] Dextrin in baked root less dextrin in raw root

Table 4—Intrinsic viscosity (INV)[a] for total dextrin extract (TDE)[b] and purified dextrins[c] in baked sweet potato varieties

	Intrinsic viscosity	
	Total extract	Dextrins
At Harvest		
Centennial	72.7	—d
Jewel	73.1	—d
Porto Rico Mutant	54.5	—d
Pelican Processor	—d	—d
31 Day		
Centennial	32.1	12.8
Jewel	33.1	17.1
Porto Rico Mutant	39.2	16.1
Pelican Processor	78.4	—d
48 Day		
Centennial	28.8	14.6
Jewel	35.8	20.5
Porto Rico Mutant	35.4	18.2
Pelican Processor	56.6	—d
71 Day		
Centennial	27.6	11.8
Jewel	33.8	18.5
Porto Rico Mutant	31.1	13.7
Pelican Processor	38.4	25.3

[a] Grams per ml in 0.1M KCl. Viscosities measured in the same concentration range for all samples.
[b] Material extracted with 10% aqueous ethanol freeze dried and reconstituted before viscosities were measured.
[c] Purified by lead acetate removal of contaminants
[d] Insufficient material for viscosity studies

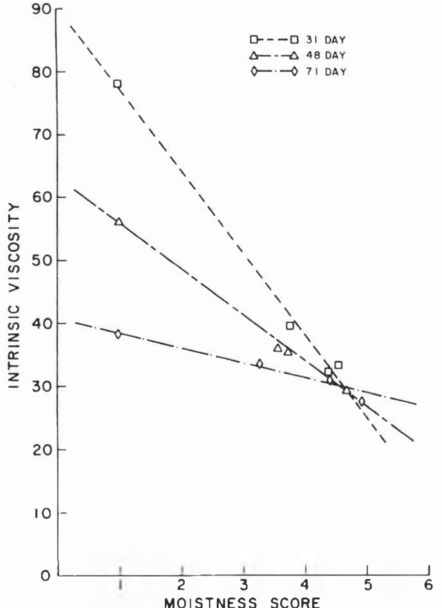

Fig. 1—Relation between organoleptic "moistness" score and intrinsic viscosity of the total dextrin extract for four baked sweet potato cultivars. Data are for three storage times at 16°C.

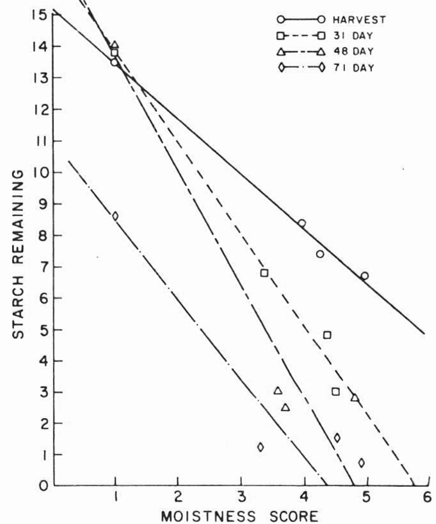

Fig. 2—Relation between organoleptic "moistness" score and starch remaining after baking for four sweet potato cultivars. Data presented are for four storage times.

combined correlation coefficient for all varieties for three panel dates is −0.793 ($P < 0.01$). Moistness scores were more highly correlated with INV than with α-amylase activities. This relation suggests that dextrins, pectins and, possibly, other components of TDE interact and thereby influence the textural differences described as "moistness" or "dryness." Others (Chen and Joslyn, 1967a, b) have reported that mono- and disaccharides or chemically-prepared dextrins added to pectin solutions interact to cause changes in the flow characteristics of the pectin solutions. Changes in this property have an impact on what humans perceive as textural properties.

A part of the mouthfeel could also be contributed by that starch which is not converted during baking. Possibly, as starch is removed by enzymatic action, the "dryness" decreases or "moistness" increases. A plot of "moistness" scores versus unconverted starch (Fig. 2) indicated that the two variables are negatively related. The combined correlation coefficient for four varieties and four panel dates is −0.859 ($P < 0.01$). Thus, both INV and starch remaining are significantly correlated with "moistness" scores.

The textural property of "moistness" or "dryness" in sweet potatoes is a complex organoleptic sensation. No one factor appears to be more important than all others. Our data indicate that mouthfeel depends on the amount of starch remaining after baking, the amounts and molecular sizes of dextrins and possibly on the amount of sugar present. All of these properties are affected by amylolytic enzymes. In addition, the unconverted starch probably had been partially degraded by amylolytic enzymes and might have contributed to the overall property of moist mouthfeel. Very probably the presence and state of pectin and cellulose fibers also exerted important influences. The effects of small changes in these variables upon mouthfeel is not known.

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FLAVOR QUALITY AND STABILITY OF POTATO FLAKES: EFFECTS OF ANTIOXIDANT TREATMENTS

INTRODUCTION

PREVIOUS STUDIES of the oxidative deterioration of potato flakes have demonstrated that product storage stability is dependent on raw material quality and on peeling, cooking, and drying conditions (Sapers et al., 1973, 1974). Dehydrated mashed potatoes are usually stabilized against oxidation by the addition of antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) at levels prescribed by the Food and Drug Administration (Code of Federal Regulations, 1973). These antioxidants may be added as components of emulsions containing other ingredients or as alcoholic sprays. One of the objectives of the current study was to determine the effects of different methods of addition on antioxidant retention and product stability.

The polyunsaturated fatty acids of potato, from which oxidative off-flavors arise, are found principally in the relatively polar glycolipid and phospholipid fractions of the potato fat (Ben-Abdelkader, 1968). The total lipids represent only about 0.7% of the dry weight of the potato (Mondy et al., 1963). Efforts to stabilize the lipids in this system must contend with the problem of dispersing fat soluble antioxidants in a largely aqueous medium. Pratt (1965) suggested that certain naturally occurring flavones and substituted cinnamic acids might have potential value as antioxidants in such low fat aqueous foods. The antioxidant activity of quercetin and other polyhydroxy flavones may be due to their ability to complex metal ions, or more likely, to act as free radical acceptors (Pratt and Watts, 1964). Quercetin and caffeic acid are considered to be relatively nontoxic compounds (Stecher, 1968; Griffith et al., 1955), although they are not currently approved as food additives in the United States. Sato et al. (1972) used caffeic acid to prevent quality deterioration in sake; Radaeva and Tyukavkina (1972) employed quercetin as an antioxidant in dry milk products. These and related compounds may be responsible for the antioxidant activity ascribed to plant extracts used to preserve dehydrated vegetables (Nestle's Products Limited, 1967). A second objective of this study was to determine the efficacy of quercetin and caffeic acid as antioxidants in potato flakes.

EXPERIMENTAL

Raw material and process

Potato flakes were prepared from Norchip tubers, harvested in North Dakota in the Fall of 1972 and stored for 3 months at 7°C followed by 1 month at 18°C prior to processing. The raw material had a specific gravity of 1.092 and contained approximately 0.024% fructose, 0.028% glucose and 0.020% sucrose (fresh weight basis).

Processing was carried out in the pilot plant of the Red River Valley Potato Research Center, East Grand Forks, Minn. The potatoes were abrasion peeled, subdivided, dipped 5–10 sec in 1% $NaHSO_3$ solution, precooked for 20 min at 71–77°C, cooled for 20 min at 10°C, steam cooked for 30 min at 100°C and mashed in a Hobart Mixer. An ingredient emulsion containing 20g mono- and diglycerides (Durkee EM 200 E), 15g sodium acid pyrophosphate, and 2.50g $NaHSO_3$ in 725 ml H_2O was added to each 22.7 kg (50 lb) batch of potatoes during mashing.

Antioxidants were added to 22.7 kg portions of mashed potatoes in the Hobart Mixer as follows:

1. Control–1.5 ml Tenox 4 (Eastman; 20% BHA and 20% BHT in corn oil) added in the ingredient emulsion.
2. Tenox 4 spray–1.5 ml Tenox 4 applied as a spray using a Freon-propelled aerosol sprayer.
3. Tenox 5–1.2 ml Tenox 5 (Eastman; 25% BHA and 25% BHT in ethyl alcohol) diluted to 100 ml with ethyl alcohol and applied with the aerosol sprayer.
4. Quercetin–0.274g quercetin (Baker) dissolved in 100 ml ethyl alcohol and applied with the aerosol sprayer.
5. Caffeic acid–0.164g caffeic acid (Baker) dissolved in 100 ml ethyl alcohol and applied with the aerosol sprayer.
6. Tenox 5 + quercetin–1.2 ml Tenox 5 and 0.274g quercetin dis-in 100 ml ethyl alcohol and applied with the aerosol sprayer.
7. Tenox 5 + caffeic acid–1.2 ml Tenox 5 and 0.164g caffeic acid dissolved in 100 ml ethyl alcohol and applied with the aerosol sprayer.

Drying was carried out using a single drum drier operating at a drum speed of 2.2 rpm, as described previously (Sapers et al., 1974). The finished flakes were packaged in polyethylene-lined fiber drums and shipped to the Eastern Regional Research Center in Philadelphia for further study.

Storage and evaluation

Samples were air- and nitrogen-packed in No. 303 and No. 10 cans and were stored at −18° and 23°C.

Initially, flakes were analyzed for moisture, equilibrium relative humidity, and sulfur dioxide; these data are summarized in Table 1. In addition, analyses were carried out for BHA, BHT and quercetin initially and after 12 months of storage. Quercetin was determined in alcoholic extracts prepared as follows: 10g potato flakes were reconstituted with 50 ml boiling water. The resulting paste was cooled and extracted with 100 ml absolute ethanol in three successive portions. Alcoholic extracts were combined, filtered through a Buchner funnel having a coarse porosity fritted disc, concentrated to 25 ml on a steam bath, cooled and extracted with 100 ml diethyl ether in three successive portions. The ether extracts were combined, concentrated to 10–15 ml on a steam bath, cooled and diluted to 100 ml with absolute alcohol. A 10-ml aliquot was analyzed by the spectrophotometric method of Naghski et al. (1951).

Table 1–Moisture and sulfur dioxide in potato flakes

Antioxidant treatment	Moisture content (%)	Equilibrium R.H. (%)	SO_2 (ppm)
Tenox 4 control	6.04	20.1	350
Tenox 4 spray	6.44	21.5	446
Tenox 5	7.17	27.8	352
Quercetin	7.02	26.3	280
Caffeic acid	6.54	23.5	207
Tenox 5 + quercetin	7.16	28.6	400
Tenox 5 + caffeic acid	6.04	19.3	341

[1] A Center operated cooperatively by the USDA North Central Region, ARS; the Minnesota Agricultural Experiment Station; the North Dakota Agricultural Experiment Station; and the Red River Valley Potato Growers Association.

Gas chromatographic (GC) determinations of volatile oxidation products in potato flake headspace vapor (lower boiling components) and in volatile concentrates prepared by steam distillation (higher boiling components) were performed using procedures described by Sapers et al. (1972). GC analyses were carried out in duplicate initially and at intervals during storage. Quantitative GC data were calculated as the ratios of individual oxidation product peak areas to the peak area of an internal standard (ethyl butyrate) which was added to each sample prior to analysis. These data were expressed more concisely as the sums of the peak area ratios for the major volatile oxidation products found in potato flakes in current and previous studies as described by Sapers et al. (1973).

Sensory evaluations were performed by a 15-member trained taste panel at the same intervals as the GC determinations. Flake samples (including a hidden standard) were compared with a standard, the nitrogen-packed frozen Tenox 4 control, using an eight point rating scale ranging from "much better than standard" (8) to "extreme off-flavor" (1). Results were expressed as mean flavor scores. The significance of differences reported by the panel was determined using Duncan's multiple range test.

All procedures and analytical methods used in this investigation have been described previously (Sapers et al., 1973, 1974) except as otherwise specified.

RESULTS & DISCUSSION

Antioxidant retention

Levels of addition of BHA and BHT in this study corresponded to concentrations of 55–60 ppm in the dehydrated potato flake products. As can be seen in Table 2, the actual antioxidant concentrations, determined by analysis, were substantially lower than the added levels, representing a recovery of only 15–35%. Initial antioxidant concentrations were lower and varied more from product to product with flakes containing Tenox 5 than with the Tenox 4 flakes. Initial levels of BHT were lower than corresponding BHA levels in the Tenox 5 samples.

These initial antioxidant losses are typical for the potato flake process and presumably result from vaporization and steam distillation during the addition of antioxidants to the hot mash and during drum drying (Ogg, 1960). Differences between Tenox 4 and Tenox 5 may be due to the volatility of the solvent (corn oil vs ethanol) rather than to the method of addition; the application of Tenox 4 as an emulsion or by spraying resulted in similar antioxidant levels. BHA and BHT concentrations obtained with Tenox 4 in the current study are comparable to those found previously (Sapers et al., 1973, 1974) and fall within FDA limits.

Variations in antioxidant concentrations within products, determined by analyses of replicate (6–8) flake samples representing several cans, were not related to the different antioxidant treatments described herein. Coefficients of variation ranged from 3–6% for BHA and from 7–12% with BHT; this difference is probably due to the method of analysis rather than to the uniformity of the flakes.

The method of antioxidant addition did not affect the retention of BHA and BHT in air-packed potato flakes stored for 1 yr at 23°C. Storage losses of BHA were negligible under these conditions. However, small losses of BHT did occur during storage, probably as a consequence of degradation rather than volatility since the samples were packaged in hermetically sealed cans.

Quercetin concentrations in the flakes containing quercetin alone, and quercetin in combination with Tenox 5 were found to be 39 and 41 ppm, respectively, representing a recovery of approximately 70%. After 12 months storage in air at 23°C, these samples contained 38 and 36 ppm quercetin, respectively.

Table 2–Retention of BHA and BHT in potato flakes stored in air at 23°C

	Concentration (ppm)				Retention (%)	
	BHA		BHT			
Antioxidant treatment	Initial[a]	12 Months	Initial[a]	12 Months	BHA	BHT
Tenox 4 control	20.3	20.4	20.8	16.8	100	81
Tenox 4 spray	19.5	19.5	18.6	14.5	100	78
Tenox 5	13.4	13.6	12.5	10.7	101	86
Tenox 5 + quercetin	14.5	13.5	10.5	7.8	93	74
Tenox 5 + caffeic acid	11.4	10.4	8.4	6.2	91	74

[a] Theoretical concentration = 55–60 ppm

Table 3–Effect of antioxidant treatments on the stability of potato flakes stored in air at 23°C

Antioxidant treatment	Storage time (months)	Mean flavor score	Sum of major volatile oxidation products: Headspace vapor	Volatile conc
Tenox 4 control	0	4.7	0.085	3.71
	6	4.1	0.122	5.85
	12	3.8	0.138	7.94
Tenox 4 spray	0	4.8	0.027	1.16
	6	3.4[a]	0.066	3.57
	12	3.1[b]	0.104	5.72
Tenox 5	0	4.8	0.052	2.62
	6	4.1	0.090	4.92
	12	3.2	0.132	9.54

[a] Significantly different from Tenox 5 at 0.05
[b] Significantly different from control at 0.05

Effect of antioxidant treatment on storage stability

Sensory and gas chromatographic data summarized in Table 3 indicate that the method of antioxidant addition had a relatively small effect on the storage stability of air-packed potato flakes containing BHA and BHT. Mean flavor scores for the three treatments were similar initially. The Tenox 4 spray treatment received significantly lower flavor scores than were obtained with the Tenox 5 treatment after 6 months and the control containing emulsified Tenox 4 after 12 months storage.

Levels of major volatile oxidation products were determined in the headspace vapor above reconstituted flakes and in the volatile concentrates prepared by distillation. Initially, oxidation product levels were lowest in flakes containing Tenox 4, applied by spraying, and were highest in the control. During storage the extent of oxidation increased in all samples. After 6 months, the initial order was unchanged, oxidation product levels increasing by about the same amount with all three treatments. After 12 months storage, oxidation product levels were highest in the Tenox 5 sample and lowest with the Tenox 4 spray treatment. However, oxidation product levels increased less in the control than in the other samples during the 6–12 months storage period. The lack of agreement between the GC and sensory data for the Tenox 4 spray sample may have been due to the presence of some unknown factor other than oxidation (i.e., a contaminant introduced during processing or canning) which influenced product flavor.

Neither the mean flavor scores nor the volatile oxidation product levels appear to be correlated with initial or final concentrations of BHA and BHT, within the narrow limits obtained in this study. There is some indication that increases in oxidation product levels during the 6–12 months storage peri-

Table 4—Effects of quercetin and caffeic acid on the stability of potato flakes stored in air at 23°C

Antioxidant treatment	Storage time (months)	Mean flavor score	Sum of major volatile oxidation products: Headspace vapor	Volatile conc
Quercetin	0	4.8	0.059	2.35
	6	3.3	0.206	12.78
	12	3.1	0.204	17.56
Caffeic acid	0	5.0	0.054	2.39
	6	3.9	0.232	11.72
	12	2.9[a]	0.251	18.83
Tenox 5 + quercetin	0	4.7	0.020	1.12
	6	4.4[b,c]	0.051	4.02
	12	3.4	0.079	6.98
Tenox 5 + caffeic acid	0	4.9	0.024	0.88
	6	4.0	0.094	5.11
	12	3.7	0.123	10.46

[a] Significantly different from control at 0.05
[b] Significantly different from quercetin at 0.05
[c] Significantly different from Tenox 5 at 0.05

od are inversely related to antioxidant concentration. Although differences between samples are small, the control treatment is recommended over the other methods of antioxidant addition since this procedure results in higher and more uniform antioxidant levels than were obtained with Tenox 5, a higher flavor score after 12 months and a smaller increase in volatile oxidation product level during storage.

Effects of quercetin and caffeic acid on storage stability

Stability data for potato flakes containing quercetin, caffeic acid and combinations of these compounds with Tenox 5 are summarized in Table 4. Initially all samples were satisfactory with respect to mean flavor scores and levels of major volatile oxidation products. During storage, flakes containing quercetin and caffeic acid alone were substantially less stable than the samples containing Tenox 4 and Tenox 5 (Table 3).

Quercetin and caffeic acid were added to potato flakes at concentrations of 5×10^{-5}M, comparable to levels found by Pratt and Watts (1964) to be effective in retarding the oxidation of roast beef slices. The poor performance of quercetin and caffeic acid in potato flakes may be due to their limited mobility in the dehydrated state or to the use of an inadequate level of addition. Much higher concentrations probably could not be used because of the low solubility, yellow color and bitter flavor of these compounds (Stecher, 1968; Seidell, 1941).

Potato flakes containing caffeic acid in combination with Tenox 5 were generally similar in storage stability to flakes containing only Tenox 5 and to the control (Table 3). Increases in levels of volatile oxidation products were higher in the former sample, possibly because of the lower concentrations of BHA and BHT obtained with this treatment. In the absence of any evidence for significant antioxidant activity or synergism, no advantage can be seen for the addition of caffeic acid to potato flakes.

Flakes containing quercetin in combination with Tenox 5 received a significantly higher flavor score than the flakes containing Tenox 5 alone after 6 months storage and had lower levels of volatile oxidation products than the Tenox 5 or control treatments throughout the study. Increases in volatile oxidation products during storage were lower in the quercetin plus Tenox 5 combination than with the Tenox 5 treatment and were comparable to the control, even though the latter flakes contained substantially higher levels of BHA and BHT.

The results show sufficient promise to warrant confirmation and further study. Higher concentrations of quercetin in combination with maximum allowable levels of BHA and BHT should be tested to determine whether true synergism occurs and to assess the potential value of this treatment in extending potato flake shelf life. Favorable results would then justify consideration of quercetin availability, cost and FDA approval.

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RELATIONSHIP BETWEEN WATER ACTIVITY AND WATER BINDING IN HIGH AND INTERMEDIATE MOISTURE FOODS

INTRODUCTION

THE CONTROL of water activity is an important aspect in the intermediate moisture food processing. A number of intermediate moisture foods have been prepared with additives such as sugar, salt and glycerol solution (Brockman, 1970; Collins et al., 1972; Kaplow, 1970). This control of water activity is based on Raoult's Law to establish and maintain an appropriate concentration of solutes throughout the aqueous phase of foods.

It is thought that by depressing water activity, water is also bound. According to Bull and Breese (1968), binding of free water molecules reduces water vapor pressure and hence water activity. However, Vrchlabsky and Leistner (1970) found that there is no statistical correlation between water binding and water activity in meat. Similar experimental results were shown by Leistner et al. (1971) in postmortem muscle.

The correlation between water binding and water activity in foods is still unresolved. Therefore, the present preliminary study was undertaken to correlate water binding and water activity.

EXPERIMENTAL

VARIOUS additives—glycerol, sodium chloride and sodium caseinate—were used to study the correlation between water binding and water activity. 5%, 10%, 15%, 20% and 25% aqueous solutions or suspensions of each component (w/w) were prepared. In addition, each concentration of sodium caseinate was mixed either with 4% and 10% of sodium chloride or 15% and 25% glycerol (w/w).

High and intermediate moisture food model systems were also prepared to study the relationship between water binding and water activity. High moisture food model systems were prepared by combining 14–22% of carboxymethylcellulose, 2–10% of casein, 1% of oil and 75% of water. Intermediate moisture food model systems were composed of 14–22% carboxymethylcellulose, 2–10% casein, 1% oil, 25% glycerol and 50% water. All the samples were analyzed for water activity and percent bound water.

The amount of bound water was determined using a Differential Scanning Calorimeter (Perkin-Elmer, Norwalk, Conn.) as described by Karmas and Chen (1974).

Water activity was determined by an Electric Hygrometer (Hygrodynamics, Inc., Silver Spring, Md.). Samples each 20g were sealed in air-tight jars in which a temperature-humidity sensor was fitted through the lid. After an equilibrium between the product and the surrounding atmosphere was reached, the equilibrium relative humidity and water activity were recorded.

RESULTS & DISCUSSION

THE CORRELATION between water binding and water activity was determined for sodium caseinate, glycerol and sodium chloride by varying their concentration in water from 5% to 25%. The correlation was also determined for sodium caseinate, in the same concentration, in combination with either glycerol or sodium chloride. The results are shown in Figure 1. Simple correlation coefficients between water binding and water activity are given in Table 1.

For aqueous sodium caseinate mixtures, there was no significant correlation between water binding and water activity. As the concentration of the protein increased, water binding increased significantly; however, water activity was depressed very little. It may be stated that proteins alone only imbibe water and leave the water activity of the imbibed aqueous phase essentially unaffected. In other words, a colloidal protein does not have as much interaction with water as a completely water-soluble solute has. Furthermore, the molecular weight of sodium caseinate is too high to change the mole fraction of water and produce a significant effect on water activity even though water binding is increased.

On the other hand, as the concentration of glycerol solutions increased, water activity was depressed significantly but the effect on water binding was almost nil. Glycerol has a relatively small molecular weight (92.1g/mole) and, according to the Raoult's Law,

$$a_w = \frac{n_w}{n_w + n_s} = \frac{p_s}{p_w}$$

where a_w = water activity
n_w = number of moles of water
n_s = number of moles of solute
p_w = vapor pressure of pure water
p_s = vapor pressure of aqueous solution,

it lowers significantly the vapor pressure of sufficiently concentrated aqueous solutions and, hence, also the water activity. However, glycerol possesses only negligible water-binding property as determined by differential scanning calorimetry. It seems that the energy of the hydrogen bonding between water and glycerol molecules is not significantly different from that existing between the water molecules and therefore no water binding is exhibited by glycerol.

For sodium chloride solution, the percentage of bound water was strongly and negatively correlated with water activity. Sodium chloride also has a small molecular weight (58.5g/mole) and, in an aqueous solution, on the basis of Raoult's Law, it depresses water activity uniformly throughout the solution. The ionic bonding between the sodium and chloride ions and water may be held responsible for the significant lowering of water activity as well as increased water binding.

Table 1—Correlation coefficients between water binding and water activity using various solutes

Sample	Bound water vs. water activity
Sodium chloride	−0.933
Glycerol	−0.198
Sodium caseinate	−0.694
Sodium caseinate with 4% sodium chloride	−0.992
Sodium caseinate with 10% sodium chloride	−0.970
Sodium caseinate with 15% glycerol	−0.952
Sodium caseinate with 25% glycerol	−0.938

Table 2–Relationship between water binding and water activity in high and intermediate moisture food model systems

Sample	Fraction of the total water bound	Water activity
High moisture food model systems		
HMF-22-2[a]	0.35	0.98
HMF-20-4	0.34	0.99
HMF-18-6	0.31	0.99
HMF-16-8	0.29	0.99
HMF-14-10	0.31	0.99
Intermediate moisture food model systems		
IMF-22-2[b]	0.29	0.84
IMF-20-4	0.27	0.85
IMF-18-6	0.26	0.85
IMF-16-8	0.25	0.85
IMF-14-10	0.24	0.85

[a] HMF-x-y: Composed of x% carboxymethylcellulose, y% casein, 1% oil and 75% water.
[b] IMF-x-y: Composed of x% carboxymethylcellulose, y% casein, 1% oil, 25% glycerol and 50% water.

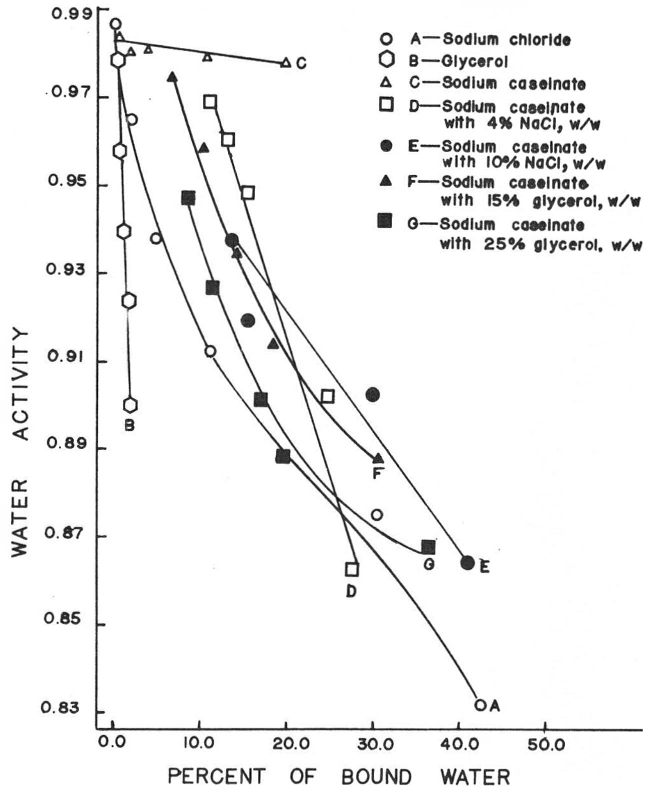

Fig. 1–Correlation between water activity and bound water using various solutes.

When either sodium chloride or glycerol was added to the aqueous sodium caseinate mixture, the correlation between water binding and water activity was significantly improved (Fig. 1). Water binding for sodium caseinate and sodium chloride combinations, for example, had a significant negative correlation with water activity (Table 1). It is particularly interesting to note that the protein-glycerol mixture also combined the outstanding water-binding and water-activity depressing properties of each substance, respectively.

The correlation between water activity and water binding in high and intermediate moisture food model systems is shown in Table 2. The results indicate that all systems had a highly significant effect ($P < 0.01$) on water binding, but no significant effect on water activity. Correlation coefficients for each model system indicate that there was no significant correlation between water binding and water activity in any of the systems.

The data in Table 2 further show that carboxymethylcellulose is a better water binder than casein. This preliminary study also indicates that the addition of glycerol lowers the water binding of the intermediate moisture food systems considerably. These findings have to be confirmed by further studies.

Since the molecular weights of carboxymethylcellulose and casein are high, they cannot lower the relatively high mole fraction of water and thus water activity. A significance could be obtained by removal of free water or by addition of large amounts of the water binders. In intermediate moisture food processing this may affect undesirably the sensory characteristics, such as texture and flavor, of the foods.

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INFLUENCE OF WATER ACTIVITY ON GROWTH AND ENTEROTOXIN FORMATION BY *Staphylococcus aureus* IN FOODS

INTRODUCTION

THE FACTORS influencing growth and enterotoxin production by *Staphylococcus aureus* in foods are many and complex. Temperature, pH, food composition, process history and moisture levels, singularly or in combination, may enhance or deter the growth of this organism. In addition to the ability of *S. aureus* to produce one or more potent enterotoxins, this organism also possesses several characteristics particularly troublesome to the food processor; the organism grows rapidly throughout a relatively wide range of pH levels (Genigeorgis et al., 1971), it is frequently found in the nasopharyngeal passages of humans and thus may have ready access to foods via food handlers (Minor and Marth, 1972), it produces toxins of remarkable stability to heat (Read and Bradshaw, 1966) and it is capable of growth at moisture conditions which prevent the growth of most other genera of bacteria (Scott, 1953).

The minimal water activity (a_w) for staphylococcal enterotoxin formation in artificial media is somewhat higher than that for growth (Hojvat and Jackson, 1969, Troller, 1971; 1972), however, results pertaining to the effect of a_w on staphylococcal growth and toxin production in foods are lacking. The extension of the earlier studies (Troller, 1971; 1972) of one of the authors to two experimental food systems, rehydrated potato doughs and shrimp slurries, is the basis for the present work.

MATERIALS & METHODS

Preparation of shrimp slurries

Peeled, frozen shrimp were thawed and cooked for 15 min prior to grinding with a Hobart grinding mill attachment (Hobart Manufacturing Corp., Troy, Ohio). Two passages through the grinder were required to obtain the desired particle size. The cooked, ground shrimp were then mixed with water (control) or glycerol solutions (Table 1) and blended in a Waring Blendor at the high speed setting for 3 min at room temperature. The resulting slurry possessed a pH level of 7.0.

The blended slurry was weighed into 300g aliquots and poured into 1 liter Ehrlenmeyer flasks followed by sterilization in an autoclave at 121.5°C for 25 min. Confirmation of sterility was obtained by inoculating 1 ml of the paste in 10 ml of Brain Heart Infusion (BHI) broth (Difco Labs, Detroit, Mich.) and incubating duplicate tubes aerobically and anaerobically for 48 hr at 37°C. Absence of growth in transfers from this broth to Total Plate Count agar (Difco) was taken as proof of sterility.

Preparation of potato doughs

Potato doughs were prepared by adding a heated (95°C) mixture of water and glycerol to commercially available dehydrated potato in the quantities indicated in Table 1. Following addition, the doughs were mixed for 5 min in a Hobart Model N-50 mixer. No attempt was made in these experiments to reduce or eliminate the normal flora of the potato flour before mixing into a dough.

Cultures and growth conditions

Two strains of *S. aureus* were used in this study. The 196E strain was employed for enterotoxin A production and the C243 strain was used for enterotoxin B production. All cultures were maintained on BHI slants at 5°C and were subcultured at 37°C in BHI broth preparatory to inoculation. One ml inocula were used throughout this study. No increase in a_w of the test systems could be detected as a result of inoculation.

The inoculated foods were incubated at 37°C for 80–100 hr (slurries) or 48 hr (doughs). Shorter incubation times were used with doughs because *S. aureus* is a poor competitor (Troller and Frazier, 1962) and tends to be overgrown by the normal potato dough flora during extended incubation. These difficulties were not encountered with the sterilized shrimp slurries because only pure cultures of the inoculated staphylococci were present. Staphylococci from both systems were counted on Baird-Parker agar (Difco) incubated at 37°C for 48 hr.

Water activity analysis

Water activity is commonly expressed as a_w or the ratio of the vapor pressure of a given solution to that of pure water at the same temperature. All a_w determinations described in these studies were obtained with a Sina model EZFBA-4X humidity probe (Beckman Instruments, Inc., Cedar Grove, N.J.) connected to a model SJT indicator of the same manufacture. Before measurements were taken, this instrument was carefully calibrated with known molarity H_2SO_4 solutions. All measurements are accurate to ± 0.002 a_w; however, readings are rounded off to the nearest hundredth a_w unit.

Following autoclaving, water activity measurements of both foods were obtained by removing 5 ml of each material to the probe sensor assembly. Readings were taken following sample equilibration.

Enterotoxin analysis

After incubation, 200g samples were removed from each flask and extracted with 200 ml of 0.2M NaCl by mixing at high speed in a Waring Blendor for 5 min. The pH was then adjusted to 7.5 with 1N NaOH and the mixture allowed to settle for 15 min before centrifuging at 45,000 × G in a Sorvall RC2B refrigerated centrifuge (Ivan Sorvall, Inc., Norwalk, Conn.). The supernatant was filtered and combined with reextracted supernatant followed by concentration overnight in dialysis tubing against a 50% solution of Carbowax 100M (Union Carbide Corp., Chicago, Ill). The contents of the dialysis tubing were removed with 20 ml of 0.02M NaCl and added to 20–25 ml of a carboxymethyl cellulose slurry. The pH of the slurry was adjusted to pH 5.5 followed by mixing at 5°C for 1 hr. The mixed slurry was then added to a glass column, rinsed with 150 ml of 0.01M phosphate buffer (pH 5.5) and eluted with 100 ml of 0.2M, pH 7.5 phosphate buffer containing 0.2M of NaCl. The eluate was extracted with 50 ml of $CHCl_3$ and the aqueous layer containing the extracted toxin was concentrated against Carbowax 100M

Table 1—Relation between glycerol content of food systems and a_w

	Slurry (g)	Glycerol (g)	a_w
Shrimp	500	0	0.99
	468	44.3	0.97
	432	80.5	0.95
	402	98.0	0.93
	372	128.0	0.91
	345	155.0	0.89
Potato dough	200	0	0.97
	188	12	0.93
	176	24	0.88

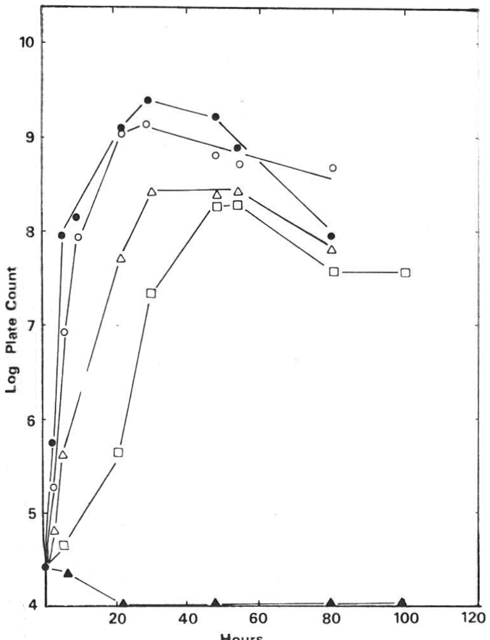

Fig. 1—Growth of S. aureus 196E in shrimp slurries at a_w: ●—● 0.99; ○—○ 0.97; △—△ 0.95; □—□ 0.93; and ▲—▲ 0.89.

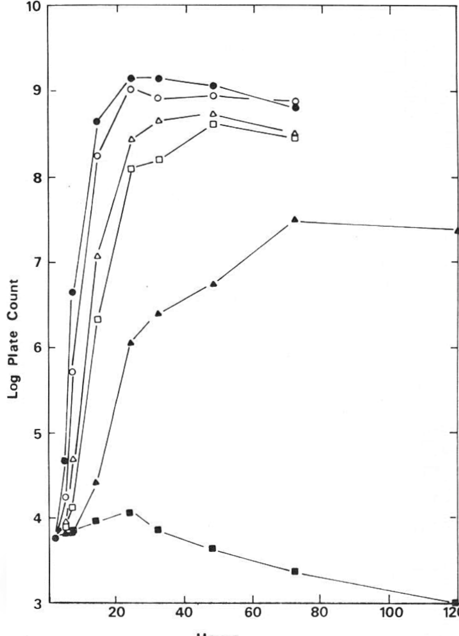

Fig. 2—Growth of S. aureus C243 in shrimp slurries at a_w: ●—● 0.99; ○—○ 0.97; △—△ 0.95; □—□ 0.93; ▲—▲ 0.91; and ■—■ 0.89.

overnight at 5°C as indicated above. The toxin was then rinsed from the dialysis sacs with 0.01M Na_2HPO_4 buffer (pH 7.5) and dialyzed for 7 hr against distilled water at 5°C. A final reconcentration against Carbowax and lyophilization followed.

Lypohilized extracts were rehydrated in 0.37% BHI broth and quantified by the method of Oudin (1952) as modified by Weirether et al. (1966). The limit of enterotoxin detection by this procedure was determined to be approximately 1 μg/ml for both enterotoxins. Antisera used in these studies were the gift of M.S. Bergdoll, University of Wisconsin, Madison, Wisc. Extraction procedures were essentially identical for potato doughs and shrimp slurries, however, in doughs inoculated with *S. aureus* 196E, the small amounts of enterotoxin A produced required a 50-fold concentration increase to obtain analyzable quantities of enterotoxin A.

RESULTS & DISCUSSION

Staphylococcal growth

Both strains of *S. aureus* utilized in this study grew in shrimp slurries adjusted to a_w levels in excess of 0.91 (Fig. 1 and 2). Water limitation did, however, suppress maximal total counts and rates of growth (Table 2) of both strains. The lowest a_w tested, 0.89, prevented growth of the 196E strain although a slight increase in count during the initial 24 hr of incubation of slurries at this a_w level was observed with the C243 strain. Maximal counts of both strains in control (a_w = 0.99) systems exceeded 10^9/ml after 20–22 hr of incubation.

As noted above, staphylococci possess a relatively high degree of osmotolerance, thus the failure of the present strains to grow at a_w levels below 0.89 was somewhat unexpected. Minimal a_w levels for staphylococcal growth have been reported to vary between 0.83 (Hill, 1973) and 0.86 (Scott, 1953) with the lowest minimal a_w levels for growth of this organism observed in foods such as cooked pork. Thus, laboratory media may not be the most ideal system to evaluate minimal a_w growth characteristics. Solute differences could account for the demonstrated failure of these organisms to grow at a_w 0.89; however, glycerol, the solute used in these studies to adjust a_w has been observed (Troller, 1971) to possess minimal, intrinsic, inhibitory properties when compared with other solutes in artificial media.

Generation times also varied as a function of a_w in shrimp slurries. The 196E strain grew somewhat slower than the C243 strain at all a_w levels tested although highest maximal counts were obtained with the 196E strain. Higher maximal counts were found with the 196E strain throughout the a_w range tested in these experiments.

Although less complete, the data describing the growth of *S. aureus* C243 and 196E in potato doughs (Table 3) indicate higher counts of staphylococci at a_w 0.88 in this product than those occurring in shrimp slurries at a_w 0.89. The growth of *S. aureus* 196E appeared to be somewhat more sensitive to limited moisture conditions than the C243 strain. It is doubtful if natural contaminants present in potato doughs were responsible for the slightly suppressed growth of staphylococci which occurred as the a_w was lowered. This is especially true at the lowest a_w tested, where the greater osmotolerance of the staphylococci would be expected to selectively favor this organism to the detriment of other bacteria in the doughs.

Table 2—Growth rate, maximal numbers and enterotoxin production by S. aureus 196E and C-243 in shrimp slurries at various a_w levels

	a_w	Generation time (min)	Maximal count X 10^8/ml[a]	Enterotoxin (μg/ml)	Enterotoxin (μg/10^8 cells)
S. aureus	0.99	45	65	4	0.06
196E	0.97	64	49	3	0.06
	0.95	88	28	1	0.04
	0.93	96	6	0	0
	0.89	No Growth	–	–	–
S. aureus	0.99	30	14[b]	22	1.57
C-243	0.97	38	11	8	0.80
	0.95	51	6	5	0.83
	0.93	75	4	3	0.75
	0.91	140	0.3	0	0
	0.89	300	0.00012	0	0

[a] Initial (0 hr) count = 2 X 10^4/ml.
[b] Initial (0 hr) count = 6 X 10^4/ml.

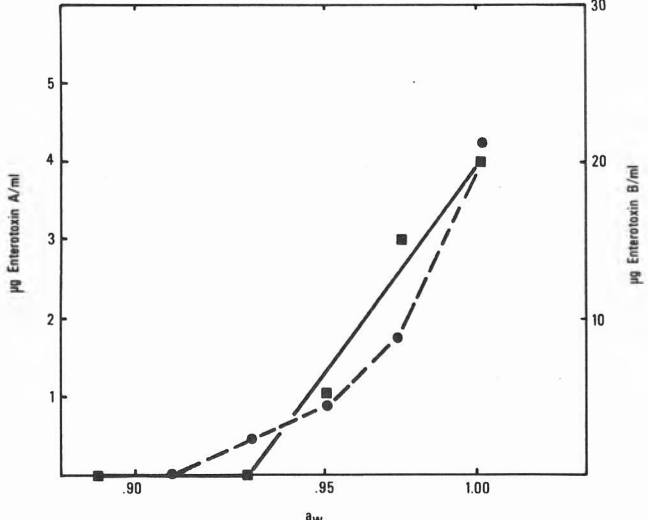

Fig. 3–Enterotoxin A and B production in shrimp slurries by, respectively, S. aureus 196E (■) and C243 (●) as a function of a_w.

Table 3–Growth and toxin formation by S. aureus 196E and C-243 in potato doughs at various a_w levels

	a_w	Organisms/ml		Enterotoxin	Enterotoxin
		0 hr	48 hr	µg/50g	µg/10^8 cells
S. aureus	0.96	1.3 X 10^3	2.4 X 10^9	A 8	A 0.007
196E	0.93	1.1 X 10^3	2.1 X 10^9	A 5	A 0.005
	0.88	4.0 X 10^3	4.8 X 10^7	A 0	A 0
S. aureus	0.97	5.1 X 10^3	4.2 X 10^9	B 10	B 0.23
C-243	0.93	3.7 X 10^3	1.5 X 10^9	B 0	B 0
	0.88	3.7 X 10^3	1.8 X 10^8	B 0	B 0

Enterotoxin production

Both strains produced enterotoxin A or B in control (a_w = 0.99) shrimp slurries. The minimal a_w level for toxin production varied to some degree with the strain employed; the 196E strain produced detectable toxin in slurries adjusted to a_w levels as low as 0.95 but not at a_w 0.93 whereas the C243 strain produced detectable levels of toxin at a_w = 0.93 but not at a_w = 0.91 (Fig. 3). Previous work in our laboratory indicated that toxin production by the C243 strain was somewhat more sensitive to water limitation than the 196E strain (Troller, 1971; 1972); however, these earlier studies were performed in laboratory media and thus substrate differences might exist. In slurries inoculated with *S. aureus* 196E, enterotoxin production did not occur at a_w 0.93 despite the presence of as many as 6 X 10^8 cells/g. Similarly, detectable toxin levels could not be found in C243 inoculated shrimp slurries at an a_w level of 0.91. The maximal count obtained at this a_w was 3 X 10^7 staphylococci per gram. In both cases, these numbers of *S. aureus* should have been sufficient to produce detectable quantities of enterotoxins.

Table 2 shows that toxin production in shrimp slurries relative to maximal counts of staphylococci was depressed as a_w levels were lowered. The greatest decrease in relative amounts of enterotoxin B appeared to occur between a_w = 0.99 and a_w = 0.97. Relative enterotoxin A levels were identical at a_w = 0.99 and 0.97 and decreased with moisture limitation at a_w = 0.95 and 0.93.

Small amounts of enterotoxin A were produced in potato doughs at a_w = 0.97 and 0.93 (Table 3); however, decreasing total and relative amounts of this toxin were noted. No enterotoxin A could be detected at a_w = 0.88. Enterotoxin B production was observed only at the highest water activity tested, a_w 0.97, with no detectable concentrations of the toxin occurring at a_w 0.93 and 0.88.

The general decrease in relative enterotoxin levels commensurate with reduction in a_w levels in both experimental foods, indicates that the toxin decline is not a result of a reduction in numbers of staphylococci present, but, more probably is related to moisture availability in the system. In the case of potato doughs, suppression of toxin production, but not growth, by competing organisms could not be ruled out.

The present data confirm earlier observations made on staphylococcal growth and enterotoxin production in artificial media and extend these findings to two food systems. The ability of staphylococci to produce toxin in additional foods adjusted to varying a_w levels with solutes other than glycerol are the subjects of current investigations; however, preliminary results appear to confirm the data reported herein. Foodborne disease outbreaks in which toxin is present but vegetative cells absent at the time of epidemiological examination are relatively common and usually are related to the extreme stability of the toxins to terminal pasteurizing treatments. Similarly, large numbers of staphylococci, capable of producing toxins under optimal conditions, have been found in foods in which toxins cannot be detected. In these situations consideration should be given to the environmental factors to which such foods were exposed and in which suppression of toxin formation could have occurred. Reduced moisture conditions appear to be one of these factors.

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GELATION AND THICKENING PHENOMENA OF VEGETABLE PROTEIN PRODUCTS

INTRODUCTION

THE UTILIZATION of concentrated seed proteins as food ingredients is largely determined by their functional properties such as solubility, emulsification, water- and fat-binding properties and foaming characteristics. In a previous study (Fleming et al., 1974), marked differences in water absorption and viscosity characteristics were observed among soy and sunflower flours, concentrates and isolates. Factors influencing slurry viscosity included concentration, pH, ionic strength, temperature and mixing time.

Many investigators have reported that heating will increase the viscosity of protein slurries (Johnson, 1970; Briskey, 1970). Circle et al. (1964) found that gels were formed by heating 8% or higher dispersions of sodium soy proteinate to temperatures of 80–125°C in sealed containers for 30 min. According to Catsimpoolas and Meyer (1970) the slurry of soybean globulins (the sol) was heated to a progel state and when cooled, formed a reversible gel. Excess heat produced a metasol which did not gel on cooling. By this procedure, Hermansson (1972) produced gels with 10% protein dispersions of Promine D and whey protein concentrate, but not with sodium caseinate. The formation of gels has not been reported for any other protein source.

Catsimpoolas and Meyer (1970) stated that the gelling ability of soybean occurred in the globulin fraction, but they did not selectively isolate this fraction. Several other workers (Saio et al., 1973, 1974) have further separated this globulin fraction and compared the properties of the 7S and 11S globulin components. They noted that the 11S fraction gave a firmer gel structure than the 7S fraction when either calcium induced or heat-induced. According to Briskey (1970), the ability of gel structures to act as a matrix to hold moisture, lipids, polysaccharides and other ingredients is of significance in many food applications. In particular sausage and wiener emulsions acquire gel-like properties when heated. It is therefore useful to evaluate new vegetable protein products for their thickening and gelling abilities.

In western Canada, field pea and fababean are being developed as new sources of high lysine protein for livestock feed. Their potential as new sources of concentrated seed protein for food uses is also under investigation. An objective of the present study was to evaluate the functional properties of field pea and fababean proteins as a gelling agent in processed foods.

The objective of the present study was to evaluate the gel forming and thickening properties of flours, concentrates and isolates from defatted sunflower, field pea and fababean, in addition to the soybean controls, under the conditions described by Circle et al. (1964). The influence of temperature, heating time, slurry concentration, ionic strength and pH-activation on gel properties were also determined. In addition, the globulin protein fractions were isolated from soybean and sunflower flour and fababean and field pea concentrates, and their gelation ability was evaluated.

EXPERIMENTAL

THE SOYBEAN PRODUCTS used in the present study were obtained from commercial sources while the preparation of sunflower flour and concentrate has been previously described (Fleming et al., 1974). The field pea and fababean concentrates were prepared by pin milling and air classification of the flour into a protein-rich fraction containing about 50% protein. All products were ground to pass through a 100 mesh screen. The protein isolates of sunflower, fababean and field pea were prepared according to the method of Sosulski and Bakal (1969). Proximate composition was determined by AOAC (1970) methods.

Aqueous dispersions of 10% protein were prepared from the flours, concentrates and isolates by stirring for 1 min in a Sorvall Omni-Mixer at 11,000 rpm. Air was removed from the slurries by centrifugation at 1,000 × G for 2 min. The experiments were conducted at pH 7 the adjustments being made with 6N HCl and 2N NaOH. The pH 12 treatment of the flours and concentrates was done by the procedure of Fleming et al. (1974) by cycling the pH of the slurry to 12.0 and back to 6.0. The apparent viscosities of the initial slurries were determined on a Brookfield viscosimeter, model LVF. The standard rotating spindles were used to measure viscosities of less than 500 cps while the T-spindles and Helipath stand were used for more viscous solutions to allow measurement of undisturbed structure at all times. The viscosimeter was operated at 6 and 12 rpm. Gelation experiments were conducted by heating the slurries in sealed stainless steel containers to 90°C for 45 min in a water bath for all protein products except fababean and field pea concentrates which required 60 min of heating to form a gel. The containers were then cooled quickly in an ice bath to 25°C, opened and the viscosity of the heated slurry or gel determined as above.

The albumin and globulin proteins were isolated by the procedures of Chen and Bushuk (1970) and Bajaj et al. (1971). The flours of soybean and sunflower and the protein concentrates of fababean and field pea were extracted in a salt solution of 0.5M NaCl buffered to pH 7.0 with 0.03M Na_2HPO_4 and 0.02M $NaH_2PO_4 \cdot H_2O$ at a solvent to meal ratio of 4:1. The dispersions were slowly stirred for 2 hr, centrifuged at 1500 × G for 15 min and the supernatant decanted. The residue was resuspended and two more extractions (1 hr, and 30 min, respectively) were conducted. The combined supernatants were dialyzed against distilled water for 72 hr and the albumin and globulin fractions were separated by centrifugation at 8,000 × G for 5 min and freeze dried. Aqueous dispersions of 10% protein in 0.5M NaCl were prepared and heated in 4 oz sealed glass jars at 90°C for 45 min, cooled, and the viscosity measured as earlier described.

RESULTS & DISCUSSION

THE PROTEIN CONTENT of soybean and sunflower flours, protein concentrates and isolates averaged 50, 65 and 85%, respectively (Table 1). By means of air classification, the protein content of the field pea and fababean flours was concentrated from less than 30%, to 49 and 56%, respectively. The standard protein isolation procedure yielded fababean and field pea products which contained about 25% of nonprotein constituents that might interfere with the functionality of the isolates. Generally, the sunflower, fababean and field pea concentrates and isolates were higher in oil content than the corresponding soybean products. The sunflower concentrates were also higher in crude fiber and ash than the legume products.

The aqueous dispersions of protein products were adjusted to 10% protein in solution and pH 7 before measurement of the viscosity before and after the gelation heating treatment. The viscosities of the initial slurries were very low except for those of the soybean isolate, Supro 610, and fababean isolate (Table 2). At this concentration, no difference could be noted in the viscosities of the slurries of the other protein products. At higher concentrations, however, the initial viscosity of the soybean products was as high or higher than those of the corresponding products for the other protein sources.

The viscosities of all slurries were increased markedly by the gelation procedure of Circle et al. (1964). The sunflower flour and concentrate −80, but not the isolate, were as viscous after heating and cooling as the soybean products (Table 2). The fababean concentrates and isolates also compared to the soybean products in their high viscosities. The soybean protein isolate, Promine D, was the only heated slurry to form a uniform but firm gel which held its shape when cut with a knife. Circle et al. (1964) and Hermansson (1972) also used Promine D to demonstrate the gelation phenomena. This gel also showed high cold viscosity (> 166,000 cps) and the nongelling protein isolate, Supro 610, was less viscous. However, viscosity was not directly associated with the gelation property since the soybean protein concentrate, Isopro, showed a high viscosity after heating but remained granular and pourable. Similarly, the high viscosity of the sunflower concentrates did not indicate gel formation since the products formed an irregular structure with a foamy appearance.

The fababean concentrate and isolate formed medium gels which were somewhat less firm than the Promine D gel and lacked the elastic property (Table 2). However, their viscosities were very high. The field pea concentrate had similar gelling properties to fababean but the isolate developed a soft gel with lower viscosity.

The slurries were also made up in 5% salt (NaCl) solutions rather than distilled water but the increase in ionic strength tended to reduce or have no effect on viscosities and gelation characteristics of most products, and the data are not reported in Table 2.

In order to improve the viscosity and gelling properties, the flours and concentrates were cycled through the same alkaline pH treatment which is applied during protein isolation. The pH 12 treatment increased protein hydration and swelling of the initial slurries but, after the gelation treatment, only soybean flour and sunflower concentrate −60 showed higher viscosities. However, the slurry of soy flour also developed the soft gel characteristics which had been obtained with the field pea isolate. The Isopro and sunflower slurries remained as smooth fluids, with very high viscosity for Isopro but low viscosities for sunflower flour and concentrate −80. The fababean and field pea concentrates again developed gels with medium structure. The fababean isolate, and concentrate after pH 12 treatment, developed dark brown colors especially after heating. Presumably, phenolic acids were present which oxidized to quinones under alkaline conditions and complexed with the proteins, as has been previously demonstrated for

Table 1–Proximate composition of protein products (moisture-free basis)

Protein Product	Crude protein (N X 5.7) %	Crude fat %	Crude fiber %	Ash %
Soybean				
Flour	50.0	3.6	4.0	6.1
Isopro	66.1	0.3	3.0	3.5
Supro 610	79.9	0.2	0.1	3.7
Promine D	84.6	0.3	0.1	3.9
Sunflower				
Flour	51.3	3.1	4.2	8.1
Concentrate −60[a]	65.7	2.1	5.5	5.7
Concentrate −80	64.0	2.6	5.4	6.9
Isolate	85.2	4.5	0.8	3.6
Fababean				
Concentrate	55.7	2.5	2.6	5.6
Isolate	76.5	3.6		
Field pea				
Concentrate	49.0	3.4	2.8	6.0
Isolate	74.3	6.0		

[a] Concentrate −60 and −80 refer to concentrates produced by extraction at 60° and 80°C, respectively.

Table 2–Apparent Brookfield viscosity of seed protein slurries before (initial) and after (final) heating at 90°C for 45 min

Protein product	pH 7 Initial (cps X 1,000)	pH 7 Final (cps X 1,000)	pH 12 treated Initial (cps X 1,000)	pH 12 treated Final (cps X 1,000)	Characteristics of final product pH 7	Characteristics of final product pH 12 treated
Soybean						
Flour	1	45	3	>166	smooth fluid	soft gel
Isopro	6	>166	14	>166	granular fluid	smooth fluid
Supro 610	70	123	–	–	granular fluid	–
Promine D	3	>166	–	–	firm gel	–
Sunflower						
Flour	1	69	4	21	smooth fluid	smooth fluid
Concentrate −60	3	93	9	143	foamy fluid	foamy fluid
Concentrate −80	3	>166	9	35	granular gel	foamy fluid
Isolate	1	49	–	–	syneresis	–
Fababean						
Concentrate	0	>166	32	>166	medium gel	medium gel
Isolate	47	>166	–	–	medium gel	–
Field pea						
Concentrate	0	>166	4	>166	medium gel	medium gel
Isolate	3	120	–	–	soft gel	–

Table 3—The viscosity of globulin protein slurries after heating at 90°C for 45 min

Globulin source	Apparent Brookfield viscosity (cps) Protein concentration 10%	15%
Soybean	>166,000	>166,000
Sunflower	—	—
Fababean	8,330	>166,000
Field pea	128,000	>166,000

sunflower flour (Sabir et al., 1974). However, the sunflower isolate and pH 12 treated flour were grey in appearance while soybean and field pea products were light yellow in color at the final pH levels of 6–7.

The heated 10% protein dispersions of the globulins in 0.5M NaCl prepared from soybean flour and field pea and fababean concentrate formed thickened gel structures whereas the sunflower globulins coagulated during heating and separated from the absorbed water, causing the protein to settle out (Table 3). The viscosity of the soybean globulin at a 10% protein concentration was in excess of 166,000 cps, whereas the viscosity from the fababean globulin was only 8,330 and that of field pea was 128,000 cps. Each gel did, however, have a smooth, homogeneous appearance with a clearness typical of the gel produced from Promine D. Whereas the soybean globulin gave a firm gel, the fababean gave a soft, pourable gel and the field pea globulin gave a medium gel capable of maintaining its shape. At 15% protein concentration the fababean and field pea globulin formed firm gels with viscosities of more than 166,000 cps.

It is interesting to note that the globulins had to be dispersed in dilute salt solution to give the gel structure since dispersions in distilled water separated and formed two distinct layers. Supposedly, the natural salts present in the commercial product, Promine D, and the salts created by pH adjustment in preparing the isolate by Catsimpoolas and Meyer (1970) would have provided such a medium.

The albumin protein fractions prepared from soybean and sunflower flour and from fababean and field pea concentrate did not show any gelation ability when heated under the conditions described for the globulin proteins. This suggests that the gelling ability of the soybean, fababean and field pea protein occurs in the globulin fraction only.

Further research should be conducted to study the influence of salt concentration in the extraction medium on the functional properties of the globulin proteins. It is possible that the salt concentration used in this study caused some irreversible protein denaturation, thus giving a structure which is less firm than could be obtained using optimum conditions. The influence of protein concentration, temperature and time of heating, and pH and ionic strength of the dispersion medium on the viscosity of the gels should also be studied.

The fababean and field pea concentrates and globular isolates, and pH 12 treated soybean flours may have application in food products which require proteins with thickening and gelling properties. Wiener and sausage emulsions form gel-like structures at specific temperatures. Custard-type puddings and sauces also require such properties.

CONCLUSIONS

THE GELLING ABILITY which is characteristic of the soybean isolate, Promine D, and the soybean globulins has also been found to occur in fababean and field pea protein products, and soy flour after pH 12 treatment. The isolated globulin proteins from these sources appeared to be responsible for the gelation phenomenon. Fababean and field pea concentrates and isolates may be useful in applications where commercially prepared soy protein with the gelling ability are now used.

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OXIDATION OF PEA LIPIDS BY PEA SEED LIPOXYGENASE

INTRODUCTION

UNBLANCHED PEAS during cold storage have been shown to develop objectionable off-flavors accompanied by large losses in the fatty acids of triglycerides and phospholipids (Lee and Mattick, 1961; Pendlington, 1962; Bengtsson and Bosund, 1966). Analysis by gas-liquid chromatography revealed the preferential hydrolysis of unsaturated fatty acids and suggested their substantial breakdown into smaller molecules detectable in storage headspace. The main enzyme responsible for lipid oxidation is lipoxygenase (formerly known as lipoxidase; E.C. 1•13•1•13, linoleate: oxygen oxidoreductase), but other enzymes may be involved as well (Siddiqi and Tappel, 1956; Wagenknecht et al., 1958). Macerated blanched peas containing added lipoxygenase and lipase showed a considerable oxygen uptake, an effect which was not apparent either with lipoxygenase alone, or when catalase and/or peroxidase were present along with lipoxygenase (Wagenknecht and Lee, 1958).

The possibility of the existence of multiple lipoxygenases in green peas was intimated by Hale et al. (1969). By electrophoresis on acrylamide gels and using the specific staining technique they separated an intermediate and a high mobility band, both with lipoxygenase activity. In a study of identification of lipoxygenase isoenzymes as carotene oxidases, Weber et al. (1973) isolated the enzyme from ripe green peas. Using DEAE-cellulose column chromatography they separated the enzyme into two well defined peaks, each with an optimum activity at pH 6.5, an elution pattern which corresponded closely to lipoxygenase-2 and -3 from soybean. Recently, from acetone powder prepared from dwarf pea seed allowed to swell in water overnight, Anstis and Friend (1974) used a carboxymethylcellulose column to separate five fractions with lipoxygenase activity, with a predominating activity in the first three fractions.

Eriksson and Svensson (1970) isolated and purified green pea seed lipoxygenase and used isoelectric focussing to detect the presence of two active components. The molecular weights determined by amino acid analysis and ultracentrifugation were 72,000 and 67,000 Daltons. Eriksson and Svensson also indicated that the enzyme activity was highest in the pea center and lowest in the skin.

In our previous study of lipoxygenase in green pea seeds (Haydar and Hadziyev, 1973a), it was found that the enzyme was present in cytoplasm, mitochondria, peroxisome-like bodies, and with only a low activity, in chloroplasts.

When pea seeds were stored in the cold for 6 months or longer a substantial bleaching of the seeds occurred. When such seeds were checked for lipoxygenase enzyme by the acrylamide gel zymogram technique, the activity was found only in one intermediate mobility band. Isolating the enzyme in the presence of the Ca^{2+} found in pea seed, gave an enzyme extract with an activity lower than the extract in which the ion interference was avoided. Thus, the Ca^{2+} response of this storage resistant enzyme simulated that of soybean lipoxygenase-3. Therefore, this study was designed to give a more detailed characterization of the storage resistant enzyme of pea seeds and its effect upon the major seed lipids responsible for the development of seed off-flavors.

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MATERIALS & METHODS

WRINKLED PEA SEEDS (*Pisum sativum* L., var. Homesteader) were used. The air-dried seeds were stored up to the fifth month at 15°C and at a RH of 20%, and then were kept at 4°C in a storage cold room. After 1 yr the skin of such seeds was substantially discolored. The viability of these seeds, determined by a germination test, was better than 90%.

Lipoxygenase isolation and purification

The enzyme preparations were extracted from nondefatted pea seeds, because the defatting treatment yielded preparations with a consistently lower activity.

The seeds, in amounts of 100g, were ground with dry ice in a Waring Blendor. The powder was extracted by stirring with 1 liter 0.1M TRIS-Cl buffer pH 7.2 for 16 hr. The slurry obtained was filtered through six layers of cheesecloth and centrifuged at 20,000 × G for 15 min. The supernatant was collected and recentrifuged at an average of 140,000 × G for 2.5 hr. The supernatant was precipitated with 25% ammonium sulfate saturation for 30 min, the precipitate was discarded and the supernatant precipitated with 50% ammonium sulfate saturation. The precipitate was collected, dissolved in 90 ml of 50 mM TRIS-Cl buffer pH 7.2 and dialyzed against 40 volumes of the same buffer for 15 hr with several changes of buffer. The resultant lipoxygenase dialyzate was further purified by Sephadex and DEAE-cellulose column chromatography.

A Sephadex G150 column (1.5 × 90 cm) was equilibrated with 50 mM TRIS-Cl buffer pH 7.2. The dialyzate, 6.7 ml, containing proteins corresponding to an absorbance of A_{280} = 400 (A_{280} units) was applied to the column and eluted with the same buffer. The elution rate was kept constant with an LKB Model peristaltic pump and 4.5 ml fractions were collected each 15 min by means of a time operated fraction collector. The absorbance at 280 nm of each fraction was determined after which all fractions having UV absorbance were assayed for lipoxygenase activity. The fractions 11–23 containing lipoxygenase were combined and then the enzyme was precipitated by 50% ammonium sulfate saturation, and pelleted at 15,000 × G for 20 min. The pellet obtained was dissolved in 10 mM TRIS-Cl buffer pH 6.5 containing 2 mM $CaCl_2$ and 1 mM 2-mercaptoethanol (TRIS-Ca^{2+}-SH). The solution was dialyzed against 40 volumes of the same buffer for 5, 10 or 15 hr with several changes of buffer.

The DEAE-cellulose column (2.5 × 45 cm) was equilibrated with the above TRIS-Ca^{2+}-SH buffer. The dialyzed lipoxygenase was applied in an amount of 30–120 A_{280} units. Elution was performed by applying the same buffer and a shallow linear salt gradient in a range of 0.0–0.2M NaCl. The gradient was controlled refractometrically by using a Zeiss Abbe refractometer. Fractions of 4.5 ml/tube were collected each 12 min and assayed for UV absorption and lipoxygenase activity.

In a second similar procedure the enzyme extraction was achieved by stirring in 10 mM Na-phosphate buffer pH 7.0 for 16 hr. The slurry obtained was filtered through cheesecloth and further clarified with low and high speed centrifugation as before. After final precipitation with 50% ammonium sulfate saturation, the pellet collected was dissolved in 90 ml of 10 mM Na-phosphate buffer pH 7.0 and dialyzed against 40 volumes of the same buffer for 15 hr. The resultant lipoxygenase dialyzate was purified on a 2.5 × 40 cm DEAE-cellulose column equilibrated in the same phosphate buffer. The solution applied to the column was 3–5 ml containing 30–120 A_{280} units. The elution was performed, also with the same buffer, using the shallow linear salt gradient of 0.0–0.2M NaCl.

All the above isolation and purification steps were carried out in a cold room at a temperature of 4°C.

Protein determinations were done according to the method of Lowry et al. (1951), using crystalline bovine serum albumin as standard.

Disc-gel electrophoresis

A mixture of 8.5% acrylamide and bis-acrylamide (Bio Rad Labs, Richmond, Calif.) in a 45:1 weight ratio was applied in gels using 8 × 100 mm tubes. The gels and the electrode reservoirs contained 50 mM Na-phosphate buffer pH 7.1 and 0.1% sodiumdodecylsulfate (SDS; Fisher Scientific Co.). The protein-SDS complexes were prepared by incubating the purified pea seed or commercial soybean lipoxygenases together, or separately but simultaneously, with protein standards (conc 3–4 mg/ml) for 10 min in a boiling water bath in the presence of 1% SDS and 0.5 mM dithiothreitol. After incubation the solution was cooled to room temperature and then 60% glycerol was added to increase the density of the protein solution. A 50 μg pea or soybean protein sample and 4 μg of each standard protein (Schwartz/Mann Co., Orangeburg, N.Y.) were applied to the top of the gels (Weber and Osborn, 1969).

Electrophoresis was performed at room temperature at a constant current of 5 mA per tube for 5 hr. The gels were stained for 2 hr in 0.2% (w/v) of Coomassie Brilliant Blue (Schwartz/Mann Co.) in an aqueous solution containing 46% methanol and 9.2% glacial acetic acid, and destained electrophoretically in a Canalco model quick gel destainer for 25 min in a solution containing in v/v 7.5% acetic acid and 5% methanol.

Isoenzyme patterns of purified lipoxygenase isolated from fresh green peas and pea seeds were obtained on polyacrylamide gels following the procedure of Ornstein and Davis (1960). However, the stacking gel was replaced with 30% glycerol. In this solution the amount of protein applied was 100 μg per tube. The small pore gel contained 1% potato amylose. The specific staining technique applied was that of Guss et al. (1967a). The enzyme extracts in 10 mM Na-phosphate buffer pH 7.0 were applied without a preliminary readjustment of their pH, and neither a preliminary or simultaneous treatment of the gels with thioglycollic acid was performed (Hale et al., 1969).

To avoid the possibility of obtaining an altered electrophoretic pattern due to an interaction of pea lipoxygenase with free fatty acids, defatted enzyme samples were also run. However, when the crude enzyme was extracted with a cold chloroform-methanol solvent (2:1 v/v), followed by acetone, the isoenzyme pattern was similar to that of unextracted enzyme. This observation agreed with the results obtained by Hale et al. (1969). Hence, the defatting step was abandoned and the zymograms reported are for the isoenzymes as present in situ.

Lipid fractionation

Isolation of pea neutral and phospholipids. The neutral lipid fraction was isolated from the seeds by hot chloroform-methanol (2:1 v/v) extraction. Nonlipid contaminants were removed by Sephadex G25 following essentially the technique of Williams and Merrilees (1970). From the purified lipid extract the neutral lipid fraction was separated and further fractionated into triglycerides, free fatty acids, sterols and plant pigments as already described (Haydar and Hadziyev, 1973b).

Phosphatidylcholine (PC), phosphatidylethanolamine (PE) and diphosphatidylglycerol (DPG) were isolated and purified in good yields from the mitochondria of germinated 4-day-old pea cotyledons (Haydar and Hadziyev, 1974).

Pea galactolipids. The isolated chloroplasts of the leaves of 10-day-old germinated pea seedlings were used as a rich source of galactolipids. The isolation of chloroplasts and their purification by a discontinuous sucrose gradient centrifugation has been described (Haydar and Hadziyev, 1973a). For galactolipids isolation the procedure described by Rosenberg et al. (1966) and by Gardner (1968) was adapted and modified for preparative scale work.

Silicic acid, 150g, was slurried in n-heptane and poured into a 4.5 × 50 cm column. Heptane was drained off and the column was washed with 1 liter chloroform. The crude chloroplast lipid extract, 7 ml (520 mg lipid), was applied to the column and eluted with 1 liter chloroform, followed by 500 ml chloroform-acetone (1:1 v/v) and then by 500 ml acetone, followed by 500 ml methanol. The separation was performed at 2–4°C in a column protected from light. Effluents were collected in 11 ml fractions, examined by thin-layer chromatography and their phosphorous and galactose content determined. Phosphorus was determined by the procedure of Bartlett (1959), and galactose by the method of Dubois et al. (1956). The chloroform-acetone effluent containing monogalactosyldiglycerides and the acetone effluent containing digalactosyldiglycerides were concentrated and subjected to one dimensional thin-layer chromatography using a 500μ layer of silica gel G, and acetone-acetic acid-water (10:2:1, v/v) as the developing solvent.

The sugar identity in the isolated galactolipids was confirmed by subjecting the lipids to methanolysis and separating the released sugars which were then silylated and analyzed by gas liquid chromatography (Sweeley et al., 1963). Trimethylsilyl derivatives of the sugar components of approximately 5 mg of the lipid were prepared by the addition of 0.5 ml of a mixture (10:2:1, v/v) of dry pyridine, hexamethyldisilazane and trimethylchlorosilane (Pierce Chem. Co., Rockford, Ill.). After 15 min, 1–5 μl of the reaction mixture was injected into a U-shaped 6-ft column, 1/4" o.d., packed with 3% OV 17 on chromosorb W, HP. The operating conditions of the gas chromatographic analysis were: injector temperature 250°C, detector temperature 270°C, nitrogen carrier gas flow rate 30 ml/min, and programmed column temperature from 120 to 200 at 4°C/min.

Fatty acid analysis. Fatty acid methyl esters were obtained by a transesterification procedure. Neutral lipids (5–10 mg) were gently refluxed in 2 ml of 1% (w/v) sulfuric acid in methanol for 2 hr, while polar lipids (5–10 mg) were esterified under nitrogen with 2 ml of 2% (w/v) sulfuric acid in methanol in sealed ampules at 65°C overnight. Subsequent isolation of methyl esters and their analysis by gas liquid chromatography has been reported (Haydar and Hadziyev, 1973b).

Enzymatic oxidation of lipid substrates

The enzymes used in this study were the purified and characterized pea seed lipoxygenase, wheat germ lipase (Nutritional Biochemicals Corp., Cleveland, Ohio), and snake venom from Ophiophagus Hannah (Sigma Chem. Co., St. Louis, Mo.) with high phospholipase A_2 and galactolipase activities.

Lipoxygenase catalyzed oxidation of the lipids was followed polarographically at 20° or 25°C using a Biological Oyxgen Monitor Model 53, equipped with a Clark electrode (Yellow Springs Instruments, Ohio).

In the course of pea lipoxygenase purification the enzyme activity was followed using linoleic acid as substrate (Fisher Scientific Co.; freshly distilled at 149–150°C under a vacuum of 0.4 mm Hg). The substrate consisted of 7.5×10^{-3}M linoleic acid emulsified with 0.25% Tween-20 in a 0.1M TRIS-Cl buffer pH 7.0.

In the substrate specificity assay the linoleic acid, its methyl ester, or trilinolein were used with a purity better than 99%, and were supplied by Hormel Institute (Austin, Minn.). For the assay of individual pea polar lipids the lipids were emulsified ultrasonically in 0.1M borate buffer pH 7.0 in the presence of 0.04% sodium deoxycholate and 2.5 mM calcium acetate. Neutral lipids were emulsified in 0.1M borate buffer pH 7.4 containing 0.4M NaCl, 5 mM $CaCl_2$ and 2 mg sodium taurocholate and 3 mg gum acacia per ml of buffer.

Preliminary work on the activity of lipoxygenase in the buffer media for lipase and phospholipase proved that the media constituents, except for Ca^{2+}, do not affect lipoxygenase activity. Also, the polarographic method of determining lipoxygenase activity was satisfactorily accurate to a linoleic acid molarity less than 1 mM in the above media. Hence, suitable amounts of the individual lipids were included in the media to give a concentration of bound linoleic acid ranging between 1 and 2.3 mM.

The substrate was divided into two aliquots, 3 ml each, one of which was incubated with the hydrolyzing enzyme while the other was used as a control. Substrates of the polar lipids were incubated with 1 mg of snake venom by shaking at 37°C for 1 hr while substrates containing neutral lipids were incubated with 10 mg lipase at 25°C for 3 hr after which the pH of 7.4 was adjusted to 7.0–7.2 by a few drops of 0.5N HCl. Both controls and incubated samples were checked for lipoxygenase activity.

RESULTS & DISCUSSION

Isolation and characterization of seed lipoxygenase

The crude extract of pea seeds, after ultracentrifugation and protein precipitation at 25–50% ammonium sulfate saturation, yielded a four times concentrated preparation, which maintained its initial lipoxygenase activity for more than a year during storage at 4°C. However, the preparation possessed a substantial activity of catalase and peroxidase enzymes.

Gel filtration on Sephadex G150 (Fig. 1) resulted in threefold purification of the lipoxygenase. This enzyme together with a major portion of catalase and peroxidase was located behind the high molecular weight protein that was first eluted.

The partially purified lipoxygenase, present in tube numbers 11–23, was further purified on a DEAE-cellulose column. This step brought about a further twofold purification and the activity peak coincided with the absorption curve of the first eluted peak. In addition, the purified material was free from any detectable catalase or peroxidase activities.

Recently, Weber et al. (1973) reported that soybean extract under similar conditions of purification on a DEAE-cellulose column brought about three peaks with lipoxygenase activity. The peaks corresponded to those obtained by Christopher et al. (1972) using DEAE-Sephadex as an anion exchanger. The enzymes under each peak were well characterized and designated in order of elution as lipoxygenase isoenzyme-3, -2 and -1 or Theorell enzyme (Christopher et al., 1970; 1972). However, when Weber et al. (1973) repeated the fractionation of an extract from fresh green peas, two lipoxygenase peaks were obtained in an elution sequence corresponding to those of soybean isoenzymes-3 and -2. The third, Theorell enzyme, was absent. This, and our similar result for fresh green peas as well as the results for stored pea seed, suggested that the green peas contain at least a storage resistant and a storage sensitive lipoxygenase. When the isolation and purification steps applied for green peas were exactly reproduced, the elution sequence of seed enzyme coincided with that of isoenzyme-3 (see Fig. 2).

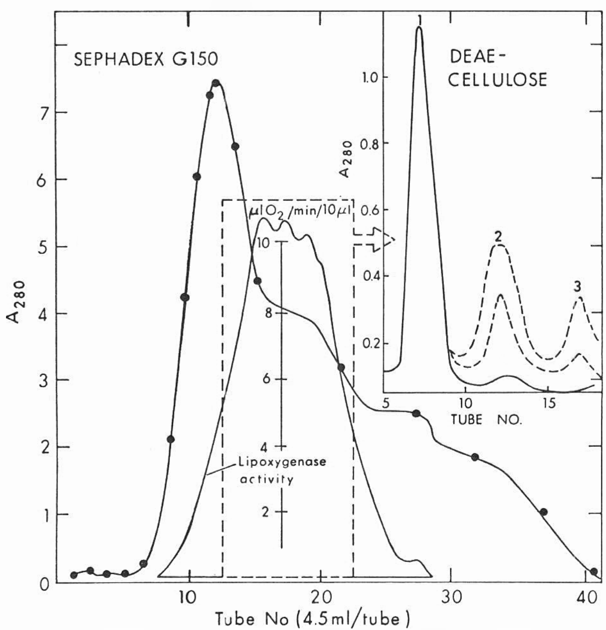

Fig. 1–Pea seed lipoxygenase purification. The fractions collected from Sephadex column were dialyzed in TRIS-Ca^{2+}-SH buffer and then applied to a DEAE-cellulose column. From this column the size of elution peaks 2 and 3 substantially decreased as the duration of the previous dialysis step was prolonged. For details see Materials & Methods.

Further, to establish the lipoxygenase peak identity, the enzyme was assayed for the relationship between its concentration and linoleic acid oxidizing activity.

This assay revealed a striking dependence of the activity on the enzyme concentration. The nonlinear relationship varied more than threefold over an enzyme range of 0.1–0.8 A_{280} units under the assay condition in which the rate of oxygen uptake by linoleic acid was proportional to reaction time. These results were similar to those of soybean lipoxygenase-3 reported by Christopher et al. (1972).

An additional assay, the response of activity to Ca^{2+}, is illustrated on Figure 3. In a concentration range of 0 to 4 mM Ca^{2+} all concentrations were inhibitory. The inhibition was significant with enzyme preparation, regardless whether it was isolated and subsequently purified in the presence or absence of Ca^{2+}. However, the initial activity and those observed with added Ca^{2+} were consistently higher for the enzyme isolated, purified and, finally, fractionated on a DEAE-cellulose column in the absence of Ca^{2+}. This finding is essentially similar to that observed recently for soybean lipoxygenase (Restrepo et al., 1973).

The enzyme activity as a function of pH using linoleic acid as a substrate showed a broad pH profile throughout the range of pH 4.5–9.5. The optimum activity was found around pH 7.0–7.2 (see Fig. 4). Under the same conditions, the linoleic acid esters such as methyl ester and trilinolein showed similar responses. This indicated that the enzyme may correspond to both a "linoleic acid" and a "triglyceride" lipoxygenase.

The enzyme assays as well as the elution pattern on the DEAE-cellulose column, as carried out in this lab (Walker, 1975), suggested that the storage resistant pea lipoxygenase is

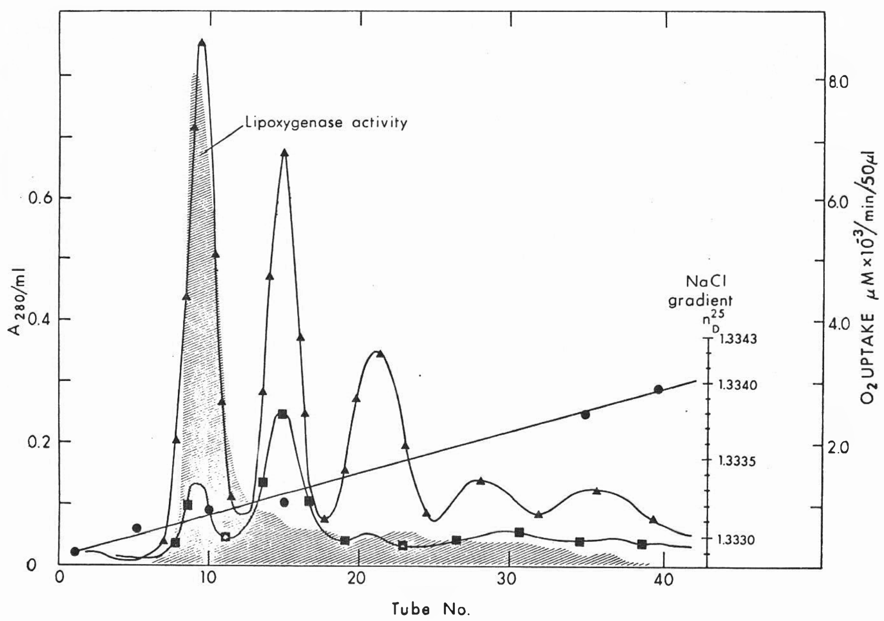

Fig. 2–Pea seed phosphate buffer extract and its purification on the DEAE-cellulose column. -▲-▲- Dialyzate applied straight to DEAE-cellulose column; -■-■- Dialyzate previously purified on a column of Sephadex G150. For details see Materials & Methods.

similar or equal to soybean lipoxygenase-3, isolated and characterized by Christopher et al. (1972).

Additional characterization of the pea seed lipoxygenase peak involved the determination of the electrophoretic mobilities of the proteins present in the peak. Sodiumdodecylsulfate (SDS) in combination with dithiothreitol (DTT) was applied. As proved by Weber and Osborne (1969) this mixture resolves the proteins into their single polypeptide chains whose electrophoretic mobilities in polyacrylamide gels are closely related to their molecular weights. However, instead of using marker proteins covering a range of 12,400–67,000 Daltons as applied previously (Haydar and Hadziyev, 1973a), Human-γ-globulin was incorporated among marker proteins. Thus, the molecular weight range was extended to 160,000 Daltons. As seen from Figure 5, the semi-log of the marker's molecular weight versus distance of migration relative to the tropomyosin (Rm = 1) is a straight line.

When the five markers together with lipoxygenase activity peak were subjected to electrophoresis, an additional five protein bands were revealed. From the molecular weight calibration curve the corresponding molecular weights were 106,000, 76,000, 29,000, 12,600 and less than 10,000 Daltons. The first, lowest mobility band of 106,000 Daltons was consistently most abundant to the extent of at least 40% of the total protein applied to the gel, with the second band close to 30% of the total (Fig. 6).

For comparison, commercial soybean lipoxygenase was also subjected to electrophoresis. As seen on Figure 6 the commercially purified soybean lipoxygenase consists of six protein bands among which 49% of the total protein is present in the single major band corresponding to a molecular weight of 106,000 Daltons. This result compares well with the molecular weights reported for commercially purified soybean lipoxygenase and for soybean lipoxygenases-2 and -3, obtained by a Sephadex G150 gel filtration technique (Christopher et al., 1972).

Thus, the storage resistant pea lipoxygenase appeared to have a molecular weight equal to that of soybean lipoxygenase-3. In addition similarity in molecular weights obtained by the gel filtration technique and by gel electrophoresis as applied in this study (Walker, 1975) suggested that neither enzyme is made up of subunits. In agreement with this, when both enzymes were incubated either at 40°C for 30 min, or 100°C for 10 min or longer in the presence of 1% SDS and 0.5 mM DTT, and then subjected to electrophoresis, a decrease in protein content present in their major bands did not occur.

Finally, the electrophoretograms of both enzymes revealed a molecular weight for the second most abundant band of 76,000 Daltons for peas and 70,050 for soybean. While the latter protein band identity is still obscure, the former pea band most likely represents the major lipoxygenase protein species of green peas which was earlier characterized by Eriksson and Svensson (1970).

The disc-gel electrophoresis runs were repeated to further clarify the identity of pea lipoxygenase proteins, but in order to preserve their enzymatic activity the proteins SDS-DTT treatment was omitted. By using the specific staining technique of Guss et al. (1967a) zymograms were obtained (Fig. 7). There was only one broad activity band for soybean lipoxygenase at Rf 0.40 relative to the dye as marker, this band coinciding with that of peas.

The zymogram patterns obtained after 1, 4, 6 and 12 months of seed storage suggested that the fresh green peas are rich in a higher mobility band to which a molecular weight of 76,000 Daltons was ascribed. The band was rather broad indicating the probability it contained more than one active component. The lower mobility upper band corresponding to 106,000 Daltons was also a broad band and upon prolonged storage only this band retained its enzyme activity.

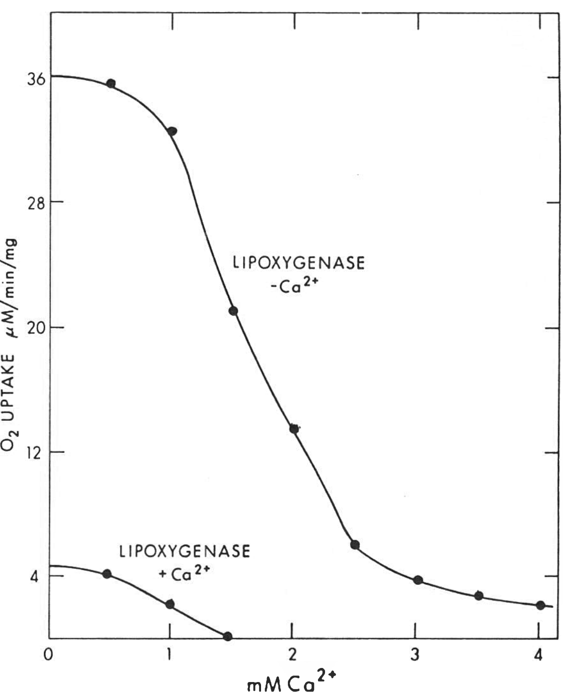

Fig. 3—Effect of Ca^{2+}-ion on purified pea lipoxygenase. The enzyme was purified on Sephadex and DEAE-cellulose columns using the TRIS-SH buffer, and in the case of lipoxygenase prepared in the presence of Ca^{2+}, also 2 mM $CaCl_2$. The results recorded at 25°C are given in μmoles O_2 min^{-1} per mg of purified enzyme protein.

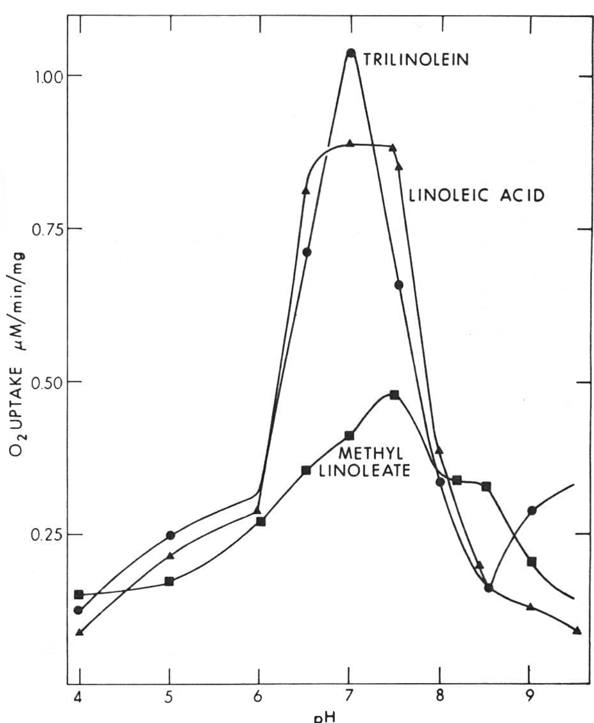

Fig. 4—Pea lipoxygenase activity as a function of pH and substrate. The linoleic acid, its methyl ester and trilinolein were used as substrates at 20, 20 and 40 mg% concentrations, respectively, in the presence of 0.25% Tween-20 as an emulsifier. Equal amounts (2 μg) of the enzyme extract were used in 50 mM buffer solutions; acetate for pH 4.0–5.0, Na-phosphate for pH 6.0–8.0 and Na-borate for 8.5–9.5. Results are given at 20°C as O_2 uptake in μmoles min^{-1} per mg of purified enzyme protein.

Pea seed neutral and phospholipids

The pea seed neutral lipids were shown by one dimensional thin-layer chromatography to consist mainly of triglycerides, small amounts of diglycerides, free fatty acids, free and esterified sterols and pigments. The nonpolar developing solvent used resulted in clear separation of pure triglycerides needed for substrate specificity studies.

The primary components of pea seed polar lipids were phosphatidylinositol, 17.1%, phosphatidylcholine, 54.3%, and phosphatidylethanolamine, 16.1% of the phospholipid-P. Their corresponding Double Bond Indices (summation of the weight percent of fatty acid, multiplied by the number of double bonds it contains per molecule and divided by 100) were 0.59, 0.78 and 0.94, respectively. However, when the same lipids were isolated from purified mitochondria their unsaturation degrees were substantially higher. Therefore, the mitochondrial lipids were chosen for substrate specificity studies (see Table 1).

Pea galactolipids

When galactolipids were isolated from bleached seeds their unsaturation degrees were low, suggesting their possible degradation during prolonged storage. The corresponding values of Double Bond Index were 0.77 for MGDG and 1.04 for DGDG. Hence, the pea leaf chloroplasts were chosen as a source of undegraded lipids.

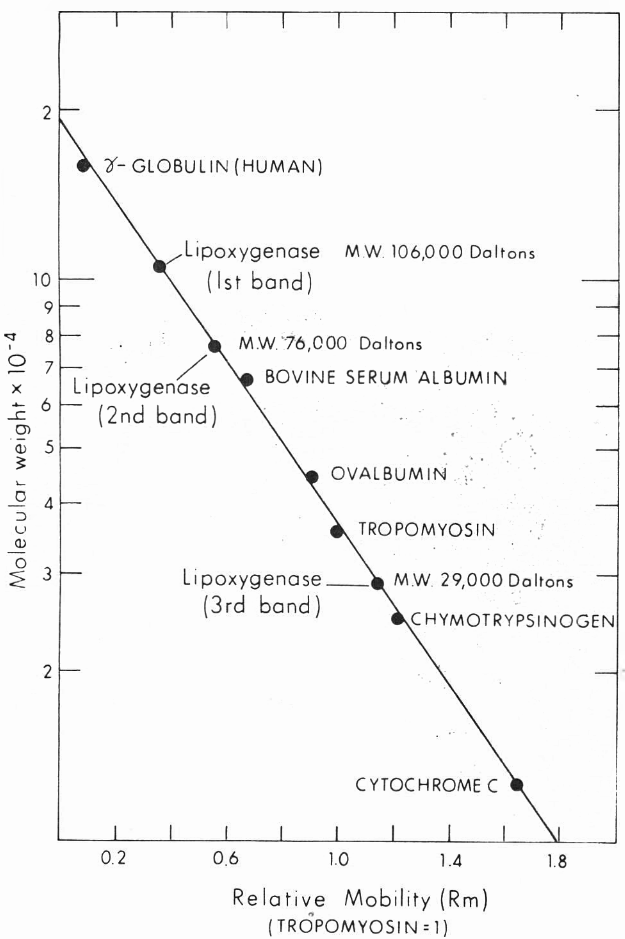

Fig. 5—Semi-log calibration curve used for determination of lipoxygenase molecular weight. The equation for the line of best fit drawn through the data points is y = mx + b; where m = −0.74658; b = 5.30288; y = log mol wt and x is the distance of protein migration relative to tropomyosin.

The results of the column chromatographic separation of pea chloroplasts galactolipids is shown in Figure 8. Chloroform-acetone (1:1) eluted the fraction containing monogalactosyldiglycerides, while acetone eluted that containing digalactosyldiglycerides. Both fractions were still contaminated with small amounts of polar lipids and/or pigments. The bulk of pigments was separated by elution with chloroform, while the majority of phospholipids was retained in the column until eluted by methanol. The traces of residual pigments and/or phospholipids were readily eliminated by subsequent preparative thin layer chromatography. The gas chromatographic analysis of the silylated sugars for both galactolipids gave three peaks. The first two corresponded to α- and β-galactopyranosides and had higher retention times than the last one which represented galactofuranosides. Despite the clear separation of these peaks it was not possible to ascertain the form of galactose present in each lipid. Thus the presence of two anomeric forms of silylated D-galactosides might reflect a possible mutarotation of the sugar occurring during methanolysis or silylation steps.

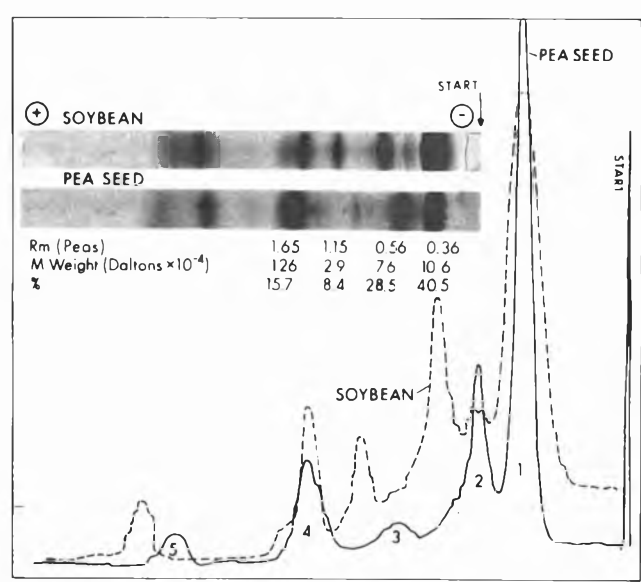

Fig. 6—Polyacrylamide gel electrophoresis of the pea seed and soybean purified lipoxygenases. Protein bands stained with Coomassie blue were scanned at 620 nm using Joyce Loebl's integrating and recording densitometer. For details see Materials & Methods.

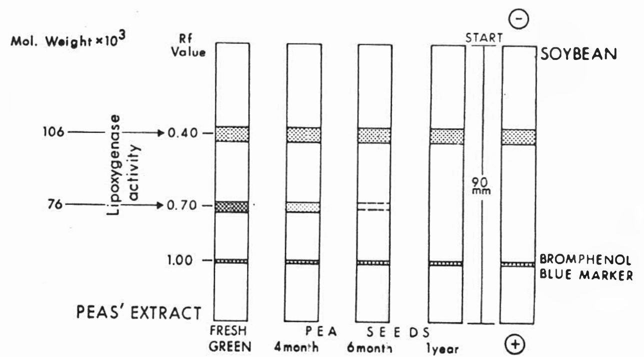

Fig. 7—Isoenzyme patterns of purified lipoxygenase isolated from fresh green peas and pea seeds. The isoenzyme patterns were not affected by potassium cyanide at a concentration of 5 × 10⁻³ M.

Enzymatic oxidation of lipid substrates

The pea seed lipoxygenase-substrate specificity study has been carried out on the isolated neutral lipids, on phosphatidylcholine, phosphatidylethanolamine and diphosphatidylglycerol as the major pea mitochondrial phospholipids, and on mono- and di-galactosyldiglycerides as the major chloroplast galactolipids. The fatty acid composition of these substrates is presented in Table 1. Replacing free linoleic acid as substrate by the above lipid samples, the oxidation results obtained with purified pea lipoxygenase are presented in Table 2. In addition, some of the oxygraph tracings are shown in Figure 9.

As seen from Table 2, oxygen consumption was negligible with galactolipids and all phospholipids but not with the neutral lipids used as substrate. The neutral lipid fraction gave a many-fold higher consumption of oxygen than any of the individual polar lipids tested. The presence of free fatty acids and mono- and di-glycerides in the neutral lipid fraction of pea seeds was suspected to be responsible for the high substrate reactivity. Thus, when enzymatic oxidation was repeated with identical amounts of pure triglycerides recovered from thin-layer plates, a decrease in the oxidation rate from 0.119 to 0.090 μM O_2/min was recorded, i.e., a decrease amounting to 25%. When the latter was preincubated with lipase, the activity of lipoxygenase, due to the release of free fatty acids, increased by 1.5 times and even exceeded by 12% the activity obtained with the unpurified neutral lipid fraction. These results suggested that the lipoxygenase oxidation is enhanced when the substrates contain both the triglycerides and the free fatty acids. As proved by TL-chromatography, the suggestion is valid, since pure triglycerides incubated with lipase still contained unhydrolized triglycerides.

The substrate specificity study with phospho- and galactolipids revealed a different trend. In the absence of free fatty acids, both mono- and di-galactosyldiglycerides had a negligible oxygen consumption rate of about 6×10^{-3} μM/min, a rate which changed only slightly when phosphatidylcholine, phosphatidylethanolamine or diphosphatidylglycerol were used as substrates. This low oxidation rate with individual polar lipids was about 20 times less than that obtained for pure triglycerides.

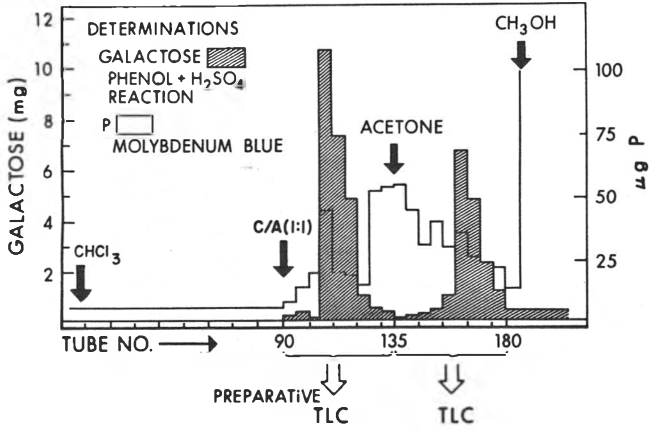

Fig. 8–Separation of monogalactosyldiglycerides and digalactosyldiglycerides from pea chloroplast lipids by column chromatography on silicic acid. For details see text.

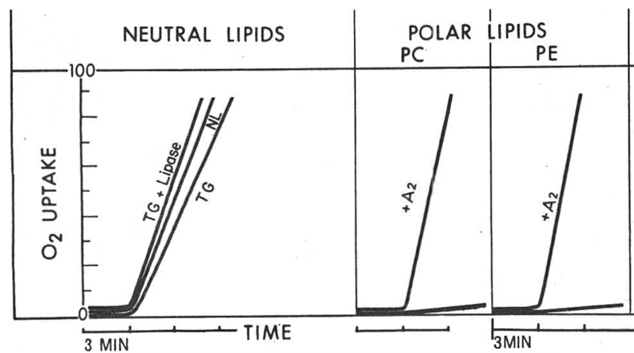

Fig. 9–Oxygraph tracings of oxygen consumption during lipoxygenase catalyzed oxidation of pea seed neutral lipids (NL), their triglycerides (TG) with and without lipase, and of pea mitochondrial phosphatidylcholine (PC) and phosphatidylethanolamine (PE) before and after their incubation with phospholipase A_2. For details see text.

Table 1–The fatty acid composition of lipids used in the pea seed lipoxygenase substrate specificity study

Fatty acid[a]	NL[b]	PC[c]	PE[c]	DPG[c]	MGDG[d]	DGDG[d]
10	tr	tr	tr	0.6	tr	tr
12	tr	tr	0.6	0.8	tr	tr
14	tr	tr	0.9	2.0	tr	0.5
15	tr	tr	tr	1.8	tr	tr
16	11.4	11.2	10.5	17.0	1.7	5.9
16:1	tr	tr	tr	3.3	0.5	tr
16:2	tr	0.6	tr	0.8	tr	tr
18	4.0	7.3	4.7	11.3	1.0	1.7
18:1	27.6	19.8	12.4	22.5	2.8	2.4
18:2	48.1	58.0	68.2	36.7	11.7	5.4
18:3	8.3	2.4	2.4	2.8	82.0	83.8
DBI[e]	1.57	1.44	1.56	1.09	2.73	2.65

[a] The results are expressed as % of total fatty acid content; tr: less than 0.5%
[b] NL, neutral lipids isolated from seeds
[c] PC, phosphatidylcholine; PE, phosphatidylethanolamine; and DPG, diphosphatidylglycerol, all isolated from pea mitochondria
[d] MGDG, monogalactosyldiglyceride; DGDG, digalactosyldiglyceride, both from pea leaf chloroplasts
[e] DBI, double bond index. These values are expressed as the summation of the weight percent of fatty acid multiplied by the number of double bonds it contains per molecule, and divided by 100.

Table 2–Oxygen uptake (μM O_2/min) by different lipid substrates in the presence of lipoxygenase, phospholipase A_2, galactolipase and triglyceride-lipase

Substrate	Control, lipoxygenase	+ Phospholipase A_2 (snake venom)	Activity increase (times)
Polar lipids			
Phosphatidylcholine	0.005[a]	0.209	42
Phosphatidylethanolamine	0.004	0.223	56
Diphosphatidylglycerol	0.006	0.162	27
Monogalactosyldiglycerides	0.006	0.382	64
Digalactosyldiglycerides	0.007	0.362	52
Neutral lipids	**Control, lipoxygenase**	**+ Lipase**	**Activity increase (times)**
Crude neutral lipids	0.119	–	–
Pure triglycerides	0.090	0.133	1.5

[a] Values are averages of at least two determinations

Although all of the polar lipids investigated contained a high content of the 1,4-pentadiene system required by lipoxygenase, they proved to be poor substrates. When enzymatic hydrolysis with snake venom phospholipase A_2 was performed, and the same system in the presence of the released free fatty acids tested for lipoxygenase action, the rates of oxidation became very high. As seen in Table 2, for the three phospholipids tested an increase in the oxidation rate to about 200×10^{-3} μM O_2/min was obtained.

A large increase of the oxidation rate for galactolipids when incubated with snake venom was also obtained. In comparison to the results of oxygen uptake before incubation, there was a 64-fold increase for mono- and a 52-fold increase for di-galactosyldiglycerides. From these observed increases, it appears that, like phospholipids, galactolipids are also poor substrates for lipoxygenase attack unless hydrolyzed.

In an earlier study Sastry and Kates (1964) found that leaves of Phaseolus had a significant galactolipid hydrolyzing activity, which was absent in soybean, a member of a closely related family. Of the galactolipase preparation from sources other than plants they found that venom of Agkistrodon piscivorus was devoid of galactolipase activity toward mono- but not di-galactosyldiglyceride. In our study, no attempt was made to isolate the leaf galactolipase. Instead the venom of Ophiophagus Hannah was used since it equally hydrolyzed both of the galactolipids.

Despite the adequate evidence in the literature suggesting the presence of lipoxygenases specific for free fatty acids and for triglycerides (Koch et al., 1958; Dillard et al., 1960), the purified pea seed lipoxygenase devoid of lipase activity can oxidize both triglycerides and free fatty acids but does not oxidize any of the polar lipids investigated. The preferential oxidation of free fatty acids over triglycerides in peas has been suggested earlier by the determination of acid and peroxide values (Wagenknecht and Lee, 1958). However, our results supported by direct evidence using purified pea lipid substrates and enzyme showed in wheat germ lipase incubated pea triglycerides a preferential oxidation of free fatty acids only to an extent of 1.5 times, and the oxidation of pure triglycerides to be considerable. Also, it is obvious that this difference should depend on pH (see Fig. 4). In addition, the adjunct action of lipase and lipoxygenase might also contribute to this difference since lipases from some seeds favor hydrolysis of linoleic acid to that of saturated acids (Berner and Hammond, 1970).

Apart from a study using as substrates galactolipids contaminated with cerebrosides and reporting negligible lipoxygenase catalyzed oxidation of these lipids (Guss et al., 1967b), no other polar lipids appear to have been studied as substrates. It was repeatedly suggested that a major portion of off-flavor development in unblanched peas is accompanied by breakdown of phospholipids but the possible additive action of phospholipases and lipoxygenase was not investigated. However, this study established that phospholipase action on three phospholipids is prerequisite to their oxidation by lipoxygenase.

There is little information about the occurrence of phospholipases A and/or B in plants (Barron, 1964), although phospholipase B has been reported in barley malt (Acker and Geyer, 1969). Recently Galliard (1970) reported the rapid formation of free fatty acids and their oxidation products from phospho- and galactolipids in potato tuber homogenates. He also described the phospholipid and galactolipid hydrolase activities which, together with lipoxygenase, were responsible for such a breakdown of endogenous lipids. In pea seeds, a high activity of phospholipase D is present and remains appreciable for at least one year after drying the seeds (Quarles and Dawson, 1969), but whether phospholipase A_2 and/or B as well as galactolipase are present in pea seeds remains to be investigated.

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EFFECT OF GAMMA-IRRADIATION ON RED GRAM (Cajanus cajan) PROTEINS

INTRODUCTION

DRY LEGUMES form the major and widely used food items of protein in average Indian diets. Though legume proteins have low biological values compared to those of animal origin, they supplement in quantity and quality the cereal proteins generally deficient in lysine (Hegsted, 1969; Hutchinson, 1970). It is known that physico-chemical properties of proteins are modified by radiation treatment. The chemical nature of damage in irradiated proteins has been explained by molecular rearrangements resulting in condensation or polymerization (Carroll et al., 1952), degradation (Schweigert, 1959), hydrogen bond disruption and cleavages of intermolecular disulfide bonds (Casarett, 1968). Such changes frequently improve the physical quality of the final products (Moore, 1961).

The presence of trypsin inhibitors in a variety of Indian legumes and vegetables is well known (Liener and Kakade, 1969). This has been isolated, purified and characterized from soyabean (Kunitz, 1947), field bean (Banerji and Sohonie, 1969) and *Lathyrus sativus* (Roy and Srinivasa, 1971). The deleterious effects of trypsin inhibitor on protein digestibility and nutritive value of a number of legumes consumed in this country have been extensively studied (Phadke and Sohonie, 1962).

We have examined the potential for use of radiation processing for improved stabilization on storage of pulses, particularly of red gram (*Cajanus cajan*). It was observed that irradiated (1 Mrad) red gram showed a reduction in cooking time, improvement in texture and better retention of B vitamins on cooking (unpublished).

In the present investigation, the effect of irradiation on red gram proteins in terms of total and free amino acid profiles was studied. Radiosensitivity as well as in vitro enzymic susceptibility of red gram proteins were assessed by characterizing the degradation products formed by irradiation and proteolytic digestion.

MATERIALS & METHODS

RED GRAM was purchased from a local market. Cytochrome C, polymixin B-sulfate and amino acids were purchased from Sigma Chemical Co., USA. Crystalline pepsin (from hog stomach mucosa, Cat. No. P-6895) and crystalline trypsin (from bovine pancreas, Type III, Cat. No. T-8253) were also Sigma products. Erepsin (2451 h, batch No. 24491) was obtained from Koch-Light Lab Ltd.

Irradiation procedure

100-g lots of the pulse were packed in polyethylene bags and exposed at ambient temperature (25°C) to a cobalt-60 source of gamma radiation (Gamma Cell 220, Atomic Energy of Canada Ltd.) having a flux of 15 Krad/min, at 1–3 Mrad dose levels. Absorption of radiation was checked with ferrous sulphate and ceric sulphate dosimetry (Weiss, 1952).

Amino acid analyses

Red gram flour (60 mesh) was hydrolyzed with 6N HCl at 110 ± 1°C, for 24 hr and excess acid was removed in vacuo. Residue was taken in 0.2M sodium citrate buffer, pH 2.2 and an aliquot used for quantitative separation of amino acids on a Beckman Unichrome Automatic Amino Acid Analyzer. Acidic and neutral amino acids were separated on a long column (0.9 × 54 cm) packed with resin PA-28 and eluted with citrate buffer pH 3.28 and 4.25, respectively. Basic amino acids were eluted from a small column (0.9 × 0.5 cm) packed with resin PA-35 at pH 5.28. A standard amino acid mixture was used to establish the resolution of individual amino acids as sharp peaks. Tryptophan was estimated colorimetrically in alkaline hydrolyzate using p-dimethylaminobenzaldehyde as a chemical reagent (Spies, 1967). To estimate free amino acids, pulse extract in 70% ethanol was passed through a Dowex 50 H^+ column (0.9 × 10 cm), eluted with 2N NH_4OH and lyophilized. Residue was taken in 0.2M sodium citrate buffer and processed as described above. Total proteins were estimated by a micro-Kjeldahl method (Oser, 1965).

In vitro enzymic digestibility

To study the stepwise enzymic digestion, red gram (≡ 5g protein) flour was suspended in 150 ml water (pH adjusted to 1.8 with 1N HCl) and incubated under toluene with pepsin (0.5 mg/ml) at 37°C for 24 hr with frequent shaking. This was followed by a further 24-hr incubation with trypsin (0.5 mg/ml, pH 8.2) (Ford and Salter, 1966). Aliquots were removed at indicated time intervals and alpha-amino nitrogen estimated in trichloroacetic acid (TCA) filtrates (Rosen, 1957). Erepsin was then added (0.5 mg/ml) and the digestion continued for a further 24 hr.

On completion of enzymic digestion, aliquot of the filtrate (≡ 25 mg N) was subjected to gel filtration on a Sephadex G-25 column (2.7 × 60 cm, pH 7.6). The position of markers of known molecular weights, like cytochrome C (mol wt 12,400), polymixin-B sulfate (mol wt 1470) and of a mixture of 18 amino acids were used to establish the resolution of the protein digest into undigested soluble proteins, peptides and free amino acids, respectively (Ford, 1965).

Separation of red gram proteins by gel filtration

Red gram flour (10g) was extracted with 180 ml AUC solvent (0.1N acetic acid, 3M urea and 0.01M cetyl trimethyl ammonium bromide) for 1 hr with constant shaking and centrifuged at 40,000 × G for 30 min (Meredith and Wren, 1966). An aliquot of the supernatant (≡ 25 mg protein) was applied on a Sephadex G-200 column (2.2 × 55 cm) and eluted with the same solvent. 2.5-ml fractions were collected using LKB automatic fraction collector; optical density was recorded at 280 nm.

Similarly, water-soluble proteins were separated on a Sephadex G-200 column and eluted with distilled water. Total proteins were estimated by a biuret method (Gornall, 1948).

Isolation and purification of trypsin inhibitor

Trypsin inhibitor was extracted by shaking 25g of red gram flour with 100 ml of 0.05N HCl for 1 hr at room temperature and centrifuging at 2,000 × G for 15 min.

Inhibitory activity was determined against trypsin following the method described by Sohonie and Bhandarkar (1955), with appropriate controls. Test extract (1 ml) and 0.2% trypsin (1 ml) were incubated at 37°C (pH 7.6) for 10 min and 5 ml of 5% skimmed milk powder (dispersed in water) was added. The reaction was stopped with 5% TCA after 10 min. Tyrosine was estimated in the filtrate colorimetrically using Folin's reagent (Lowry et al., 1951). Antitryptic activity was expressed as trypsin inhibitor units (T.I.U.), calculated according to Borchers et al. (1947).

RESULTS

Effect of irradiation on amino acid profile

Total proteins (22.97%) (N × 6.25) in red gram were not changed appreciably by radiation treatment up to 3 Mrad. No significant changes in the total amino acid profiles of unirradiated and irradiated (1 Mrad) red gram (Table 1) were observed.

Table 1–Effect of irradiation on total amino acids in red gram[a]

Amino acid	Control	Mrad 1	Mrad 2	Mrad 3
		(g/16g N)		
Aspartic acid	7.73 ± 0.52[b]	7.76 ± 0.41[b]	7.77 ± 0.35[b]	7.63 ± 0.56[b]
Threonine	3.36 ± 0.21	3.34 ± 0.13	3.28 ± 0.20	3.13 ± 0.19
Serine	4.36 ± 0.17	4.31 ± 0.26	4.12 ± 0.31	4.12 ± 0.30
Glutamic acid	23.71 ± 1.91	23.52 ± 1.21	22.76 ± 1.14	23.47 ± 1.08
Proline	3.54 ± 0.31	3.41 ± 0.15	3.17 ± 0.12	2.78* ± 0.32
Glycine	3.02 ± 0.20	2.96 ± 0.19	2.76 ± 0.09	2.95 ± 0.08
Alanine	3.88 ± 0.14	3.33 ± 0.22	3.52 ± 0.19	3.91 ± 0.23
Cysteine	trace	trace	trace	trace
Valine	3.55 ± 0.27	3.17 ± 0.24	3.38 ± 0.28	3.61 ± 0.30
Methionine	0.79 ± 0.09	0.85 ± 0.06	0.84 ± 0.01	0.81 ± 0.02
Isoleucine	3.14 ± 0.22	3.14 ± 0.25	3.04 ± 0.18	3.02 ± 0.23
Leucine	6.03 ± 0.67	5.77 ± 0.38	5.66 ± 0.32	5.15* ± 0.33
Tyrosine	1.45 ± 0.18	1.68 ± 0.07	2.04* ± 0.21	1.79 ± 0.11
Phenylalanine	8.79 ± 0.71	8.28 ± 0.53	8.43 ± 0.44	8.74 ± 0.53
Lysine	6.29 ± 0.32	6.22 ± 0.48	6.45 ± 0.37	6.84 ± 0.43
Histidine	3.22 ± 0.15	2.85 ± 0.11	3.23 ± 0.21	3.65 ± 0.24
Arginine	4.87 ± 0.28	4.93 ± 0.33	4.81 ± 0.40	5.46 ± 0.39
Tryptophan	1.25 ± 0.09	1.22 ± 0.07	1.22 ± 0.09	1.20 ± 0.02

[a] Red gram flour (≡ 20 mg protein) was hydrolyzed with 6N HCl at 110°C for 24 hr. Excess HCl was evaporated under vacuum at 50°C. The residue was taken in 20 ml 0.2M citrate buffer (pH 2.2) and an aliquot analyzed for total amino acids. Corrections were made in the values for serine and threonine to account for losses during acid hydrolysis. Values are averages of three independent analyses.
[b] Mean ± standard error
* Not significant

Tyrosine values in irradiated (1–3 Mrad) samples were consistently higher than in the control. A possible explanation can be that perhaps radiation treatment accentuates the release of tyrosine during acid hydrolysis. Glutamic acid contributed about 24% of the total values. However, free (nonprotein) amino acid values estimated in alcohol extracts were increased by about 15% at 1 Mrad (Table 2). The rates of release were not similar with different amino acids, e.g., significantly more lysine, phenylalanine and tyrosine were liberated due to radiation treatment, whereas, no appreciable increase in methionine level was observed.

In vitro enzymic digestibility

Results on in vitro enzymic digestion of red gram proteins are plotted in Figure 1. Susceptibility of proteins to successive 24 hr action of pepsin and trypsin was increased due to irradiation; comparatively more alpha-amino nitrogen was liberated from irradiated samples. However, differences were more pronounced in the peptic digests than in those where peptic action was followed by trypsin digestion. The digestive products were further separated as three distinct fractions on Sephadex G-25 column (Table 3). At the end of 72 hr digestion, a negligible amount of undigested soluble proteins was present in both the samples. However, about 22% more free amino acids, expressed in terms of leucine equivalents was recovered from irradiated (1 Mrad) samples with concomitant decrease in peptides. This suggests that amino acids arise primarily from peptide bond splitting. But the possibility of increased digestibility due to breakage of disulfide bonds cannot be completely ruled out.

Elution pattern of irradiated red gram proteins on Sephadex G-200 column

Distribution of red gram total proteins (extracted with AUC) according to their molecular weights, was obtained by gel filtration (Fig. 2). The elution curve contained four main peaks, viz. globulin, glutenin, albumin and nonprotein nitrogen, identified according to Tawde and Cama (1961). When the areas under the protein peaks were measured by planimeter, the combined areas under peaks A to C and under peak D were 74.3% and 25.7%, respectively, with the unirradiated control sample. On irradiation (1 Mrad) there was a shift in the distribution of protein peaks, the area under peaks A to C decreasing by 9%, with a concomitant increase in nonprotein

Table 2–Effect of radiation treatment on free amino acid contents of red gram[a]

Amino acid	Control	1 Mrad
	(mg/g nitrogen)	
Aspartic acid	2.52 ± 0.07	3.1 ± 0.24
Threonine	13.96 ± 0.80	14.7 ± 0.69
Serine	–	–
Glutamic acid	53.03 ± 3.2	58.95 ± 4.6
Proline	–	–
Glycine	10.89 ± 0.76	11.32 ± 0.68
Alanine	10.27 ± 0.34	10.04 ± 0.84
Cystine	traces	traces
Valine	2.16 ± 0.13	2.58 ± 0.10
Methionine	4.89 ± 0.21	4.93 ± 0.21
Isoleucine	100.90 ± 3.7	112.12 ± 2.8
Leucine	11.88 ± 0.92	13.36 ± 0.86
Tyrosine	0.6 ± 0.06	1.06 ± 0.08*
Phenylalanine	0.5 ± 0.02	1.11 ± 0.08**
Lysine	2.26 ± 0.13	3.49 ± 0.26*
Histidine	4.51 ± 0.19	3.78 ± 0.21
Arginine	23.31 ± 0.93	20.20 ± 0.38
Tryptophan	–	–

[a] 5-g samples of unirradiated and irradiated (1 Mrad) red gram flour were extracted thrice with 70% ethanol. Extracts were passed through cation exchanger columns and eluted with 2N NH_4OH. Amino acids were analyzed in the eluates. Values are means of three independent determinations, expressed as mg/g of original red gram N.
*$P < 0.01$
**$P < 0.001$

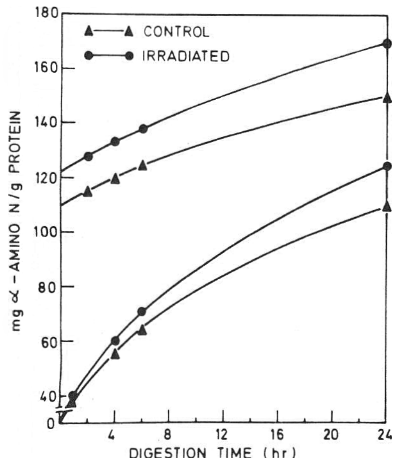

Fig. 1—In vitro digestibility of red gram proteins: Red gram flour (≡ 5g protein) was digested with pepsin (5 mg) followed by trypsin (5 mg) for 24 hr each at 37°C. Alpha-amino nitrogen liberated was measured at indicated time intervals and expressed in terms of leucine equivalent. The lower and upper sets of curves are for peptic and peptic followed by tryptic digestion, respectively.

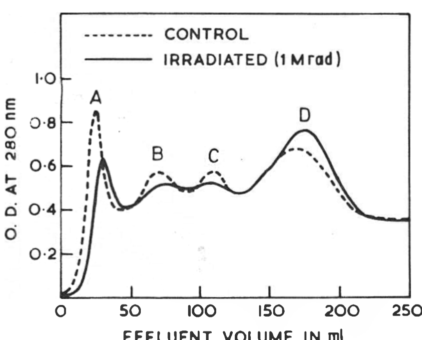

Fig. 2—Effect of radiation on the elution pattern of red gram proteins (total): An aliquot (≡ 25 mg protein) of red gram flour extracted with AUC, was applied on a Sephadex G-200 column. Optical density was recorded automatically at 280 nm; Peaks A, B, C and D tentatively represent globulin, glutenin, albumin and nonprotein nitrogen, respectively.

value in peak D. Resolution of water-soluble proteins from control and irradiated (1–3 Mrad) samples are shown in Figure 3. They were separated into two sharp peaks, contributing 47% proteins and 53% nonprotein entities, respectively (Table 4) in nonirradiated samples. In irradiated samples, a progressive decrease with increasing dose levels was observed in the area under peak 1 with a concomitant increase in that of peak 2. The continued shift of the elution volume of peak 2 (Fig. 3) from 75 ml in the control to 50 ml in the sample irradiated at 3 Mrad could be attributed to the changes in molecular weight of protein due to radiation treatment.

Activity of the trypsin inhibitor

Results compiled in Table 5 indicate that the antitryptic activity in red gram was not affected by irradiation up to 3 Mrad. Trypsin inhibitor was also found to be thermo-resistant. Activity was not affected even after 30 min of heating at 100°C (data not given).

DISCUSSION

RADIATION TREATMENT could be expected to bring about certain gross and subtle changes in foods. Total proteins and amino acid profiles of red gram were not changed appreciably due to irradiation up to 3 Mrad (Table 1). The amino acid composition of raw red gram obtained in the present study is comparable to the reported values (Hanumantha Rao and Subramanian, 1970). Radiation processing did not affect total proteins and amino acid contents of wheat (1 Mrad) (Srinivas et al., 1972), wheat bran (5 Mrad) (Moran et al., 1968) and semi-dried (40% moisture level) shrimp (0.25 Mrad) (Srinivas et al., 1974). Some specific and nonspecific amino acid damages have, however, been reported in irradiated wheat (Patten and Gordy, 1964). Sulphydryl (–SH) and disulfide (–S–S) groups in proteins are apparently more susceptible to irradiation (Lee, 1962). However, no appreciable loss in methionine, one of the limiting amino acids in red gram, was observed in irradiated samples up to 3 Mrad. At very high doses of 10 Mrad, reduction in cystine content of wheat has been reported (Doguchi, 1969). The observed increase of some free amino acids in irradiated red gram (Table 2) could be due to a breakdown of proteins. A similar release of free amino acids following radiation treatment has been reported with wheat (Srinivas et al., 1972), casein (McArdle and Desrosier, 1955) and shrimp (Srinivas et al., 1974).

Of the several characteristics that determine the nutritive quality of protein, its digestibility may be of prime importance. In vitro enzymic digestibility of red gram proteins was increased due to irradiation (Fig. 1). A similar increase in the liberation of free amino acids from irradiated wheat subjected to autolysis or proteolytic digestion (Srinivas et al., 1972) has been reported. Trypsin digestion of irradiated egg albumin or casein, also exhibited such increase (McArdle and Desrosier, 1955). However, radiation processing did not show any adverse effects on the protein efficiency ratio (PER) of wheat (20 Krad) (Vakil et al., 1973), pepsin digestibility ratio (PDR) index of turkey and pork (Sheffner et al., 1957) as well as true digestibility or biological value of gluten (2.8 Mrad) (Metta and Johnson, 1959). Increase in net protein utilization in chicks fed irradiated (0.4 or 5.0 Mrad) wheat has been observed (Moran et al., 1968).

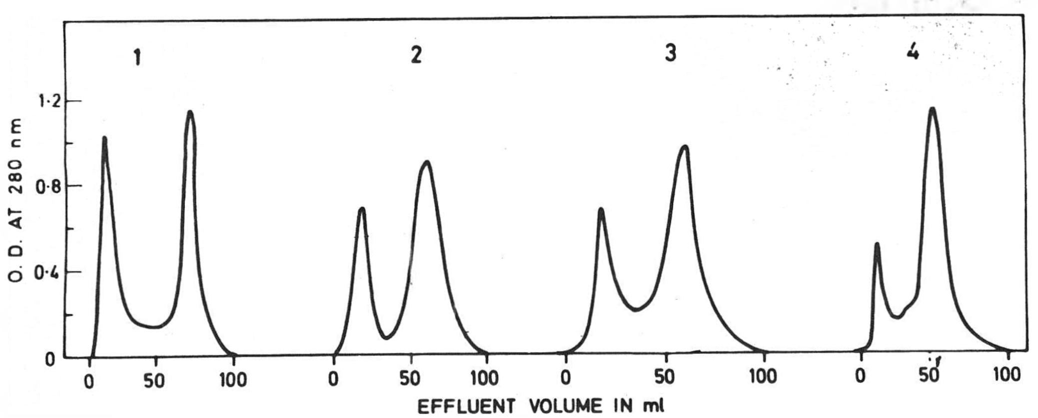

Fig. 3—Effect of irradiation on water-soluble proteins of red gram: Red gram flour (5g) was extracted with 25 ml of distilled water for 2 hr. An aliquot (≡ 16 mg protein) was applied on a Sephadex G-200 column equilibrated with water and having a flow rate of 2 ml/min. 2.5 ml fractions were collected in each tube using LKB fraction collector. Curves 1, 2, 3 and 4 represent control and samples irradiated at 0, 1, 2 and 3 Mrad, respectively.

Table 3–Fractionation of enzymic digests of red gram proteins[a]

Fraction	Control	Irradiated (1 Mrad)
	(mg leucine equivalent/25 mg N)	
Soluble proteins	0.48 ± 0.01	0.44 ± 0.02
Peptides	69.5 ± 2.3	57.7 ± 1.6*
Free amino acids	81.0 ± 2.7	98.7 ± 3.8

[a] 6 ml (≡ 25 mg N) of combined enzyme digest was separated on a Sephadex G-25 column, eluted with 0.02M phosphate buffer and effluent collected (7.5 ml each) on LKB fraction collector. Position of each fraction was established by using markers as described in the text. Combined eluates from each peak were pooled separately, hydrolyzed with 6N HCl for 18 hr and alpha-amino nitrogen estimated. Values are expressed in terms of leucine equivalent and are means of three determinations.
* $P < 0.01$

Table 4–Separation of water-soluble proteins of red gram by gel filtration

Treatment (Mrad)	Protein distribution[a] Peak 1	Peak 2
	(% of total area)	
0	47 ± 1.4	53 ± 2.1
1	34 ± 1.9	66 ± 2.4*
2	23 ± 2.1	77 ± 3.6
3	16 ± 1.2	84 ± 2.5

[a] Total area of protein peaks (Fig. 3) for unirradiated and irradiated (1, 2, 3 Mrad) samples (Curves 1 to 4) was measured by planimeter. Area of two individual peaks in each curve was calculated and expressed as % of total area.
* $P < 0.001$

Table 5–Effect of irradiation on the activity of trypsin inhibitor[a]

Treatment (Mrad)	Trypsin inhibitor units (T.I.U./g)
0.0	312.6×10^{-4}
0.5	318.3×10^{-4}
1.0	310.2×10^{-4}
2.0	308.9×10^{-4}
3.0	312.3×10^{-4}

[a] Trypsin inhibitor from control and irradiated (0.5 to 3 Mrad) red gram was extracted with 0.05N HCl and its activity determined as described in text. Tyrosine released due to action of trypsin in presence or absence of trypsin inhibitor was determined and T.I.U. calculated.

Thus, the observed enhancement in proteolytic digestion may be attributable either to the degradation of proteins present in the legume, making it more susceptible to enzyme action or to the partial destruction of trypsin inhibitor. Fragmentation of red gram proteins to low molecular weight entities was revealed when AUC or water soluble proteins were separated by molecular seiving on a Sephadex column (Fig. 2 and 3). Similar degradation of wheat proteins on irradiation has been reported (Srinivas et al., 1972). The presence of heat resistant anti-tryptic factor in red gram has been demonstrated. Tawde (1961) has shown that partially purified trypsin inhibitor from this legume reacts stoicheometrically with trypsin, the combination ratio being one. It is known that the thermostability of the antiproteolytic factors varied widely from the highly heat labile one of soyabean (Kunitz, 1947) and wheat flour (Shyamala and Lyman, 1964) to the moderately heat-labile one of field bean (Sohonie and Ambe, 1955) and to the extremely heat stable factor of lima beans (Tauber et al., 1949) and green gram (Honawar and Sohonie, 1959). It was observed in the present studies that the activity of trypsin inhibitor was comparable in control and irradiated red gram samples (Table 5). Beneficial effects of radiation treatment on soyabean protein utilization was attributed to the inactivation of trypsin inhibitor (Moore, 1961). This observation also indicates a close relationship between heat lability and susceptibility to radiation of the trypsin inhibitor. Thus, the observed degradation of proteins in the irradiated pulse was responsible for the increased enzymic digestibility, largely by pepsin rather than by trypsin, the presence of the trypsin inhibitor masking trypsin action.

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BOUND PHENOLIC ACIDS IN Brassica AND Sinapis OILSEEDS

INTRODUCTION

THE MAJOR PHENOLIC constituent of *Brassica* and *Sinapis* oilseeds is sinapine, a choline ester of sinapic acid. Sinapine was isolated from seed of *B. napus* and *B. campestris* (Clandinin, 1961; Austin and Wolff, 1968) and from the related crucifer *Crambe abyssinica* (Austin and Wolff, 1968). Other such polyphenols as flavonol 7- and 3,7-glycosides were found in the leaves and flowers of 26 species and cultivars of *Brassica* and kaempferol 7-glucoside-3-sophoroside in the seed extracts by Durkee and Harborne (1973). Flavonoid tannin in the seed coat of dark-seeded varieties of *Brassica* was suggested by Durkee (1971).

There is, however, little information on other phenolic compounds in oilseeds. Harborne and Corner (1961) found glucose esters of *p*-coumaric acid, ferulic acid and caffeic acid in the leaves of *Raphanus sativus* and 1-sinapoylglucose in the leaves of *B. oleracea.* The latter ester was also isolated from seed extracts of *Crambe abyssinica* by Austin and Wolff (1968). Recently Geissman and Neukom (1973) found that ferulic acid was bound to insoluble pentosans in wheat flour, and Maga and Lorenz (1974) claim that free vanillic and syringic acids occur in soya flour, peanut and cottonseed. Sunflower seed meal was shown to contain caffeic acid esters (Mikolajazek et al., 1970), especially chlorogenic acid (Brummett and Burns, 1972).

The present investigation was undertaken to survey the free and bound phenolic acids of the seed meals and hulls of some of the more common species and varieties of rape and mustard, as a part of a study of the polyphenol composition in cruciferous oilseeds. The phenols are an important consideration in relation to taste, color and nutritive value of processed rapeseed meal (Maga and Lorenz, 1973; Brummett and Burns, 1972).

MATERIALS & METHODS

Materials

The seeds used in this study were from certified Canadian rape and mustard varieties. Seed coats and meals were prepared from *Brassica campestris* L Var. Echo; *B. campestris* L Var. Span; *B. napus* L Var-Oro; and white mustard, *Sinapis alba.* The hull was separated from the seed meal by an air-classification procedure used at the Food Research Institute. The seed meals were defatted by a cold extraction with hexane. The coats were not defatted, but were ground to a finer particle size in a Waring Blendor before extraction. Whole defatted crushed seed of *B. campestris* L Var. Yellow Sarson was also examined but there was difficulty in separation of the yellow hulls from the kernels and therefore no separate hull sample was available for analysis.

Extraction

The hulls and meal portions were extracted with 70% ethanol by first bringing to a boil and then allowing the mixture to stand overnight at room temperature. The centrifuged supernatant was evaporated down for the chromatography of the free phenolic acids. For the determination of soluble bound phenolic acids, the supernatant or portion of it was subjected to acidic and basic hydrolysis using 2N HCl and 2N NaOH respectively. The residue of meal or hulls remaining after extraction with 70% ethanol was washed with more ethanol and finally distilled water and then hydrolyzed to obtain the insoluble bound phenolic acids.

Hydrolysis of the extracts

Acid hydrolysis was accomplished by heating for 30 min on the water bath (100°C) and alkaline hydrolysis was done at room temperature for 4 hr, followed by acidification to pH 2.0. The phenolic acids were extracted from the aqueous hydrolysates with diethyl ether. The ether extracts were dried with anhydrous sodium sulfate, filtered and taken to dryness. The residues were dissolved in a small amount of 70% ethanol for chromatography.

Chromatography of phenolic acids

Solvents used for paper chromatography were: (1) toluene-acetic acid-water (TAW) 4:1:5 (duplicate samples were developed by the descending method for 5 1/2 hr and 48 hr) essentially according to the method of Ribereau-Gayon (1972); (2) 6% acetic acid (6% HOAc); (3) 2% acetic acid (2% HOAc); (4) benzene-propionic acid-water (BPW) 2:2:1; and (5) butanol-acetic acid-water (BAW) 4:1:5. For 2-dimensional TLC on MN 300 cellulose-coated plates, 2% HOAc was used for the first direction and BPW for the second. All papers and plates were examined under long-wave UV light before spraying. The sprays used were diazotized sulfanilic acid (DSA) (Block et al., 1958), and diazotized *p*-nitroaniline (DPN) (Ribereau-Gayon, 1972). A recently developed new spray consisting of a mixture of 10% sodium tungstate, 5% trichloroacetic acid, 0.5N HCl and freshly prepared 5% sodium nitrite (6:6:3:6) was used as a first spray (Bhatia et al., 1973) in some instances. All these chromogenic reagents were followed by a second spray with either dilute NaOH or sodium carbonate. All compounds in Tables 1 and 2 were identified by comparison with authentic substances and co-chromatography in more than one solvent system and more than one spray reagent.

Identification of sinapine and chlorogenic acid

Sinapine was isolated as the thiocyanate salt from *B. campestris* L Var Echo by the method of Clandinin (1961) and used as a marker for comparison of the chromatograms of unhydrolyzed extracts of the other species and cultivars.

In order to detect chlorogenic acid, the seed was extracted at room temperature with acetone-water (8:1). After concentration (rotary evaporator 45°C) to remove acetone, ethyl acetate was added. The ethyl acetate phase contained chlorogenic acid, leaving the sinapine in the aqueous phase. The chlorogenic acid was then identified by its behavior on two-dimensional chromatograms (BAW and 6% HAc). Elution of the spot and hydrolysis with acid yielded quinic acid and caffeic acid, which were identified by comparison with authentic substances in two solvent systems.

β-Glucosidase

A portion of the dry 70% ethanol extract was taken up in distilled water and a small amount of β-glucosidase (Sigma Chem. Co.) was added. The mixture was incubated at room temperature for 4 hr.

Degradation of sinalbin (Aldrich Chem. Co.)

Sinalbin was treated with acid and/or alkali (as previously described under hydrolysis) and the products of hydrolysis and degradation were chromatographed on paper. Development was carried out in TAW for 48 hr and also in 6% acetic acid.

RESULTS & DISCUSSION

Seed meal (soluble bound-phenolic acids)

Chromatograms of the 70% ethanolic extract in both BAW and TAW solvent systems gave no indication that free phenolic acids were present in amounts detectable by our methods.

Bound phenolic acids liberated after hydrolysis of the extracts prepared from three varieties of *B. campestris*, one of *B.*

napus and one of *Sinapis alba* are summarized in Table 1. The Rf values and color reactions of the compounds are found in Table 2.

It is clear from Table 1 that sinapic acid is the prominent acid in all species and cultivars, followed by much smaller amounts of *p*-coumaric acid, ferulic acid, *p*-hydroxybenzoic acid and caffeic acid. All these were liberated by alkaline hydrolysis and to some extent by acid. Table 3 shows that sinapic acid, *p*-coumaric acid and *p*-hydroxybenzoic acids were obtained as a result of enzymic hydrolysis (β-glucosidase). These results suggest that the bound phenolic acids occur chiefly as glucose esters rather than β-glucosides or glycosides since alkaline hydrolysis is effective.

Meal prepared from white mustard (*Sinapis alba*) contains other related substances (Table 2) such as: *p*-hydroxybenzyl alcohol, *p*-hydroxybenzyl nitrile and two unknowns (unknowns #1 and #2). These substances were found after hydrolysis together with large amounts of *p*-hydroxybenzoic acid. The occurrence of these compounds was found to be due largely to the breakdown of *p*-hydroxybenzyl glucosinolate. When sinalbin (the sinapine salt of the glucosinolate) was treated with 2N NaOH for 4 hr *p*-hydroxybenzoic acid was identified as one of the products, and some was *p*-hydroxybenzyl alcohol. Treatment with 2N HCl yielded *p*-hydroxybenzyl nitrile and mainly unknown #1. The latter compound was not stable in alkali and if eluted from the chromatogram and treated with alkali, *p*-hydroxybenzoic acid was one of the products identified. Unknown #1 has the same color reaction with DSA as that reported for *p*-hydroxybenzyl isothiocyanate; however, the isothiocyanate was not available for comparison purposes. The two other hydroxybenzyl derivatives (alcohol and nitrile) were previously reported by Kawakishi and Muramatsu (1966) and Kawakishi et al. (1967) as degradation products formed after enzymic hydrolysis with thioglucosidase.

However, some *p*-hydroxybenzoic acid was found after alkaline and enzymic hydrolysis of the seed meal extracts of the *Brassica* which contain mere traces of the glucosinolate (Josefsson, 1970). It is likely that this acid occurs as an ester in the *Brassica* meals. This is the first report on *p*-hydroxybenzoic acid as a constituent of the *Cruciferae*. Its presence was confirmed by both one-dimensional paper chromatography and 2-dimensional TLC on MN 300 cellulose. Color reactions with DSA and DPN agreed with the authentic compound, as well as co-chromatography in two solvent systems.

Seed coats (soluble bound-phenolic acids)

The same acids only in much smaller quantities were identified in the seed coat extracts after hydrolysis. *B. napus* L Var Oro coats contained protocatechuic acid, which was not detected in the meal. This was confirmed by streaking a band of the hydrolysate on 3 MM paper and downward development in TAW (4:1:5) for 48 hr, after which the band at low Rf 0.03 was located by spraying a portion with DSA. The band was eluted with 2% acetic acid, spotted on paper and then developed in 2% acetic acid for 5 hr. Color tests using DSA and the new spray developed by Bhatia et al. (1973) together with co-chromatography with authentic protocatechuic acid proved the compound to be this acid. There was some evidence from two-dimensional paper chromatograms that vanillic acid was a constituent present in the seed coat of most species and cultivars.

Table 1—Phenolic acids in hydrolysates of the extracts from seed meals and hulls of Brassica and Sinapis species and cultivars[a]

	Brassica campestris						B. napus			
	(Span)		(Echo)		(Y. Sarson)		(Oro)		S. alba	
Substance	1	2	1	2	1	2	1	2	1	2
Sinapic acid	+++	+	+++	+	+++	+	+++	+	+++	+
p-coumaric acid	+	—	+	—	+	—	+	—	+	—
Ferulic acid[b]	+	—	+	—	+	—	+	—	+	—
Caffeic acid	+	+	+	+	+	+	+	+	+	+
p-hydroxybenzoic acid	+	—	+	—	+	—	+	—	++	+
Protocatechuic acid							+	+		
Vanillic acid	—	—	+	+	+	+	+	—	+	+
p-hydroxvbenzyl nitrile	—	—	—	—	—	—	—	—	—	+
p-hydroxybenzyl alcohol	—	—	—	—	—	—	—	—	+	—
Unknown #1	—	+	—	—	—	—	—	Trace	+	+++
Unknown #2	+	—	+	—	+	—	+	—	+	—

[a] 1 = NaOH hydrolysis; 2 = HCl hydrolysis, (+++) = very intense spot.
[b] (+) Ferulic acid liberated by NaOH from the insol. residue, mere traces of soluble ferulic were found.

Table 2—Rf values and color reactions of identified phenolic acids

	Color[a]				Rf Values[b]				
Substance	DSA	DPN	ST	UV	TAW(5)	TAW(48)	6% HOAc	2% HOAc	BPW
Sinapic acid	purple	red—buff	purple—brown	fl	0.30	—	0.30 0.53	0.26	0.86
p-coumaric acid	red	yellow—blue	—	—	0.15	0.80	— —	0.40,0.64	0.74
Ferulic acid	purple	pink—blue	yellow—pink	fl	0.50	—	— —	0.36,0.65	0.88
Caffeic acid	brown	buff	brown	fl	0.00	0.02	— —	0.28,0.50	0.33
p-hydroxybenzoic acid[c]	yellow	yellow-red	faint yellow	—	0.08	0.32	0.53	0.60	0.63
p-hydroxybenzyl nitrile[c]	orange	red	—	—	0.56	—	0.65	—	—
p-hydroxybenzyl alcohol[c]	yellow	red	yellow	—	0.00	0.15	0.70	—	—
Vanillic acid[d]	yellow	yellow—purple	yellow	—	0.48	—	—	0.52	0.87
Protocatechuic acid[d]	red	blue	yellow—pink	—	0.00	0.05	—	0.53	—
Unknown #1	red	—	—	—	0.05	0.26	0.80	—	—
Unknown #2	orange	—	—	—	0.00	0.15	—	—	—

[a] Color tests—See Methods Section. ST = new spray, UV = long wave, fl = fluorescence, (—) between colors indicates first and second spray.
[b] See Methods Section for solvent systems used.
[c] These compounds obtained also after treatment of sinalbin with acid or alkali treatments.
[d] Found only in the seed coat—tentative identification of vanillic acid.

Table 3—Phenolic acids liberated after β-glucosidase hydrolysis

Substance	Relative amount
Sinapic acid	+++
p-hydroxybenzoic acid	+
p-coumaric acid	+
Caffeic acid[a]	+

[a] Caffeic acid was tentatively identified on two-dimensional TLC (MN 300 cellulose) which suggests that the acid occurs both as a glucose derivative and as quinic acid ester (chlorogenic acid).

Insoluble-bound phenolic acids

The 70% ethanol insoluble residues of the coats and seed meals after hydrolysis yielded appreciable amounts of the same phenolic acids. Ferulic acid was quite noticeable on the chromatograms of the coat fractions, but absent in the seed meal. The quantity of sinapic acid was much lower than in the ethanol-soluble fraction of the meal. The results after alkaline hydrolysis indicated the presence of insoluble complex substances esterified with phenolic acids.

Soluble esters

Two esters were identified in unhydrolyzed extracts as sinapine and chlorogenic acid (see Methods). The identification of chlorogenic acid in *B. campestris* seeds confirms the work of Lo and Hill (1972), who identified caffeic and chlorogenic acid, based mainly on spectra. These workers attributed the discoloration of rapeseed meals to chlorogenic acid. Sinapine appears to be present in all the *Brassica* and *Sinapis* seeds tested, regardless of the presence of *p*-hydroxybenzyl glucosinolate.

Discussion

With the exception of *p*-hydroxybenzoic acid and protocatechuic acid the bound phenolic acids of *Brassica* and *Sinapis* seeds differ little from those of the leaves of *B. oleracea, Raphanus sativus* (Harborne and Corner, 1961) and swede root (Rhodes and Wooltorton, 1973).

Since free phenolic acids were not found in the rapeseed meal, it is difficult to establish whether these substances have any effect on the flavor, unless they are liberated in final processing steps or baking procedures. Maga and Lorenz (1973) gave no threshold values for phenolic esters, and therefore it might be of some value to isolate some of these and have sensory evaluation tests done. Chlorogenic acid and sinapine are readily available; the latter being well-known as a bitter principle. Further work on an unknown phenolic substance of rapeseed meal which resembles a tannin is in progress now. This substance can be removed from solution with polyclar AT and may contribute to astringency.

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PROTEIN PRODUCTION FROM CRUDE LACTOSE BY *Saccharomyces fragilis*. Continuous Culture Studies

INTRODUCTION

USING THE INFORMATION obtained following batch cultivation of *S. fragilis* (Vananuvat and Kinsella, 1975). studies were performed to determine the requirements and productivity of *S. fragilis* in continuous culture and to assess the feasability of continuously producing yeast protein from crude lactose obtained by reverse osmosis/ultrafiltration (Zall, 1971). Continuous culture, which is commonly used in the production of food yeast *Candida (Torula) utilis*, has been discussed (Ricica, 1958).

MATERIALS & METHODS

THE YEAST (*Saccharomyces fragilis*) culture preparation, composition of medium, fermentation conditions and analytical procedures were described in the preceding paper (Vananuvat and Kinsella, 1975). Continuous cultivation was carried out in a fermentor equipped with a Continuous Culture Console Model XCMF-10, and Sigma pumps (New Brunswick Scientific Co., New Jersey). A working volume of 7.2 liters was used in all experiments. Initially six liters of medium containing 2% lactose and the supplemental ingredients described earlier, (Vananuvat and Kinsella, 1975) were placed in the fermentation jar. The medium was inoculated with 1.2 liter of the yeast inoculum prepared as described (Vananuvat and Kinsella, 1975). The pH, temperature and air supply were kept constant at 5.0, 30°C, and 1 VVM respectively. Agitation was maintained at 700 rpm. Continuous cultivation was started following 7-hr batch growth of *S. fragilis.* Fresh medium was then supplied at constant rates. Cells and broth were withdrawn continuously to maintain a constant volume in the fermentor. Criteria of steady state in the continuous cultivation jar were the maintenance of pH, temperature, flow rate and cell population.

In studying the effect of dilution rate (0.1, 0.18 and 0.23 per hr), fresh media containing 2% lactose were used during the continuous flow. In the study of the effect of lactose concentration, media containing 4.7 and 5.9% lactose were used. The flow of fresh media was maintained at the dilution rate of 0.18 per hr. During continuous cultivation, samples of culture medium were withdrawn and analyzed in the same manner as used in batch studies (Vananuvat and Kinsella, 1975). Yeast cells were harvested after 15 hr when the steady state had been reached.

Definitions

Dilution rate refers to the fraction of the culture volume replaced by fresh medium per hr of continuous cultivation. Productivity, expressed as gram per liter per hour, is the dry weight of yeast (g) produced per liter of the culture medium per hour of continuous cultivation. The lactose, Kjeldahl and Lowry protein consumption (expressed as percent), represents the amount of lactose, Kjeldahl and Lowry protein depleted from the medium during cultivation. The yeast concentration (g per liter) is the dry weight of yeast cells per liter of culture medium. The yeast yield (expressed as percent) is the dry weight of cells produced from 100g of lactose substrate during yeast cultivation. The Kjeldahl protein, Lowry protein and nucleic acid content (expressed as percent) represent the amount of Kjeldahl protein (N × 6.25), Lowry protein and the difference between these values respectively per 100g of dry yeast. The Kjeldahl protein, Lowry protein and nucleic acid yield (expressed as percent) are the weight of Kjeldahl protein, Lowry protein and nucleic acid in yeast, produced per 100g of lactose substrate depleted during cultivation. The chemical oxygen demand (COD) reduction (expressed as percent) is the amount of COD depleted from the medium during yeast cultivation. Kjeldahl (K) protein (N × 6.25) Lowry (L) protein and nucleic acid were quantified as described in preceding paper (Vananuvat and Kinsella, 1975).

RESULTS

CONTINUOUS CULTIVATION studies were carried out to determine the optimum lactose concentration and dilution rate and to evaluate their individual and combined effects on yeast yield, productivity protein and nucleic acid content of yeast. From previous batch experiments (Vananuvat and Kinsella, 1975), it was observed that yeast, and protein yields were high after 7 hr of batch cultivation. Thus continuous flow of fresh medium was begun after batch cultivation had been carried out for 7 hr.

Effect of dilution rate

In preliminary studies of continuous cultivation at dilution rates higher than 0.23 per hr, great variability in performance was obtained indicating that under these cultivation conditions a steady state of cultivation was difficult to obtain in our fermentation system. Thus this study was limited to three dilution rates viz. 0.10, 0.18 and 0.23 per hr. The results of different dilution rates on the various parameters during steady state continuous cultivation (with 2% lactose in the medium) are summarized in Figures 1 and 2.

The consumption of lactose, Kjeldahl (K), nitrogen and protein from the medium dropped as the dilution rate increased. COD reduction was high (78%) at 0.1 dilution rate compared to 54% at 0.23 dilution rate. This was because at the higher dilution rates a smaller percentage of the available substrate was utilized. Kjeldahl (N × 6.25) and L-protein content of yeast showed the highest values at dilution rate of 0.18. Nucleic acid content of yeast decreased slightly as dilution rate increased.

Yeast concentration decreased from 12.94 to 10.45g/liter as dilution rate increased (Fig. 2). However, the productivity rose with dilution rate. The highest yields of yeast, K-protein (N × 6.25) and L-protein were 72.55, 36.25 and 29.20%, respectively, at the dilution rate of 0.18 per hr. At this dilution rate, COD reduction was 61.20%, and the consumption of lactose, K- and L-protein from the medium was 78.5, 30.0 and 20.5% respectively (Fig. 1).

Effect of lactose concentration

Using a dilution rate of 0.18 per hr, the effect of increasing lactose concentrations (2.0, 4.7 and 6.0%) during continuous cultivation of *S. fragilis* was studied (Fig. 3).

Lactose and protein consumption and COD reduction were highest when the lactose concentration was 4.7%. K-protein (N × 6.3) consumption from the medium increased with lactose concentration and this was higher than L-protein consumption by about 6–10% at all three lactose concentrations. The K- and L-protein content of yeast rose slightly as the lactose concentration increased while the nucleic acid content was fairly constant at all the three concentrations.

Yeast concentration and productivity increased as the concentration of lactose increased but tended to level off at higher concentrations, i.e., between 4.7 and 5.9% (Fig. 4). Yield of yeast, K- and L-protein and nucleic acid decreased concomitantly (Fig. 4). Thus the continuous cultivation of *S. fragilis* at the dilution rate of 0.18 per hr with lactose concentration higher than 2% was not optimal in terms of yields of yeast and

protein even though the K- and L-protein content of yeast grown above 2% lactose showed slightly higher values. *S. fragilis* grown continuously at the dilution rate of 0.18 per hr in the medium containing 2% lactose had 50% K-protein (N × 6.25), 40% L-protein and 10% nucleic acid content. The yeast yield and productivity under these conditions were 72.5% and 2g/liter/hr respectively.

DISCUSSION

DURING CONTINUOUS cultivation of *S. fragilis* at different dilution rates, the yeast concentration dropped quite sharply as dilution rate increased. Nucleic acid content decreased slightly from 11 to 9% as dilution rates increased from 0.1 to 0.23 per hr. This suggested that during continuous flow cultivation the growth rate is considerably below the possible maximum. Two reasons may account for this, i.e., nutritional deficiency in the medium and/or insufficient aeration, i.e., inadequate oxygenation. The possible nutrient deficiency in the medium was alluded to earlier (Vananuvat and Kinsella, 1975), and Fencl and Burger (1958) indicated that minor nutrient deficiencies scarcely detectable in batch microbial culture become fully apparent during the intensified biosynthesis of cells during continuous culture. Some of the effects of deficiencies of nutrients in medium during continuous cultivation have been discussed in relation to baker's yeast (Beran, 1958).

Optimum aeration during continuous cultivation depends on the dilution rates. At dilution rates less than those required for the maximum specific growth rate, the growth rate of microorganisms is proportional to dilution rate when oxygen supply is in excess. When oxygen supply is inadequate, though constant, growth rate is expected to be constant and independent of dilution rate.

S. fragilis grown continuously at a dilution rate of 0.18 on 2% crude lactose gave the highest yield in our studies (72.5%). The highest yields obtained with *T. utilis* were 40–45% from wood hydrolyzate and potato starch wastes respectively (Harris et al., 1948). The optimum productivity obtained in this study was 2g/liter/hr which is comparable to *T. utilis* grown on potato wastes (Reiser, 1954) and *Candida lipolytica* on gas oil (Munk et al., 1969). Baker's yeast grown on glucose medium (Maxon and Johnson, 1953) gave lower yeast yield (42%) but higher productivity (9.4g/liter/hr) than did *S. fragilis* in present experiments, probably because these workers used higher concentrations of substrate and higher dilution rates.

In studying the effect of substrate concentration at the dilution rate of 0.18 per hr, yeast protein content increased but the yeast yield decreased as the lactose concentration increased. This is similar to the performance of *S. cerevisiae* on wood hydrolyzate (Harris et al., 1948). *Candida lipolytica* also gave lower yield when concentration of gas oil was increased (Dostalek et al., 1969). As observed by other workers, for

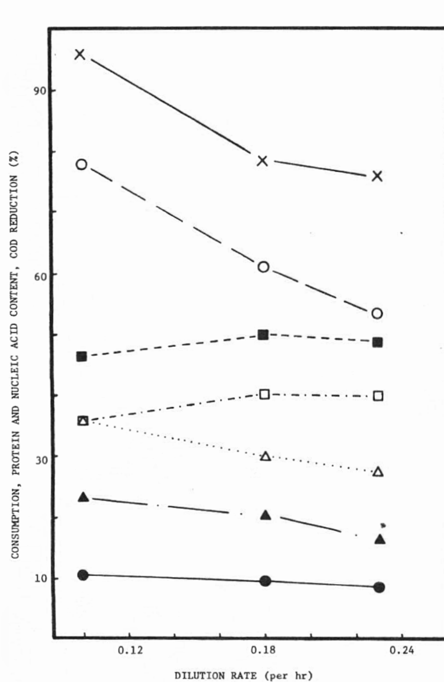
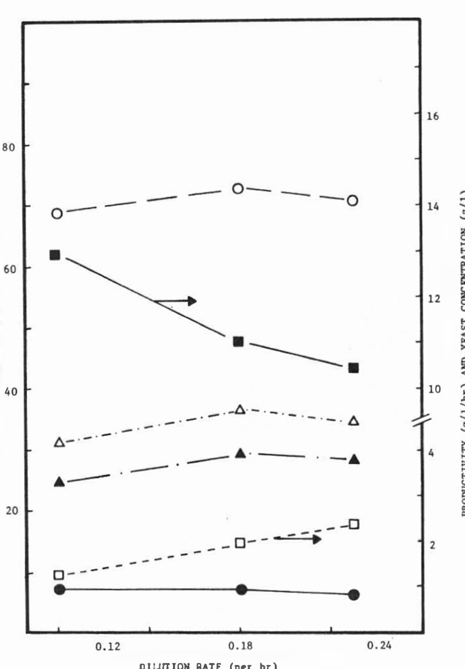


Fig. 1—Effect of dilution rate on consumption of lactose and protein from the medium; reduction of chemical oxygen demand and protein and nucleic acid content during continuous cultivation of S. fragilis on 2% lactose. Cultivation conditions: aeration – 1 VVM; pH 5.0; temperature 30°. (X lactose consumption from medium; △ K-protein (N × 6.3) consumption from medium; ▲ L-protein consumption from medium; ■ K-protein (N × 6.25) content of yeast; □ L-protein content of yeast; ● nucleic acid content of yeast; ○ COD reduction.)



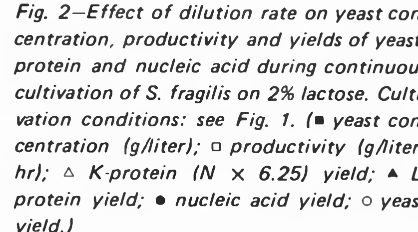


Fig. 2—Effect of dilution rate on yeast concentration, productivity and yields of yeast, protein and nucleic acid during continuous cultivation of S. fragilis on 2% lactose. Cultivation conditions: see Fig. 1. (■ yeast concentration (g/liter); □ productivity (g/liter/hr); △ K-protein (N × 6.25) yield; ▲ L-protein yield; ● nucleic acid yield; ○ yeast yield.)

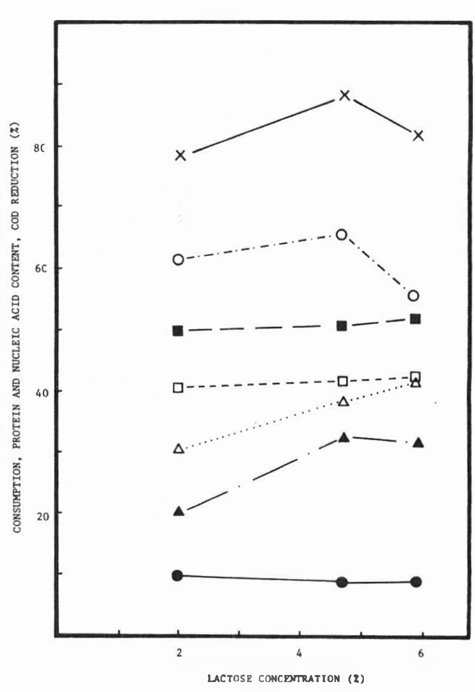


Fig. 3—The effects of lactose concentration on consumption of lactose and protein; reduction of chemical oxygen demand, and on protein and nucleic acid content of S. fragilis during continuous cultivation at dilution rate or 0.18 per hr. Cultivation conditions: see Fig. 1. (X lactose consumption; △ K-protein (N × 6.3) consumption from medium; ▲ L-protein consumption from medium; ■ K-protein (N × 6.25) content of yeast; □ L-protein content of yeast; ○ COD reduction; ● nucleic acid content of yeast.)

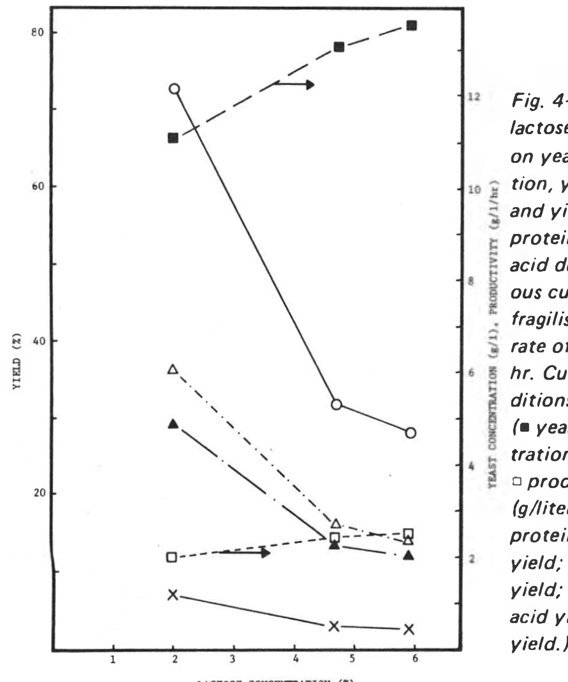


Fig. 4—Effect of lactose concentration on yeast concentration, yeast productivity and yields of yeast, protein and nucleic acid during continuous cultivation of S. fragilis at dilution rate of 0.18 per hr. Cultivation conditions: see Fig. 1. (■ yeast concentration (g/liter); □ productivity (g/liter/hr); △ K-protein (N X 6.25) yield; ▲ L-protein yield; X nucleic acid yield; ○ yeast yield.)

other microbes the protein yield decreased with increasing substrate concentrations (Bough et al., 1972; Updegraff, 1971). The lower yield at higher concentration of substrate may be the result of insufficient aeration because oxygen requirement for microbial growth increased with increasing substrate concentration and the viscosity of the medium (Updegraff, 1971). The increased viscosity may decrease the oxygen transfer rate. It is also possible that further increases in the substrate concentration may not give highest yeast yield but cause yeast to produce more alcohol (Fencl and Burger, 1958). In this respect, when yeasts are grown for the production of cells (protein), the continuous culture is controlled in such a way that the sugar added to the vessel should be immediately assimilated to maintain low concentrations not exceeding 0.1% (Fencl and Burger, 1958).

Continuous culture of *S. fragilis* under present optimum conditions caused about 60% COD reduction. *Torula utilis* grown on potato starch waste gave 60% COD reduction (Reiser, 1954). Knight et al. (1972) have reported that the inoculum volume influences growth rate and showed that higher inoculum volumes gave faster maximum growth and higher COD reduction.

Further studies are required to define the nutrient requirements and optimum oxygenation conditions for improving growth rate and yield of yeast grown under continuous conditions for production of protein.

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GROWTH OF FUNGI AND BOD REDUCTION IN SELECTED BREWERY WASTES

INTRODUCTION

PREVIOUS INVESTIGATORS have examined a number of waste products as substrates for fungal growth. Although *Candida utilis* has been employed in many studies, numerous other fungi have been grown successfully on a variety of waste products.

Fungi can be used advantageously in the treatment of certain processing wastes due to (a) nutritive value of the cells or mycelia produced, and/or (b) the ability of fungi to reduce the Biochemical Oxygen Demand (BOD) of wastes which present problems in conventional treatment systems. The use of fungi for food and feed, and the nutrient content of numerous fungi are well-documented (Mateles and Tannenbaum, 1968). Two recent examples of fungi used to degrade food processing wastes include the use of a mixture of Fungi Imperfecti for reducing the BOD of corn and pea canning wastes (Church et al., 1973), and inoculation of *C. utilis* into sauerkraut waste to reduce the BOD and produce yeast invertase (Hang et al., 1973).

At present municipal facilities are commonly used for the treatment of brewery effluents. However, in the future this situation may change due to stricter antipollution laws and the possibility of surcharges being levied on industrial wastes sent to municipal treatment plants. This investigation was undertaken to determine the ability of various fungi to grow in selected brewery wastes to produce microbial protein and reduce to BOD.

MATERIALS & METHODS

THE CULTURES used in this investigation are listed in Table 1. All of the cultures were maintained at 25°C on yeast extract-malt extract (YM) agar slants (Wickerham, 1951).

Preparation of wastes

Quantities of three brewing wastes were obtained from a Michigan Brewery. Prior to use, the wastes were frozen in Cry-O-Vac bags (W.R. Grace Co., Simpsonville, S.C.) and held at −20°C. The bags, containing approximately 4-liter portions, were thawed as needed by holding them at 10°C for 24 hr. In order to obtain accurate assessments of the dry weight of cells grown on the wastes, as much solid material as possible was removed.

Spent grains, recovered from the mashing process, and trub, a precipitate from the hot wort tank, were transferred to closely woven nylon bags. Spent grain and trub press liquors were prepared with the aid of a sausage stuffer (F. Dick Co.) and filtered through several layers of cheesecloth to remove contaminating particles. Sludge from the fermentation tank was treated by batch centrifugation at 10,000 × G, followed by vacuum filtration through Hy-Flo Supercel (Johns-Manville Corp., Manville, N.J.). This served to remove most of the spent yeast and cell debris from the sludge.

The waste liquors were adjusted to pH 5.3 using 1N NaOH or 1N HCl. 100-ml aliquots of the wastes were transferred to 250-ml Erlenmeyer flasks and heated in flowing steam for 10 min prior to use.

In studies with yeasts, substrates were inoculated with 1 ml of a 72-hr YM broth culture. For the mushroom cultures, mycelial spheres from 8-day YM broth cultures were filtered through a fine wire screen and transferred to flasks containing sterile glass beads and 50 ml deionized water. These flasks were vigorously shaken for 1 hr to break up the mycelia and 1-ml aliquots of the resultant suspensions were used for inoculation. Cultures were incubated at 25°C on a gyrotary shaker (New Brunswick Scientific) operating at 200 rpm.

Yeast cells were harvested by centrifugation for 10 min at 10,000 × G and suspended in deionized water. Mushroom cultures which formed discrete mycelial balls were harvested by filtration through a fine wire screen. (Occasionally mushroom cultures grew in a dispersed form, and the mycelia were harvested by centrifugation at 10,000 × G for 10 min.)

The harvested cells and mycelia were washed with 50 ml of deionized water, placed in tared aluminum weighing dishes, dried to a constant weight in a convection oven at 60°C (ca. 48 hr), and transferred to a dessicator for 24 hr prior to weighing.

Analytical techniques

All wastes were analyzed before and after microbial growth. Reducing sugars were determined according to the methods of Somogyi (1945) and Nelson (1944), and the pH of samples was measured using a Beckman Research pH meter. Nitrogen was determined, except for the following modifications, by the micro-Kjeldahl method of AOAC (1970): Four-ml aliquots of a digestion mixture containing 5g $CuSO_4 \cdot 5H_2O$, 5g SeO_2 and 500 ml conc H_2SO_4 was added to a micro-Kjeldahl flask containing 2 ml of undiluted samples of wastes or 0.1g of dried cells. When the sample cleared during heating, the sides of the flask were rinsed with deionized water, 1 ml of 30% H_2O_2 was added, and heating was continued for another hour.

Chemical oxygen demand (COD) and biochemical oxygen demand (BOD) were determined according to the methods described in *Standard Methods for Examination of Water and Wastewater* (APHA, 1971). Primary effluent obtained from the East Lansing, Mich., Sewage Treatment Plant was used at a concentration of 2 ml/liter as a seeding agent. Dissolved oxygen was determined by the azide modification of the iodometric method (APHA, 1971), and a standard containing 150 mg/liter of both glucose and glutamic acid was analyzed with each set of samples.

Data presented represent the means of duplicate determinations.

Table 1—Fungal cultures grown in brewery wastes

Organisms	Strain	Source
Yeasts		
Candida steatolytica	70-40	UCD[a]
Candida utilis	C-25	UCD
Saccharomyces cerevisiae	Y-25	MSU-1[b]
Saccharomyces uvarum	ATTC9080	MSU-1
Mushrooms		
Agaricus bisporus		MSU-2[c]
Calvatia gigantea		MSU-1
Morchella esculenta		MSU-2
Pleurotus ostreatus		MSU-2

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RESULTS & DISCUSSION

THE CHARACTERISTICS of the brewery wastes prepared for this investigation are given in Table 2. As noted by other investigators (Thiel and duToit, 1965; Ault, 1969) brewery effluents such as Grain Press Liquor (GPL) and Trub Press Liquor (TPL) contain large amounts of fermentable organic matter and tend to be low in nitrogen. Thus GPL and TPL have ratios of reducing sugars to nitrogen of ca. 70:1. In contrast, FSL, obtained from fermentation wastes, has a ratio of reducing sugar to nitrogen of 3.5:1. Grain press liquor was previously reported to have a BOD of 39,000 mg/liter (Ault, 1969) and FSL has a BOD of ca. 69,000 mg/liter (Dietrich, 1961). We are unaware of any reports on the BOD of TPL. As shown in Table 2, the BOD values of GPL and FSL used in this investigation were within the range reported by previous investigators. Of particular note is the extremely high BOD of TPL.

The BOD:COD ratios demonstrate a difference in composition of the wastes. Trub has a BOD:COD ratio of nearly 0.8, indicating a high percentage of biologically degradable organic matter. GPL and FSL have BOD:COD ratios of about 0.6 and 0.5, respectively, implying a greater content of nonbiologically utilizable compounds.

All of the wastes were capable of supporting microbial growth, although there was a considerable difference in the yield obtained with each of the substrates. A significant variation in yield among different species grown on the same substrate was also noted. There is evidence to suggest that some organisms are utilizing other carbon sources in the substrates in addition to reducing sugars. Where significant quantities of carbon sources other than reducing sugars are assimilated, the expression of yield in terms of g cells/g reducing sugar is of questionable value. This calculation is included in Table 3, however, as an aid in comparing these data with those of previous investigators who measured yields in this manner.

All the yeast cultures were harvested after a 72-hr incubation period since preliminary growth studies with *S. cerevisiae* and *C. steatolytica* indicated that both cultures had similar growth patterns and produced maximum yields at ca. 72 hr. The mushroom cultures, however, showed considerable variation in growth rate, as had been noted by a number of investigators. Maximum growth of *A. campestris* in agitated culture has been obtained over incubation periods ranging from 8 days (Reusser et al., 1958a) to 12 days (Falanghe, 1962). Optimum incubation times for *Morchella* species have been reported as 5 and 7 days for *M. esculenta* and *M. crassipes*, respectively (Litchfield, 1967), and 8 days for *M. hybrida* (Reusser et al., 1958a). In this investigation, incubation of the mushroom cultures was limited to 8 days. It was felt that even though greater yields of some organisms might have been obtained by longer incubation, the excessive time interval would preclude their use in commercial operation.

With respect to time requirements for commercial production, it should be noted that such operations normally use large fermentors which can be operated on a continuous flow basis. Maximum mycelial yields under these conditions are obtained after considerably shorter periods than those required for agitated flasks. Incubation times as short as 30 hr have been reported (Block, 1960).

The net dry weights of cells and mycelia ranged between 3.28 and 6.28 g/liter for organisms grown in GPL (Table 3). Similarly, reducing sugar utilization varied from 69.2–89.4%. With the exception of *C. gigantea* which produced a dry mycelial weight of 6.25 g/liter, the mushrooms generally produced smaller cell masses than the yeasts, although the overall difference in utilization of reducing sugar was less marked. *C. steatolytica* produced the greatest yeast cell mass, 6.28 g/liter of dry cells.

Trub press liquor proved to be the best substrate for all the organisms (Table 3). Dry cell and mycelial weights ranged from 8.94–27.72 g/liter, with the mushroom cultures producing higher yields than the yeasts. *C. gigantea* produced the greatest net dry weight of all the organisms, yielding 27.72 g/liter. All mushroom cultures, however, produced four to five times the mycelial mass produced in grain press liquor. The cell mass did not increase as dramatically with the yeast cultures, although significant gains were made. *C. steatolytica* produced 10.56 g/liter, the largest dry cell mass among the yeasts. The percentage of reducing sugar utilization, varying from 52.7–73.5%, was generally lower in TPL than in GPL.

Cell and mycelial yields ranging from 2.45–6.46 g/liter were obtained from FSL. These were not as small as might have been predicted from the low sugar content of the medium. As shown in Table 3, *S. cerevisiae*, *C. steatolytica* and *A. bisporus* essentially grew as well in FSL as they had in GPL.

Table 2—Characteristics of brewery wastes

Waste	Reducing sugar[a] (mg/liter)	Nitrogen (mg/liter)	COD (mg/liter)	BOD (mg/liter)	pH[b]
GPL	30,000	434	56,100	31,800	5.96
TPL	55,000	764	169,000	132,000	5.30
FSL	9,900	2,870	133,000	67,500	4.43

[a] Expressed as glucose
[b] pH prior to adjustment

Table 3—Reducing sugar utilization and yields of organisms grown on selected brewery wastes

	Grain press liquor			Trub press liquor			Fermentation sludge liquor		
	Reducing sugar utilization	Yield (Dry wt basis)		Reducing sugar utilization	Yield (Dry wt basis)		Reducing sugar utilization	Yield (Dry wt basis)	
Organism	(%)	(g/liter)	(g/g sugar)	(%)	(g/liter)	(g/g sugar)	(%)	(g/liter)	(g/g sugar)
S. cerevisiae	88.5	5.02	0.189	66.4	8.94	0.245	83.3	5.21	0.631
S. uvarum	89.4	4.94	0.177	73.5	8.95	0.221	84.5	4.02	0.480
C. utilis	73.5	5.09	0.231	58.1	9.86	0.308	78.7	3.65	0.468
C. steatolytica	74.2	6.28	0.282	63.6	10.56	0.302	81.0	6.46	0.805
P. ostreatus	69.2	3.81	0.184	52.7	20.05	0.691	74.7	3.42	0.462
M. esculenta	78.3	3.28	0.139	53.6	16.88	0.570	72.2	2.45	0.343
A. bisporus	83.2	3.45	0.138	54.5	11.32	0.377	87.1	3.38	0.392
C. gigantea	83.8	6.25	0.248	63.6	27.72	0.749	78.8	3.04	0.390

Sludge liquor proved to be the least suitable waste for the growth of the other organisms; however, of particular note is the relatively poor growth of *C. gigantea*, from which the highest yields had been obtained on the other substrates. Reducing sugar utilization varied from 72.2–87.1%, approximately the same range observed in GPL.

Yields obtained with several organisms on nonsupplemented GPL, TPL, and FSL compare favorably with those reported by previous investigators on a variety of enriched substrates. Reusser et al. (1958a) reported yields of 3.4 and 17.1 g/liter for *A. campestris* grown on sulfite liquor and a beet molasses medium, respectively. The same organism generated 7.2 g/liter of dry mycelia on a malt syrup, cane molasses medium (Moustafa, 1960), while a yield of 11.32 g/liter of *A. bisporus* mycelia was obtained on TPL in the present experiments.

M. esculenta yielded 7.92 g/liter of dried mycelia when grown on pumpkin canning waste (Litchfield and Overbeck, 1965). Significantly greater yields, up to 16.88 g/liter, were obtained in the current study. While Sugihara and Humfeld (1954) used a synthetic medium to produce up to 25 g/liter of *P. ostreatus*, the highest yield of this organism obtained in these experiments was 20.05 g/liter in TPL.

Using a culture identified as *Torula*, Gray et al. (1964) obtained 8.2 g/liter of dried cells from a synthetic medium. However, in much of the available data on yeast production, yields are expressed in terms of g cells/g reducing sugar. Harris et al. (1948) reported obtaining 0.296–0.392g cells/g sugar of *C. utilis* from sulfite liquor. Yields of another strain of *C. utilis*, grown on wood sugar stillage, ranged from 0.53–0.63 g cells/g sugar (Kurth and Cheldelin, 1946). Inskeep et al. (1951) reported yields of 10 g/liter for *C. utilis* grown on sulfite liquor. Agarwal et al. (1947) obtained yields of *S. cerevisiae*, grown on beet molasses, ranging from 0.427–0.543g cells/g sugar. In the present study, *S. cerevisiae* and *C. utilis* produced maxima of 8.94 and 9.86 g/liter, repectively, and maximum yields of 0.631 and 0.468 g/g reducing sugar utilized, respectively. These values for g cells/g reducing sugar may be misleading since the organisms are also assimilating nonreducing carbon sources.

Table 4 lists the protein concentration in the cells grown on grain and trub press liquors (calculated as Kjeldahl N × 6.25). As noted by previous investigators (Reusser et al., 1958a; Litchfield et al., 1963a; Litchfield, 1967) mushroom mycelia generally have lower protein concentrations than yeast cells although this varies markedly with the organism. The protein contents of all organisms were highest when trub press liquor was the substrate. *C. steatolytica* had the lowest protein concentration of the yeasts in GPL and TPL, although it produced essentially the same quantity of total protein due to its greater yield.

Table 4—Protein content of organisms grown on grain press liquor and trub press liquor

Organism	Protein content: Grain press liquor (%)	Protein content: Trub press liquor (%)
S. cerevisiae	26.77	32.91
S. uvarum	26.40	27.74
C. utilis	27.11	28.67
C. steatolytica	21.30	23.49
P. ostreatus	6.88	7.14
M. esculenta	22.50	27.12
A. bisporus	20.31	22.28
C. gigantea	13.69	17.50

Considerably more variation in protein content was observed among the mushrooms. *P. ostreatus*, as previously noted, was extremely low in protein, containing a maximum of only 7.14% on TPL. Mycelia of *C. gigantea* also contained relatively low concentrations of protein, but total protein production equaled or exceeded that of all other organisms. *M. esculenta* and *A. bisporus* both produced good yields of protein, with the former organism actually exceeding the yeast *C. steatolytica* in protein content.

Overall there appeared to be little difference in total protein production between the yeasts. Among the mushrooms, *M. esculenta* and *C. gigantea* were approximately equal in total protein produced, both yielding significantly greater quantities than *P. ostreatus* and *A. bisporus*.

The organisms grown on GPL, TPL and FSL had consistently lower protein contents than those reported in the literature. *C. utilis* and *S. cerevisiae*, for example, have both been reported to contain 45–55% protein when grown under optimum conditions (Aries, 1946; Bunker, 1963). On TPL they had protein contents of only 28.67 and 32.91%, respectively. The mycelia of *A. bisporus* similarly were found to consist of only 22.28% protein in contrast to reported protein contents ranging from 35–45% (Humfeld and Sugihara, 1949). Of the eight test organisms, only *M. esculenta*, with a protein content of 27.12%, attained a concentration of protein near its suggested maximum of 31.1% (Litchfield et al., 1963b). However, other investigators have shown that the mycelial protein concentration of a variety of organisms could be increased markedly by supplementation of the substrates with various nitrogen sources (Humfeld and Sugihara, 1952; Reusser et al., 1958b; Falanghe, 1962; Litchfield et al., 1963a, b).

Table 5—Effectiveness of yeasts and mushrooms in reducing COD and BOD of brewery wastes

Organism	Grain press liquor: COD Reduction (%)	Grain press liquor: BOD Reduction (%)	Trub press liquor: COD Reduction (%)	Trub press liquor: BOD Reduction (%)	Fermentation sludge liquor: COD Reduction (%)	Fermentation sludge liquor: BOD Reduction (%)
S. cerevisiae	30.5	34.4	14.7	42.4	35.3	25.9
S. uvarum	30.3	34.0	17.1	43.2	30.1	21.5
C. utilis	28.6	28.0	18.3	45.5	26.3	20.0
C. steatolytica	30.4	28.2	20.7	45.5	33.1	24.4
P. ostreatus	19.6	21.9	18.7	43.9	24.1	17.0
M. esculenta	19.7	22.7	18.0	42.5	20.3	15.5
A. bisporus	33.9	34.4	24.3	49.2	33.1	24.4
C. gigantea	57.1	56.2	27.8	56.1	34.6	25.9

The reductions in BOD and COD achieved by growing the organisms in the wastes are shown in Table 5. The organisms grown on GPL reduced the BOD by approximately 22–34%, with the exception of *C. gigantea* which reduced the BOD by 56.2%. In TPL the BOD was reduced about 45%. There was essentially no difference among the organisms with the exception of *C. gigantea* which removed 56.1% of the BOD. Lower BOD reductions, in the area of 16–26% were obtained in FSL.

The reductions in BOD obtained in these wastes were substantially less than the maximum values obtained by other investigators (Church et al., 1973; Hang et al., 1973), while the cell yields were adequate. Since the nitrogen contents of GPL and TPL were low, nitrogen supplementation of these wastes could yield improved BOD removal with a concomitant increase in cell yield and protein. The growth of *C. gigantea* and *C. steatolytica* in supplemented wastes will be the subject of another investigation.

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GROWTH OF Calvatia gigantea AND Candida steatolytica IN BREWERY WASTES FOR MICROBIAL PROTEIN PRODUCTION AND BOD REDUCTION

INTRODUCTION

PREVIOUSLY we reported on the growth of four yeasts and four mushrooms in selected brewery wastes (Shannon and Stevenson, 1975). Although cell yields were satisfactory, reductions of BOD were only 20–45% in most cases. Wiley (1954) reported that the nonfermentable carbon compounds in the medium and the metabolic wastes produced by the organisms were factors in determining the amount of BOD reduction. In addition, several investigators, e.g., Reusser et al. (1958b), Falanghe (1962) and Litchfield et al. (1963), have reported that addition of nitrogen to substrates increases the growth of fungi, and Helmers et al. (1952) and Reiser (1954) have reported that the concentration of nitrogen is important in determining the effectiveness of organisms in reducing the BOD of a given substrate.

Candida steatolytica and *Calvatia gigantea* were the yeast and mushroom cultures which previously produced the greatest yields in nonsupplemented brewery wastes. This investigation was undertaken to determine the effects of nitrogen supplementation on growth of these two organisms in brewery wastes.

MATERIALS & METHODS

DETAILS concerning the sources and maintenance of the cultures, and the sources and characteristics of the grain press liquor (GPL), trub press liquor (TPL), and fermentation sludge liquor (FSL) used in this investigation were described previously; procedures used for the determination of reducing sugars, nitrogen, biochemical oxygen demand (BOD) and chemical oxygen demand (COD) were also given previously (Shannon and Stevenson, 1975). In this investigation, GPL and TPL were supplemented with 0.05, 0.10 and 0.15% nitrogen using either $(NH_4)_2SO_4$ or FSL as the source of nitrogen; this corresponded to 2.36, 4.72 and 7.08g $(NH_4)_2SO_4$, and 174, 349 and 523 ml FSL, respectively, per liter of the press liquors. All of the supplemented wastes contained 3 g/liter of potassium phosphate and were adjusted to pH 6.0.

RESULTS & DISCUSSION

SUPPLEMENTATION WITH FSL significantly decreased the concentration of reducing sugars and changed the BOD of the wastes; the characteristics of the resulting waste solutions are given in Table 1. The addition of $(NH_4)_2SO_4$ to GPL and TPL had essentially no effect on the reducing sugar content or the BOD of the wastes.

Yield

Significantly greater yields of *C. gigantea* were obtained when the wastes were supplemented with nitrogen (Table 2). The improvement in growth was most pronounced in GPL, and the greatest yields were obtained in samples supplemented with $(NH_4)_2SO_4$. In general, maximum growth in both substrates, with both nitrogen sources was obtained at a level of 0.05% added nitrogen. At nitrogen concentrations exceeding this, decreases in yield were observed. With $(NH_4)_2SO_4$ supplementation this may indicate that the substrate did not have sufficient buffer capacity to maintain suitable pH ranges at higher nitrogen levels since $(NH_4)_2SO_4$ tends to increase acid production in the medium (Reusser et al., 1958b). At higher concentration of FSL nitrogen, lower yields are at least partly related to substrate dilution. However, the yields in all samples containing FSL were in excess of the amounts which would be expected from the sum of the yields on the separate components of the sample.

The reducing sugar:N ratios which afforded maximum yield of *C. gigantea* were found to be independent of the nitrogen source. In GPL, the best growth was observed at reducing sugar:N ratios in the area of 30:1 while the figure for TPL was closer to 40:1. The utilization of reducing sugar was essentially the same as in nonsupplemented wastes.

Improvements in the growth of *C. steatolytica* were not as marked as those observed with *C. gigantea* (Table 2). A maximum of 8.1 g/liter of dry cells was obtained in GPL, while total yields up to 12.7 g/liter were recorded in TPL. In contrast to the results shown in Table 2, in GPL growth of *C. steatolytica* using FSL nitrogen was equivalent to or greater than that obtained with ammonium nitrogen. It would be tempting to attribute this to the presence of growth factors or other required nutrients in FSL. If this is the case, however, these growth factors are evidently present at optimum levels in TPL, since the addition of FSL actually results in decreases in net yield (assumed to be due to substrate dilution as noted with *C. gigantea*). Apparently $(NH_4)_2SO_4$ is the preferred N-source for *C. steatolytica*. Maximum yield was obtained on GPL at reducing sugar:N ratios in the area of 20:1 to 25:1. On TPL, optimum production occurred at reducing sugar:N ratios of 30:1 to 35:1.

Since *C. gigantea* has not been extensively studied and the first published description of *C. steatolytica* appeared only recently (Yarrow, 1969), there are no data available on their growth characteristics in other substrates. However, yields reported by previous investigators working with fungi indicate

Table 1—Composition of grain press liquor and trub press liquor with and without supplementation using various concentrations of fermentation sludge liquor (FSL)

Sample	Reducing sugar (mg/liter)	BOD (mg/liter)
Grain press liquor (GPL)	30,000	56,100
GPL + 0.05% N (17.4% FSL)	26,000	58,000
GPL + 0.10% N (34.9% FSL)	23,000	59,000
GPL + 0.15% N (52.3% FSL)	20,000	61,900
Trub press liquor (TPL)	55,000	132,000
TPL + 0.05% N (17.4% FSL)	47,000	121,000
TPL + 0.10% N (34.9% FSL)	39,000	109,000
TPL + 0.15% N (52.3% FSL)	32,000	98,000

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Table 2–Reducing sugar utilization, yields, and protein content of Calvatia gigantea and Candida steatolytica grown on brewery wastes supplemented with $(NH_4)_2SO_4$ and fermentation sludge liquor (FSL)

Waste[a]	Reducing sugar:N ratio	Reducing sugar utilization		Yield (dry wt basis)				Protein content	
		C. gigantea (%)	C. steatolytica (%)	C. gigantea (g/liter)	C. gigantea (g/g sugar)	C. steatolytica (g/liter)	C. steatolytica (g/g sugar)	C. gigantea (%)	C. steatolytica (%)
Grain press liquor (GPL)	69.1:1	74.2	83.8	6.25	0.248	6.28	0.282	13.69	21.30
GPL + 0.05% N as $(NH_4)_2SO_4$	32.1:1	76.7	90.3	15.19	0.660	6.51	0.283	29.40	28.75
GPL + 0.10% N as $(NH_4)_2SO_4$	20.9:1	78.3	86.7	13.03	0.501	7.89	0.336	35.62	40.13
GPL + 0.15% N as $(NH_4)_2SO_4$	15.5:1	76.0	73.3	10.17	0.462	5.45	0.239	37.37	34.87
GPL + 0.05% N as FSL	28.4:1	79.2	83.0	10.67	0.498	8.08	0.384	33.54	27.75
GPL + 0.10% N as FSL	16.0:1	80.4	87.0	9.96	0.498	7.84	0.424	37.94	34.87
GPL + 0.15% N as FSL	10.1:1	82.1	82.1	9.23	0.576	6.93	0.433	44.40	34.65
Trub press liquor (TPL)	72.0:1	63.6	68.4	27.72	0.749	10.56	0.302	17.50	23.49
TPL + 0.05% N as $(NH_4)_2SO_4$	43.5:1	68.1	70.9	39.74	0.883	10.71	0.286	28.56	27.75
TPL + 0.10% N as $(NH_4)_2SO_4$	31.2:1	70.0	76.4	36.61	0.872	12.70	0.330	38.27	44.25
TPL + 0.15% N as $(NH_4)_2SO_4$	24.3:1	65.4	74.5	15.65	0.382	11.03	0.306	29.70	34.81
TPL + 0.05% N as FSL	37.3:1	72.3	81.8	31.44	0.816	10.25	0.301	40.27	37.16
TPL + 0.10% N as FSL	22.6:1	71.8	83.9	28.21	0.881	9.06	0.324	44.23	33.60
TPL + 0.15% N as FSL	13.9:1	71.0	85.5	25.52	0.945	8.04	0.365	44.52	29.56

[a] All samples buffered with 3 g/liter of potassium phosphate and adjusted to pH 6.0

the yields obtained in this study are quite high. Maximum yields of mushroom mycelia reported previously include 26.6 g/liter for *Agaricus blazei* (Block et al., 1953) and 29.6 g/liter for *Morchella hybrida* (Reusser et al., 1958a). The yield of 39.7 g/liter of *C. gigantea* is considerably higher than these values. Yields of yeast cultures are usually on the order of 8–10 g/liter (Inskeep et al., 1951; Gray et al., 1964). Again the yield of 12.7 g/liter of *C. steatolytica* compares well with these figures.

Protein production

As shown in Table 2, significant increases in protein content were achieved with nitrogen supplementation. Maxima of 44.3% and 44.5% protein were obtained in *C. steatolytica* and *C. gigantea*, respectively. Protein concentrations obtained in TPL were higher than those obtained in GPL.

In GPL *C. steatolytica* produced maximum protein concentrations at 0.10% added nitrogen using either nitrogen source. Optimum protein concentrations were obtained in TPL in 0.10% added NH_4^+-nitrogen and at 0.05% added FSL-nitrogen. With both wastes, the greatest total yields of protein, 3.17 and 5.62 g/liter in GPL and TPL, respectively, were obtained with 0.10% NH_4^+-nitrogen (as calculated from the net dry weight and protein content given in Tables 2 and 3).

The maximum protein concentrations in *C. gigantea* were attained in substrates supplemented with 0.15% FSL-nitrogen. The differences in mycelial protein concentration between substrates with 0.10% and 0.15% added nitrogen were generally small. For total protein production, the highest yields, 4.64 and 14.01 g/liter in GPL and TPL, respectively, were obtained in both wastes at 0.10% added NH_4^+-nitrogen.

The highest protein concentration obtained in *C. steatolytica* was 44.25%, which is at the lower range of protein concentrations most investigators have observed in yeast cells (Agarwal et al., 1974; Aries, 1946). A similar protein content of 44.52% was attained by *C. gigantea*. The latter figure compares quite favorably with the values usually reported for mycelial protein concentrations. Falanghe (1962) has reported 44.6% protein in a strain of *A. campestris* and 48.3% protein in *Morchella hybrida*, while Reusser et al. (1958a) have reported a protein content of 53.8% for *Cantharellus cibarious* mycelia.

BOD reduction

Table 3 shows the effects of nitrogen supplementation on BOD reduction. In general, *C. gigantea* was found to be much more efficient than *C. steatolytica* in this respect. While the addition of $(NH_4)_2SO_4$ to GPL had a negligible effect on the removal of BOD by *C. steatolytica*, addition of FSL yielded BOD reductions up to 42%, a 12% increase over nonsupplemented GPL. In TPL there was very little difference between the nitrogen sources in effecting reductions in BOD. Maximum BOD reductions obtained with *C. steatolytica* were in the area of 55% which is less than the 60% BOD reductions reported for *C. utilis* by Inskeep et al. (1951) and Reiser (1954).

Table 3–Effect of nitrogen supplements on BOD reduction in grain press liquor and trub press liquor

Waste[a]	BOD reduction C. steatolytica (%)	BOD reduction C. gigantea (%)
Grain press liquor (GPL)	28.2	56.2
GPL + 0.05% N as $(NH_4)_2SO_4$	28.1	75.0
GPL + 0.10% N as $(NH_4)_2SO_4$	31.2	67.2
GPL + 0.15% N as $(NH_4)_2SO_4$	29.7	66.4
GPL + 0.05% N as FSL	40.0	55.3
GPL + 0.10% N as FSL	41.8	65.1
GPL + 0.15% N as FSL	39.2	59.1
Trub press liquor (TPL)	45.5	56.1
TPL + 0.05% N as $(NH_4)_2SO_4$	50.7	65.2
TPL + 0.10% N as $(NH_4)_2SO_4$	54.5	62.1
TPL + 0.15% N as $(NH_4)_2SO_4$	50.7	55.3
TPL + 0.05% N as FSL	52.1	54.5
TPL + 0.10% N as FSL	50.0	63.3
TPL + 0.15% N as FSL	48.5	59.8

[a] All samples buffered with 3 g/liter of potassium phosphate and adjusted to pH 6.0

For *C. gigantea*, supplementation with 0.05% nitrogen using $(NH_4)_2SO_4$ permitted the largest reductions in BOD, 75% and 65% in GPL and TPL, respectively. With FSL supplements, maximum BOD reductions were obtained at 0.10% added nitrogen. As noted in the growth studies, the effects of addition of FSL on BOD removal were significantly greater than could be accounted for on a simple additive basis.

This investigation has shown that addition of nitrogen, from both $(NH_4)_2SO_4$ and FSL, to GPL and TPL produced a significant increase in cell yields, protein contents, and reductions of BOD. On the basis of the results, it would appear that further experiments employing *C. gigantea* to reduce the BOD of brewery wastes and produce microbial protein are warranted.

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ROLE OF THE LYSINE, TYROSINE AND TRYPTOPHAN RESIDUES IN THE ACTIVITY OF MILK LYSOZYMES

INTRODUCTION

SINCE IT APPEARS that each amino acid in a protein chain has a specific role in the function of the molecule, it should be possible to elucidate the nature of this function by physical and chemical methods. The x-ray crystallographic studies of Blake et al. (1965, 1967) have yielded a three dimensional projection of egg white lysozyme (EC 3.2.1.17) and virtually every side chain residue which is susceptible to chemical modification has been studied in regards to its role in the conformation and activity of the enzyme.

The lysine residues of egg white lysozyme (EWL) have been studied extensively but their role in the action of the enzyme is not yet clearly established. Chemical modifications such as guanidination which retain the positive charge of these residues do not affect lytic activity (Geschwind and Li, 1957; Parsons et al., 1969); whereas, modifications such as acetylation which remove the positive charge result in progressive inactivation at neutral pH (Parsons et al., 1969; Davies and Neuberger, 1969; Yamasaki et al., 1968a). Furthermore, the loss of lytic activity due to acetylation has been shown to be directly proportional to the number of acetyl groups introduced and the lowering of the isoelectric point of EWL. Complete acetylation of the lysozyme molecule does not seem to affect chitinase activity (Davies and Neuberger, 1969; Hayashi et al., 1968; Yamasaki et al., 1968b).

Atassi and Habeeb (1969) nitrated lysine residues 20 and 23 with tetranitromethane (TNM), and observed that the nitrated EWL possessed only 50% activity and subsequent reduction of the nitro group restored only slight activity. Both modifications caused conformational changes which were probably responsible for the loss in activity.

The involvement of the tryptophan residues in the activity of EWL has been previously reported. Hayashi et al. (1963, 1964, 1965) noted that 80% of the lysozyme activity was lost upon oxidation of tryptophan 62 by N-bromosuccinimide (NBS). Hartdegen and Rupley (1964, 1967) found that iodine oxidation of tryptophan 108 destroyed most of the lytic activity of EWL. The crystallographic studies of Blake et al. (1965, 1967) and Phillips (1967) have confirmed the involvement of tryptophan residues 62 and 63 as well as tryptophan 108 in the substrate binding of EWL.

Inhibition studies involving N-acetylglucosamine (NAG) and imidazole compounds have also shown the involvement of tryptophan in substrate binding of EWL. Neuberger and Wilson (1967) related changes in the tryptophan difference spectrum of lysozyme to the inhibitory power of NAG and its derivatives. Shinitzky et al. (1966a) observed that the fluorescence of lysozyme was affected by its binding with NAG and its polymers. They proposed that the spectral shifts were due to conformational changes of the protein that bring hydrophobic groups closer to the affected indole rings. Shinitzky et al. (1966b) also noted the formation of charge-transfer type complexes between imidazole and indole derivatives in model systems and postulated that the lysozyme inhibition by compounds containing a protonated imidazole ring may be due to the formation of a charge-transfer complex with the tryptophan residues in the enzyme.

Except for the information available on EWL, little is known about the role of specific amino acids in lysozymes from other sources. Human milk lysozyme (HML) and bovine milk lysozyme (BML) have been isolated in this laboratory. Chemical modification of certain amino acid residues of BML and HML and comparison with data available on EWL could yield valuable information about the role of these amino acids in the milk lysozymes. In the present study the lysine, tyrosine and tryptophan residues in BML and HML were selectively modified and the effect of these modifications on lytic or chitinase activity was determined.

MATERIALS & METHODS

BML AND HML USED in this study were isolated in an electrophoretically and ultracentrifugally pure form as previously reported (Chandan et al., 1965; Dalaly et al., 1970). EWL, used as a control enzyme, was a three times recrystallized enzyme (Lot No. 7812) obtained from Nutritional Biochemicals Corp.

Acetylation of lysine residues

The three lysozymes were acetylated with various amounts of acetic anhydride (5–10 μl/mg protein), according to the method of Fraenkel-Conrat (1957). Lytic activity was determined during the course of the reaction and the modified product was isolated, lyophilized and retained for further analysis. The decrease in free amino groups as a result of acetylation was determined on the lyophilized protein by the ninhydrin assay method (Spies, 1967).

Nitration of the tyrosine residues

The tyrosine residues were nitrated with tetranitromethane (TNM) according to the procedure of Atassi and Habeeb (1969) and the number of moles of 3-nitrotyrosine per mole of protein was determined by the method of Sokolovsky et al. (1966).

Oxidation of tryptophan residues

N-bromosuccinimide oxidation of tryptophan was carried out according to the procedure of Spande and Witkop (1967). The number of tryptophan residues oxidized in milk lysozymes was determined by the method of Patchornik et al. (1958).

Assays for enzymatic activity

Lysozyme activity toward cell walls of *Micrococcus lysodeikticus* was determined by the method of Parry et al. (1965). Determination of activity on whole chitin was based on the procedure of Howard and Glazer (1967), and was followed by the reducing sugar method of Park and Johnson (1949).

RESULTS & DISCUSSION

Acetylation of lysine residues

While determining the effect of acetylation of lysine residues on the activity of BML and HML, the effect of acetylation on EWL was also determined for comparison (Table 1). During acetylation the lytic activity of the lysozymes decreased progressively, and the rate of inactivation was proportional to the amount of acetic anhydride added. The extent of

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acetylation varied for each of the lysozymes since the amount of acetic anhydride was varied from 5–10 μl/mg protein. In addition, native BML contains 11 free amino groups which are available for acetylation while HML and EWL contain 6 or 7 (Parry et al., 1969; Eitenmiller et al., 1975). In order to make a valid comparison, therefore, between the acetylation of three lysozymes, the percent change in activity divided by the percent change in free amino groups was calculated. This value was approximately equal to one for all three enzymes which indicated that for every one percent modification of the free amino groups, there was a corresponding one percent decrease in activity regardless of the extent of acetylation. It was noted, however, that inactivation of EWL was somewhat more rapid than that of HML or BML.

Like EWL and many other lysozymes, BML and HML also possess chitinase activity in addition to the lytic activity toward bacterial cell walls. It is possible that the effects of lysozyme upon chitin are closely related to the effects on bacterial cell walls since both the substrates are structurally similar. The cell wall mucopolysaccharide contains alternate units of N-acetylglucosamine and N-acetylmuramic acid residues joined by β-1, 4 linkages while chitin contains only N-acetylglucosamine residues. We, therefore, investigated whether the decrease in lytic activity of BML and HML due to acetylation is accompanied by a decrease in chitinase activity. As shown in Table 2, there was no loss in activity toward chitin after acetylation with acetic anhydride.

Several workers (Davies et al., 1969; Yamasaki et al., 1968a, b; Kronman et al., 1967) suggested that the basic lysine residues facilitate contact of the lysozyme with the negative charged microbial cell wall. This electrostatic attraction force is progressively lost with acetylation – a charge modifying procedure. Therefore, lytic activity decreases while chitinase activity, which is independent of the charge on the lysozyme molecule, remains constant. From the present study it appears that the lysine residues of not only EWL but also of the milk lysozymes do function only in contributing to the total basicity and do not have a direct role in enzymatic activity.

Nitration of the tyrosine residues

The effect of nitration on the activity of the milk lysozymes and EWL is summarized in Table 3. Nitration of the lysozymes with TNM decreased lytic activity about 50%. This is in agreement with the findings of Atassi and Habeeb (1969) for EWL. The percent loss in chitinase activity was similar to the loss in lytic activity and in both cases there seems to be no direct relationship between percent change in activity and percent change of tyrosine residues due to nitration. The loss of enzymic activity therefore was probably due to conformational changes, making it difficult to assess the role of the tyrosine residues in the activity of the milk lysozymes. It is interesting to note that other workers have reported that acetylation of the tyrosine residues of EWL does not alter lytic activity and that these residues probably play no role in the enzymic activity (Parsons et al., 1969). Similar studies on the milk lysozymes could conceivably clarify the role of the tyrosine residues.

Oxidation of the tryptophan residues

When the single tryptophan residue in BML was oxidized with NBS, only slight loss of activity occurred. However, as shown in Figure 1, the oxidation of only one tryptophan residue in HML leads to almost complete loss of activity. This loss of activity in HML is similar to the observed effect of NBS oxidation on EWL (Hayashi et al., 1963, 1964, 1965; Takahashi et al., 1965) and suggests that tryptophan is involved in the substrate binding site of HML but not of BML. When HML was subjected to NBS oxidation in 8.0M urea, five of the six tryptophan residues were oxidized, indicating that in the native state at least one residue may be buried in a hydrophobic region of the HML molecule. Kronman et al. (1967) criticized the NBS method for its apparent lack of tryptophan specificity. The authors reported, using EWL as a standard, that at NBS to protein molar ratios of greater than 9:1, the NBS reagent was no longer specific for tryptophan since oxidation of tyrosine and histidine residues may occur. It was also found that conformational changes occurred within the EWL molecule so that evaluation of the degree of tryptophan exposure to reagent must be viewed with caution. However, we believe the HML data suggest that tryptophan is involved in the sub-

Table 1—Effect of acetylation of lysine residues on the lytic activity of lysozymes

Lysozyme	Lytic activity (%)	No. of free amino groups	% Free amino groups after acetylation	Change % activity / Change % amino groups
BML				
Native	100	11.0	100	–
Acetylated	17	1.8	16	0.99
HML				
Native	100	6.0	100	–
Acetylated	63	3.7	62	0.96
EWL				
Native	100	7.0	100	–
Acetylated	9	1.3	19	1.12

Table 2—Chitinase activity of native and acetylated lysozymes

Lysozyme	μg/ml reducing substance[a]
BML	
Native[b]	110
Acetylated	110
HML	
Native	140
Acetylated	140
EWL	
Native	138
Acetylated	138

[a] Glucose equivalent
[b] Assay conditions were 5 mg/ml powdered chitin suspended in 0.2M sodium acetate buffer, pH 5.5, at enzyme concentration of 13.3 μM for the BML, HML or EWL. Incubation was at 37°C for 24 hr.

Table 3—Activity of native and nitrated lysozymes

Lysozyme	No. of tyrosines	No. of nitrated tyrosines	% Activity Lytic	% Activity Chitinase
BML				
Native	7	0	100	100
Nitrated	4	3	57	40
HML				
Native	5	0	100	100
Nitrated	2	2–3	41	–[a]
EWL				
Native	3	0	100	100
Nitrated	1	2	50	53

[a] Insufficient material for activity determination

strate binding since almost complete loss of activity occurred when 1 mole of tryptophan was oxidized at a NBS to lysozyme ratio of 5:1.

N-acetylglucosamine inhibition of tryptophan

The data on the effects of N-acetylglucosamine (NAG) upon BML and HML are shown in Table 4. BML was activated at all NAG concentrations with maximum activation of 38.4% occurring at 0.20M concentration of NAG. HML, on the other hand, was inhibited with maximum inhibition of 59.1% occurring at 0.0125M and 0.025M concentrations of NAG. Inhibition was slightly less at higher NAG concentrations of 0.10 and 0.20M. This indicates a difference in the substrate-binding sites of BML and HML and supports the NBS titration data which show tryptophan to be involved in the activity of HML but not of BML.

Table 4–Effect of N-Acetylglucosamine on the activity of milk lysozymes

N-Acetylglucosamine (M)	% Relative activity	
	HML	BML
0	100	100
0.00625	61.4	110
0.0125	40.9	110
0.025	40.9	110
0.10	42.6	130.8
0.20	46.3	138.4

Table 5–Effect of histamine on the activity of milk lysozymes and egg white lysozyme

Histamine (M)	% Inhibition		
	BML	HML	EWL
0.04	2.5	37.5	12.5
0.08	25.0	79.2	62.5

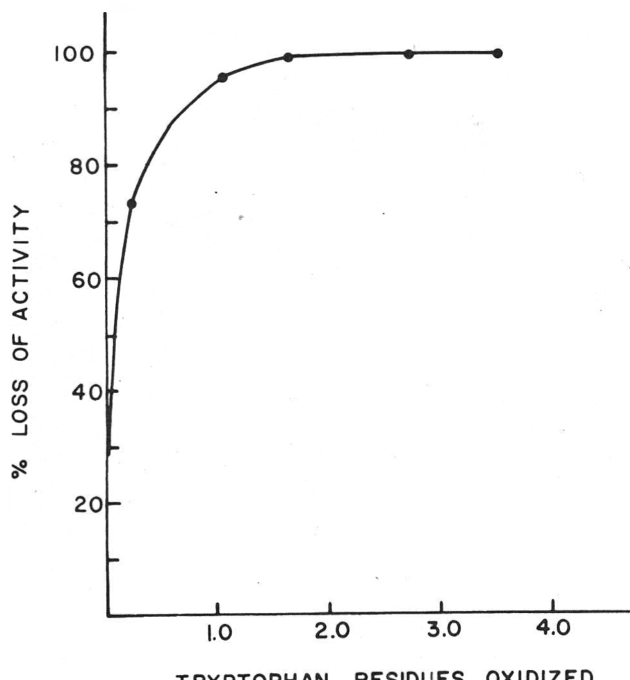

Fig. 1–N-bromosuccinimide oxidation of tryptophan residues in HML.

Histamine inhibition

Histamine and other imidazole compounds also have been shown to be potent inhibitors of EWL. As can be seen in Table 5, histamine inhibits HML and EWL to a similar extent, 79 and 62%, respectively, at 0.08M histamine, but does not inhibit BML to any significant extent.

It appears that the results of the NBS oxidation and the inhibition studies with NAG and histamine show tryptophan to be involved in the substrate-binding site of HML as is the case for EWL. The fact that BML is not inhibited by the above treatments makes it fairly certain that the single tryptophan residue in the BML molecule is not involved in the substrate binding by the enzyme. In this respect, BML differs from most lysozymes of animal origin and appears to resemble plant lysozymes of fig and papaya in which tryptophan is reportedly not involved in the substrate-binding (Howard and Glazer, 1967; Kronman et al., 1967).

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STABILITY OF L-ASCORBATE 2-SULFATE AND L-ASCORBATE IN WHEAT FOODS AND MILK

INTRODUCTION

L-ASCORBIC ACID has been added to some foods for years because of its functional properties and/or its vitamin potency (Bauernfeind and Pinkert, 1970). In more recent years, animals other than primates and the guinea pig have also been shown (Chatterjee, 1973) to depend on dietary sources of vitamin C. These include fish, flying mammals, and highly evolved birds. It is interesting to note that L-ascorbic acid is not always a desirable component of foods. For example, the color of cranberry (Starr and Francis, 1973) and grape juice (Skalski and Sistrunk, 1973) are adversely affected by L-ascorbic acid.

The instability of L-ascorbic acid under oxidative (Hay et al., 1967) or hot acidic conditions (Feather and Harris, 1973) is well recognized. Vitamin C is destroyed especially rapidly in aerated foods that contain biological or inorganic catalysts such as the enzyme L-ascorbic acid oxidase or the metallic ions of iron and copper. For example, fortification of baked foods with vitamin C is a problem, since these foods are leavened, and since wheat flour contains both types of oxidative catalysts.

Several years ago we became interested in possibly using L-ascorbate 2-sulfate as a more stable form of vitamin C in foods and feeds. The sulfate ester of L-ascorbic acid apparently occurs widely in animals and marine life. It was first discovered in brine shrimp (Mead and Finamore, 1969), and since then has been found in the urine of man, rat, guinea pig, trout and coho salmon (Baker et al., 1971) as well as in tissues of the rat and trout (Tolbert and Baker, 1974; Mumma and Verlangieri, 1972; Hornig et al., 1973). Enzymes which synthesize or hydrolyze L-ascorbate 2-sulfate have likewise been found in rat, guinea pig, monkey, and trout (Tolbert and Baker, 1974). No one has reported the occurrence of L-ascorbate 2-sulfate in plants.

L-Ascorbate 2-sulfate is readily available in pure crystalline form. The compound can be synthesized in high yield (80%) by sulfation of L-ascorbate at pH 9.5–10.5 using trimethylamine-sulfur trioxide (Seib et al., 1974). L-Ascorbate 2-sulfate is stable in aqueous acid and alkali (Seib et al., 1974; Tolbert et al., 1971). Furthermore, it is considerably more difficult to oxidize than L-ascorbate (Seib et al., 1974; Ford and Ruoff, 1965). The sulfate group is, however, rapidly lost from L-ascorbate 2-sulfate in acidic methanol.

The vitamin C potency of L-ascorbate 2-sulfate appears to be species-dependent; it prevents scurvy in fish (Halver et al., 1972), but not in the guinea pig (Kuenzig et al., 1974; Campeau et al., 1973; Bond, 1972). The important question concerning its potency in primates has not yet been answered.

In this communication we present an assay procedure for determining L-ascorbate 2-sulfate, and compare the stabilities of L-ascorbate 2-sulfate and L-ascorbate in several foods. The assay procedure could also be applied to the determination of L-ascorbate 2-sulfate in living tissue. In addition, a rapid method has been developed to measure L-ascorbic acid in vitamin C-fortified milk at levels greater than 180 ppm.

EXPERIMENTAL

L-ASCORBIC ACID (10 mg, 77 mg, or 212 mg) was added to wheat flour (100g) and pup loaves were subsequently baked at 218°C for 15 min using a straight dough procedure (Finney and Barmore, 1945). In one bake test, ferrous sulfate heptahydrate (20 mg) was also added along with L-ascorbic acid (212 mg) to the flour (100g). Assuming the flour contained 1.3 mg of naturally occurring iron per 100g of flour, the total level of iron in the pup loaf was estimated to be 16 mg of iron per pound of bread.

An extruded product was prepared from the identical ingredients used to make bread, except water was reduced from 66% (based on flour) to 25%. The moistened meal was cooked using a Brabender Extruder (Model 250). The extruder's geometry is as follows: diameter 0.75 in., length to diameter ratio 25:1, normal-flighted screw with a feed-depth of 0.15 in. and a compression ratio of 5:1, and a rod-type die 3/16 in. in diameter. The extruder is fitted with three heating zones. During extrusion, the final (metering) zone was heated to 175°C; no external heat was applied to the first two zones.

Pancakes were prepared from flour (100g) containing added L-ascorbic acid (297 mg). Portions (50g) of the batter were heated on a griddle (250°C) for 30 sec on each side. While pasteurized milk and low-fat (2%) milk were fortified with L-ascorbic acid (24 mg per 100 ml); the samples were stored at 10°C.

In a second series of experiments, the foods were prepared using fortification with dipotassium L-ascorbate 2-sulfate (DAS) instead of L-ascorbate. DAS was prepared as previously described (Seib et al., 1974) and had m.p. 96–7°, $[\alpha]^{25}_{D}$ + 50° (*c* 1.0, water), and water solubility of 0.8g $(ml)^{-1}$ at 25°C. DAS (formula weight 332.2) was added in amounts equivalent on a molar basis to the quantities of L-ascorbic acid. The levels were as follows: bread, 19, 150 and 400 mg of DAS per 100g of flour; extruded product, 19 mg per 100g of flour; pancake, 503 mg per 100g of flour; and milk 46 mg per 100 ml of milk.

Prior to analysis, solid foods were air-dried and ground in a Wiley mill to pass a 60-mesh screen. Pancakes were defatted with ethyl ether in a Soxhlet before grinding.

L-Ascorbic acid in the cereal products was determined by a microfluorometric method (Deutsch and Weeks, 1965), whereas, in the vitamin C-fortified milk L-ascorbate was determined by the following UV procedure. To 10.0 ml of milk was added 30 mg of ammonium sulfate, and the mixture was dialyzed 12 hr against 5% aqueous metaphosphoric acid (90.0 ml). In a control experiment using water instead of milk, diffusion of L-ascorbic acid through the dialysis bag was shown to be at equilibrium in a maximum of 8 hr. Duplicate experiments showed the concentration of L-ascorbate in the dialyzate was equal to the theoretical value. After dialysis of the milk, 20 ml of the solution outside the bag was pipetted into a 100 ml volumetric flask and made to volume with 5% aqueous metaphosphoric acid. The absorbance of the solution was read at 245 nm against a blank. To test the reproducibility of the method, four samples of milk fortified to 240 ppm were assayed immediately after addition of L-ascorbic acid. Recovery of L-ascorbic acid was 83.9 ± 1.5%. Since the molar extinction (ϵ) of L-ascorbic acid at pH 2 is 1×10^4, the assay procedure can be used on milk containing ⩾ 180 ppm of L-ascorbic acid.

L-Ascorbate 2-sulfate in cereal products was determined as follows. Duplicate samples containing 1–20 mg of DAS were weighed directly into two separate 50 ml volumetric flasks. Water was added and the mixture shaken 5 min and allowed to stand 1 hr. The mixture was filtered through a small, dry, filter-aid pad of Celite (Hyflo SuperCel, Johns Manville, Denver, Colo.) previously prepared on top of a glass-

fiber mat and dried. An aliquot (20.0 ml) of the clear filtrate was placed on top of a column (6 × 80 mm) containing 200 mg of a weakly basic, ion-exchange resin (BioRad AG-3, chloride form, 200–400 mesh, BioRad Laboratories, Richmond, Calif.). After washing the column with water (100 ml) and 0.1M aqueous hydrochloric acid (100 ml), L-ascorbate 2-sulfate was eluted with 1M aqueous sodium sulfate (95 ml). The elute was adjusted to volume (100 ml) and to pH 7, and its absorbance was read at 255 nm on a spectrophotometer (Beckman, Model DB-G). A standard curve was constructed from the absorbance of solutions containing 5–16g $(ml)^{-1}$ of DAS (ϵ = 16,300 at pH 7.0). Control samples were prepared by addition of DAS (15–500 mg) to ground bread and pancake crumbs (90g), and analysis gave quantitative recovery of DAS. The widest range in the values of duplicate analyses was 7%.

L-Ascorbate 2-sulfate (DAS) in milk was determined essentially as described for the cereal products, except DAS was first separated from some components of milk by dialysis. To a sample (10.0 ml) of milk was added ammonium sulfate (30 mg), and the solution was dialyzed 12 hr against 90.0 ml of water that had been previously purged of oxygen by a stream of nitrogen (addition of ammonium sulfate was essential for complete recovery of DAS during dialysis in water). 10 ml of the dialyzate was then added to the weakly basic anion exchange resin (200 mg), and the remaining steps were carried out as previously described.

Table 1–Stability of L-ascorbic acid and dipotassium L-ascorbate 2-sulfate (DAS) in selected foods

Food	L-ascorbic acid (mg)[a]	Recovery (%)	DAS (mg)[a]	Recovery (%)
Bread	10	10	19	86
Bread	77	11	150	86
Bread	212	35	400	86
Bread (ferrous sulfate)	212	26	–	–
Extruded product	10	21	19	88
Pancake	297	0	503	96
Milk, whole pasteurized	24[b]	68[b]	46[b]	95[b]

[a] Amount added to 100 ml of milk or to 100g of flour used to make bread, extruded product and pancake. 10 mg of L-ascorbic acid is equivalent to 19 mg of dipotassium L-ascorbate 2-sulfate (DAS).
[b] Stored 15 days at 10°C

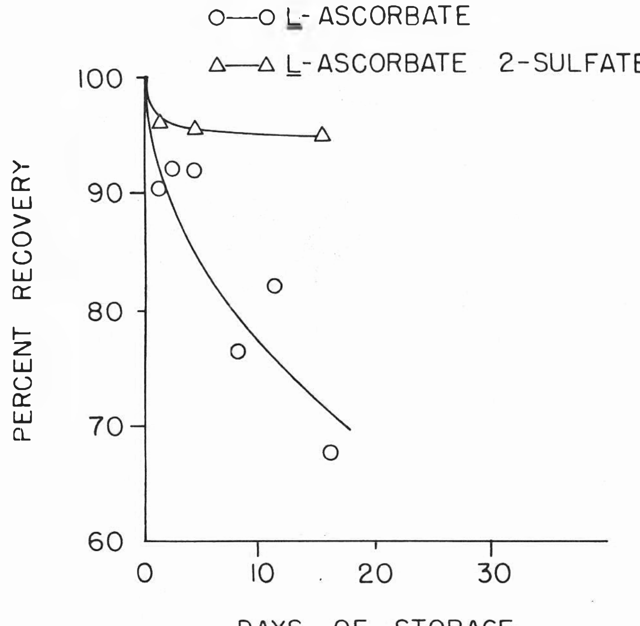

Fig. 1–Recovery of L-ascorbate and L-ascorbate 2-sulfate from whole, pasteurized milk stored at 10° C.

RESULTS & DISCUSSION

THE RECOVERIES of L-ascorbic acid and dipotassium L-ascorbate 2-sulfate (DAS) from various foods are given in Table 1. The data shows DAS is generally more stable than L-ascorbate in bread, milk, pancake and an extruded product.

In bread the loss of DAS (14%) was independent of its concentration, whereas, the loss of L-ascorbate (65–90%) was dependent on concentration. These results are in accord with the behavior of the pure compounds in model systems. Kinetic studies (Quadri et al., 1973; Seib et al., 1974) showed L-ascorbate 2-sulfate degrades by a zero-order reaction in boiling water in the presence or absence of oxygen. This explains why the loss of DAS is independent of concentration in bread.

On the other hand, loss of L-ascorbate in bread is concentration dependent because wheat flour contains L-ascorbic acid oxidase (Bauernfeind and Pinkert, 1970). This enzyme catalyzes the conversion of L-ascorbic acid to the reactive intermediate dehydro-L-ascorbic acid, which rapidly decomposes and thereby leads to irreversible loss of L-ascorbate. Thus, L-ascorbate may be destroyed in bread not only by oxygen and heat, but also by a more rapid route involving enzyme action. However, because dough contains limited amounts of both oxygen and the enzyme, at some concentration of L-ascorbate the enzyme system will become saturated with substrate. Consequently a greater percentage of L-ascorbate will survive the bake as the levels of L-ascorbate are increased in bread. Apparently, L-ascorbate 2-sulfate is not a substrate for L-ascorbic acid oxidase.

One other possible explanation of the greater recovery of L-ascorbate at its higher concentration in bread can not be ruled out. All L-ascorbate may have been destroyed, and the fluorometric assay of bread may have instead measured a decomposition product of L-ascorbate. Present evidence indicates this explanation is unlikely, since the principal decomposition product of L-ascorbate in bread at a level of 10 mg per 140g of flour has been found to be L-threonic acid (Thewlis, 1971), which would not interfere in the fluorometric procedure (Deutsch and Weeks, 1965), but more evidence is needed to clear up this point.

From the recovery data in Table 1, one can estimate that to obtain their recommended daily allowance of vitamin C, an adult would have to consume approximately 130g of the bread (five slices) prepared from flour originally containing 212 mg of L-ascorbic per 100g of flour. In contrast, only two slices would be required (assuming equal vitamin potency) of bread baked with equivalent levels of L-ascorbate 2-sulfate. The standards of identity for bread presently permit only 200 ppm of L-ascorbic acid in flour (Pyler, 1973).

Because ferrous ions accelerate the oxidation of L-ascorbic acid in aqueous solution, we decided to examine the effect of ferrous sulfate enrichment on the stability of L-ascorbic acid in bread. At a level of 16 mg iron per pound of bread, 75% of which was added as ferrous sulfate, only a minor decrease in recovered L-ascorbate was found (Table 1). These results indicate L-ascorbate is probably destroyed in bread mainly through the enzymatic system.

L-Ascorbic acid could not be detected in a pancake made from flour containing 297 mg of added vitamin C per 100g flour, whereas, 96% of L-ascorbate 2-sulfate was recovered from pancakes. The loss of L-ascorbic acid probably occurs during mixing when air is incorporated into the alkaline pancake batter (pH 7.2). It is well known that L-ascorbic acid in an acidic medium is less rapidly oxidized by air than in an alkaline solution (Herbert et al., 1933).

Fresh whole milk generally contains 23 ppm of L-ascorbic

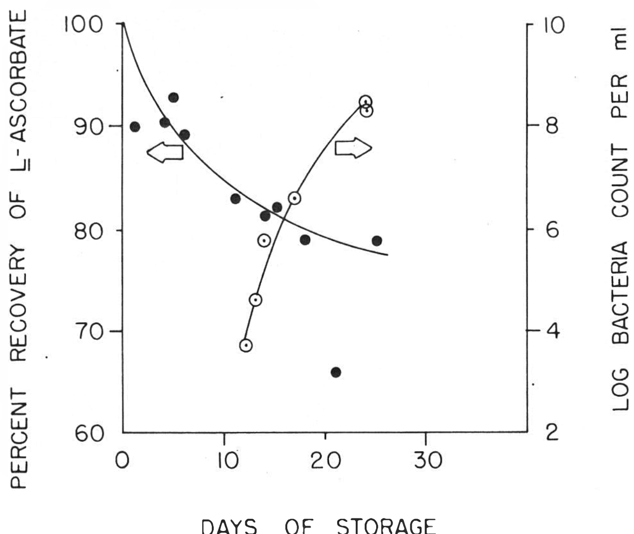

Fig. 2—Recovery of L-ascorbate and bacterial count of low-fat, pasteurized milk stored at 10°C.

acid, but the vitamin is slowly destroyed upon storage (Hartman and Dryden, 1965). Our results (Table 1 and Fig. 1) confirm the general instability of L-ascorbate in milk; 32% of added L-ascorbate was lost after 15 days at 10°C. Under the same conditions, only 5% of an equivalent level of L-ascorbate 2-sulfate was lost. However, we hasten to add one inconsistency. When whole milk was fortified to 240 ppm with L-ascorbic acid, only 84 ± 1.5% was recovered from milk when it was dialyzed immediately. On the other hand, recovery of L-ascorbic acid from this same milk amounted to 90%, 92% and 92% on standing 1, 2 and 4 days, respectively, at 10°C. These results remain unexplained.

In another experiment involving milk, we wondered whether the loss of L-ascorbate might be due to its serving as a substrate for growing population of microorganisms. The results in Figure 2 show that the microbes in milk do not consume added L-ascorbate. During the storage period of the 12 to the 14 day of storage, the number of microorganisms increased by four orders of magnitude, while only 3.5% of L-ascorbate was lost. On the other hand, very few microorganisms were present in milk during the storage period of 0—12 days; still 17% of L-ascorbate was lost.

Simulated pasteurization (63°C for 30 min) of milk fortified with 240 ppm of L-ascorbic acid or 500 ppm of DAS resulted in a 20% loss of L-ascorbate, whereas no loss was found for L-ascrobate 2-sulfate.

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YIELDS FROM CHYMOTRYPSIN AND LYSOZYME UNDER FLUCTUATING TEMPERATURE TREATMENTS

INTRODUCTION

REACTION under fluctuating temperature may show different results from those under the constant mean temperature because the integration of fluctuated rates has been proven to be greater than the rate at the constant mean temperature (Hicks, 1944; Schwimmer et al., 1955; Powers et al., 1965). Enzymic reactions are affected by fluctuating temperature differently from simple chemical reactions because activation, deactivation, reactivation and possibly certain patterns of temperature adaptation unique to enzyme molecules influence the rate.

The effect of fluctuating temperature on lipase activity was studied by Chang and Powers (1974), and on invertase activity and stability by Wu et al. (1974). Results of these investigations indicated that fluctuating temperature reaction rates depended upon the relationship of the temperature region under study to the transition temperature of the enzyme, the cycling-mode (up or down-shifted), the frequency, and amplitude of cycling.

In this study chymotrypsin and lysozyme were studied to determine effects of various fluctuating temperature treatments on reaction rates. The data were analyzed to determine the mode of temperature adaptation that may occur in enzymes subjected to temperature fluctuation.

EXPERIMENTAL

Materials

Bovine pancreatic α-chymotrypsin (peptidylpeptide hydrolase, E.C. 3.4.4.5.) and egg white lysozyme (N-acetylmuramide glycanohydrolase, E.C. 3.2.1.17.) were salt-free, crystalline products purchased from Worthington Biochemical Corp. (Freehold, N.J.). Lysozyme assay sets, benzoyl-L-tyrosine ethyl ester (BTEE), and other substrates were also purchased from Worthington.

The aluminum thermogradient bar for controlling temperature was described by Howell et al. (1971).

Methods

Temperature cycling. The fluctuating temperature was controlled as described in a previous paper (Wu et al., 1974). The modes of temperature fluctuation were also described in that paper and were a combination of square-wave and sine-wave types of fluctuation.

Constant and fluctuating temperature treatment on chymotrypsin. Chymotrypsin activity was measured by determining the rate of hydrolysis of benzoyl-L-tyrosine ethyl ester (BTEE) to benzoyl-L-tyrosine (BT) which was monitored by the change in absorbance at 256 nm (Hummel, 1959; Worthington Biochem. Corp., 1972). A stock solution containing 0.1 mg of chymotrypsin/ml in 0.001M HCl was prepared. The final concentration of chymotrypsin in the reaction solution was 0.0005 mg/ml. This concentration enabled a steady reaction rate throughout the 48 min period required for the temperature treatments. Substrate and buffer solutions were the same as specified in the Worthington Enzyme Manual.

Buffer and substrate solutions (1.5 × 1.4, v/v) were mixed prior to the experiment. Aliquots of 2.9 ml of the mixture were pipetted into 10 ml test tubes and placed into the wells of the thermogradient bar to bring the solution to the desired temperatures. Then, 0.1 ml of enzyme solution (0.0015 mg of chymotrypsin/ml in 0.001M HCl) was pipetted into the substrate solution. Absorbance at 256 nm was measured at 6 min intervals after transferring the reaction solution into a 1 cm quartz cuvette.

Three sets of conditions were chosen: 15 ± 5°C with up and down cycling modes, 15 ± 10°C with up and down cycling modes and 15 ± 15°C with up and down cycling modes. Cycling frequency was 24 min/cycle in every case. "Cycling up" refers to the fluctuation starting from low temperature and "cycling down" refers to fluctuation starting from the high temperature.

Constant and fluctuating temperature treatments on lysozyme. The lysozyme reaction rate was measured by determining the decrease in turbidity of a *Micrococcus lysodeikticus* cell suspension at 520 nm (Shugar, 1952). The analysis procedure was similar to that given in the Worthington Manual except that the final lysozyme concentration was 0.16 μg/ml during low temperature studies and 0.04 μg/ml when studies were conducted in the high temperature region. Dilution and incubation were done as described previously in the chymotrypsin section. Two temperature regions around 20 and 50°C were chosen for this study. In each region, comparisons were made involving two amplitudes, two frequencies and cycling-up or cycling-down modes.

Theoretical plots of product accumulation during the fluctuation temperature treatment. Plots of rates vs temperatures were first determined by assaying chymotrypsin at 5–30°C and lysozyme from 10–60°C. In the case of lysozyme where activities were not constant, activity curves were divided into several zones of constant-activity regions depending on the reaction time. According to the temperature-time curves that showed the temperature of the solution at any time during the fluctuation, the corresponding rate at that temperature could be found from the activity plots. An integration plot was obtained (using a Wang-700 computer plotter) by integrating the corresponding rates determined previously vs time. The integration plot thus obtained represented the yield-time plot of a specific enzyme under a fluctuating temperature treatment.

Statistical methods of analysis. Differences of rates in each cycling period between the experimental and mathematically calculated results were compared by the paired-t test. Final yields at each complete cycle under different treatments were compared by Duncan's new multiple range test (Dixon, 1973). Linear regression analysis was also used to compare the average ratio of different treatments (Barr and Goodnight, 1972).

RESULTS & DISCUSSION

Activity of chymotrypsin under constant temperatures

The production of benzoyl-L-tyrosine (BT) from benzoyl-L-tyrosine ethyl ester (BTEE) through catalysis by chymotrypsin at constant temperatures from 0–30°C at 5° intervals and at 40, 50 and 55°C was studied. The production of BT under the experimental condition (enzyme concentration 0.003 μg/ml; BTEE 0.00107M with 0.08M Tris, pH 7.8 and 0.1M $CaCl_2$) was fairly linear for 48 min in the region between 10 and 30°C. At 5°C, chymotrypsin catalysis was enhanced after 24 min; at 0°C, activity could not be detected.

Effect of fluctuating temperature on chymotrypsin activity

Figure 1 represents the yield-time curves obtained at 15 ± 10°C. Similar results were observed at 15 ± 5°C and 15 ± 15°C. The yield-time curves obtained under all conditions were similar to those of the mathematically predicted curves. When analyzed by the "paired-t" test, the slopes of all experimental curves from up-shifted periods were significantly higher (at 1% significance level) than those from corresponding theoretical values. The slopes of all curves from down-shifted periods were significantly lower (at 1% significance level) than those from theoretical data. The theoretical plots were calcu-

lated under the assumption that the temperature response of chymotrypsin belongs to the category of "no adaptation" (Christophersen, 1967) which assumes that the rate of catalysis is proportional to the temperature change and equals the rate whenever the reaction is held constant at the corresponding temperature. Thus, this experiment suggests that chymotrypsin shows an over-response to the temperature changes, which leads to the conclusion that the temperature response of chymotrypsin is a type of "inverse compensation" (Christophersen, 1967). The phenomenon of inverse compensation is possibly controlled by the overshoot or undershoot of enzyme activity occurring at the moment the temperature is up-shifted or down-shifted, respectively. The undershoot phenomenon was not pronounced in the first down-shifted periods in all experiments, which was probably due to the fact that in the beginning of each treatment the enzyme was immediately removed from the ice bath and the period at the high temperature was different from those of the following cycles. This phenomenon was not observed when the temperature was up-shifted.

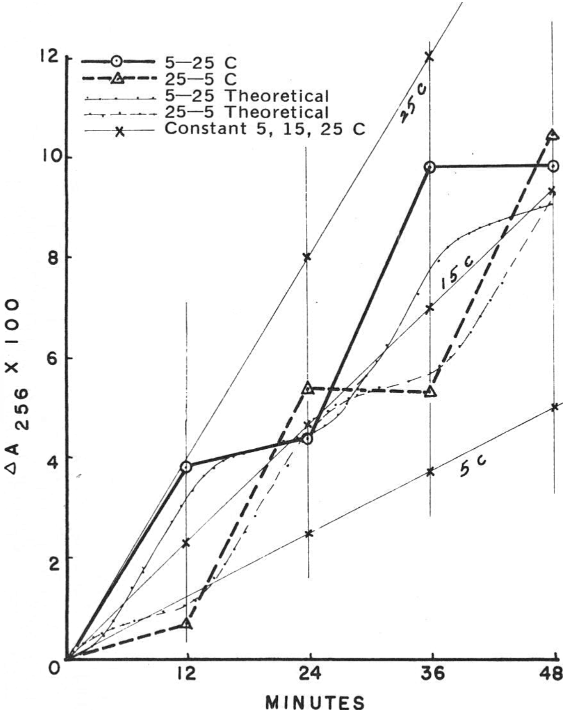

Fig. 1–Comparison of the theoretical and the experimental product yields of chymotrypsin catalysis under the fluctuating temperature treatments (15 ± 10°C with 24 min/cycle).

Table 1–Effect of fluctuating temperature treatments[a] (around 15°C temperature region) on chymotrypsin activity (expressed as P_f/P_c)[b]

Temp	15°C	15 ± 15°C		15 ± 10°C		15 ± 5°C	
Mode	Constant	Up	Down	Up	Down	Up	Down
P_f/P_c	1	1.02	1.31	1.03	1.09	1.00	1.17
	1	1.02	1.31	1.07	1.09	1.00	1.17
	1	1.04	1.29	1.00	1.12	1.02	1.12
	1	1.00	1.33	1.00	1.07	1.02	1.12
Mean	1	1.02	1.31	1.03	1.09	1.01	1.15
Rank	(7)	(5)	(1)	(4)	(3)	(6)	(2)

Duncan's Multiple Range Test (Numbers are the above rankings):
(1) (2) (3) (4) (5) (6) (7)

[a] Fluctuation conditions included three amplitudes, two cycling modes (up or down), and one frequency (24 min/cycle). Reaction time was 48 min.
[b] The P_f/P_c ratio is the ratio of the final yield under the fluctuating temperature treatments to that at the constant mean temperature treatment.

Comparison of the final yields after six sets of different treatments (three amplitudes, two modes and one frequency) around the 15°C region are summarized in Table 1. Slight batch-to-batch variation of enzyme activity was corrected by using the ratio, P_f/P_c, which represents the ratio of final yield from fluctuating temperature to that of constant temperature treatment. Analysis by Duncan's Multiple Range Test based on 5% significance level showed that all three treatments of the cycle-up mode with 15 ± 15°C, ± 10°C and ± 5°C showed no significant difference from the constant treatment, while all three treatments with cycle-down and three amplitudes were higher than cycle-up and constant treatments. There were no significant differences between 15 ± 10°C and 15 ± 5°C in the cycle-down mode; whereas the rate at 15 ± 5°C was statistically higher.

Rates of deactivation were very rapid at temperatures higher than 40°C. For this reason, the study of reaction rates at temperature above 40°C became difficult and inaccurate; therefore, fluctuation temperature studies were not conducted on chymotrypsin catalysis at temperatures above 40°C.

Lysozyme activities under constant temperatures

Rupture of *Micrococcus lysodeikticus* cells by lysozyme was followed at 10 different temperatures in the region from 0–65°C. The final concentration used in the 0–30°C range was 0.16 μg/ml, four times higher than that used in 40–65°C range (0.04 μg/ml). In the temperature region between 10 and 60°C, the lysozyme activity showed a general pattern of constant rate for the first 5–20 min then reaching an inflection point where the rate decreased and remained constant until the end of 48 min. Heating of blank substrate solutions at each temperature showed fairly constant absorbance reading throughout the experiment. Significant heat inactivation was not apparent until 65°C was reached. No activity could be detected at 0°C.

Effect of fluctuating temperature on lysozyme activity

Figures 2, 3, 4 and 5 show the results of the 20°C and the 50°C studies. Patterns similar to those observed with chymotrypsin were obtained. The comparison by the paired-t test also showed the same pattern as chymotrypsin; the slopes of up-shifted periods were greater than those from theoretical data, and the slopes of down-shifted periods were smaller. Thus, inverse compensation is apparent in the response of lysozyme to temperature change. After the inflection points were reached, the reaction rates were relatively unaffected by the fluctuation. The enzyme during the first down-shifted period in the 20°C region showed a greater activity than predicted by theory while in the 50°C region lower activity was observed. The cycling frequency affected the magnitude and the occurrence of overshoot and undershoot. Similar results were obtained in treatment at 20 ± 5°C and 50 ± 5°C.

The comparison of final yields after treatments for 48 min showed that at the 20°C region, slower frequency (24 min/cycle); and greater amplitude of fluctuation generally created greater yield (Table 2). Cycle-up treatment showed no significant difference from cycle-down treatments. All the yields under fluctuating temperature treatments were higher than those obtained under constant mean temperature. The highest yield was obtained at 20 ± 10°C with the cycling-up mode and 24 min/cycle frequency.

In the 50°C region (Table 3), Duncan's Multiple Range Test

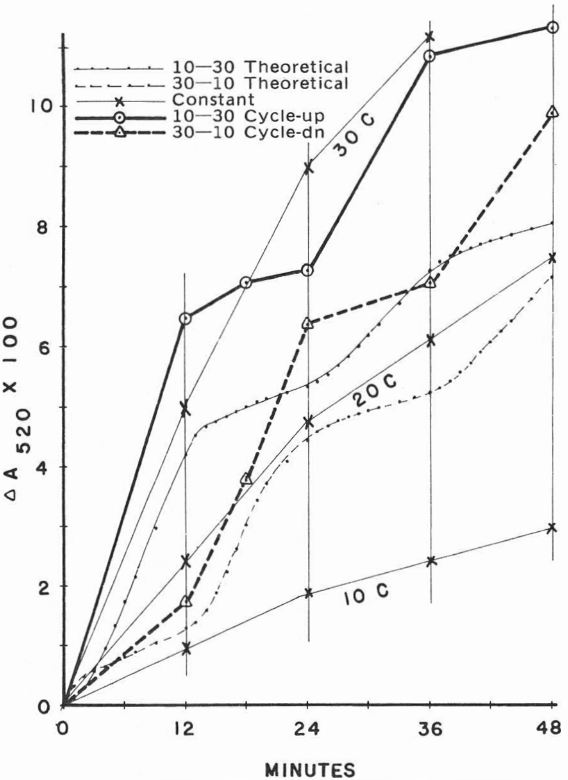

Fig. 2–Comparison of the theoretical and the experimental product yields of lysozyme catalysis under the fluctuating temperature treatments (20 ± 10°C with 24 min/cycle).

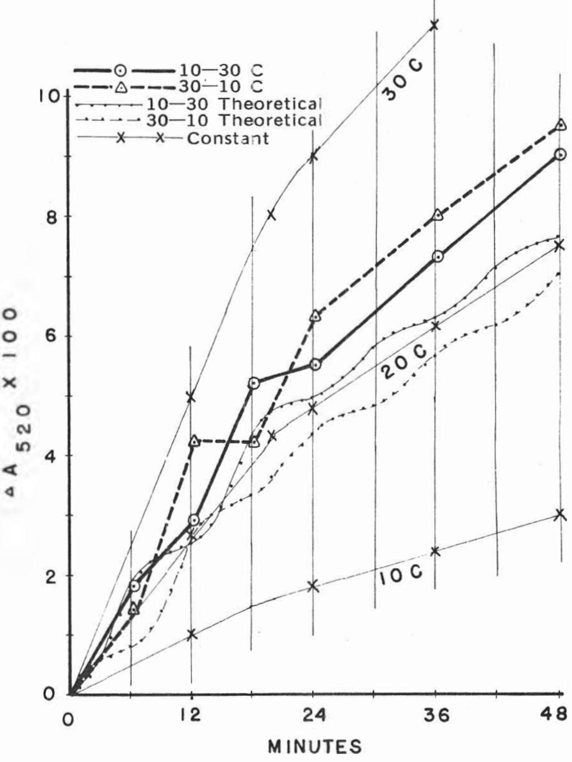

Fig. 3–Comparison of the theoretical and the experimental product yields of lysozyme catalysis under the fluctuating temperature treatments (20 ± 10°C with 12 min/cycle).

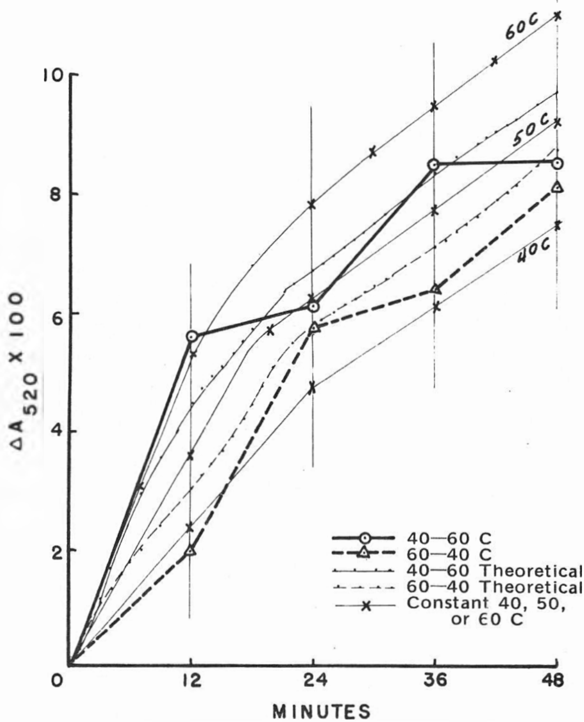

Fig. 4–Comparison of the theoretical and the experimental product yields of lysozyme catalysis under the fluctuating temperature treatments (50 ± 10°C with 24 min/cycle).

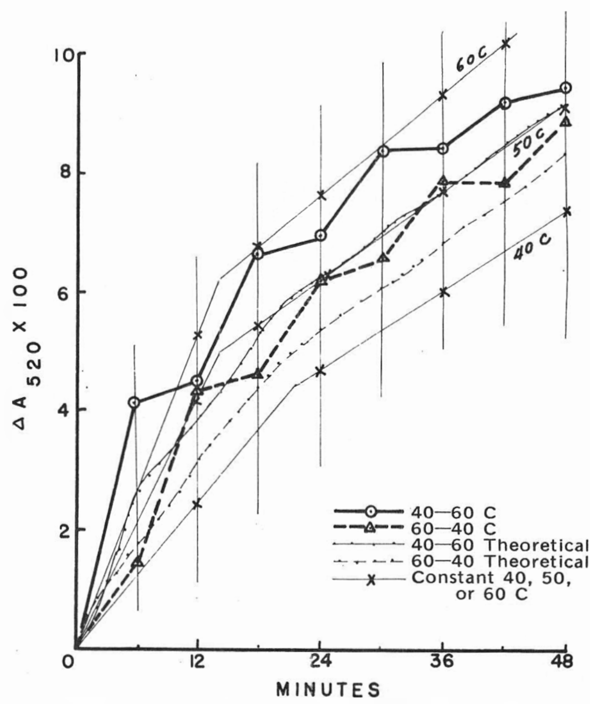

Fig. 5–Comparison of the theoretical and the experimental product yields of lysozyme catalysis under the fluctuating temperature treatments (50 ± 10°C with 12 min/cycle).

showed that treatments with faster frequency (12 min/cycle) created higher yields than those with slower frequency (24 min/cycle). Cycle-up modes created higher yields than cycle-down modes. Smaller amplitude (± 5°) created greater yields than greater amplitude (± 10°). The highest yield was created by the treatment at 50 ± 5°C with the frequency of 12 min/cycle and the cycle-up mode. Most of the fluctuating temperature treatments created smaller yields than that at the constant mean temperature. The slopes in the first down-shifted periods in the 50°C region were lower than theoretical values, differing from observations noted in the 20°C region. This is probably due to the heat denaturation when the temperature of lysozyme solution is brought to the higher limit prior to the addition of substrate.

From the above investigation, it is apparent that overshoot and undershoot phenomena do occur for enzyme reactions when temperature changes occur; as a result, inverse compensation is shown. The final product yield after subjecting an enzyme to a fluctuating temperature treatment depends on the compensation between the overall magnitude of overshoot and undershoot. The cycle-down mode and the slower frequency of fluctuation seem to create a greater influence on the rate changes. In the absence of heat inactivation, e.g., in the 15°C region for chymotrypsin and the 20°C region for lysozyme, faster rates and thus higher yields resulted. When heat inactivation was apparent, e.g., 50°C region for lysozyme, the slower frequency and the cycle-down mode resulted in greater inactivation as well as greater activation; yet, the former effect can probably overshadow the latter effect and result in a lower yield. This is supported by the previous finding that the effect of fluctuating temperature on heat inactivation can overshadow the effect on heat activation (Chang and Powers, 1974; Wu et al., 1974). The effect of cycling amplitude coincides with previous findings with invertase (Wu et al., 1974).

The mechanism for overshoot or undershoot of an enzyme reaction was not determined in this study. Whether the two effects were short term adaptation or persistent throughout the whole shifted period is not known.

Table 2—Effect of fluctuating temperature treatments[a] (around 20°C temperature region) on lysozyme activity (expressed as P_f/P_c)[b]

Temperature	20°C	10–30°C		30–10°C		15–25°C		25–15°C	
Frequency (min/cycle)	—	12	24	12	24	12	24	12	24
P_f/P_c	1	1.24	1.50	1.31	1.31	1.13	1.09	1.02	1.13
	1	1.24	1.50	1.31	1.31	1.13	1.09	1.02	1.13
	1	1.24	1.50	1.31	1.31	1.13	1.09	1.02	1.13
Mean	1	1.24	1.50	1.31	1.31	1.13	1.09	1.02	1.13
Rank	(8)	(3)	(1)	(2)	(2)	(4)	(6)	(7)	(5)

[a] Fluctuation conditions included two amplitudes, two frequencies and two cycling modes (up or down). Reaction time was 48 min.
[b] The P_f/P_c ratio is the ratio of the final yield under the fluctuating temperature treatment to that of the constant mean temperature treatment.

Table 3—Effect of fluctuating temperature treatments[a] (around 50°C temperature region) on lysozyme activity (expressed as P_f/P_c)

Temperature	50°C	40–60°C		60–40°C				55–45°C	
Frequency (min/cycle)	—	12	24	12	24	12	24	12	24
P_f/P_c	1	1.02	0.92	0.94	0.88	1.01	0.91	0.98	0.99
	1	1.02	0.92	0.94	0.88	1.01	0.91	0.98	0.97
	1	1.00	0.91	0.93	0.84	0.92	0.90	0.97	0.99
	1	1.03	0.93	0.95	0.91	1.04	0.92	0.99	0.98
Mean	1	1.02	0.92	0.94	0.88	1.00	0.91	0.98	0.98
Rank	(3)	(1)	(7)	(6)	(9)	(2)	(8)	(5)	(4)

Duncan's Multiple Range Test (Numbers are the above rankings):
(1) (2) (3) (4) (5) (6) (7) (8) (9)

[a] Fluctuation conditions included two amplitudes, two cycling modes (up or down), and two frequencies. Reaction time was 48 min.

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AN IMPROVED DYED AMYLOSE FOR PLANT α-AMYLASE ASSAY

INTRODUCTION

CHROMOGENIC SUBSTRATES have been used extensively for α-amylase assays in medical research (Klein et al., 1970; Thoma et al., 1971), but have received only limited attention in agricultural areas (Walter and Purcell, 1973; Bärwald et al., 1970). Since the procedures for determination of α-amylase (α, 1→4-glucan 4-glucanohydrolase, EC 3.2.1.1) in plants (AOAC, 1970; Ikemiya and Deobald, 1966) are time consuming, improved chromogenic substrates could be advantageous for plant α-amylase assays, e.g., in sweet potato flake production where processing conditions are altered relative to the α-amylase activity of the roots (Hoover, 1967; Deobald et al., 1969). Because chromogenic substrates have been reported to be nonreactive with β-amylase (Thoma et al., 1971), and the assay procedures are simple and rapid, effort was directed towards synthesis of a dyed amylose which would be a sensitive substrate for determination of plant α-amylase.

MATERIALS & METHODS

AMYLOSE, Type 1, from potato was purchased from Sigma Chemical Company, and Amylochrome from Scientific Products. For analysis of tobacco α-amylase, the Amylochrome was washed with water and air dried to remove added buffer. South Carolina tobacco (1971), which had been flue-cured and redried, was freeze dried prior to the amylase extract preparation. Tobacco enzyme was extracted in phosphate buffer (0.04M, pH 6.2, containing 0.001M $CaCl_2$) in the presence of Polyclar AT (Loomis and Battaile, 1966) by the procedure of Perez et al., (1971). Sweet potatoes were obtained from the fresh market. A mixture of sweet potato juice plus Celite (about 1.5g/30 ml) was centrifuged, and the extract diluted with buffer.

Synthesis of Amylose-Cibachron Blue F3GA

The initial steps in the substrate preparation were based on the procedure of Klein et al. (1969), with modifications in the amount of Na_2SO_4 (370g/125g amylose) and reaction temperature (60°C). The washed dyed amylose was incubated in water at 50°C for 3-3/4 hr, centrifuged, and the supernatant decanted. The product was incubated at 44°C for 1-1/2 hr in 0.04M phosphate buffer (pH 6.2) containing 0.001M $CaCl_2$, centrifuged, washed with water until the washings were practically colorless, and freeze dried. Three active preparations of the substrate, which is stable at room temperature, were made.

Alpha-amylase assays

New substrate method. Substrate (70 mg) was incubated with 0.2 ml sweet potato extract in 2.3 ml of 0.04M phosphate buffer (pH 6.0) containing 0.001M $CaCl_2$ at 40 ± 0.2°C, as described by Klein et al. (1970). The reaction was terminated with 4.5 ml of 1.8% TCA. Celite (about 0.1g) was added to the supernatant prior to centrifugation. Reaction of 20 mg substrate with enzyme in 1.0 ml incubation volume and 4.2 ml final volume was linear up to an absorbance of 0.35 (625 nm); hence, 20 mg substrate could be used with dilute enzyme extracts. The color of the supernatant after removal from substrate remains stable indefinitely.

Tobacco enzyme was incubated with substrate in phosphate buffer (pH 6.25) containing 0.001M $CaCl_2$ at 37°C; and 0.1M acetate buffer (pH 3.5) was effective in terminating the reaction. Human saliva in 0.9% NaCl was reacted with substrate at 37°C in phosphate buffer (pH 7.0) containing 0.02M NaCl, and the reaction terminated with the acetate buffer. Sample absorbance readings were converted to dye units by reference to a calibration curve constructed as described by Ewen (1973). One unit of enzyme activity is defined as the amount of enzyme which results in liberation of 0.01 micromole of dye per minute under the conditions defined for assay.

Amylochrome procedure. Tobacco α-amylase was determined using water washed Amylochrome following the procedure described for the reaction of the new dyed amylose with tobacco enzyme, except that a 2.0 ml incubation volume was used. The total final volume was 7.0 ml. In the sweet potato enzyme assay, an Amylochrome tablet was dispersed in 2.0 ml of 0.001M $CaCl_2$ at 40°C, the enzyme added, incubated, and the reaction terminated with 2.2 ml of 2.6% or 5.0 ml of 1.8% TCA. Salivary amylase plus water to make 2.0 ml was incubated with an Amylochrome tablet at 37°C, and the enzyme activity terminated with 2.2 ml of acetate buffer, pH 3.5.

AOAC procedure. The AOAC (1970) method for α-amylase modified for use with sweet potatoes (Ikemiya and Deobald, 1966) was followed, except that a dextrin-iodine solution as described by Hasling et al. (1973) was substituted for the comparator.

RESULTS & DISCUSSION

DUPLICATES agreed to ±0.004 absorbance units using 20 mg substrate and ±0.008 absorbance units with 70 mg substrate. Dye units (DU) calculated for a sweet potato sample assayed using 70 mg substrate (0.234 DU/μl) and 20 mg substrate (0.247 DU/μl) were similar. Reaction rates were linear for at least 20 min.

As is evident in Figure 1, good correlation was observed by comparison of results obtained for sweet potato samples assayed using the AOAC (1970) method (Ikemiya and Deobald, 1966) and the new dyed amylose procedure. β-Amylase from sweet potato (Sigma) and β-amylase (Wallerstein) used in preparation of β-limit dextrin did not react with the new substrate when incubated at pH 4.8, 37°C for 15 min, which could be expected from the good agreement found between the AOAC α-amylase procedure and the new method.

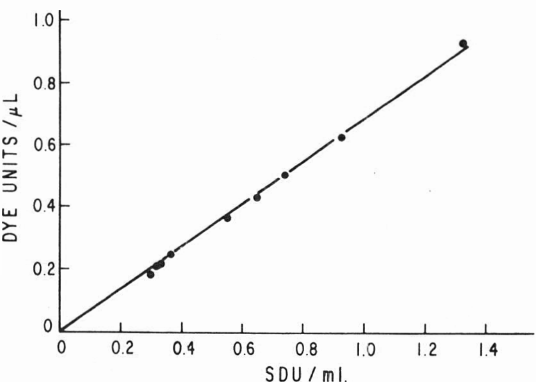

Fig. 1—Comparison of α-amylase activities in sweet potato samples determined by modified AOAC (SDU) and New dyed amylose methods.

The tobacco enzyme released about 45 times as much dye from the improved substrate (Table 1). A manuscript is in preparation on serial tobacco sample analyses. About seven to ten times greater response was obtained with 20 mg of the new substrate than found with Amylochrome tablets (containing 200 mg substrate) in reaction with salivary and sweet potato α-amylases (Table 2). The fact that an absorbance of about 0.03 units was obtained at "zero" enzyme concentration on reaction of sweet potato enzyme with Amylochrome (Fig. 2) in addition to the lower sensitivity observed makes the new substrate more attractive than the commercially available dyed amylose for determination of plant α-amylase.

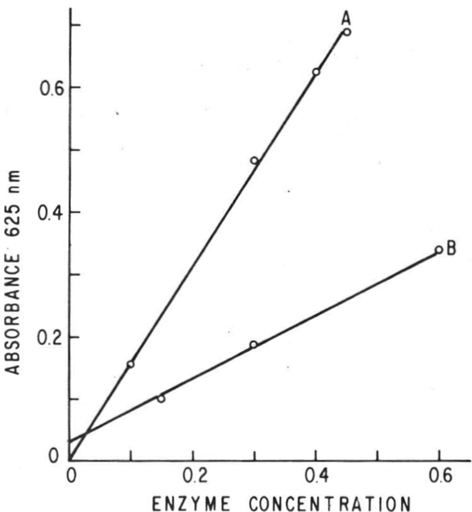

Fig. 2–Reaction of sweet potato α-amylase with increasing enzyme concentration using new substrate (A) and Amylochrome tablets (B).

Initial studies were made using substrate prepared as described by Klein et al. (1969) for Amylochrome, which gave the same results with tobacco enzyme as those obtained with commercially available Amylochrome. Work on development of the improved substrate included use of starch or amylose from several sources, variations in temperature (50–60°C), and incubation in water or in buffer containing $CaCl_2$. The substantial improvement in substrate was obtained only after treatment with buffer containing $CaCl_2$. Possibly incorporation of calcium into the substrate induced a change in conformation of the substrate which provided energetically favorable binding at the enzyme active sites.

Recently, Walter and Purcell (1973) used the chromogenic substrate Amylopectin Azure (Calbiochem) for analysis of sweet potato α-amylase. However, at 40°C incubation, extrapolation to "zero" enzyme concentration gave an absorbance of about 0.03 units (595 nm), and the linear portion at 40°C extended only to 0.225 absorbance units. At 60°C linearity was observed only up to 0.45 (595 nm). Sweet potato α-amylase shows linearity with the new dyed amylose at least to an absorbance of 0.70 (625 nm). The new substrate procedure was about 3.5 times more sensitive than the Amylopectin Azure method described by Walter and Purcell (1973) for sweet potato enzyme. Since good correlation with the established α-amylase procedure (Ikemiya and Deobald, 1966) was obtained using the dyed amylose (Fig. 1), much time and effort could be saved by using the chromogenic substrate in assaying α-amylase for sweet potato flake production.

The stability of the dyed amylose is a decided advantage over the substrate used in the AOAC procedure. Because of the possible presence of color or inhibitors in plant extracts, the dilute enzyme samples which can be measured provides a particular advantage. The substrate, which is effective with tobacco, sweet potato, and human salivary α-amylases, may have application for enzymes from other sources, e.g., cereal α-amylases. This precise, simple, rapid method offers advantages over established procedures for determination of plant α-amylase.

Table 1–Flue-cured tobacco α-amylase activity observed with Amylochrome[a] and with the new substrate

Substrate	mg Substrate	ml E[b]	Incubation time (min)	Absorbance (625 nm)	Units ml E 15 min
Amylochrome	200	0.20	30	0.065	1
Amylochrome	200	0.40	30	0.113	0.8
Amylochrome	50	0.20	30	0.008	0.1
New dyed amylose	50	0.10	15	0.738[c]	44.7

[a] The Amylochrome was washed with water and air dried prior to use.
[b] Enzyme extract
[c] The amount of dyed amylase used was not sufficient for maximum activity at this level.

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Table 2–Yam, sweet potato and human salivary α-amylase activity obtained with Amylochrome tablets and with the new substrate

	Amylochrome (200 mg substrate)				New substrate (20 mg)			
Sample	ml E[b]	E dil.	Absorbance (625 nm)	Units 1.0 ml juice or saliva	ml E[b]	E dil.	Absorbance (625 nm)	Units 1.0 ml juice or saliva
1. Yam	0.30	1→100	0.024[a]	30.0	0.10	1→100	0.118	461
2. Yam	0.40	1→100	0.063	56.0	0.10	1→100	0.170	671
3. Sweet potato	0.10	1→25	0.116	104.5	0.10	1→250	0.101	995
4. Human saliva	0.20	1→20,000	0.070	25,000	0.10	1→20,000	0.244	170,000
5. Human saliva	0.20	1→20,000	0.063	22,400	0.10	1→20,000	0.211	147,000

[a] Since on reaction of sweet potato enzyme with Amylochrome, extrapolation to "zero" enzyme concentration gave an absorbance of about 0.03 units, a correction factor (0.03) was used for the sweet potato and yam results.
[b] Enzyme extract

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EFFECTS OF POLYPHOSPHATES ON THE FLAVOR VOLATILES OF POULTRY MEAT

INTRODUCTION

IN THE PAST few years, the use of polyphosphates in meat to improve the binding properties and water-holding capacities has been reported (Furia, 1972). A mixture of polyphosphate salts has also been used to minimize the oxidative deterioration of cooked poultry (Mahon, 1962; Spencer et al., 1963). May et al. (1963) reported that the addition of polyphosphates to simmer broiler meat enhanced the chickeny flavor. Smith and Bowers (1972) found that treating turkey roulades with polyphosphates resulted in an increase in desirable flavor. Marinating of cut-up broiler quarters in polyphosphates before baking and frozen storage has also been shown by Landes (1972) to decrease the rancid flavor and the warmed over flavor scores.

Sulfur-containing volatiles and volatile carbonyls are important for the flavor of poultry meat. Minor et al. (1965) indicated that the carbonyl compounds are responsible for the "chickeny" aroma of cooked chicken while the meaty aroma is due to the presence of sulfur volatiles. However, little or no information was reported concerning the effect of polyphosphates on the flavor volatiles of poultry meat.

This study was conducted to determine the effects of adding polyphosphates on the quantities of the major flavor volatiles of the canned poultry meat. The effects of polyphosphates on the carbonyl and sulfur volatiles during cooking process of broiler meat and depot fat were also investigated.

EXPERIMENTAL

Canning and processing

Breast and thigh meat from fresh broilers was hand deboned and cut into approximately 3/4-in. chunks. The 303 × 406 tin coated cans were filled with 350g of the broiler meat chunks and 80 ml of polyphosphate (KENA, Calgon Corporation, Pittsburgh, Pa.) solution was added to yield a final concentration of 0, 0.5, 1.0, 1.5 and 2.0% of polyphosphates.

The cans were steam-flow closed by an American Can Steam-Flow closing machine and processed in a steritort (Model 600-10, Food Machinery Corp.) for 55 min at 121°C.

Aroma evaluation

Canned chicken chunk samples with 0, 1.0 and 2.0% of the added polyphosphates were used for the study. The samples were stored for 1 wk at room temperature after canning and processing. The samples were then blended in a Waring Blendor for 2 min and the aroma of the products was evaluated and compared by eight experienced panelists. Methods as described by Larmond (1970) were used. Aroma evaluation was repeated three times and the data were statistically analyzed.

Volatile components analyses

The canned broiler meat samples were blended for 2 min in a Waring Blendor. Hydrogen sulfide and methyl mercaptan contents of the samples were determined as described by Luh et al. (1964). The saturated and unsaturated carbonyl contents of the samples were assayed according to the methods described by Henick et al. (1954). Thiobarbituric acid values (TBA values) were obtained by a distillation method (Tarladgis et al., 1960). A modified AOAC procedure as described by Brooks (1973) was used for the determination of free ammonia. The pH values of the products were measured using a Sargent-Welch pH meter.

Sample preparation for cooking study

The breast and thigh meat of fresh broiler carcasses was hand deboned and separated from skin. The pooled meat samples were thoroughly mixed and ground through a Sears Kenmore meat grinder plate with 2.4 mm diameter holes and packaged separately in 150g portions in polyethylene bags.

The depot fat samples were obtained by removing fat that surrounded the gizzard and lay between the abdominal muscles and the intestines of 8-wk old broilers. The fat samples were prepared using the procedures similar to those for the meat samples. All prepared samples were stored at −18°C immediately after preparation.

Cooking and the collection of volatiles

The frozen 150g meat or depot fat samples were thawed and mixed with 150 ml of 0, 2.5 and 5.0% polyphosphate solutions. The well-mixed slurry was transferred into a 1-liter three-neck flask and boiled for 13 hr. Prepurified air was purged through the system at a rate of approximately 400 ml/min to favor oxidative conditions as well as to entrain and sweep volatile components into the trapping reagents. The air was purified by passing it through a series of reagent traps similar to those used for the capture of volatiles.

An absorption apparatus was made using two 250 ml Erlenmeyer flasks joined together with glass tube fittings. The absorption train was then connected to a distillate condenser. The first trap contained 150 ml of saturated lead acetate solution while the second trap contained 150 ml of 2,4-dinitrophenylhydrazine (2,4-DNPH) solution (2g 2,4-DNPH per liter of 2N HCl) (Pippen et al., 1958).

After 13 hr of oxidative cooking, the 2,4-dinitrophenylhydrazones (2,4-DNPH's) and the lead derivatives were filtered, washed several times with distilled water and dried at 60°C under 5-in. vacuum. The 2,4-DNPH's and lead derivatives were weighed using a Mettler balance.

RESULTS & DISCUSSION

Alteration of aroma preference scores by polyphosphates

Addition of polyphosphates enhanced the meaty aroma of canned poultry meat. Broiler meat canned with 1% polyphosphates received better average aroma preference scores than the control and the 2% polyphosphates-treated samples (Table 1). All panelists commented that the samples with 2% polyphosphates had an extremely strong meaty odor. This excess meaty odor resulted in lower aroma preference ratings. The hydrogen sulfide contents of these samples (Fig. 1) can be used to explain these preference scores. Johnson and Vickery (1964) reported that low concentrations of hydrogen sulfide probably contributed to the flavor of heated proteinaceous food such as beef, chicken, eggs, fish and milk while high concentrations render these foods unacceptable due to the distinctive odor and flavor.

Table 1—Mean aroma scores of the canned broiler meat treated with various levels of polyphosphates

% Added polyphosphates	Mean aroma scores[a]
0	3.19b
1	1.95a
2	3.00b

[a] Means not followed by the same letter differ significantly from one another ($P < 0.01$). Possible scores ranged from 1 to 9 with lower scores indicating greater acceptability.

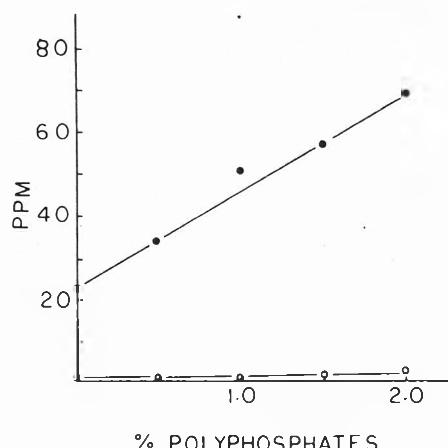

Fig. 1–Effects of polyphosphates on the hydrogen sulfide and methyl mercaptan concentrations of the canned chicken meat. (–●–●– hydrogen sulfide; and –○–○– methyl mercaptan.)

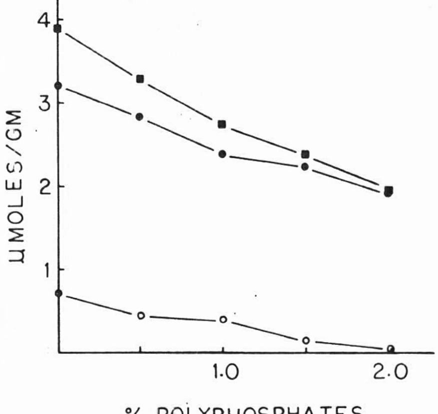

Fig. 2–Effects of polyphosphates on the carbonyls of the canned broiler meat. (–■–■– Total; –●–●– saturated; and –○–○– unsaturated.)

The acceptable level of phosphates in food varies with their application. According to the Handbook of Food Additives (Furia, 1972), the acceptable level of phosphates in meat products is 0.5%. However, higher levels were used to investigate the effects of this additive on flavor volatiles.

Effects of polyphosphates on the flavor volatiles of canned broiler meat

Polyphosphates increased the concentrations of both hydrogen sulfide and methyl mercaptan in the canned broiler meat (Fig. 1). Two percent added polyphosphates tripled the concentration of hydrogen sulfide in the product. Kagan (1961) reported that the destruction of methionine and accumulation of hydrogen sulfide were the main chemical changes during the processing of beef at 120 and 150°C. Mecchi et al. (1964) reported that cysteine and cystine were the principal sources of hydrogen sulfide in chicken meat. The possible catalytic effect of polyphosphates for the breakdown of these sulfur containing amino acids during heat processing should be of interest to food scientists.

As we indicated in the earlier part of this report, the polyphosphates-treated samples were more meaty in aroma than those of the controls. Minor et al. (1965) reported that the sulfur containing compounds were responsible for meaty flavor. It was assumed that the increased production of sulfur-containing volatiles due to the addition of polyphosphates enhanced the meaty flavor of the product.

Addition of polyphosphates greatly decreased the carbonyl volatiles in the canned broiler meat (Fig. 2). A greater effect for the unsaturated carbonyls than the saturated carbonyls was calculated. Persson and von Sydow (1973) reported that some of the aldehydes and ketones have burnt, pungent, moldy and sickly odors. They further added that none of the ketones except 2,3-butadione and 2,3-pentandione seems to contribute to the aroma of canned beef. The flavor enhancement of canned or cooked poultry meat by the addition of polyphosphates might also be due to the decreasing level of certain carbonyls which are undesirable in aroma.

Polyphosphates also decreased the TBA values of the canned broiler meat (Fig. 3). TBA value was used as an indicator for the oxidative rancidity of a product. Since TBA value correlates with the quantity of malonaldehyde present in the sample, the lower TBA values of the treated samples indicated lower concentration of malonaldehydes in the sample. It was interesting to note that the adding of polyphosphates beyond 0.5% did not further decrease the TBA values of the products. In another experiment, when polyphosphates were added to the fresh ground broiler meat at different levels prior to the analyses, it was found that the TBA values of the samples were not affected by the presence of polyphosphates during the tests. It was assumed that the polyphosphates prevented the formation of malonaldehyde during heat processing. These lower malonaldehyde levels in the polyphosphates-treated products might also have improved the aroma scores.

The pH of the canned poultry meat increased linearly from 6.25 to 6.68 as the polyphosphates increased from 0 to 2%. The results of other experiments in our laboratory have indicated that the changes in the production of flavor volatiles by the addition of polyphosphates were not due to changes in the pH of treated samples. However, the presence of polyphosphates reduced the concentration of free ammonia in the product (Fig. 3).

Effect of polyphosphates on the sulfur and carbonyl volatiles during cooking process

Polyphosphates greatly affected the formation of sulfur-containing and carbonyl volatiles during the cooking process

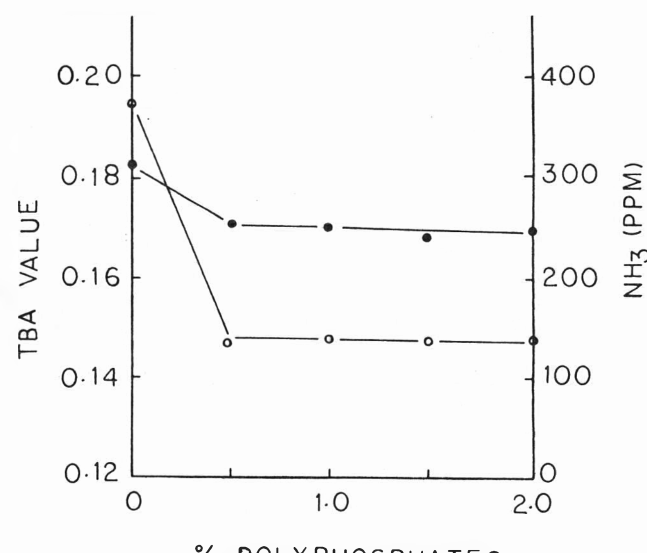

Fig. 3–TBA values and the ammonia content of canned broiler meat with various levels of polyphosphates. (–○–○– TBA value; and –●–●– ammonia.)

Table 2—Effects of polyphosphates on the yield of carbonyl and sulfur volatiles during cooking[a]

Treatment	Mean weight of volatiles produced[b]	
	Carbonyl volatiles as mg 2,4-DNPH's	Sulfur volatiles as mg lead sulfate
Broiler meat		
Control	71.50a ± 3.70	194.93c ± 4.62
With 1.25% polyphosphates	13.17b ± 1.06	227.17b ± 7.95
With 2.50% polyphosphates	4.47c ± 1.55	284.44a ± 5.03
Depot fat		
Control	140.41a ± 1.57	Nondetectable
With 1.25% polyphosphates	115.94b ± 3.46	Nondetectable
With 2.50% polyphosphates	71.75c ± 4.06	Nondetectable

[a] Cooking processes were conducted by boiling 150g of sample with 150 ml of distilled water for 13 hr. Compressed air was purged through the system at a speed of 400 ml/min to remove the volatiles.

[b] Each mean represents the average of six observations. Means within a column not followed by the same letter differ significantly from one another ($P < 0.01$).

(Table 2). Broiler meat cooked with polyphosphates was more meaty in aroma than the controls.

Polyphosphates significantly ($P < 0.01$) increased the yields of sulfur-containing volatiles and decreased the carbonyls. The reduction in yields of 2,4-DNPH's was greater in meat than in depot fat samples. No sulfur-containing volatile derivatives were detected for either the treated or the control depot fat samples.

A distinct color difference was noticed in the collected 2,4-DNPH's. The 2,4-DNPH's from the polyphosphates-treated samples were brick red in color while an orange-yellow colored 2,4-DNPH's were obtained from the controls. This difference in the color of the 2,4-DNPH's indicated a possible qualitative change in the carbonyls.

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POSTMORTEM GLYCOLYSIS IN PRERIGOR GROUND BOVINE AND RABBIT MUSCLE

INTRODUCTION

THE POSTMORTEM METABOLISM of glycogen to lactate (glycolysis) plays a leading role in the conversion of muscle to meat and in meat quality. It has been intensively studied in the intact (unground) muscles of meat animals, but has not been thoroughly studied in coarse ground muscle. The coarse grinding of prerigor bovine muscle is often used, sometimes in combination with salting and/or freezing procedures, in the production of meat for certain sausage products (Hamm, 1972). Examination of the levels of metabolites postmortem can be used to determine how and where glycolysis is controlled, as previously shown in intact muscle by Kastenschmidt et al. (1968) and Newbold and Scopes (1967). We desired to study the metabolite levels and glycolytic control mechanisms in ground muscle to determine if they are similar to those reported for intact muscle and furthermore, to examine muscles and species where the changes in all glycolytic metabolites have, to our knowledge, not been reported. We also examined the question of metabolite levels since earlier work in our laboratory had shown a nonstoichiometric (55–70%) conversion of glycogen into lactate in ground, ground/salted, and intact bovine muscle (Hamm and van Hoof, 1971; van Hoof and Hamm, 1973). A preliminary report by Hamm et al. (1973) on some of the research covered in this paper agreed with this conclusion, but these results were in disagreement with those of Bendall (1973b), who concluded that glycogen is stoichiometrically (=100%) converted into lactate in intact bovine muscle. Follet and Ratcliff (1969) have also shown a low nonstoichiometric conversion of glycogen into lactate in ovine and bovine muscle. The nonstoichiometric conversion of glycogen into lactate in our preliminary study (Hamm et al., 1973), and also in that of Follet and Ratcliff (1969) could not be accounted for by changes in intermediates since there was a decrease in the sum of glycolytic metabolites during the postmortem period. Reports from other laboratories have also indicated varying results concerning the sum of metabolites in intact muscle. In porcine muscle, Bodwell et al. (1966) and Charpentier (1968) found both stoichiometric (= initial) and nonstoichiometric (< initial) sums of glycogen, reducing sugars and lactate during glycolysis in different groups of animals, while Kastenschmidt et al. (1968) reported a loss of metabolites only in "slow-glycolysing" muscle during the first hour postmortem. Bodwell et al. (1965) found a stoichiometric sum of glycogen, reducing sugars and lactate in bovine muscle. In light of these inconclusive findings and the lack of information about the sum of metabolites in coarse ground bovine and rabbit muscle, it was the purpose of this experiment to evaluate all glycolytic metabolite changes in ground muscle, looking especially at the conversion of glycogen into lactate and intermediates, and at the sum of all metabolites during the postmortem period between shortly after death and 24 hr.

EXPERIMENTAL

MUSCLE SAMPLES from the bovine longissimus and sternomandibularis, and rabbit white muscle were obtained within 1 hr after death, trimmed of fat and connective tissue, coarsely ground through a meat grinder (once through a plate with 6 mm holes), and stored at 2°C. Muscle samples were taken immediately and at various times up to 24 hr postmortem (6 samples 0, 1, 3, 6, 9, 24 hr postmortem; 22 samples 0 and 24 hr postmortem only). The samples were homogenized and extracts prepared as described previously (Dalrymple and Hamm, 1973). Analysis for glycogen was conducted according to Dalrymple and Hamm (1973) and for all other metabolites according to procedures in Bergmeyer (1970). Duplicate analyses not agreeing within ± 10% were rerun. Biochemicals, chemicals and enzymes were obtained from Boehringer Mannheim GmbH, Mannheim, Germany; Zellstoff-Fabrik Waldhof, Wiesbaden, Germany and Firma E. Merck, Darmstadt, Germany.

RESULTS & DISCUSSION

Metabolite levels

Figures 1 to 15 show the postmortem changes in the levels of glycolytic metabolites in the ground muscles. The changes for glycogen and lactate show that the rabbit muscle had the fastest glycolytic rate, the bovine longissimus the slowest, with the sternomandibularis being intermediate. This glycolytic rate is accelerated by the grinding process, so they may not be representative of the rates and differences found in intact muscle (Newbold and Scopes, 1971; Hamm and van Hoof, 1971). All muscles showed an increase in free glucose which was expected and is in agreement with Kastenschmidt et al. (1968) and Newbold and Scopes (1971). The levels of intermediates (glucose-1-phosphate through pyruvate) in the sternomandibularis are similar to those presented for this muscle (unground) by Newbold and Scopes (1967). The effect of grinding on these metabolites, as found by Newbold and Scopes (1971), was not evident in our work, due probably to our less intense, one time coarse grinding and storage at 2°C as opposed to their twice fine grinding and storage at 20–25°C. The longissimus glucose-6-phosphate level is similar to that found by Hamm and van Hoof (1971) and van Hoof and Hamm (1973) in both ground and unground longissimus. The postmortem levels of all glycolytic metabolites in the longissimus and rabbit muscle are, to our knowledge, presented in the literature for the first time. They are relatively similar to each other, more so than to porcine muscle with its exceptionally high level of fructose diphosphate (Kastenschmidt et al., 1968; Beecher et al., 1969) and ovine muscle with its higher levels of fructose diphosphate, dihydroxyacetone phosphate and glyceraldehyde-3-phosphate (Follet and Ratcliff, 1969). The sum of all metabolites (Table 1), expressed as glucose equivalents, is similar to that given by Bodwell et al. (1965) for the sum of glycogen, reducing sugars and lactate in the bovine longissimus. Glycogen, glucose, glucose-6-phosphate, fructose-6-phosphate and lactate make up more than 95% of the total glycolytic metabolites in muscle at all times postmortem. Our results and literature values show that different muscles and species can have large variations in glycolytic rate and also in metabolite levels.

Cessation of postmortem glycolysis

The cessation of normal glycolysis results from the inacti-

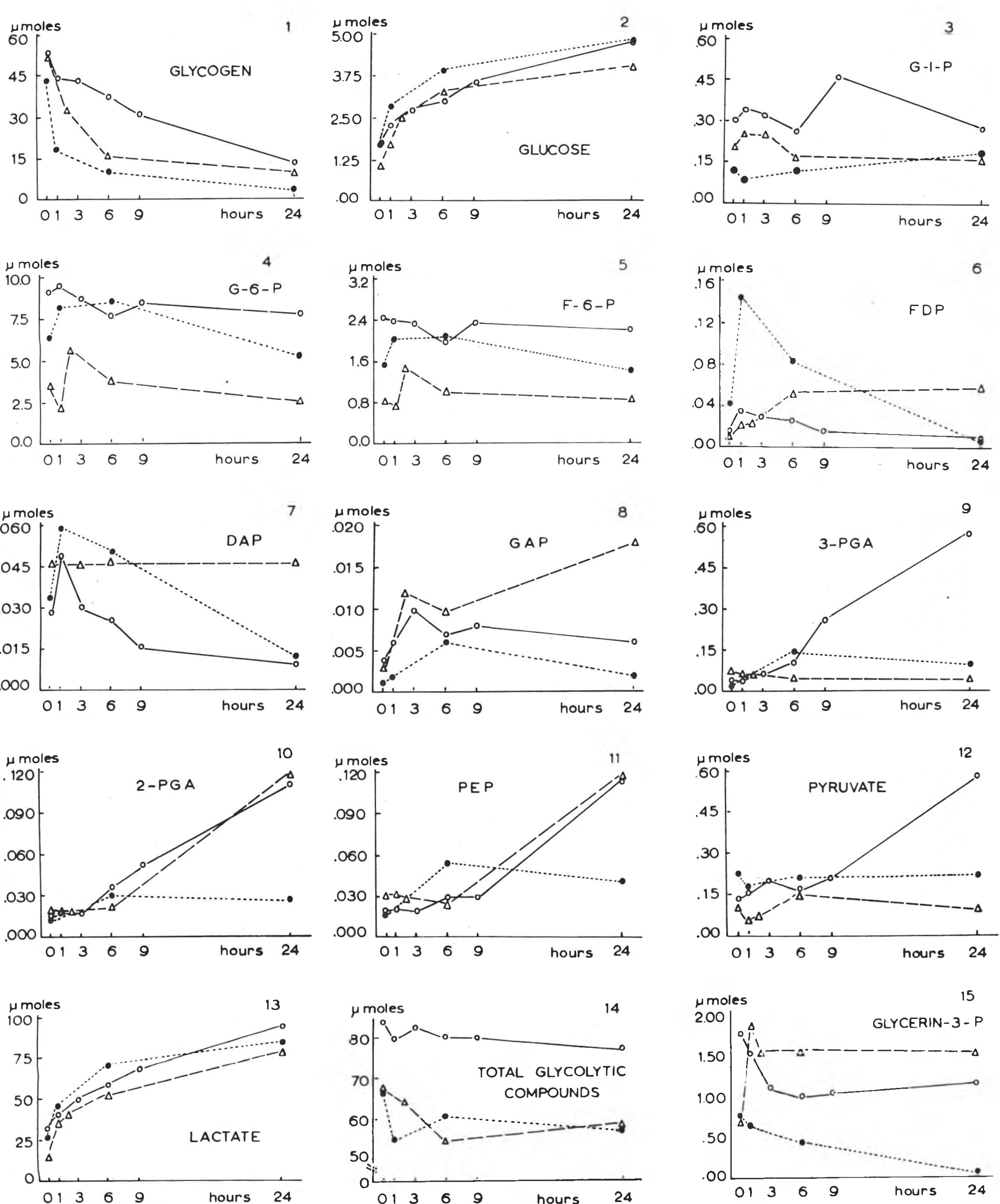

Fig. 1–15—Comparison of the concentrations of glycolytic metabolites in the prerigor ground muscles from animals of the bovine and rabbit species. (○ represent means from two bovine longissimus muscles; △ represent means from two bovine sternomandibularis muscles; ● represent means from two rabbit white muscles.)

vation of phosphorylase (EC 2.7.1.1) and phosphofructokinase (PFK-EC 2.7.1.11) as evidenced by the remaining glycogen and normal hexose monophosphate levels. This inactivation results from low pH and/or limiting levels of adenine nucleotides (Bendall, 1973a; Kastenschmidt et al., 1968; Newbold and Scopes, 1967).

Control of active postmortem glycolysis

The glycolytic control enzymes in intact skeletal muscle (phosphorylase, PFK, glyceraldehyde-3-phosphate dehydrogenase GAPDH-EC 2.7.1.12, and pyruvate kinase PK-EC 2.7.1.40) have been elucidated by Kastenschmidt et al. (1968) and Newbold and Scopes (1967). The reactions of these enzymes, except GAPDH, are irreversible and the separate gluconeogenic enzymes have low activity in muscle (Scrutton and Utter, 1968). High and constant levels of glucose- and fructose-6-phosphate, in comparison to the concentration of metabolites (except lactate) after PFK, demonstrate that mainly PFK and to a lesser extent phosphorylase play the major role in the control of metabolite levels in ground muscle, phosphorylase through control of glycogen breakdown and PFK through control of the flux of metabolites into the energy yielding steps. No large changes in intermediate levels during glycolysis indicate a steady state metabolism. The maintenance of metabolite concentrations far from equilibrium reveals that there is still some control at the GAPDH and PK steps, in agreement with Kastenschmidt et al. (1968), although these steps appear to be of lesser importance in controlling metabolite levels postmortem. It may be concluded that glycolysis in ground muscle is controlled similar to that in intact muscle. However, it should be emphasized that the glycolytic control enzymes and the rate of glycolysis are actually governed by the adenosine triphosphate (ATP) turnover rate (ATPase activity) through their response to the changing adenine nucleotide levels (Bendall, 1973a).

Metabolite recovery

Table 1 summarizes the recovery of total metabolites, and of glycogen as lactate, or as intermediates and lactate in a total of 22 muscle samples from this experiment and from additional experiments (Dalrymple and Hamm, 1974a, b, c). The mean values for glycogen recovered as lactate, and as intermediates and lactate indicated a general stoichiometric relationship, although in several muscles this was not so as indicated by the large standard deviations, which were evident in each of the muscle types studied. A large deviation for these same values is also found in the literature. A preliminary investigation in the same three muscles, reported by Hamm et al. (1973), had given lower values than those reported here, 82.8 ± 19.7% and 83.6 ± 13.4% for glycogen recovery as lactate, and intermediates and lactate, respectively. Hamm and van Hoof (1971) and Van Hoof and Hamm (1973) found glycogen to lactate recovery values of 55–70% for ground, ground/salted and intact bovine longissimus. Follet and Ratcliff (1969) reported average glycogen recovery values of 57–67% for both the ovine and bovine semimembranosus, with the changes in intermediates not fully accounting for the discrepancy. Kastenschmidt et al. (1968) noted that in the porcine longissimus of two "slow-glycolyzing" breeds the recovery of glycogen and intermediates as lactate was only 75 and 85% between the "0" and 60-min samples. However, the recovery for "fast-glycolyzing" muscles and for the two slow types after 60 min was slightly greater than 100%, therefore resulting in average recoveries of about 100% for the entire period in all muscles. Bendall (1973b) reported for bovine muscles a conversion of glycogen to lactate, after allowing for glucose production, of 95.8 ± 30%, a value which is very much in line with our present results. In rabbit muscle, the recovery of glycogen as lactate was generally low (mean 81.2%) for the four samples, but the change in intermediates could account for this low value in all but one sample, thus being in general agreement with the linear relationship between pH and glycogen or lactate reported by Bendall (1973a) for muscle from this species. The slight decrease in intermediates between the initial and end samples accounts for the lower percent recovery of glycogen as intermediates and lactate in comparison to the recovery as lactate alone. This decrease was the result of adding NaCl or diphosphate to some of the samples, therefore causing a large decrease in glucose- and fructose-6-phosphate, which more than compensated for the build-up of glucose (Dalrymple and Hamm, 1974a, b).

The sum of metabolites showed some decrease between the initial and end samples. The mean percentage recovery can be considered as stoichiometric when considering the limitations of the analytical analyses and a possible aerobic conversion of some glycogen to CO_2 in the ground, aerated samples. Although there was some variation, the large majority of samples demonstrated a stoichiometric sum of metabolites, which is in agreement with the results reported by Bodwell et al. (1965) for the sum of glycogen, reducing sugars and lactate in the bovine longissimus, and Bodwell et al. (1966) and Charpentier (1968) for the same metabolites in the porcine longissimus in one of two groups of pigs. However, the second group in both experiments showed a nonstoichiometric sum of these metabolites.

We conclude that in general a stoichiometric recovery of (1) glycogen as lactate, (2) glycogen as intermediates and lactate, and (3) total metabolites takes place postmortem in bovine longissimus and sternomandibularis, and rabbit white muscles, although the finding of a nonstoichiometric recovery of (1) and/or (2) is often possible in these muscles.

Table 1–Recovery of total metabolites, glycogen as lactate and glycogen as intermediates plus lactate in 22 muscle samples of the bovine longissimus (8) and sternomandibularis (10), and rabbit white muscle (4)

Metabolite	Concentration[a]			% Recovery
	Initial	End	Change	
Glycogen	39.7 ± 13.0	7.3 ± 6.6	−32.4 ± 9.4	
Lactate	15.2 ± 7.7	44.4 ± 5.8	+29.2 ± 5.3	94.1 ± 24.6
Intermediates and Lactate	27.0 ± 8.9	54.8 ± 6.4	+27.8 ± 5.8	89.1 ± 15.9
Total Metabolites	66.7 ± 9.2	62.1 ± 8.1	− 4.6 ± 5.3	93.4 ± 7.5

[a] All concentrations are expressed in μmoles of glucose equivalents per gram of wet muscle.

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STABILITY OF RAINBOW TROUT (Salmo gairdneri) MUSCLE LYSOSOMES AND THEIR RELATIONSHIP TO RIGOR MORTIS

INTRODUCTION

REVIEWING existing literature, Goll et al. (1970) concluded that cathepsins were not factors in either the "slippage" of the actin-myosin interaction or the dissolution of the Z line proteins that are believed to promote the macroscopic textural changes in aging muscle. However, evidence is available that suggests cathepsins may be involved in postmortem textural changes in muscle. Suzuki et al. (1969) found hydrolysis of actomyosin by rabbit muscle cathepsins and Eino and Stanley (1973a) reported activity by bovine muscle cathepsins on the water soluble, salt soluble and insoluble muscle fractions. Fragmentation of the Z-line of isolated fibers required the presence of cathepsins or other soluble cellular materials (Penny, 1968; Fukazawa et al., 1969). Fish myofibrils incubated with cathepsins fragmented more than myofibrils incubated in water, although not to the extent of myofibrils from intact muscle (Tokiwa and Matsumiya, 1969). Incubation of rabbit muscle fibers in crude cathepsin preparations accelerated the decreases in tensile properties over unsoaked controls (Eino and Stanley, 1973b).

Catheptic and other hydrolytic enzymes were shown to be within a subcellular organelle termed the lysosome in muscle tissue (Parrish and Bailey, 1967; Canonico and Bird, 1970; Caldwell and Grosjean, 1971), including fish muscle (Bird et al., 1969; Milanesi and Bird, 1972; Reddi et al., 1972). Lysosomal stability has not been studied in fish muscle which is subject to more extensive postmortem softening than mammalian muscle (Siebert, 1962). This report relates the release of lysosomal cathepsins to the textural changes of postmortem fish muscle and studies some of the factors affecting lysosomal stability.

MATERIALS & METHODS

Rainbow trout

Rainbow trout (*Salmo gairdneri*) were maintained on an ad lib diet based on fish protein concentrate and herring oil (47% protein, 20% oil) under conditions described by Lee et al. (1967). The fish, which were 9–15 mo of age and weighed 150–400g, were captured in a net and stunned by a blow on the head, immediately beheaded, eviscerated and packed in ice while transported to the laboratory. Rigor state of the muscle was determined by tactile observation (Tomlinson et al., 1961). The muscle was aged on the unskinned carcass at 4 or 15°C. At the latter temperature a 100 ppm chlortetracycline dip minimized bacterial growth (Tarr, 1961; Penny, 1968).

Lysosome extraction

Skin and red muscle from the lateral line area were removed. The white muscle was expressed through an extruder and 4.0g were extracted with 16.0 ml of a cold 0.25M sucrose/0.175M KCl/1 mM EDTA solution (Ono, 1971). After blending for 2 sec with a Tissumizer blender (Tekmar Co., similar in design to that of Caldwell and Grosjean, 1971), the slurry was homogenized by one up and down pass with a Potter-Elvehjem homogenizer. This homogenate was centrifuged at 1000 × G for 10 min. The pellet material was resuspended in 10.0 ml of the extracting solution, given a 0.5 sec burst with the Tissumizer blender, and recentrifuged under the same conditions. 2g of this pellet, designated the sediment fraction, were resuspended in 8.0 ml of extracting solution. The two supernatants from the above extraction were combined, weighed and centrifuged at 27,000 × G for 20 min to form the lysosomal pellet. This pellet was resuspended in extracting solution by a short burst with the blender. The supernatant was used to determine soluble catheptic activity. All procedures were carried out at 4°C. Each of the three fractions were made 0.2% with Triton X-100 and a minimum of 30 min was allowed for lysosomal disruption.

In vitro stability of lysosomes

Lysosome suspensions, prepared similarly as described above, were incubated for the desired time, aliquots were removed, and were centrifuged immediately at 20,300 × G at 4°C for 20 min. The supernatant was decanted and the pellet was resuspended in a volume of distilled water equal to that of the supernatant. All samples were made 0.2% with Triton X-100 and 30 min were allowed to disrupt the lysosomes. If the enzyme analyses were not made within a few hours, the samples were held at −23°C until assayed.

Stability of the lysosomes was defined on the basis of sedimentation at 23,300 × G. The amount of lysosomal breakage, termed soluble enzyme, was expressed as the percentage of supernatant (soluble) enzyme activity to the sum of the activities of the supernatant and pellet.

Enzyme assays

The cathepsin assay was based on the hemoglobin degradation method as modified by Ting et al. (1968). The assay mixture consisted of 3.0 ml of 2% hemoglobin (pH 3.5), 3.0 ml of 0.4M acetate (pH 3.5), and 1.0 ml of enzyme preparation. After a 2-hr incubation of 37°C the reaction was stopped by the addition of 10 ml of 5% trichloroacetic acid (TCA). In the blank the TCA was added 30 sec after the enzyme. Activity was expressed as the absorbance difference at 280 nm of the TCA filtrates per ml of enzyme preparation per 2 hr.

α-Glucosidase (EC 3.2.1.20) has been shown to be localized in the lysosome, including rainbow trout muscle lysosomes (Milanesi and Bird, 1972). The assay, as outlined by Barrett (1972) with substrate and pH conditions of Milanesi and Bird (1972), contained 0.50 ml of 0.035M p-nitrophenyl-α-D-glucopyranoside, 0.50 ml of 0.10M acetate buffer (pH 4.0) and 0.50 ml of enzyme preparation. After incubation at 37°C for 2 hr, the reaction was terminated with 1.50 ml of 3.3% TCA and the mixture was centrifuged. 2 ml of the supernatant were combined with 1.0 ml of 0.5M $NaHCO_3$-0.5M Na_2CO_3 and measured at 420 nm against a blank prepared by adding TCA before the enzyme. Activity was expressed as the absorbance difference per ml enzyme preparation per 2 hr.

RESULTS & DISCUSSION

Lysosomal stability in vivo

Preliminary experiments indicated that no significant differences ($p > 0.05$) existed between the distribution of catheptic enzymes in different portions of the muscle, although the total catheptic activity appeared to decrease slightly toward the posterior. An extreme blending treatment of 60 sec with the Tissumizer and three cycles with the Potter-Elvehjem homogenizer reduced the percentage of catheptic activity in the lysosomal fraction of fresh muscle from 23 to 3.4%, and freezing and thawing the muscle four times reduced the lysosomal cathepsin yield to 7.9%. These experiments indicated that the procedures were capable of measuring the rupture of lysosomes and release of cathepsins.

Muscle from nine trout aged at 4°C were sampled four

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times postmortem from anterior to posterior. Samples of muscle were taken at 1 hr (prerigor), 20 hr (rigor mortis), 44 hr (post-rigor) and 92 hr (very soft flesh). Onset and dissolution of rigor mortis approximated the pattern described by Tomlinson et al. (1961); the trout were in rigor by 15 hr post-mortem and were soft and flexible by 40 hr. The results (Figure 1) show 23% of the catheptic activity was found in each of the lysosomal and supernatant fractions and 54% in the sediment at zero time. Attempts to increase the catheptic activity in the lysosomal fraction by increasing the homogenization time were unsuccessful. When the homogenization time was increased from 2 to 25 sec, catheptic activity in the lysosomal and sediment fractions decreased 29 and 14%, respectively, and increased 85% in the supernatant. Therefore, extraction of a greater proportion of the lysosomes from the myofibrils and cellular debris caused a release of catheptic activity from the lysosomes. Percentage of the total catheptic activity in the sediment fraction remained constant throughout the aging period despite the large textural changes that had taken place in the muscle. This indicates that the extraction of lysosomes was unaffected by the physical changes of the muscle. Percentage of catheptic activity in the lysosomal pellet decreased significantly ($p < 0.01$) from 23.3 to 14.7% in the 92-hr aging period with a corresponding significant ($p < 0.01$) increase in supernatant catheptic activity. Therefore, approximately one-third of the catheptic activity was released in 92 hr and the major portion of this release occurred prior to the resolution of rigor mortis.

The experiment was repeated with eight fish aged at 15° and a similar pattern resulted (Fig. 2). Rigor mortis was evident from 12 to 25 hr and sampling was at 1, 12, 24 and 47 hr postmortem. Percentage of catheptic activity in the sediment fraction remained constant during the onset and dissolution of rigor mortis ($p > 0.05$) although decreasing slightly at the last sampling. The lysosomal fraction showed a significant ($p < 0.01$) decrease in catheptic activity through the rigor mortis period followed by little subsequent change. Again, about one-third of the catheptic activity was released from the lysosomes with a corresponding significant ($p < 0.01$) increase in supernatant catheptic activity.

These results differed from Ono (1971) who reported that the release of three noncatheptic lysosomal enzymes from bovine muscle was nearly complete after aging 10 days. These enzymes were used as markers to determine lysosomal rupture and release of cathepsins. Other workers, however, have shown the assumption that all the lysosomal enzymes were released simultaneously is not necessarily valid (Sawant et al., 1964a, b; Romeo et al., 1966; Caldwell and Grosjean, 1971). Lutalo-Bosa (1970) found the percentage of cathepsin D activity liberated from bovine muscle lysosomes was initially near zero and increased to only 15–20% during the first 2 days with little subsequent change. Cathepsin B was 25–30% soluble at 2 hr postmortem and increased to 35–40% with aging, while cathepsin C was 50% soluble soon after slaughter and showed no subsequent change (Lutalo-Bosa, 1970). The results of Eino and Stanley (1973a) show that the specific activity of bovine muscle cathepsins increased during the first 6 days postmortem. This was followed by no change in specific activity on water soluble bovine muscle proteins and a decrease in specific activity on salt soluble proteins. With aging, a partial release from 2.5 to 15% of cathepsin D into the expressed juice of ultracentrifuged bovine muscle was observed by Valin (1970).

Lysosome stability in vitro

To determine the stability of lysosomes under defined conditions, the lysosomal pellet was resuspended in solutions of various osmotic pressure, salt, pH and temperature. Suspended in 0.0, 0.1, 0.25 or 0.5M sucrose solutions at 4°, the lysosomes showed a rapid initial response to their environment with relatively little change over the remaining 158 hr of incubation (Fig. 3). The total activities during the incubation period were relatively constant, which indicates that enzyme inactivation did not occur. Maximum stability of the lysosomes was observed in 0.5M sucrose with decreasing stability with decreasing osmotic pressure. This relationship was similar to short term stability studies (Appelmans and de Duve, 1955; Gianetto and de Duve, 1955; Parrish and Bailey, 1967). The observed stability with time (Fig. 3) differed from the results of Sawant et al. (1964a, b) who found rat liver lysosomes released their enzymes during extended incubations.

Lysosomes were suspended in fish saline (Holmes and Stott, 1960) at 4°C for 3 hr to determine whether physiological salt concentrations would provide protection. The lysosomes in the saline released 60% of their catheptic activity, which is similar to the 65% released in distilled water (Fig. 3). In 0.25M sucrose 38% of the catheptic activity was released, while saline with 0.25M sucrose gave greater protection (27% release) than sucrose alone. These data show that saline alone could not be considered as capable of stabilizing the lysosomes, but increases the ability of sucrose to stabilize fish muscle lysosomes.

The effect of pH on lysosomal stability was determined by altering the pH of the 0.1M sucrose-lysosome suspension with 0.1N HCl or NaOH, incubating for 2 hr at 4°, and readjusting to the initial pH. The maximum stability was at approximately

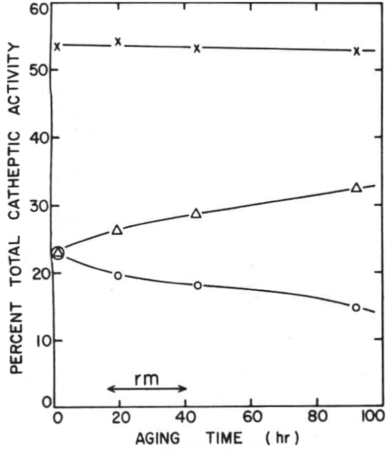
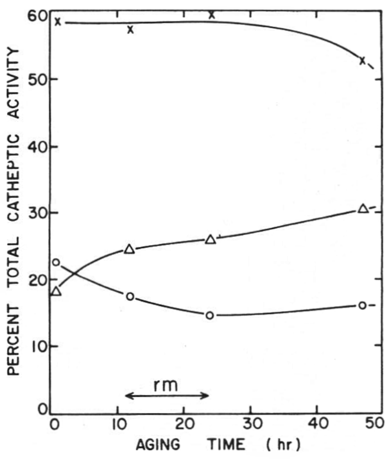


Fig. 1—(Left) Percentage of cathepsin in three fractions from trout muscle aged at 4°C (sediment, X——X; lysosome, ○——○; supernatant, △——△; rigor mortis, ←rm→). Each point represents the mean of nine replications in duplicate.

Fig. 2—(Right) Percentage of cathepsin in three fractions from trout muscle aged at 15°C (sediment, X——X; lysosome, ○——○; supernatant, △——△; rigor mortis, ←rm→). Each point represents the mean of eight replications in duplicate.



pH 6 (Fig. 4). Protein insolubility at the lower pH values increased variation, but the pH of minimum release was clearly evident and agreed with reports in the literature of other muscle lysosomes (Sawant et al., 1964a; Parrish and Bailey, 1967; Caldwell and Grosjean, 1971).

Temperature of incubation of lysosomes in 0.1M sucrose had only a slight effect on cathepsin release. Prior to incubation, 19.6% of the cathepsin was unsedimentable, after 2 hr at 4°C this increased to 25%, at 15°C it was 29%, and at 37°C it was 32%. An increasing release with increasing temperature was also noted in rat liver lysosomes by Rahman (1964), Sawant et al. (1964b) and Dingle (1961).

Lysosomal stability under different sucrose concentrations was restudied measuring cathepsin and α-glucosidase activities (Table 1). For both enzymes the rapid adjustment to the environment was again observed. Incubation increased release somewhat and higher osmotic pressure provided more protection. Approximately twice as much α-glucosidase was released as cathepsin. Similar differential releases of enzymes under various conditions were reported by Sawant et al. (1964a, b), Stagni and de Bernard (1968), Lutalo-Bosa (1970) and Caldwell and Grosjean (1971).

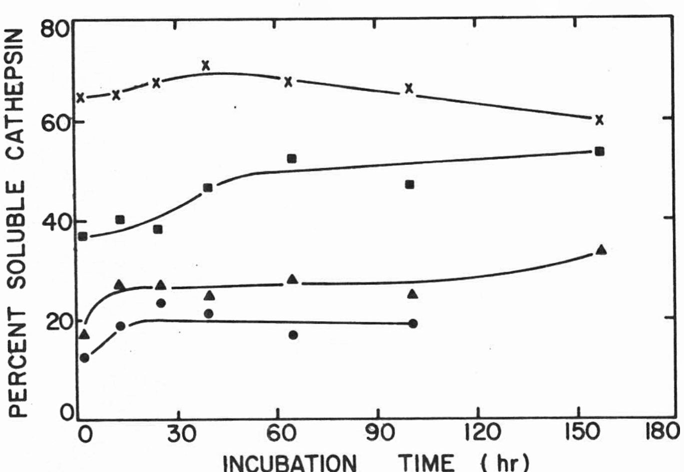


Fig. 3—Release of cathepsins from extracted lysosomes with time in different sucrose concentrations at 4°C (0.0 sucrose, X——X; 0.1M sucrose, ■——■; 0.25M sucrose, ▲——▲; 0.5M sucrose, ●——●). Each point represents the mean of duplicates.

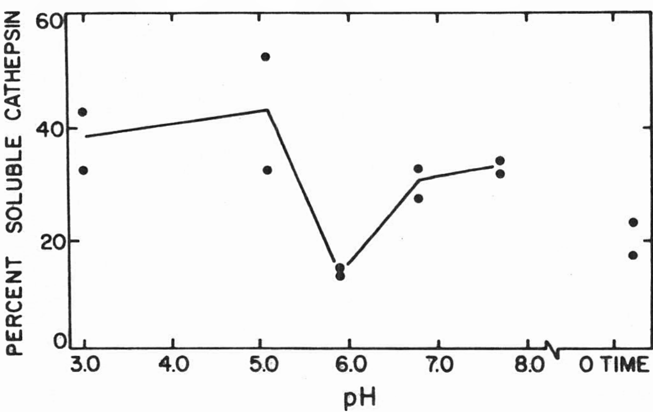


Fig. 4—Release of cathepsins from extracted lysosomes in relation to pH in 0.1M sucrose for 2 hr at 4°C. Zero time values represent the initial percentages of soluble enzyme before pH alteration and incubation. Each point represents the mean of duplicates.

To determine the effect of salts on the releases of cathepsin and α-glucosidase, lysosomal suspensions were prepared with 0.25M sucrose and 0.3M NaCl or 0.04M $CaCl_2$ and incubated for 2 hr at 4° (Table 2). The results showed that NaCl had no effect on the release of cathepsin but reduced the solubilization of α-glucosidase. The presence of $CaCl_2$ reduced the solubilization of both enzymes. These observations agreed with the suggestion of Sawant et al. (1964a) and Verity et al. (1968) that salt linkages were involved in binding of the enzyme to membrane.

These in vitro stability studies show a basic similarity between fish muscle lysosomes and those of other tissues and species toward osmotic pressure, pH, temperature and salts. The different solubilizations of cathepsin and α-glucosidase to osmotic pressure and to the presence of salts suggests that either a heterogeneous population of lysosomes existed (those with cathepsin being more resistant to disruption) or that the lysosome was ruptured and the enzyme-membrane binding was important in the solubilization of the enzymes. The results of the experiment with NaCl and $CaCl_2$ indicate the latter, although data from the literature support both situations (Sawant et al., 1964a, b; Romeo et al., 1966; Futai et al., 1972).

Cathepsin and α-glucosidase release in vivo

The lysosomal enzyme release in aging muscle was measured by following the solubilization of cathepsin and α-glucosidase to determine whether the differences observed in vitro would be apparent in the muscle. Fish muscle was aged at 4°C and extracted as before at 1, 22 and 47 hr postmortem. The decrease in catheptic activity in the lysosomal fraction was similar to the earlier trial and the lysosomal recovery of α-glucosidase was not significantly different ($p > 0.05$) from the

Table 1—Release of cathepsin and α-glucosidase from trout muscle lysosomes with varying osmotic pressure[a]

Conditions		Catheptic activity (% soluble)	α-Glucosidase activity (% soluble)
0.1M sucrose	0 hr	30.3	58.6
0.1M sucrose	2 hr	33.3	63.6
0.25M sucrose	0 hr	9.6	24.4
0.25M sucrose	2 hr	12.0	27.4
0.1M sucrose	0 hr	23.0	46.9
0.1M sucrose	5 hr	27.2	50.9
0.1M sucrose	0.5 hr	17.2	47.9

[a] Each value represents the means of two replications for 0, 2 and 5 hr and three replications for 0.5 hr. Each assay was in duplicate.

Table 2—Release of cathepsin and α-glucosidase from trout muscle lysosomes with different salts[a]

	Soluble enzyme	
Sample[b]	Cathepsin (%)	α-Glucosidase (%)
Control	12.0	27.4
0.3M NaCl	12.4	12.2
0.04M $CaCl_2$	5.1	6.7

[a] Each value represents the means of two replications in duplicate.
[b] Each sample contained 0.25M sucrose.

Table 3—Percentages of cathepsin and α-glucosidase present in the fractions of trout aged at 4°C[a]

Time isolated (hr)	Rigor state	Cathepsin Sed. (%)	Cathepsin Lys. (%)	Cathepsin Super. (%)	α-Glucosidase Sed. (%)	α-Glucosidase Lys. (%)	α-Glucosidase Super. (%)
1	Prerigor	64.3	20.4	15.3	58.9	21.3	19.7
22	Rigor	58.9	19.7	21.4	59.5	18.1	22.4
47	Post-rigor	65.8	13.8	20.4	59.2	14.2	26.5

[a] Each value represents the mean of three replications in duplicate.

cathepsin (Table 3). The percentages of both enzymes in the lysosomal fraction decreased approximately one-third. The percentage of α-glucosidase may have been slightly lower in the sediment and higher in the supernatant, but the differences were slight relative to those of the in vitro experiments and were nonsignificant ($p > 0.05$).

This suggested that in muscle the lysosomes ruptured and released both enzymes almost simultaneously. If the lysosomes in aging muscle were ruptured with cathepsin bound to the membrane, it is unlikely that the bound protease could penetrate into the highly structured myofibrillar proteins to cause hydrolysis.

Extraction of only one-quarter of the lysosomal enzymes into the lysosomal fraction 1 hr postmortem necessitates caution in making inferences on the entire population, but the difficulty in extracting the labile organelle from the muscle tissue makes greater lysosomal yields improbable. A histochemical approach to the problem of lysosomal stability may provide additional evidence towards the stability of the entire lysosomal population.

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STABILITY AND ACCEPTANCE OF INTERMEDIATE MOISTURE, DEEP-FRIED CATFISH

INTRODUCTION

INNOVATIONS for lowering the chemical activity of water to control microbial growth in foods have made possible the development of a new class of products known as intermediate moisture (IM) foods (Christian, 1963; Kaplow, 1970; Labuza et al., 1970; Potter, 1970; Rockland, 1969; Scott, 1957). There are similarities between this type product and conventional foods such as fruit cakes, country hams and jellies, which are preserved for long periods of time due to their low water activity (Aw). Foods of this new class with Aw lower than 0.6 are essentially free of microbial growth, however, such foods closely resemble their dry counterparts. Also, they usually possess flavors that are highly unacceptable due to the presence of large amounts of solutes incorporated to depress Aw. Foods with Aw greater than 0.85 readily support growth of certain microorganisms. Thus, the Aw range for developing an acceptable food product is about 0.6–0.85, but certain organisms, especially molds, grow at these Aw's. By incorporating an effective antimycotic in foods of this Aw range, good storage stability at ambient temperatures should be assured (Bone, 1969; Brockmann, 1970; Christian, 1963). This has created a strong interest in adapting a wide range of food products to the IM condition to take advantage of the potentially excellent storage stability.

Food technologists have attempted to develop IM foods for human consumption ever since the commercial success of IM pet foods (Bone, 1973). Results have been discouraging. One major problem is that essentially all solutes used for lowering Aw yield products with objectionable flavor. To overcome this, Bone (1973) suggested several areas of study, one being the control of Aw at lower solute concentrations to reduce the amount of solute contributing to unacceptable flavor.

Other attributes of IM foods should be considered. These include stability of Aw, moisture level, pH, color, and freedom from rancidity and microbial growth (Labuza et al., 1970; 1972a, b).

Collins et al. (1972) reported efforts to produce an IM food from fish flesh and indicated fish flesh might have utility for producing an IM product of excellent storage stability. However, samples were not subjected to sensory evaluation. A more extensive investigation was subsequently conducted to determine the effect of an accelerated storage treatment (37.8°C up to 4 mo) on some physical, chemical and sensory attributes and on the microbial conditions of the IM fish samples.

This paper presents the results of efforts to obtain the necessary Aw by combining deep frying of the product with the use of selected solutes. Data are presented showing changes in Aw, moisture content, pH, color, thiobarbituric acid (TBA) values and levels of bacteria, yeasts and molds of samples as influenced by initial Aw and storage at 37.8°C. Crude protein, crude lipid and ash contents are presented for freshly prepared samples.

EXPERIMENTAL

FROZEN FILLETS of ocean catfish, *Arridae* family, were purchased locally. The fish were originally supplied by a processor in Moncton, New Brunswick, Canada. The fillets were thawed in tap water and cut into 0.9 × 1.3 × 1.9 cm pieces (about 2g each); then submerged in an infusion solution (Table 1) (1g flesh: 1 ml solution) and held 3 min at 105°C to yield a product possessing Aw's of 0.86 or 0.9. Infusion was carried out in a 16-qt home-type pressure cooker.

The infused flesh was drained 15 min then coated with batter and bread (Golden Dipt Co., Millstadt, Ill.) specifically prepared for fried fish. The batter had 1% NaCl and 0.1% potassium sorbate added. The weight ratio of infused flesh to bread coating was 1.6:1. The pieces were deep fried in vegetable oil 2 min at 170°C and cooled on absorbent paper. Infused flesh of 0.86 or 0.9 Aw was lowered further to 0.76 or 0.8, respectively, by frying.

Within each of three replications and each of two initial Aw levels all fried fish pieces were combined and 200g were placed into 355 ml sterile glass jars and sealed. Thirty jars per replication and initial Aw were prepared of which 28 were placed in a forced air incubator for holding at 37.8 ± 0.5°C up to 4 mo. At 15-day intervals two previously designated jars per replication and initial Aw were removed from storage and the product was analyzed for Aw, moisture content, pH, color, TBA values and bacteria, yeast and mold counts. At 30-day intervals two preselected jars of sample per replication and initial Aw were re-

Table 1—Composition (%) of infusion solutions used to lower water activity of fish flesh

Components	Source	Initial water activity	
		0.86	0.9
Water		53.9	65.5
Sorbitol	Charles Pfizer Co., New York	31.0	19.4
NaCl		6.1	6.1
Sucrose		2.9	2.9
Potassium sorbate	Charles Pfizer Co., New York	0.7	0.7
Propylene glycol	J.T. Baker Co., Philadelphia	2.4	2.4
Monosodium glutamate	Commercial Solvents Corp., New York	2.0	2.0
Nucleotide	Takeda Inc., New York	0.5	0.5
Hickory smoke flavor	Hickory Specialists, Inc., Ocala, Fla.	0.5	0.5

moved from storage for sensory evaluation of texture, moistness, flavor and overall acceptance. Control samples were those tested at zero days storage, thus numerical values were compared. Two jars of freshly prepared product per replication and initial Aw were analyzed for crude protein, crude lipid and ash contents. Two observations were made per jar for each objective test, but the mean of these values was used for statistical analysis. Eight evaluations for each sensory test were obtained per jar of sample; the mean was used for analysis.

An electric hygrometer (Hygrodynamics, Inc., Silver Spring, Md.) (Mossell and Kuijk, 1955) was used to measure Aw. The sensors were checked against standard salt solutions for accuracy. 30g of sample were placed into a 237 ml jar, the sensor of the appropriate range was inserted through the lid and the reading was recorded after equilibration at 21–22°C. The equilibrium relative humidity value was calculated then divided by 100 to yield Aw values (Christian, 1963; Labuza, 1968).

A 10-g sample was blended 30 sec with 100 ml of deionized water, then the mixture was stirred while pH was measured by a pH meter.

Color was measured on the external portion of 40-g samples by a Hunter Color/Difference Meter (model D25D2M, Hunter Associates Laboratory, Fairfax, Va.) using the X, Y and Z values of the Commission International de l'Eclairage (CIE) system. A yellow tile standard (Hunter No. D25 C2-138) was used for calibration. The three dimensions of the CIE color solid, dominant wavelength (DW), purity and luminosity, were obtained (Hardy, 1936).

The TBA method of Bernheim et al. (1948) adapted to studies on fish by Sinnhuber and Yu (1958) and Yu and Sinnhuber (1957) was used to measure the extent of oxidative rancidity of the samples. Samples (10g) were blended with 50 ml of distilled water according to Tarladgis et al. (1964). The absorbancy values were read from the Hatachi Spectrophotometer (Coleman model 124) at 535 nm. A standard curve was constructed by using 1,1,3,3-tetraethoxypropane. The TBA value is defined as mg of malonaldehyde per 1000g sample.

For microbiological tests 20g of sample were blended 1 min with 180g of sterile distilled water. Aliquots were taken for the standard plate count (SPC) at 32°C for 48 hr (APHA, 1960) and for the yeast and mold count on rose bengal agar at 21°C for 96 hr (Overcast and Weakley, 1969).

Sensory evaluation of samples was conducted by using difference scoring for texture and moistness, and preference scoring for flavor and overall acceptance (Larmond, 1970). Beginning at the 30-day storage period, samples were also tested for overall acceptance after being covered with sauce consisting of pureed fruits, dextrose, NaCl, vinegar, garlic extract and caramel color. The scoring systems with their corresponding values are presented in the legends of the respective figures. The panel consisted of five male and three female members of the Dept. of Food Technology & Science.

At each sitting a panelist was presented three similar sets of samples, each consisting of 3–4 pieces at ambient temperature and identified by a 3-digit code. One set was used for scoring of texture, moistness and flavor; the second set for overall acceptance evaluation; and the third set was served with sauce and scored again for overall acceptance. Cool, white fluorescent lights provided illumination.

Moisture content was determined by cutting 2g of pieces into small bits and drying at 70°C for 16 hr at 200 torr (AOAC, 1965).

The crude lipid content was determined on 2g of ground, oven-dried material by extracting 16 hr with petroleum ether in a Goldfisch apparatus (AOAC, 1965).

The Kjeldahl method (N × 6.25) was utilized for determining crude protein content in a 2g sample of defatted material (AOAC, 1965).

Ash content was determined by the dry method for 2g of ground sample heated 6 hr at 600°C (AOAC, 1965).

The data were analyzed by the analysis of variance as a factorial design. Significance among means was determined by Duncan's Multiple Range Test (Li, 1957). The relationship between a dependent variable and days of storage is depicted graphically through the use of a polynomial. For TBA values and moistness, the second order polynomial was employed; for all other variables the third order polynomial was used (Sanders, 1974). Correlation coefficients were calculated for selected variables.

RESULTS & DISCUSSION

SAMPLES OF IM fish flesh of 0.76 and 0.8 Aw were placed in storage at 37.8°C and held up to 4 mo. At the end of this period the Aw was not different from that of freshly prepared samples (Fig. 1). Within each Aw group there were no values during storage that were different from the initial Aw value. However, for the lower Aw group there was a trend for a lowering of Aw. The mean Aw (0.755) for this group was lower ($P < 0.01$) than the mean (0.799) for the higher Aw group.

The moisture content of both groups was reduced ($P < 0.05$) during storage (Fig. 2). Samples of the 0.8 Aw group lost 6.4% moisture (mean moisture = 25.2%); samples of the 0.76 Aw group lost 6.7% (mean moisture = 21.3%). Collins et al. (1972) using glycerol to lower Aw in ocean catfish, reported a mean moisture content of 36.4% for a product of 0.86 Aw. A loss of 5.6% moisture occurred during storage at 26.7°C. They reported also that cod samples of 0.84 Aw had a mean moisture content of 30.2%, but lost only 1.1% moisture. In earlier work by Chen (1970) catfish exhibited a mean 38.0% moisture for samples of 0.80 Aw. These samples lost 1% moisture at 37.8°C. All data were collected under similar laboratory conditions; however, there were three factors that might have been responsible for a difference of the moisture content in relation to Aw of samples of this study compared to samples

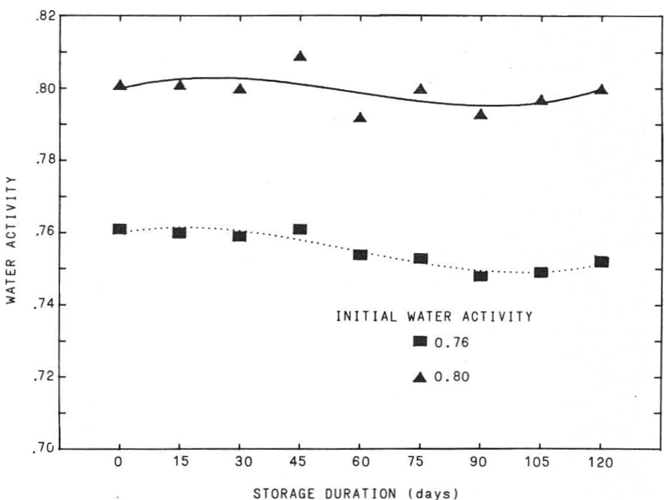

Fig. 1–Water activity of intermediate moisture, deep-fried catfish held at 37.8°C. Means of six observations.

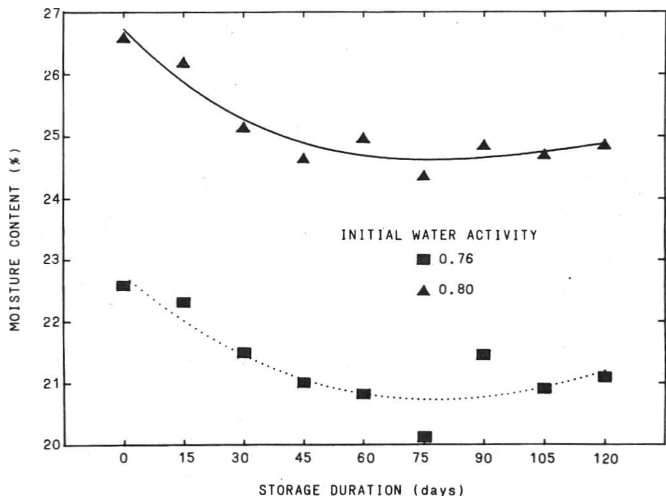

Fig. 2–Moisture content of intermediate moisture, deep-fried catfish held at 37.8°C. Means of six observations.

of earlier workers. Firstly, Chen (1970) and Collins et al. (1972) used glycerol as the primary Aw reducing substance; secondly, the frozen fish were of different lots; and thirdly, the storage temperatures were not always at 37.8°C. Physical-chemical conditions of the fish flesh change during storage (Dyer, 1968; Labuza, 1971) and undoubtedly exhibit a significant influence on the Aw-moisture relationship of items prepared at the IM range. For example, protein alterations result which reduce water holding capacity (Connell, 1968). It seems logical to conclude, therefore, that freshly caught fish would yield an IM deep-fried product of 0.75–0.85 Aw with a higher moisture content than the frozen fish yielded.

A portion, at least, of the moisture lost during storage could have been utilized in hydrolytic reactions which likely occurred among components of the samples. Since moisture losses occurred in earlier studies (Chen, 1970; Collins et al., 1972), it seems that there was an absolute loss of moisture.

Although the moisture content of the samples decreased during storage, there was no significant concomitant decrease in Aw. In this study the correlation coefficient (r) was 0.407 between these factors and −0.214 for similar data presented by Collins et al. (1972).

pH of the 0.76 Aw samples decreased from an initial 6.49 to 6.32 at 45 days with no further change (Fig. 3). The pH of the higher Aw samples decreased at a gradual rate from 6.51 to 6.35 at 120 days. The mean pH values, 6.36 for the 0.76 Aw group and 6.44 for the 0.8 Aw group, were different ($P < 0.01$). Collins et al. (1972) found that the pH of IM catfish was reduced from 6.33 to 6.13 when held 5 wk at 26.7°C. They also found that the pH of IM cod decreased from 6.30 to 6.16.

DW, purity and luminosity values for samples of 0.76 and 0.8 Aw when stored 120 days were not different from values at the beginning of the study (Table 2). DW means were not different between Aw groups. Purity was greater ($P < 0.01$) for the 0.8 Aw group while luminosity was greater ($P < 0.01$) for the 0.76 Aw group. DW and luminosity values agree closely with those obtained by Chen (1970) for IM catfish. His values for purity were lower with a mean of 35. Studies by other workers show that nonenzymatic browning may occur in IM foods due to lipid oxidation and reaction between proteins and oxidative products (Labuza et al., 1970). Neither browning nor other color changes occurred in the outer portion of the samples during storage.

The TBA values (Fig. 4) for the samples were low and may be misleading. Sinnhuber and Yu (1958) reported that canned and frozen fish of good quality yielded TBA values of less than 3; products of poor quality yielded TBA values between 4 and 27. Since the fish flesh used in this study was commercially processed and marketed through regular channels, one would expect oxidative rancidity of greater magnitude than reported here. However, there is another point to be considered. Malonaldehyde, the reactant in the TBA test, reacts with protein and other components during storage and consequently, was probably unavailable for reaction with TBA (Kwon et al., 1965; Sinnhuber and Yu, 1958; Sinnhuber et al., 1958). Since the TBA values did increase during storage ($P < 0.01$), although to a small extent, one may conclude that oxidation continued to occur. After 120 days of storage there were no TBA values greater than 1. Chen (1970) reported a maximum mean TBA value of 1.5 for IM catfish stored 5 wk at 37.8°C. Samples of the 0.8 Aw group had the higher TBA values ($P < 0.05$) with a mean of 0.77; samples of the 0.76 Aw group had a mean of 0.70. One possibility for the low values might be the procedure used. A water extract of the flesh was prepared

Table 2–CIE color values of intermediate moisture deep-fried catfish flesh of two initial water activities when held at 37.8°C

Storage duration (days)	CIE color indices[a]					
	Dominant wavelength (nm)		Purity (%)		Luminosity (%)	
	0.76 Aw	0.8 Aw	0.76 Aw	0.8 Aw	0.76 Aw	0.8 Aw
0	583	583	63	66	38	36
15	583	583	65	66	38	37
30	584	582	63	69	36	37
45	584	583	66	67	38	37
60	583	583	67	71	37	35
75	584	583	59	70	38	36
90	582	583	66	70	37	36
105	583	583	61	68	38	36
120	582	583	61	66	39	37
Mean[b]	583 a	583 a	63 e	68 d	38 t	36 u

[a] Means of six observations

[b] Means of 54 observations. Means within each color term not followed by the same letter are significantly different ($P < 0.01$).

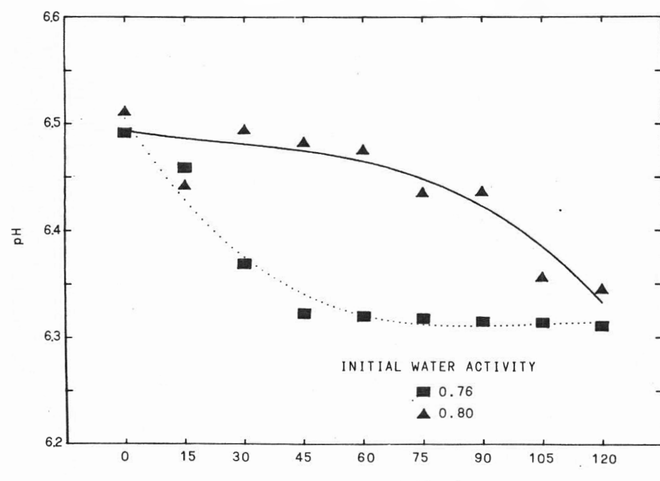

Fig. 3–pH of intermediate moisture, deep-fried catfish held at 37.8°C. Means of six observations.

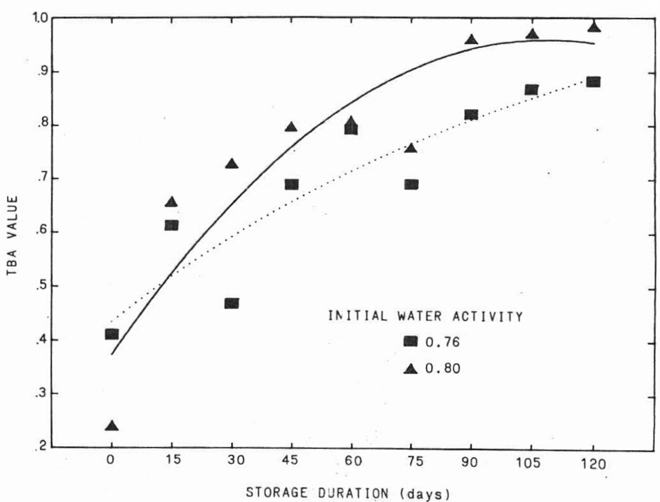

Fig. 4–Thiobarbituric acid values of intermediate moisture, deep-fried catfish held at 37.8°C. Means of six observations.

(Tarladgis et al., 1964), but according to Kwon et al. (1965), such a procedure may be useful only in the initial stages of lipid oxidation when insoluble TBA-protein complexes are unlikely to occur.

The samples were essentially free from microorganisms. The 0.76 Aw samples had SPC's less than 100 per g of sample. At the higher Aw, samples at 0 and 15 days storage had SPC's less than 300 per g; at all other periods the count was less than 100 per g. Only three samples at 0.76 Aw had positive counts for yeast and mold, the highest being 17 per g. Six samples of 0.8 Aw had yeast or mold; the highest value was 43 per g.

Samples of the lower Aw were tougher ($P < 0.01$) with a mean score of 4.70 (between slightly and moderately tough) for this group and 4.00 (slightly tough) for the 0.8 Aw group (Fig. 5). As the period of storage was lengthened, samples of both groups became progressively tougher. Upon termination of storage, all samples were tougher ($P < 0.01$) than freshly prepared samples.

Moisture content of a food product is often related to its texture. The correlation coefficient ($r = -0.771$) between these variables indicates that a significant (1% level) relationship existed between moisture decrease and toughening.

All samples became less moist during storage according to the panel (Fig. 6). Those of the 0.76 Aw group were less moist (mean 4.88) than samples of the 0.8 Aw group (mean 4.43) ($P < 0.01$) and exhibited the greater rate for loss of moistness. Samples at 120 days storage were less moist ($P < 0.05$) than samples before storage. A significant (1% level) r of −0.888

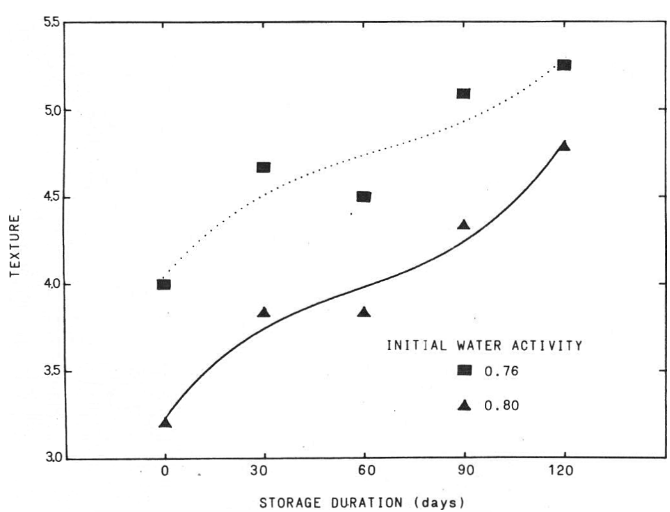

Fig. 5—Panel scores for texture of intermediate moisture, deep-fried catfish held at 37.8°C. Means of six observations. Scale: 1 = very soft; 2 = moderately soft; 3 = slightly soft; 4 = slightly tough; 5 = moderately tough; 6 = very tough.

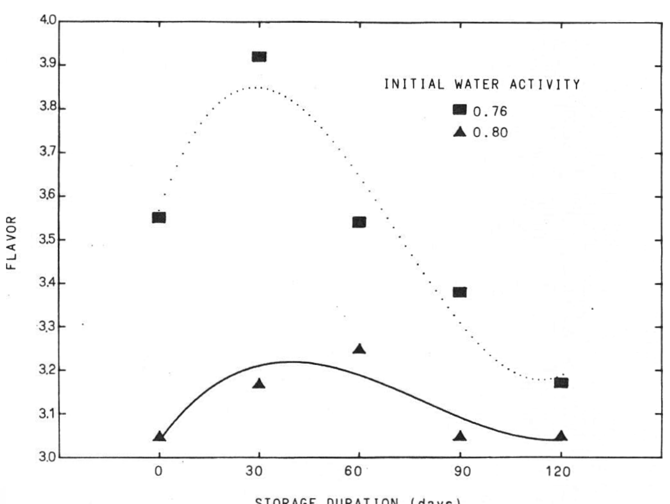

Fig. 7—Panel scores for flavor of intermediate moisture, deep-fried catfish held at 37.8°C. Means of six observations. Scale: 1 = like very much; 2 = like moderately; 3 = like slightly; 4 = dislike slightly; 5 = dislike moderately; 6 = dislike very much.

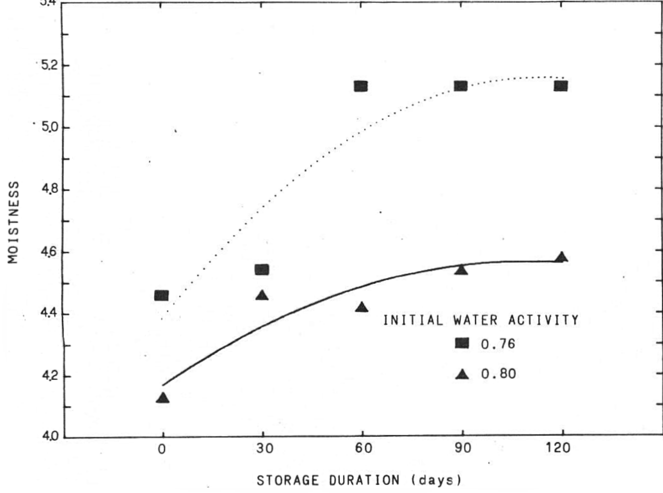

Fig. 6—Panel scores for moistness of intermediate moisture, deep-fried catfish held at 37.8°C. Means of six observations. Scale: 1 = extreme excess moistness; 2 = moderate excess moistness; 3 = slight excess moistness; 4 = slightly dry; 5 = moderately dry; 6 = very dry.

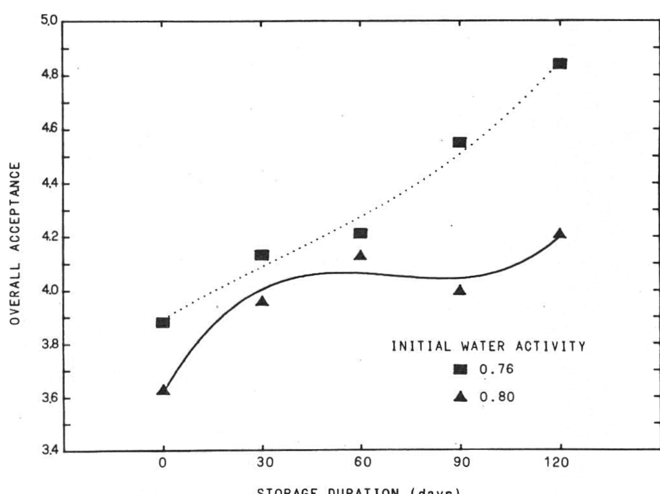

Fig. 8—Panel scores for overall acceptance of intermediate moisture, deep-fried catfish held at 37.8°C. Means of six observations. Scale: 1 = like extremely; 2 = like very much; 3 = like moderately; 4 = like slightly; 5 = dislike slightly; 6 = dislike moderately; 7 = dislike very much; 8 = dislike extremely.

was found between moistness (trend toward becoming drier) and moisture loss. The relationship between decrease in moistness and softness values of stored samples was significant (1% level) with r = 0.863.

Flavor scores (Fig. 7) between the slightly liked and slightly disliked categories were given to all samples, but the 0.8 Aw samples received the more acceptable flavor scores ($P < 0.01$). The mean score for this group was 3.11 (liked slightly); the mean score for the 0.76 Aw group was 3.51 (midpoint between slightly liked-slightly disliked). These results are consistent with those of other workers, who have reported lower flavor scores for products with the higher solute concentration. At 120 days storage the samples were not significantly different in flavor than samples not stored. Incidentally, none of the panelists mentioned rancidity when tasting the samples.

For freshly prepared samples of both Aw levels, overall acceptance scores (Fig. 8) were between the moderately and slightly liked categories. A progressive drop in overall acceptance was observed during storage; at 120 days storage samples were less acceptable than samples before storage ($P < 0.05$). Samples of the 0.8 Aw group were scored as more acceptable ($P < 0.01$). Mean score for the 0.8 Aw group was 3.99 (liked slightly); for the 0.76 Aw group, 4.32 (below liked slightly).

Samples evaluated after being covered with sauce received slightly more favorable scores (Fig. 9) for overall acceptance when compared to samples not covered with sauce. Even though the values of the two acceptance tests cannot be compared statistically, one may conclude that the sauce improved acceptance of the product. Improved acceptance was probably due to the more acceptable sauce flavor and to the addition of moisture. Samples of 0.8 Aw received the more acceptable score (mean 3.57) ($P < 0.01$) and were about midway between the moderately and slightly liked categories, while samples of 0.76 Aw were in the slightly liked category (mean 4.00). Within each Aw group, the score for the sample stored 120 days was not significantly different from the score for the sample stored 30 days, although there was a trend for lower acceptance as the storage duration was extended. When all scores are considered the 0.8 Aw sample stored 30 days received a significantly more acceptable score than all samples of the 0.76 Aw group. Scores for all samples other than that for the 0.8 Aw sample stored 30 days were not different. When sauce was added to samples of 0.76 Aw, a more favorable score was obtained which was similar to the score received for samples of 0.8 Aw without sauce.

The fish flavor of all samples was quite pronounced and this might have been a contributing factor leading to the relatively low scores for flavor. There was also a slight bitterness, probably due primarily to the presence of sorbitol. In exploratory studies for this paper, a fairly strong bitterness was observed in samples prepared without MSG and nucleotide making the product inedible. When these ingredients were added the bitterness was not evident in many cases. Thus, it is apparent that these materials were influential in masking much of the bitter taste. Flavor of the IM deep-fried catfish samples might best be described as being slightly sweet and possessing a strong fish flavor.

Table 3 presents mean values for moisture, crude protein, crude lipid and ash content of samples at the time of preparation.

This study indicated that catfish flesh can be prepared as an IM deep-fried product; that samples at 0.76 and 0.8 Aw were essentially free of bacteria, yeasts and molds; that oxidative rancidity was not sufficient to cause flavor deterioration; and that the samples did not exhibit color changes. The samples were slightly to moderately tough and dry. Flavor was indicated to be between slightly liked and disliked. Evaluation for overall acceptance placed the samples between the slightly liked and disliked categories. The addition of sweet sauce improved overall acceptance. Samples were reasonably shelf stable during the accelerated storage treatment.

This product could probably be improved by selecting fish fillets that would exhibit a greater water binding capacity, thus the IM product should be more moist and tender (Connell, 1968; Dyer, 1968; Labuza, 1971). When frozen fish are used, the storage time should be relatively short. The poor flavor presents a more difficult problem which needs further investigation.

Table 3–Composition of freshly prepared intermediate moisture, deep-fried catfish flesh[a]

Initial water activity	Moisture %	% of Total solids[b]: Crude protein	Crude lipid	Ash[c]
0.76	22.6 ± 0.01	38.3 ± 0.06	15.7 ± 0.06	5.8 ± 0.04
0.80	26.6 ± 0.01	36.5 ± 0.02	15.2 ± 0.06	5.8 ± 0.07

[a] Means of six observations
[b] Values do not constitute 100% of solids.
[c] Includes NaCl furnished by infusion solution

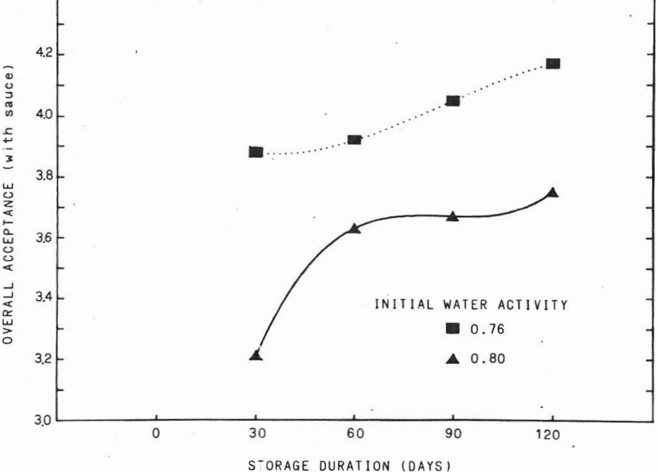

Fig. 9–Panel scores for overall acceptance of intermediate moisture, deep-fried catfish held at 37.8°C and served with sauce. Means of six observations. For scale see legend of Fig. 8.

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EFFECTS OF SULPHYDRYL BLOCKING ON THE THINNING OF EGG WHITE

INTRODUCTION

IN SPITE OF recent intensive study (Baliga et al., 1970; Dam, 1971; Robinson and Monsey, 1972a) the thick white of stored shell eggs undergoes a thinning reaction of unknown origin. The observation that reducing agents cause rapid dissipation of the gel in broken out or shell eggs (MacDonnell et al., 1951a) has led to the proposal that naturally occurring reducing substances thin albumen by splitting disulfide (SS) bonds in ovomucin. However, significant amounts of reducing substances in egg white have not been demonstrated. The possibility that the sulphydryl (SH) groups of ovalbumin are involved in the thinning reaction has been suggested by Smith and Back (1962) who have shown that ovalbumin undergoes a transition on heating or on storage resulting in a more stable protein (Smith, 1964; Smith and Back, 1965). Shifting of the SS bond in ovalbumin by SS interchange was suggested as a mechanism for this transition but the connection between the transition and albumen thinning is nebulous.

Recent work has concentrated upon the changes ovomucin undergoes during aging of albumen. Reduced, alkylated ovomucin consists of two components (Dam, 1971; Robinson and Monsey, 1971) which undergo shifts in relative concentration during aging (Robinson and Monsey, 1972a). As demonstrated by ultracentrifugation, α-ovomucin increases from 71.9% of the total schlieren pattern area to 93.1% while β-ovomucin decreases from 28.6% to 6.8% over 47 hr at 37°C. Kato et al. (1970, 1971; Kato and Sato, 1971) obtained similar results by free boundary electrophoresis of 2-mercaptoethanol reduced ovomucin. These latter authors have proposed a mechanism of thinning involving rupture of SS bonds in ovomucin.

The viscous properties of albumen have also been used to detect the thinning reaction. Tung et al. (1970, 1971) reported fresh albumen to exhibit shearing time and shear rate dependent viscometric parameters and to be pseudoplastic in its equilibrium flow behavior—the data fitting a power law equation. No differences between the power law parameters of fresh and aged albumen were demonstrated; however, the stress decay parameters showed significant changes on aging. Robinson and Monsey (1972a) fitted their flow behavior data to a power law equation and reported that power law parameters changed with aging—the apparent viscosity decreasing and the flow behavior approaching that of a Newtonian fluid.

The present study describes the effect of SH blocking and oxidation on the thinning of egg white as detected by rheological and ultracentrifugal measurements.

MATERIALS & METHODS

EGGS WERE OBTAINED on the day of lay from the University farm, broken open and the thick white obtained and pooled as described by Robinson and Monsey (1972a). All equipment was autoclaved and all operations were carried out in a laminar flow cabinet. The thick white was blended in a Sorvall Omni-mixer Homogenizer for 20 sec at speed setting 2.5. Thinning was induced by incubation of the thick white in sterile flasks at 37°C and the reaction was stopped by immersing the flasks in crushed ice. The pH of the white was 8.6–8.7 and remained constant throughout the experimental period regardless of the treatment applied.

Treatment with iodate, bromate and cysteine

KIO_3 (0.963g), 0.751g $KBrO_3$ and 0.180g L-cysteine (Schwartz Mann Lot No. W 1024) were each dissolved in 15 ml of distilled water. 10 ml of each solution were added to 400 ml of egg white and mixed thoroughly by inversion. The control consisted of 400 ml of egg white treated with 10 ml water.

Treatment with $HgCl_2$ and p-chloromercuribenzoate

Prepared white (500 ml) placed in autoclaved dialysis bags was dialyzed for 18 hr at 4°C against 2 liter of Tris-KCl buffer [0.01M Tris, 0.09M KCl, ionic strength similar to that of egg white (Donovan et al., 1972)] (pH 8.6) containing 0, 0.05, 0.1 or 0.2 mg $HgCl_2$/ml.

p-Chloromercuribenzoate (PCMB) treatment was done similarly except that the concentrations in the dialysis buffer were 0, 0.1, 0.2 or 0.3 mg/ml.

Sulphydryl determination

Performed as described previously (Beveridge et al., 1974). Protein was determined by the biuret method standardized against protein nitrogen values obtained from Kjeldahl analysis (Buttkus, 1971). Egg white protein was assumed to contain 16% nitrogen.

Preparation of ovomucin

Prepared according to Robinson and Monsey (1971) except that 2% (w/v) NaCl replaced 2% KCl.

Analytical ultracentrifugation

Sedimentation experiments were performed in a Beckman L2-65B preparative ultracentrifuge equipped with a schlieren optics accessory. Egg white was diluted approximately 1:10 (protein concentration 1%) with 0.01M Tris buffer containing 0.2M KCl, pH 8.6. Purified ovomucin, 20 mg, was dissolved in 2 ml 0.01M phosphate buffer, pH 7.5, containing 5M guanidine HCl (Schwartz/Mann Ultra Pure), 0.1M NaCl and 2.3% (v/v) 2-mercaptoethanol. Solution was complete in 30 min and the samples were run immediately in a standard double sector cell (Filled Epon, 12 mm thick), buffer being placed in the "blank" side of the cell. Photographic patterns obtained for ovomucin were printed; the enlarged peaks were cut out and weighed to obtain the relative amounts of the two components. Six prints of two pictures taken at different running times were processed to obtain a total of 12 measurements for each experimental condition. Relative viscosity measurements were performed in a capillary viscometer having a flow time of 217 sec for water at 20°C. Densities were determined pycnometrically.

Viscosity measurements

Viscosity measurements were carried out as described by Tung et al. (1971) with a Haake rotational viscometer equipped with an MV1 spindle providing a maximum shear rate of 1370 sec^{-1}, the output of which was fed to a strip chart recorder. Each sample at a temperature of 1.0 ± 0.5°C was first tested for the time dependent characteristic at a constant shear rate of 1370 sec^{-1}. After the shear stress decay curve had reached an apparent equilibrium value (about 5 min) the equilibrium flow curves were obtained. Data were collected on duplicate samples at each experimental condition, pooled and treated as a single sample.

Analysis of viscosity data

Shear stress decay data were sampled from the strip chart by a method suggested by Cramer and Marchello (1968). The shear stress range between the maximum and apparent equilibrium value was divided into 10 equal logarithmic intervals and the corresponding shear

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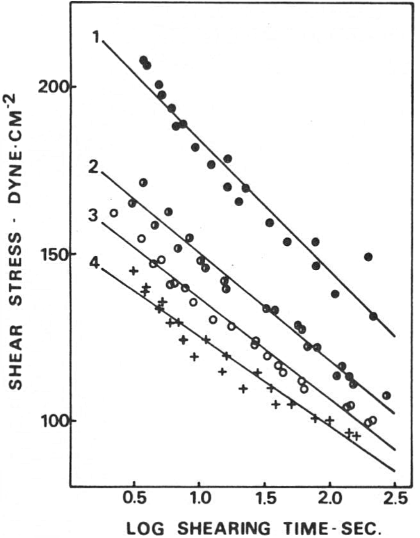


Fig. 1—Shear stress decay curves for egg white held at 37°C. Line 1 (●): holding time = 0 hr, correlation coefficient of the regression line = 0.94; Line 2 (○): holding time = 48 hr, correlation coefficient = 0.97; Line 3 (○): holding time = 72 hr, correlation coefficient = 0.97; Line 4 (+): holding time = 120 hr, correlation coefficient = 0.93.

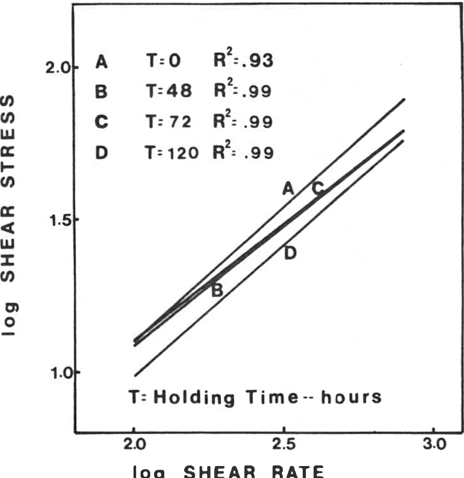


Fig. 2 (right)—Equilibrium flow behavior curves for thick egg white held at 37°C for the indicated time periods.

stresses and times recorded. The data were fitted by a function of the form (Tung et al., 1971)

$$A = A_o - n \log t \quad [1]$$

where t is time in seconds, A is the shear stress in dynes · cm^{-2}, A_o is a constant which reflects the initial shear stresses as shear begins (Tung et al., 1970) and n is a constant reflecting the rate of structural breakdown.

Equilibrium flow data were fitted to a form of the power law

$$B = K \cdot X^{n_1} \quad [2]$$

where B is the shear stress in dynes · cm^{-2}, X is the shear rate in sec^{-1} and n_1 and K are the flow behavior index and consistency index, respectively (Tung et al., 1970).

Fitting of the data was accomplished by a least squares method and testing of the linear regressions for difference in slope and intercept was accomplished by a covariance method (Snedecor, 1965).

RESULTS

Viscosity as a measure of thinning

Preliminary work suggested that obtaining flow behavior curves for thick white from which apparent viscosity values could be obtained for comparison purposes was difficult or impossible due to the rheodestructive nature of the thick albumen gel. Consequently, shear stress decay and equilibrium flow behavior were evaluated as measures of the thinning reaction. The results of a typical experiment are shown in Figures 1 and 2. Figure 1 shows the changes in shear stress decay behavior which occur in thick egg white held at 37°C. The levels of the four lines differ significantly and line 1 has a significantly different slope ($P \leq 0.01$). It is apparent that the level of the lines decreases in a regular manner as a function of incubation time. Figure 2 shows the comparable curves for equilibrium flow behavior. Lines B and C do not differ, but lines A and D differ from each other and from lines B and C ($P \leq 0.01$). The lines do not change in a regular fashion as a function of incubation time.

Figure 3 is a plot of the intercept value A_o (Eq. 1) as a function of incubation time. A_o is a reflection of the initial value of shear stress and would be expected to reflect the amount of thick gel structure originally present in the sample. On this basis, A_o should be a useful index to use as a measure of thinning. The value of A_o (Fig. 3) drops smoothly as expected from the lines of Figure 1 suggesting the use of this index as a measure of thick gel structure for comparison of various treatments.

Effect of bromate, iodate and cysteine

The effects of adding the oxidizing agents potassium bromate and iodate and the reducing agent cysteine are compared in Figure 4 by plotting the intercept values A_o obtained at each experimental condition as suggested previously (Fig. 3). The cysteine points are significantly ($P \leq 0.01$) different from either the control or oxidized samples, the low values indicating little or no gel structure present. This is in agreement with reported data (MacDonnell et al., 1951b). The line for the bromate treatment lies somewhat below the control line, the 83-hr point notably being different from the control. The iodate line lies above the control after 24 hr incubation. Analysis of sulphydryl groups for these treatments indicated that iodate lowered the SH content of thick egg white about 8% whereas bromate did not affect the levels as compared to the control.

Effect of $HgCl_2$ and PCMB

It is known that mercury compounds react with the SH groups of egg white and ovalbumin under mild conditions

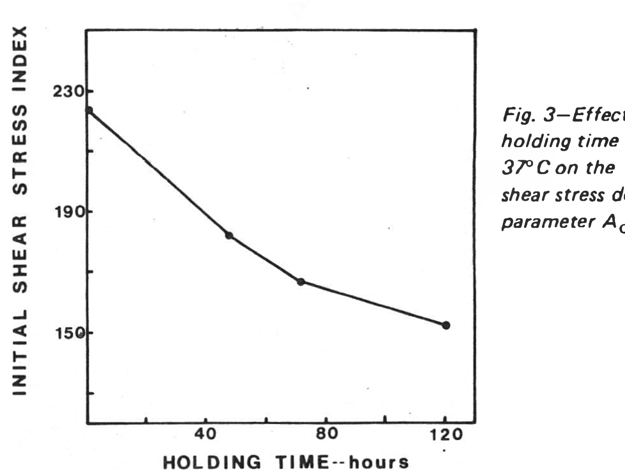


Fig. 3—Effect of holding time at 37°C on the shear stress decay parameter A_o.

(Cunningham et al., 1957; MacDonnell et al., 1951b). However, direct mixing of the compounds PCMB and $HgCl_2$ into egg white caused irreversible, localized precipitation of the proteins. This difficulty was overcome by dialyzing the mercury compound into the protein solution. Complete blockage of SH was impractical because of gelation reactions which began after about 80% of the SH had been blocked. However, extensive blocking is possible without gross visible changes occurring in the albumen.

Comparison of the intercept values (A_0) of the $HgCl_2$ blocking experiment is shown in Figure 5, and the SH levels and total solids of the treated and untreated egg white for both the $HgCl_2$ and PCMB experiments are shown in Table 1. The disruption of the normal thinning pattern observed previously (Figs. 3 and 4) is clearly shown. Two points may be noted from this figure and from Table 1. First the control, dialyzed against Tris-KCl buffer, showed a decrease of about 15% in SH groups and failed to show the typical thinning pattern. Secondly, more extensive blockage of SH groups caused a large increase in the A_0 value followed by a steady decline.

The intercept values for the PCMB blocking experiment are shown in Figure 6. The dialyzed control showed a decrease of about 15% in SH groups, and an altered thinning pattern and more extensive blockage was associated with a large increase in A_0 followed by a steady decline. In addition to the altered thinning pattern shown by the dialyzed control, blockage of 32% of the SH groups of egg white (0.1 mg/ml PCMB treatment) showed almost no thinning reaction in 72 hr at 37°C. The variation observed in initial A_0 values cannot be ascribed to differences in total solids among treatments since despite a tendency of total solids to decrease during dialysis, there were essentially no differences in total solids among PCMB treatments.

An attempt was made to determine the reason for the marked increase in A_0 caused by relatively extensive blocking of SH groups. Since the mercuric cation has a very high affinity for SH groups and is bifunctional (Steinberg and Sperling, 1967) it is possible that dimerization or polymerization of ovalbumin through SH groups crosslinked by divalent mercury was responsible for the increased apparent gel structure. Since PCMB is monofunctional, the increase in apparent gel structure was unexpected; however, both reagents are capable of precipitating egg white proteins under appropriate conditions so that nonspecific aggregation reactions cannot be eliminated as a possible cause. In the ultracentrifuge, Steinberg and Sperling (1967) were able to demonstrate extensive aggregation of ribonuclease treated with mercuric chloride.

Table 1–Sulphydryl levels and total solids in egg white treated with $HgCl_2$ and PCMB

Sample	Sulfhydryl		Total
	µM/g Protein	% of Original	Solids (%)
$HgCl_2$ treatment			
Untreated	60.4	100	11.9
Dialyzed against			
0 mg/ml	51.2	85	10.4
0.05 mg/ml	45.6	75	10.5
0.10 mg/ml	34.2	57	10.2
0.20 mg/ml	17.2	28	10.2
PCMB treatment			
Untreated	62.5	100	12.2
Dialyzed against			
0 mg/ml	53.0	85	11.5
0.10 mg/ml	43.0	68	11.5
0.20 mg/ml	38.0	61	11.5
0.30 mg/ml	30.6	49	11.7

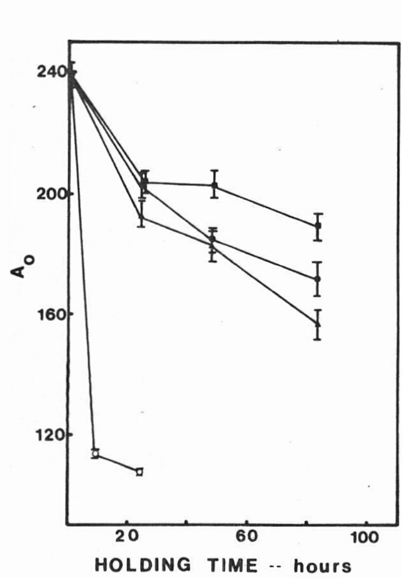


Fig. 4–The effect of treatment of egg white with cysteine, bromate and iodate on the intercept value A_0. The bars represent ± 1 standard error associated with the intercept. (● Control; ■ Iodate; ▲ Bromate; ○ Cysteine.)

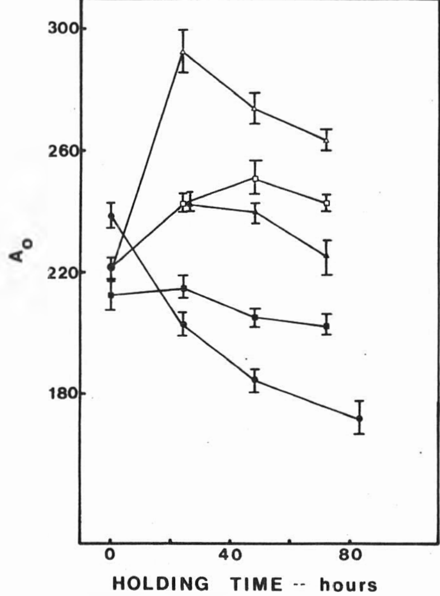


Fig. 5–The effect of blocking SH groups of egg white with $HgCl_2$ on the intercept value A_0. The bars represent ± 1 standard error associated with the intercept. (● Undialyzed control; ■ Dialyzed control; □ 0.05 mg/ml $HgCl_2$; ▲ 0.1 mg/ml $HgCl_2$; △ 0.2 mg/ml $HgCl_2$.)

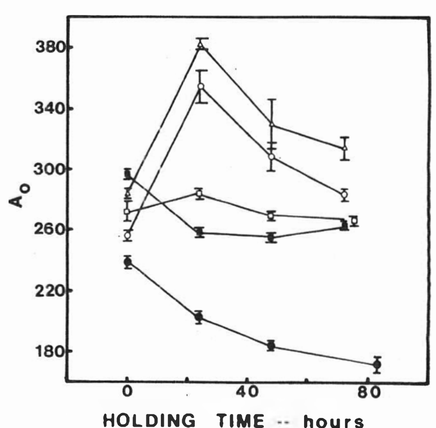


Fig. 6–The effect of blocking SH groups of egg white with PCMB on the intercept value A_0. The bars represent ± 1 standard error associated with the intercept. (● Undialyzed control; ■ Dialyzed control; □ 0.1 mg/ml PCMB; ○ 0.2 mg/ml PCMB; △ 0.3 mg/ml PCMB.)

Figure 7 shows the ultracentrifugal pattern obtained after dialysis of egg white against 0.2g/liter $HgCl_2$. No evidence of aggregation was demonstrated and the sedimentation coefficient of the peak was 3.7S indicating the molecular weight to be about 40,000 (Chervenka, 1969). This molecular weight is in agreement with that of ovalbumin and suggests that aggregation or polymerization reactions did not occur in the presence of $HgCl_2$. Similar results were obtained for all of the PCMB or $HgCl_2$ treatments used, the sedimentation coefficients varying between 3.68S and 3.88S.

In view of the failure to demonstrate any aggregation of the egg white, the reason for the sharp increase in A_o values after 24-hr incubation is difficult to explain. However, it is possible that a mercury crosslinked gel structure existed in the concentrated protein solution that was disrupted when the solution was diluted for ultracentrifugal analysis.

It has been observed by Robinson and Monsey (1971, 1972a) that shifts in the relative amounts of α- and β-ovomucin occur as egg white ages and thins. The two components may be detected in isolated ovomucin by ultracentrifugation in the presence of dissociating and reducing agents. The results of analytical ultracentrifugation of ovomucin isolated from fresh and aged white are shown in Figure 8. The shift in the relative areas of the two components are clearly demonstrated as reported by Robinson and Monsey (1972a). For fresh ovomucin, $S_{20,w}$ values of 4.81 for the fast (β-ovomucin) component and 3.43 for the slow (α-ovomucin) component were obtained. These values are lower than the $S_{20,w}$ values of 4.1 and 8.9 reported by Robinson and Monsey (1971) for their reduced, alkylated samples of α- and β-ovomucin, respectively. Furthermore, with aged white ovomucin, $S_{20,w}$ values of 6.33 and 3.02 for the fast and slow components respectively, were obtained. The reason for the disagreement with the values reported by Robinson and Monsey (1971) is not known; however, components in ovomucin with $S_{20,w}$ values of 6.4 and 2.9 have been reported by Donovan et al. (1970).

The relative area of the slow peak in untreated, aged and SH blocked egg white is shown in Figure 9. The vertical bars on the points represent 95% confidence intervals computed on the basis of the multiple photographic measurements made. The literature values (Robinson and Monsey, 1972a) and the values obtained for untreated egg white in this study lie in the same region of the graph and show reasonable agreement. Further, in all cases shown, reduction in SH level decreased the rate of change in the relative areas of the two peaks. Similar effects on egg white were observed with the PCMB treatment. It seems clear from this figure that blockage of some SH groups inhibits but does not completely stop the changes in ovomucin which are probably associated with thinning reactions.

DISCUSSION

PRELIMINARY STUDIES suggested that the time and shear rate dependent thinning exhibited by egg white (Tung et al., 1970, 1971) made single point apparent viscosity measurements at one shear rate difficult to use as a measure of age thinning. This difficulty was overcome by evaluation of the time dependent shear thinning behavior using an empirical equation, and using the intercept value (A_o in Eq. 1) as an index of thick gel breakdown. However, use of this index has two major drawbacks. First, a large amount of data must be collected and fitted by the equation to obtain just one value of

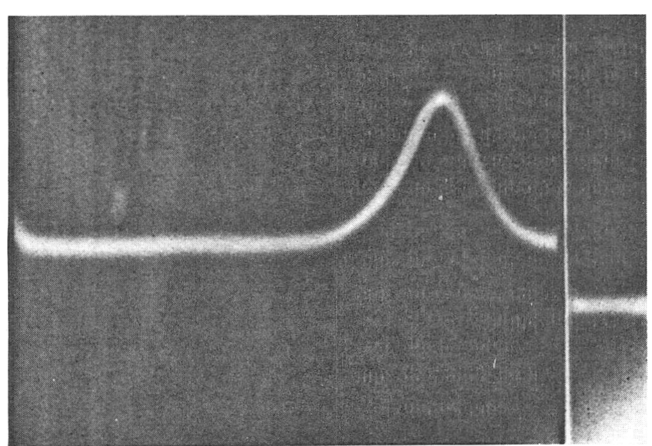
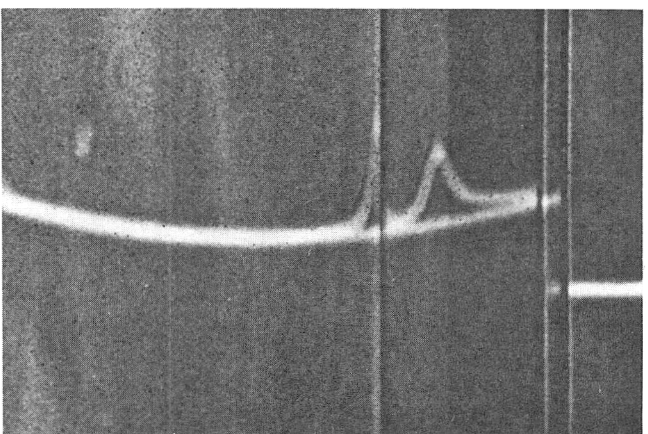
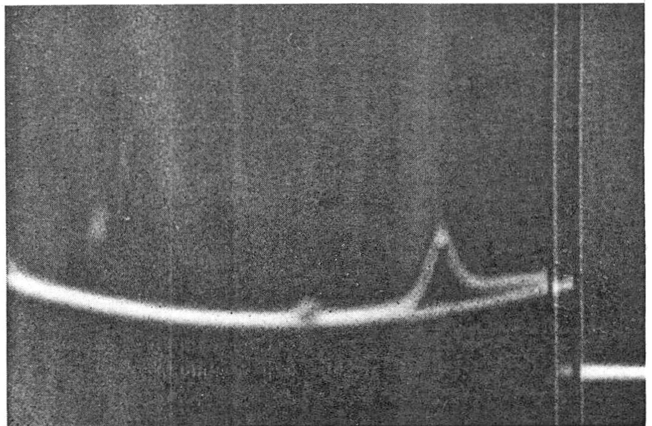


Fig. 7–Schlieren pattern of 1% egg white after treatment with 0.2 mg/ml $HgCl_2$ obtained 60 min after reaching 59,600 rpm. Temperature 20°C; phase plate angle 68°.





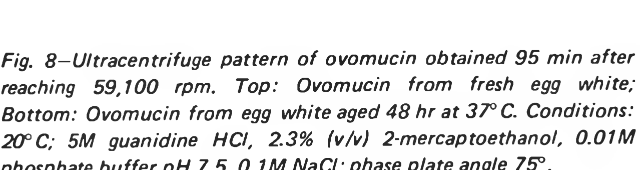


Fig. 8–Ultracentrifuge pattern of ovomucin obtained 95 min after reaching 59,100 rpm. Top: Ovomucin from fresh egg white; Bottom: Ovomucin from egg white aged 48 hr at 37°C. Conditions: 20°C; 5M guanidine HCl, 2.3% (v/v) 2-mercaptoethanol, 0.01M phosphate buffer pH 7.5, 0.1M NaCl; phase plate angle 75°.

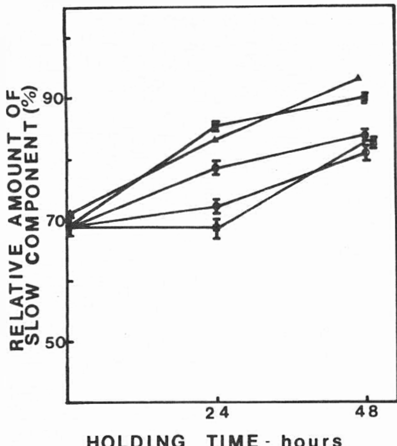


Fig. 9–Effect of blocking SH groups on the relative amount of α-ovomucin (slow peak) in the ovomucin isolated from egg white after incubation at 37°C for 24 and 48 hr. [■ Undialyzed control; ● Dialyzed control; ○ 0.05 mg/ml $HgCl_2$; □ 0.2 mg/ml $HgCl_2$; △ Literature values (Robinson and Monsey, 1972).]

A_0. Secondly, the index is sensitive to disturbances caused by gelation or aggregation reactions such as those which occurred when SH groups were extensively blocked with PCMB or $HgCl_2$.

The chemical changes which occur during the natural thinning of egg white are not understood and several mechanisms have been proposed as explanations of the phenomenon (Feeney and Allison, 1969). Prominent among these are (1) ovomucin is depolymerized by reduction of disulphide bonds; (2) a lysozyme-ovomucin complex is responsible for the rigidity of the gel and that this complex is dissociated; or (3) lysozyme complexes with ovomucin in such a way as to change the physical state of the ovomucin molecules and destroy the gel structure (Robinson, 1972). Two observations made in this study appear to support the first of these proposals. Whenever SH groups are decreased and complications due to aggregation are minimal, viscosity data indicate a disruption of normal thinning patterns and a decrease in the rate of thinning. Also, the rates at which the components in ovomucin isolated from fresh, aged and SH blocked albumen undergo shifts in their relative concentrations are reduced when SH groups are blocked. The reason for the loss of SH groups in the two dialyzed control samples is not known; however, heavy metal contaminants normally present in reagent grade chemicals have been shown to be responsible for a loss of SH in myosin preparations (Buttkus, 1971).

On the other hand, the results presented here do not exclude the possibility that lysozyme-ovomucin interactions play a role in egg white thinning. Robinson and Monsey (1972b) have shown that increasing the ionic strength of egg white with either NaCl or magnesium salts inhibited natural thinning processes. This was suggested as being due to dissociation of lysozyme-ovomucin complexes by excess lysozyme as proposed by Robinson (1972). In our experiments, replacement of natural albumen salts with Tris-KCl buffer was also effective in inhibiting natural thinning. It is possible that the observed thinning inhibition was due to a salt effect on the lysozyme-ovomucin interaction. Also, Robinson (1972) has indicated that Hg^{+2} enhances the interaction between lysozyme and ovomucin so it is possible that the inhibition of thinning is due to enhancement of lysozyme crosslinked ovomucin complexes (Robinson, 1972). If such enhancement did occur, it might explain, in part, the aggregation or polymerization reaction observed during the course of this study.

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EFFECTS OF ADDING 2% FREEZE-DRIED EGG WHITE TO BATTERS OF ANGEL FOOD CAKES MADE WITH WHITE CONTAINING EGG YOLK

INTRODUCTION

ANGEL FOOD CAKES have frequently been used to evaluate egg quality, especially quality of egg whites. Barmore (1936) observed that fresh eggs for use in angel food cakes should not have been at room temperature more than 4 days. Harns et al. (1952) reported a definite quality loss in albumen for angel food cakes after 1 wk of storage at room temperature (22.2°C). Angel food cake volume was down more than 11% and taste panel score by 10%. Rolfes et al. (1955) found that freeze-dried egg albumen had no detrimental effect on cake quality and resulted in a slight increase in cake volume. Morgan et al. (1970) observed no significant differences in comparing freeze-dried, frozen, foam-spray and spray-dried albumen for use in meringues. However, Funk et al. (1971) found that foams made from either foam-spray or spray-dried egg white were more stable than foams from freeze-dried egg white. Zabik (1968b) observed that gels made with freeze-dried albumen were not significantly weaker than those from frozen egg white.

The whipping aids, sodium lauryl sulfate and triethyl citrate, significantly improved the functional properties of spray-dried egg white containing 0.05% yolk according to Anderson et al. (1971). Angel food cake volume and taste panel rating were improved to levels comparable with those made from spray-dried egg white without yolk. Functional properties of egg white without yolk were not improved by use of the whipping aids. Sauter and Petersen (1972) reported that addition of 2.0% freeze-dried egg white to egg white containing up to 3.0% egg yolk resulted in significant improvement in both volume and stability of foams.

The study reported here was undertaken to determine whether addition of freeze-dried egg white would alleviate the detrimental effects of limited amounts of yolk in egg white used for angel food cakes.

EXPERIMENTAL

ANGEL FOOD CAKES baked in small loaf type pans (7.5 cm × 12 cm × 5.5 cm deep) were used to evaluate the effects of adding 2% by weight of freeze-dried egg white to fresh egg white containing from 0.1–0.75% of egg yolk contamination. This level of freeze-dried egg white was selected because in a previous study (Sauter and Petersen, 1972), 2% FDEW consistently improved both volume and stability of foams made from yolk-contaminated egg white.

The formula of Slosberg et al. (1948) was doubled for preparation of batters for angel food cakes as follows: egg white 122g, sugar 125g, cake flour 45g, cream of tartar 1.8g and NaCl 0.6g. Eggs were equilibrated to and egg white held at room temperature (22° ± 2°C) during preparation of cake batter. Sufficient egg white from fresh (day old–84 Haugh units) eggs was weighed, and blended without foaming to uniform consistency with a Kitchen-Aid model K45 mixer at speed 1 (70 rpm). Egg yolk was added in required amounts (0.1–0.75%) at that time. 122g of blended egg white was placed in the bowl of a Kitchen Aid model K45 mixer and whipped at speed 10 (545 rpm) for 30 sec. Cream of tartar and salt were added 10 sec after starting the mixer. Freeze-dried egg white, when used, was also added during this time. Mixer speed was reduced to 8 (440 rpm) and most of the sugar (100g) was added over a period of 15 sec after which the mixer was stopped and sides of the bowl scraped with a flexible kitchen spatula. Mixing was then continued for 10 sec. The balance of the sugar and the flour were blended in at speed 1 for 10 sec and the bowl scraped and mixing continued for an additional 10 sec. Waxed paper was placed in the bottom of the pans and 60g of batter weighed into each pan. Four cakes were baked from each batter. Cakes were baked for 27 min at 170°C in a preheated Frigidaire model RCD-71L oven, then inverted on a rack and cooled. Volume of all cakes was measured by seed displacement after which the cakes were removed from the pans, wrapped first in waxed paper, then in aluminum foil and held overnight before being served to the taste panel in a multiple comparison test. Evaluation of the angel food cakes by a 10-member panel was for color, flavor, moistness, tenderness and texture as well as for general acceptability. Scoring was from 0 to 6 in each category with the control or reference equal to four. Three cakes from each batter were used for taste panel evaluation and one cake used for tenderness testing with an Instron Universal testing machine model TM-M. Shear compression force was determined at a full load setting of 10 kg at a speed of 10 cm/min. Two samples from each cake were used. For testing, the cakes were cut into 5 cm squares. This procedure permitted trimming on the four sides of the cake. The full height of the cake was used in each sample. Batters were replicated six times per treatment.

Freeze-dried egg white used in this study was prepared from fresh (day-old) eggs broken out and separated from the yolks in the laboratory. Egg white was quick frozen in the pans and placed in a Repp model 15 sublimator for 24 hr. The freeze-dried egg white was then removed from the sublimator pans, sealed in polyethylene bags and stored at −17.8°C to −23.3°C until used. Freeze-dried egg white averaged 0.52% moisture.

Data were analyzed by the analysis of variance (Snedecor, 1956), and Duncan's (1955) multiple range test. Taste panel acceptability data and Instron values were compared by use of correlation analysis.

RESULTS & DISCUSSION

2% BY WEIGHT of freeze-dried egg white (FDEW) when added to fresh egg white containing egg yolk resulted in significant increases in volume of angel food cakes. Results indicate that up to 0.3% yolk can be effectively counteracted by adding 2% FDEW without loss of cake volume as compared to fresh egg white. These results are summarized in Table 1.

Overall acceptability of cakes as judged by a taste panel rated the cakes with up to 0.3% egg yolk and 2.0% FDEW slightly higher than the known reference made from fresh egg white but slightly lower than unidentified fresh egg white cakes. These results are shown in Table 2.

Panel evaluation of flavor characteristics indicate only minor differences in flavor score resulting from addition of 2.0% FDEW to angel food cake batter. A summary of these findings are shown in Table 2.

Evaluation of cake color by the panel resulted in a sharp break in rating between cakes with 0.3% yolk or less and cakes having 0.4% or more of egg yolk. Differences resulting from addition of 2% FDEW, while statistically significant in some cases were minor as compared with decreased scores which resulted from use of more than 0.3% egg yolk in the egg white.

These results are also shown in Table 2.

Texture rating by the taste panel are shown in Table 3. There was a slight improvement in texture apparent from addition of FDEW in angel food cake batters from egg white containing up to 0.25% yolk. No improvement was indicated with 0.3% yolk although the cakes were judged acceptable on a texture basis. As indicated by panel scoring, moistness of the angel food cakes was generally reduced by addition of FDEW due possibly to competition for moisture by increased egg protein. This was particularly apparent for cakes containing lower levels of egg yolk. These results are summarized in Table 3.

Taste panel ratings for tenderness are summarized in Table 3. Addition of FDEW to angel food cake batter resulted in reduced tenderness ratings by the panel. Reduction was significant in all cases except in cakes made from egg white containing 0.1% egg yolk. Average shear compression force necessary for penetration through the cake samples is shown in Table 4. Reduced tenderness indicated by both the panel and the Instron could be the result of increased egg protein in cakes containing FDEW.

Instron values agree quite closely with the taste panel evaluation for general acceptability of the angel food cakes. Correlation between panel acceptability data summarized in Table 2 and Instron data summarized in Table 4 resulted in a correlation coefficient of $r = 0.859$. These results indicate that the Instron Universal testing machine could be used effectively for evaluation of general acceptability of angel food cakes.

The mode of action of FDEW in overcoming the effects of yolk contamination is not clear. It may be simply a case of additional ovoglobulin, ovomucin and/or lysozyme-ovomucin complex replacing protein fractions complexed by egg yolk. If this is true, then other forms of dehydrated egg white might work equally well if prepared from high quality eggs and there is no subsequent damage to functional properties during processing. Other types of dehydrated egg white were not used in this study; however, previous work indicated that commercial spray-dried egg albumen was relatively ineffective as compared

Table 1—Average angel food cake volume obtained from 60g of batter made from egg white containing various levels of egg yolk and 2% freeze-dried egg white (FDEW)[a]

Egg White Treatment	Cake volume No FDEW (ml)	Cake volume 2% FDEW (ml)
Fresh egg white	364c	382a
Fresh egg white + 0.1% yolk	334e	368bc
Fresh egg white + 0.25% yolk	319f	374b
Fresh egg white + 0.3% yolk	310f	365c
Fresh egg white + 0.4% yolk	318f	356d
Fresh egg white + 0.5% yolk	274h	291g
Fresh egg white + 0.75% yolk	200j	230i

[a] Values having different letters are significantly different ($P < 0.05$).

Table 2—Average scores[a] for acceptability, flavor and color of angel food cakes made from egg white containing different levels of yolk with and without FDEW[b]

Egg white treatment	Acceptability No FDEW	Acceptability 2% FDEW	Flavor No FDEW	Flavor 2% FDEW	Color No FDEW	Color 2% FDEW
Fresh egg white	4.2b	4.3a	4.2a	4.1b	4.0c	4.3a
Fresh egg white + 0.1% yolk	3.2f	4.2b	4.2a	4.2a	4.1c	4.2b
Fresh egg white + 0.25% yolk	3.2f	4.1c	3.7d	3.7d	4.3a	4.1c
Fresh egg white + 0.3% yolk	3.1g	4.1c	4.0c	4.1b	4.1c	4.2b
Fresh egg white + 0.4% yolk	2.7i	3.7d	3.2f	3.3e	2.5e	2.7d
Fresh egg white + 0.5% yolk	2.2j	3.5e	2.9g	3.1fg	2.0g	2.3f
Fresh egg white + 0.75% yolk	2.1k	2.9h	2.9g	3.0g	2.0g	2.1g
S.D.	0.06	0.06	0.06	0.06	0.06	0.06

[a] Scored on a basis of 0–6 with reference equal to 4.0.
[b] Values for any factor having different letters are significantly different ($P < 0.05$).

Table 3—Average scores[a] for tenderness, texture and moistness of angel food cakes made from egg white containing different levels of yolk with and without FDEW[b]

Egg white treatment	Tenderness No FDEW	Tenderness 2% FDEW	Texture No FDEW	Texture 2% FDEW	Moistness No FDEW	Moistness 2% FDEW
Fresh egg white	4.1a	3.9c	4.0b	4.2a	4.0c	3.9d
Fresh egg white + 0.01% yolk	4.1a	4.0b	4.0b	4.2a	4.2b	4.2b
Fresh egg white + 0.025% yolk	3.9c	3.7d	4.0b	4.3a	4.3a	3.4f
Fresh egg white + 0.3% yolk	3.6d	3.0f	4.0b	4.0b	3.5e	3.5e
Fresh egg white + 0.4% yolk	3.2e	3.0f	3.0f	3.9c	3.6de	4.0c
Fresh egg white + 0.5% yolk	2.9g	2.3h	3.5d	3.2e	3.6de	3.7d
Fresh egg white + 0.75% yolk	2.2h	2.0i	2.9h	3.0f	3.0g	3.3f
S.D.	0.06	0.06	0.06	0.06	0.06	0.06

[a] Scored on a basis of 0–6 with reference equal to 4.0.
[b] Values for any factor having different letters are significantly different ($P < 0.05$).

Table 4—Average shear compression force for angel food cakes made from egg white containing various levels of egg yolk with and without FDEW[a]

Egg white treatment	Force–g/cm² No FDEW	Force–g/cm² 2% FDEW
Fresh egg white	133c	157d
Fresh egg white + 0.1% yolk	115b	150d
Fresh egg white + 0.25% yolk	114b	151d
Fresh egg white + 0.3% yolk	121bc	170e
Fresh egg white + 0.4% yolk	94a	125bc
Fresh egg white + 0.5% yolk	100a	116b
Fresh egg white + 0.75% yolk	98a	101a

[a] Values having different letters are significantly different ($P < 0.05$)

to FDEW for counteracting effects of yolk contamination in egg white used for foams, Sauter and Petersen (1972). Differences observed could have been due to initial egg white quality or damage to functional properties during processing.

From a practical standpoint, 2.0% of FDEW maintained both volume and overall acceptability of angel food cakes made from egg white containing up to 0.4% egg yolk. Under conditions of this experiment, 0.1% egg yolk was detrimental to both volume and acceptability. Freeze-dried egg white had little practical effect on flavor, color, texture, or moistness of cakes as rated by the taste panel. Differences in tenderness were slight but in all cases the panel rated cakes made with FDEW as less tender than similar cakes without FDEW.

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FATTY ACIDS IN THE TISSUES OF FOUR GENERATIONS OF MALE AND FEMALE RATS FED SEVERAL FOOD FATS WITH AND WITHOUT ADDED CHLORINATED HYDROCARBON INSECTICIDES

INTRODUCTION

THE ABILITY of dietary fats to influence the composition of the body lipids has long been recognized. However, the fatty acid composition of the tissues does not necessarily duplicate the composition of the dietary fat, and there is increasing evidence that the body exerts some control over the extent to which the composition of the tissues reflects that of the diet. Thus, Tove and Smith (1960) provided evidence that the deposition of fatty acids in depot fat is a regulated process with a marked specificity of substitution of one acid for another. Kaunitz et al. (1965) found that liver lipids, although influenced by dietary fat, have their own characteristics. Okey et al. (1961) found marked differences in the extent to which different fatty acids are retained in the liver. Tinsley and Lowry (1972) report substantial changes in fatty acid composition of liver lipids with diets containing DDT. Little information is available on the influence of the maternal diet on the fatty acids in the tissues of the young except for the report of Smith and Abraham (1970) based on a short feeding period with lactating mice. Studies of the composition of the lipids in liver, kidney and adipose fat by Privett et al. (1965) provide evidence that all lipid components are in equilibrium with each other in a highly regulated manner in relation to the fatty acid composition of the dietary fat. Although Privett et al. (1965) found that the normal turnover of lipids in the rat is a gradual process requiring 6 months or more following a change of diet, much of the information available on tissue lipids is based on the use of relatively young animals and on short feeding periods.

This report deals with an investigation to determine (1) the possible influence of feeding male and female rats diets containing the chlorinated hydrocarbon insecticides (CHI) on the fatty acid composition of their tissue lipids, and (2) the effect of feeding food fats differing considerably in composition on the fatty acids in the tissues of weanling and adult rats when high levels of these fats are fed continuously for several generations.

EXPERIMENTAL

Diets

Rats were fed, from weaning, nutritionally adequate synthetic type diets similar to those which have been shown previously to permit good growth and survival (Poling et al., 1970). The 14 diets fed were identical in all respects except for the kind of fat and the presence in seven of the diets of added chlorinated hydrocarbon insecticides (CHI). The fats provided 20% of the diet by weight or 38% of the calories. Analyses of the diets gave average values as follows in percent: H_2O, 3.1; protein, 28.6; fat, 23.2; and ash, 3.8.

The fats studied included four commonly used dietary fats—cottonseed salad oil (CS), lard (L), soybean oil (SB) and hydrogenated vegetable oil shortening (S). A composite blend of each fat was prepared by mixing aliquots of three prominent commercially available brands. To remove the cyclopropenoid fatty acids which are present in small amounts in cottonseed oil and which have been reported to delay sexual maturity in the rat (Sheehan and Varich, 1965), a fifth fat was prepared by hydrogenating a portion of CS just enough to give a negative Halphen test and to contain no detectable cyclopropene fatty acids (CS-CP). Two additional fats, heated cottonseed salad oil (HCS) and heated lard (HL) were prepared by heating aliquots of CS and L at 182°C for 120 hr under conditions previously described (Poling et al., 1970). A mixture of CHI was added to portions of each of the five unheated fats, and portions of CS and L containing the added insecticides were heated under the same conditions as the control fats. The CHI mixture was blended with the test fats in amounts sufficient to produce five times the concentration desired in the experimental diets. Each control fat and each fat containing added insecticides was prepared in sufficient quantities to complete the experiment and was stored until needed in filled sealed glass containers at −18°C or below.

The "market basket" survey of total diet samples by Williams (1964) served as the basis for the kinds and proportions of the insecticides selected, but the levels were increased 200-fold to provide concentrations close to tolerances permitted at the time this research was initiated. The fats contained the following average concentrations of insecticides in ppm: DDT and metabolites, 7.8; dieldrin, 1.7; perthane, 2.4; lindane, 0.7; BHC, 0.1; heptachlor epoxide, 0.3; and methoxychlor, 0.5. The levels of insecticides in HCS and HL were reduced by heating to an average of 5.0 ppm DDT and metabolites, 0.7 ppm dieldrin, 0.4 ppm methoxychlor, and less than 0.1 ppm of the other insecticides. The analytical procedures used are described in a previous publication (Adams et al., 1974).

Animal procedures

All rats were supplied water and the appropriate diet ad libitum from weaning until death. For the parent generation (F_0), 350 weanling rats (Holtzman albinos) were randomized among the 14 diet groups-9 males and 16 females per group. After 12 wk, the animals were mated. Femals that had weaned a litter were rested for about 2 wk before being remated for a second litter. First litters were discarded after a record was made of the number of each sex weaned, the individual weaning weight, and the apparent health status of each animal. Randomly selected weanlings (F_1) of both sexes from second litters were reared for mating and the production of F_2 weanlings. This procedure was continued until there were three generations of second litter weanlings for each diet. Litter origin was not known for the F_0 parents, but F_1 and F_2 males were selected to minimize the mating of siblings to the extent possible.

After the second litter was weaned, adult males and females of each generation, F_0, F_1 and F_2, were sacrificed. The average age of the males was 295 days and of the females 244 days. The F_3 rats were not mated and were sacrificed at an average age of 125 days. Approximately three-fourths of the liver and a sample of body fat were removed from randomly selected adults representing each diet group and each generation. The fatty tissue consisted of perirenal fat generally supplemented with retroabdominal fat to supply adequate samples for the analyses desired. When available, at least two male and two female weanlings from each experimental group were sacrificed and saved for

insecticide and fatty acid analyses. The tissues from the adults and weanlings were stored frozen until analyzed. At the time of analysis, the weanling rats were allowed to thaw in the refrigerator for approximately 24 hr. The head was separated from the body, the feet removed at the ankle, the tail removed at the spinal column, the torso skinned, and the liver removed. The remaining carcass was ground. Pooled samples of weanling carcasses and of weanling livers were prepared for each group, and all tissues were thoroughly homogenized prior to analysis.

Fatty acid analysis

Tissue lipids were extracted twice with a 2:1 mixture of chloroform-methanol in the presence of anhydrous sodium sulfate. The extracts were filtered through anhydrous sodium sulfate and evaporated to dryness prior to extraction with petroleum ether and evaporation to dryness under nitrogen. The residues were stored frozen, ready for fatty acid analysis. Fatty acid composition was determined on methyl esters by gas-liquid chromatography (GLC) with a Barber-Coleman Flame Ionization Model 5320 and a 244 × 0.4 cm column packed with 15% liquid phase diethylene glycol succinate on Chromosorb W, mesh size 80–100, at 193°, and a carrier gas flow of 80–100 ml/minute (AOCS Tentative Methods, 1972a). The cyclopropenoid fatty acids in the cottonseed oil products were measured by the stepwise hydrogen bromide titration procedure with the heated oil converted to the methyl esters (Harris et al., 1964). The concentrations of these fatty acids in the tissues were determined by a method suited to the low levels and small size of samples available (Schneider et al., 1968). This method consists of reacting the methyl esters with silver nitrate in methanol to form ether and ketone derivatives and separation from the normal esters by alumina chromatography prior to GLC. The trans-fatty acids of the dietary fats were determined by the official AOCS method (1972b). Student's t test of significance was used to evaluate group differences when there were sufficient comparable data to warrant statistical analysis. Probability levels equal to or less than 0.05 were considered significant, and levels equal to or less than 0.01, highly significant.

RESULTS & DISCUSSION

DIETARY FAT with or without added CHI had little influence on food intake or on body weight except for the low weanling weights of rats fed HCS, in conformity with previous observations regarding growth of weanling rats fed HCS (Poling et al., 1970). Average weight in grams of the weanlings of rats receiving HCS was 45 for males and 42 for females in contrast to the significantly larger weights, 59 and 56 respectively, for weanlings fed the other fats. In spite of the small weanling weights, the average body weights of the F_0, F_1 and F_2 adults fed HCS (571g for males; 308g for females) differed little from the corresponding weights of 588g and 333g for those rats fed the other fats. The size of the livers of these rats was also little influenced by diet except when HCS was the fat fed. The average weight of the livers of rats fed HCS was 18.5g for males and 17.7g for females, significantly greater than the corresponding weights of 16.2g and 13.4g for rats fed the other diets. Moreover, no significant differences in the liver size of males and females were observed with HCS in contrast to significant sex differences observed with all the other fats. Although data for the younger rats of the F_3 generation were limited, a similar trend for larger livers was observed when the dietary fat was HCS. Average weight of the livers of these rats fed HCS was 16.0g for males and 11.3g for females; the corresponding weights with the other dietary fats were 14.3g and 8.8g. In contrast to the results with older rats, the livers of younger males fed HCS weighed significantly more than those of females fed this fat.

The dietary fat was fed at a relatively high level, close to that frequently consumed by humans and at levels higher than that present in the diet ordinarily consumed by stock rats. The concentrations of the fatty acids in each of the seven experimental fats are summarized in Table 1. The results represent the weight percent of the fatty acids volatile by the GLC procedure. They do not differentiate between cis- and trans-isomers nor do they provide information on the polymerized fatty acids in the heated fats. The distribution of fatty acids in the dietary fats differed considerably: total saturates ranged from 15–40%, oleic acid from 18–61%, and total polyunsaturates from 10–60%. Linoleic acid was present in all diets at levels in excess of requirement.

Influence of insecticides

Under the conditions of this investigation, the presence of a mixture of the chlorinated hydrocarbon insecticides in the diet had no significant influence on the fatty acids in the tissues regardless of diet, sex or generation. Table 2 presents, therefore, the overall averages of the principal fatty acids found in the tissues of weanling and adult rats fed diets with and without added CHI. Failure to find any influence of CHI on the fatty acids of liver lipids in contrast to results reported by Tinsley and Lowry (1972) for DDT may be due to the high level of DDT (150 ppm) and the relatively low level of linoleic acid which these investigators fed in comparison with the levels fed in the study reported here. The presence of a mixture of insecticides in the present study may also be contributive to the differences observed.

Influence of kind of dietary fat

The concentrations of fatty acids in the fat of weanling carcasses and in the depot fat of adult rats fed the experi-

Table 1—Fatty acid composition of dietary fats[a]

Dietary fat[c]	Fatty acids — percent by weight[b]						
	C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3
CS	0.86	21.2	0.66	2.3	18.0	57.0	–
HCS	1.07	27.4	1.00	3.0	21.9	44.6	–
CS-CP	0.92	20.7	0.64	2.6	30.3	44.7	0 13
L	1.43	23.4	2.42	12.4	47.5	11.8	1.30
HL	1.37	24.0	2.44	14.2	48.6	9.3	0.84
SB	0.15	10.4	–	4.4	25.0	52.3	8.06
S	0.24	13.5	0.07	12.6	60.7	14.1	0.91

[a] Free fatty acid content of the fats was low, 0.05% or less except in HCS, L and HL which contained 0.03, 0.27 and 0.50%, respectively. Trans-acids were present in CS, CS-CP, HCS and S in concentrations of 4.6, 12.4, 5.0 and 35.2%, respectively, calculated as elaidic acid. Cyclopropenoid fatty acids were present only in CS at a level of 0.34% of the fat calculated as sterculic acid.

[b] Means are based on results of three separate analyses.

[c] Diet codes—CS, cottonseed oil; HCS, heated cottonseed oil; CS-CP, lightly hydrogenated cottonseed oil; L, lard; HL, heated lard; SB, soybean oil; and S, shortening.

Table 2—Fatty acid composition of tissues from rats fed diets with and without added insecticides

Tissue with or without added CHI[a]	Fatty acids — percent by weight				
	C16:0	C18:0	C18:1	C18:2	C20:4
Weanling carcass	21.2	6.6	35.2	24.4	2.3
+ CHI	21.3	6.4	34.9	24.7	2.1
Weanling liver	21.3	13.6	25.7	22.9	13.3
+ CHI	21.4	13.2	25.7	23.5	13.8
Adult depot fat	18.7	4.8	41.9	29.1	–
+ CHI	18.2	4.9	41.7	30.0	–
Adult liver	21.1	16.7	23.0	20.3	15.8
+ CHI	20.7	17.2	22.1	20.5	15.4

[a] Chlorinated hydrocarbon insecticides

mental diets are summarized in Table 3. Excluded from this table are the data for individual saturated fatty acids present in concentrations of 0.4% or less and for the low molecular weight fatty acids C10:0 and C12:0 present only in the fat of weanling carcasses. Total saturates, however, include these fatty acids.

In addition to the lack of differences due to the presence of CHI in the diet, no significant differences related to generation or sex were found. The results in Table 3, therefore, represent for each fat the mean of all the values obtained for the F_1, F_2 and F_3 generation weanlings and the mean of all values for the depot fat of adults of the F_0, F_1 and F_2 generations. No tissues were available for the F_2 generation of adult males fed HCS but with generation and sex differences small, comparison of the results for this diet with the other diets was possible.

Comparable data for weanling livers are presented in Table 4. Because adult livers showed some highly significant differences related to sex, the data for adult males and females are reported separately. Generation differences to be considered later in this report were not related specifically to the fatty acid composition of the diet and thus had little influence on the general response to the dietary fats. Excluded from Table 4 are the results for C17:0 and C18:3 present occasionally in low levels in the livers of weanlings and adults and C14:0 present in levels varying from 0.4–0.7% and unrelated to intake. Because of lack of data for F_2 generation males fed HCS, the means for males and females fed this fat are for the F_0 and F_1 generations.

Although the relative concentration of the individual fatty acids in the livers differed from those in body fat, the influence of dietary fat on the composition of the livers of weanling and adult rats was similar in many respects to that observed in weanling carcasses and in adult depot fat. The major differences appeared to be related chiefly to the presence of phospholipids in the liver.

The concentrations of oleic and of linoleic acid in the tissues consistently reflected dietary intake. In adult depot fat and in weanling carcasses the levels of C18:1 generally equaled or exceeded the concentration of this fatty acid in the dietary fat. In the livers of weanlings and male adults, the concentration of this fatty acid averaged 70% of intake. When the fat contained high levels of C18:2 (45–57%), the concentrations of this fatty acid in relation to intake were relatively constant for any one tissue and were consistently lower in the livers than in the body or carcass fat. When intake levels were low, 9–15%, the concentrations of C18:2 in the fat of all the tissues were close to the concentration of this fatty acid in the dietary fat.

Although the levels of C16:0 in the tissues did reflect dietary intake, the results suggest that a saturation level is reached when the dietary fats contain high levels of this fatty acid (21–24%). At these dietary levels, the concentrations of C16:0 in the fat of weanling carcasses and of weanling and adult livers were similar (22–23%) and were relatively close to intake. In contrast, when the dietary fat provided only 10–14% C16:0, the concentrations of this fatty acid in carcass and in liver fat were 16–19%, levels significantly higher than in the dietary fat. Depot fat contained significantly less C16:0 than did the other tissues.

The response to dietary C18:0 by both weanlings and adults was similar but followed a pattern in relation to intake that differed from the other fatty acids. When dietary fat contained 12% C18:0 or more, body fat levels were lower than intake; when dietary fat levels were 4% or less, body fat levels were higher than intake. In contrast to the results for adult depot fat and weanling carcasses, the 2–14% variation in the concentration of C18:0 in the dietary fat had little influence on the concentration of this fatty acid in weanling or adult livers. When dietary levels were low, liver levels not only exceeded intake but were close to those observed in the livers of rats fed diets containing high levels of this fatty acid. Although

Table 3–Influence of dietary fat on distribution of fatty acids in fat of weanling carcasses and in adult depot fat

Dietary fat	Fatty acids – percent by weight								
	C14:0	C16:0	C16:1	C18:0	Total Saturates	C18:1	C18:2	C18:3	C20:4
					Weanling carcass				
CS (8)[a]	2.5 ± 0.2[b]	23.1 ± 0.5	1.19 ± 0.04	5.7 ± 0.2	34.5 ± 0.6	20.6 ± 0.4	39.5 ± 0.4	0.7 ± 0.1	2.11 ± 0.14
HCS (11)	3.8 ± 0.3	24.4 ± 0.4	2.54 ± 0.12	5.4 ± 0.2	38.2 ± 0.3	25.1 ± 0.7	28.4 ± 0.3	0.6 ± 0.1	3.28 ± 0.28
CS-CP (12)	2.5 ± 0.1	22.2 ± 0.3	2.00 ± 0.09	4.5 ± 0.1	32.0 ± 0.4	28.8 ± 0.4	31.9 ± 0.4	1.0 ± 0.1	2.47 ± 0.23
L (12)	2.2 ± 0.1	23.2 ± 0.3	3.52 ± 0.09	7.8 ± 0.1	35.6 ± 0.3	45.6 ± 0.5	10.8 ± 0.2	1.5 ± 0.1	1.82 ± 0.25
HL (12)	2.4 ± 0.1	23.7 ± 0.2	3.53 ± 0.09	8.2 ± 0.2	36.8 ± 0.6	46.7 ± 0.7	9.3 ± 0.2	1.0 ± 0.1	1.96 ± 0.24
SB (11)	2.3 ± 0.1	15.8 ± 0.3	1.26 ± 0.09	5.8 ± 0.1	27.3 ± 0.4	26.8 ± 0.4	38.0 ± 0.5	3.7 ± 0.5	1.82 ± 0.11
S (12)	1.7 ± 0.1	16.3 ± 0.2	2.17 ± 0.13	7.9 ± 0.2	27.9 ± 0.2	51.3 ± 0.7	13.7 ± 0.5	1.4 ± 0.1	1.85 ± 0.16
					Adult depot fat				
CS (24)[c]	0.84 ± 0.04	19.5 ± 0.2	0.97 ± 0.06	3.9 ± 0.2	24.4 ± 0.4	23.5 ± 0.5	49.6 ± 0.6	0.14 ± 0.03	0.21 ± 0.04
HCS (18)[d]	1.20 ± 0.07	21.6 ± 0.4	3.68 ± 0.26	3.9 ± 0.1	26.9 ± 0.4	32.4 ± 0.4	37.0 ± 0.8	0.54 ± 0.08	0.23 ± 0.05
CS-CP (22)	0.99 ± 0.05	19.3 ± 0.3	1.92 ± 0.14	3.4 ± 0.1	23.8 ± 0.4	33.6 ± 0.6	38.3 ± 0.8	0.20 ± 0.04	0.20 ± 0.04
L (25)	1.00 ± 0.03	20.4 ± 0.3	2.99 ± 0.09	6.5 ± 0.2	28.0 ± 0.4	56.1 ± 0.5	11.2 ± 0.2	0.64 ± 0.09	0.07 ± 0.02
HL (25)	1.13 ± 0.05	21.5 ± 0.2	3.40 ± 0.15	6.5 ± 0.2	29.3 ± 0.3	56.9 ± 0.4	8.9 ± 0.3	0.53 ± 0.08	0.07 ± 0.02
SB (25)	0.52 ± 0.03	12.9 ± 0.3	1.27 ± 0.08	4.1 ± 0.2	17.7 ± 0.4	30.5 ± 0.4	46.1 ± 0.6	3.14 ± 0.15	0.19 ± 0.05
S (27)	0.59 ± 0.03	13.4 ± 0.2	2.94 ± 0.14	5.9 ± 0.2	19.9 ± 0.4	59.7 ± 0.4	15.4 ± 0.4	0.99 ± 0.10	0.05 ± 0.01

[a] The numbers in parentheses represent the number of individual tissues analyzed from adult rats or the number of pooled samples of weanling carcasses analyzed (two carcasses per composite).

[b] Mean ± SEM.

[c] The cyclopropenoid fatty acids were present only in the depot fat of the F_0 generation rats fed CS. Six of the eight samples analyzed (3 males and 3 females) contained 0.015–0.04% as sterculic acid.

[d] Females, F_0, F_1 and F_2 generations but males only F_0 and F_1 generations.

the total saturated fatty acids in the liver were influenced by the level of C16:0 in the diet, it is also apparent that the ability of the body to maintain high levels of C18:0 in the livers, regardless of the concentration in the diet, is responsible for minimizing differences in the total saturated fatty acids in the livers.

Arachidonic acid, generally absent from the triglycerides, was present in negligible amounts in depot fat. It was present in weanling carcasses in concentrations generally less than 3%, was little influenced by diet and its presence was probably due to the phospholipid containing skeletal muscle in weanling carcasses (Century et al., 1961). Differences in the concentrations of C20:4 in the livers were generally small and were not influenced by the levels of C18:2 in the diet.

The concentrations of C16:1 in the tissues showed little relationship to intake levels. Its concentration tended to be low when levels of dietary linoleic acid were high in agreement with the results of Tove and Smith (1960). Linolenic acid was found consistently in adult depot fat and in weanling carcasses but was found consistently only in the livers of rats fed SB. Its concentration in the livers of weanling and adult males fed this fat was 70% and in the livers of adult females 30% of that found in the corresponding body fat, suggesting a difference in the distribution of this fatty acid in the phospholipid and triglyceride fractions. In addition to the fatty acids present in the other tissues, weanling carcasses contained small amounts of the low molecular weight fatty acids C10:0 and C12:0 (1.1 and 1.9%, respectively). Their presence was undoubtedly due to the high concentration of these fatty acids in the milk fat of the rat (Rees et al., 1966; Smith et al., 1968). The relatively high concentration of C14:0 in the lipids of rat milk may also be responsible for the relatively high level of this fatty acid in weanling carcasses. The more rapid rate of oxidation, as well as differences in the mode of transport of these short chain fatty acids (Kirschner and Harris, 1961) may explain the low levels of these fatty acids found in weanling carcasses in comparison with their concentration in rat milk. These low molecular weight fatty acids along with slightly higher levels of C16:0 and C18:0 were responsible for significantly higher levels of total saturates in weanling than in adult body fat. In agreement with the results of Smith and Abraham (1970) the results reported here provide further evidence that the fatty acid composition of the maternal diet exerts considerable influence on the fatty acid composition of the weanling. It is also apparent that the interaction among individual fatty acids responsible for maintaining relatively constant levels of C18:0 and C20:4 regardless of intake are as effective in weanling rats as in adults.

Generation differences

Generation differences related to dietary fat were generally small and insignificant except in the livers of adult rats. Although the concentrations of C18:0 and C20:4 in the livers were generally influenced little by the composition of the dietary fat, the levels of these fatty acids showed consistent generation differences. The results for these fatty acids by generation are summarized in Table 5. The data represent averages for all the diets combined with the following exceptions. The data for females of the F_0 generation fed CS are excluded because of the high concentration of C18:0 in the livers of these rats. The results for males and females fed HCS as well as those for F_3 generation males fed HL differed from those observed with the other fats and are also excluded. Although

Table 4—Influence of dietary fat on the distribution of fatty acids in the liver fat of weanling and adult rats

Dietary fat	Sex	Fatty acids — percent by weight						
		C16:0	C16:1	C18:0	Total saturates	C18:1	C18:2	C20:4
				Weanling livers				
CS (8)[a]	M + F	22.8 ± 0.3[b]	0.11 ± 0.07	13.7 ± 0.9	37.1 ± 0.9	12.5 ± 0.6	35.4 ± 0.6	13.9 ± 0.7
HCS (11)	M + F	24.2 ± 0.2	0.85 ± 0.22	14.5 ± 0.8	39.3 ± 0.7	17.8 ± 0.6	21.6 ± 0.6	19.4 ± 0.5
CS-CP (12)	M + F	21.5 ± 0.4	0.56 ± 0.15	10.9 ± 0.4	33.3 ± 0.4	20.7 ± 0.5	30.0 ± 0.3	14.3 ± 0.4
L (12)	M + F	23.5 ± 0.6	1.73 ± 0.09	13.6 ± 0.4	37.9 ± 0.9	37.8 ± 0.6	12.0 ± 0.5	9.5 ± 0.6
HL (12)	M + F	23.3 ± 0.6	1.78 ± 0.10	14.8 ± 0.4	38.8 ± 0.7	36.6 ± 0.6	10.4 ± 0.5	12.3 ± 0.6
SB (11)	M + F	16.8 ± 0.5	0.58 ± 0.13	12.8 ± 0.6	30.6 ± 0.9	16.4 ± 0.2	38.5 ± 0.7	10.8 ± 0.8
S (12)	M + F	17.3 ± 0.5	2.29 ± 0.09	13.8 ± 0.6	31.9 ± 0.8	38.5 ± 0.9	14.5 ± 0.5	12.1 ± 0.4
				Adult livers				
CS (12)	M	22.8 ± 0.4	0.58 ± 0.14	13.5 ± 1.1	37.8 ± 1.1	12.5 ± 0.4	33.0 ± 1.3	15.9 ± 0.9
(11)	F	21.0 ± 0.8	0.74 ± 0.16	23.3 ± 1.2	45.2 ± 1.7	10.3 ± 0.6	24.5 ± 1.2	19.1 ± 1.5
HCS (7)[c]	M	23.0 ± 0.7	1.67 ± 0.32	15.7 ± 2.5	39.4 ± 2.3	17.3 ± 1.8	24.2 ± 2.4	16.2 ± 2.1
(7)[c]	F	21.5 ± 0.4	1.54 ± 0.18	20.4 ± 1.9	42.4 ± 1.7	16.8 ± 1.3	17.8 ± 0.8	20.0 ± 0.8
CS-CP (11)	M	22.7 ± 0.5	1.34 ± 0.09	11.1 ± 1.0	34.3 ± 0.8	20.4 ± 0.8	29.3 ± 1.0	12.8 ± 1.2
(12)	F	21.9 ± 0.9	1.49 ± 0.26	19.1 ± 1.1	41.0 ± 0.7	18.2 ± 0.8	21.2 ± 0.9	16.9 ± 1.4
L (12)	M	22.4 ± 0.3	1.96 ± 0.17	13.9 ± 1.0	36.8 ± 0.8	32.6 ± 1.0	11.8 ± 0.5	14.9 ± 1.0
(13)	F	21.2 ± 0.5	2.41 ± 0.14	21.2 ± 0.9	43.4 ± 0.8	27.8 ± 0.9	11.2 ± 0.3	15.3 ± 0.7
HL (13)	M	22.9 ± 0.3	2.11 ± 0.14	13.8 ± 0.7	37.2 ± 0.8	34.9 ± 0.8	11.1 ± 0.5	12.9 ± 0.7
(12)	F	21.1 ± 1.1	2.18 ± 0.19	21.1 ± 1.3	43.0 ± 1.1	27.2 ± 0.7	10.0 ± 0.7	16.5 ± 1.1
SB (13)	M	17.8 ± 0.3	1.29 ± 0.10	12.8 ± 0.7	31.2 ± 0.5	17.3 ± 0.7	33.7 ± 0.9	13.5 ± 0.9
(12)	F	19.2 ± 0.6	1.17 ± 0.09	21.1 ± 1.1	41.2 ± 0.9	14.7 ± 0.7	26.1 ± 1.0	15.6 ± 1.2
S (13)	M	17.8 ± 0.6	3.14 ± 0.10	12.1 ± 0.4	30.6 ± 0.5	35.5 ± 1.0	16.1 ± 0.5	13.2 ± 0.8
(14)	F	17.7 ± 0.8	2.80 ± 0.12	17.3 ± 0.6	35.9 ± 0.8	31.2 ± 1.2	14.7 ± 0.7	14.9 ± 1.3

[a] The numbers in parentheses represent the number of individual tissues analyzed from adult rats or the number of pooled samples of weanling carcasses analyzed (two carcasses per composite).

[b] Mean ± SEM.

[c] F_0 and F_1 generations only.

data for F_3 rats were limited (generally two rats per diet), the differences between individual values were small and the levels of C18:0 and C20:4 in the livers of F_3 generation rats were also little influenced by the composition of the dietary fats.

Generation differences were smaller with males than with females, but tended to follow patterns similar to those observed with females. The concentrations of C18:0 were higher in the livers of rats of the F_1 generation than in those of the F_0 or F_2 generation and were consistently higher in the livers of F_3 females than in any of the rats of the preceding generations. In the F_1 generation, the high levels of C18:0 were generally paralleled by a decrease in the concentration of C18:2 in the livers of males and by a decrease in the levels of C16:0 and C18:2 in the livers of females. The increase in concentration of C20:4 in F_2 generation rats was generally accompanied by a decrease in C18:0. There was a marked elevation of C18:0 in the livers of females of the F_3 generation accompanied by a decrease in the concentration of C16:0.

Although diet generally had little influence on the generation differences reported, there were a few exceptions. In spite of the low level of C18:0 in CS, the concentration of this fatty acid in the livers of F_0 females fed this fat was high, 26.7%, in contrast to an average of 17.3% for the livers of females fed the other fats. In succeeding generations, however, the levels of this fatty acid were similar to those in the livers of females fed the other fats. When the dietary fats contained 20–27% C16:0, generation differences were small. When the fat contained 10–14% C16:0 (SB and S), the concentration of this fatty acid in the livers of females decreased consistently with succeeding generations from 20.7% in the F_0 generation to 14.5% in the F_3 generation. No such differences were observed with males.

Generation differences similar in many respects to those reported here for C18:0 were observed in the concentration of DDT and metabolites in the tissues of these rats. The differences, however, were more apparent in depot fat than in liver. These insecticides in the depot fat were present in higher concentrations in the F_1 generation rats than in the F_0 or F_2 generation rats; the highest levels were found in F_3 females. Although the concentrations of these insecticides were low in the liver, similar generation differences were apparent, especially with DDT. As with the fatty acids, these differences were not related to the composition of the dietary fat (Adams et al., 1974).

There is no obvious explanation for the generation differences observed either in the fatty acids or in the insecticide content of the tissues. The rats were raised under carefully controlled environmental conditions. No differences related to body weight, food intake or liver weight were apparent. The results for the fatty acids showed no relation to any differences in the reproductive performance of the females. One possible explanation for the generation differences observed is a slow adaptation to the continued consumption of the high level of dietary fat, a level much higher than is normally consumed by the rat. The gradual decrease with generations in the concentration of C16:0 in the livers of females fed SB and S and the shift from a high concentration of C18:0 in the F_0 generation females fed CS to levels comparable to those obtained with the other fats in the following generations suggest that adaptation to dietary fat may be slow. Although a slow adaptation to high-fat diets could explain the gradual shifts in composition observed with generations in the older rats and the maintenance of a relatively high level of C20:4 in the F_3 females, some further explanation seems to be needed for the consistently high concentrations of C18:0 in the F_3 females (Table 5). Results previously reported for the insecticide content of the tissues of these rats suggested a loss of insecticides from the tissues of the older rats during lactation (Adams et al., 1974). Johnston et al. (1957) have reported results suggesting that during lactation the mothers mobilize considerable quantities of depot fat, which may be used in the production of milk fat. The differences observed between the concentration of C18:0 in the livers of unmated females of the F_3 generation and the older rats of the F_2 generation may be due to the effect of lactation on the lipid metabolism of the female rat.

Table 5–Percent C18:0 and C20:4 in the fatty acids in the livers of four generations of adult rats

Sex	Generation	Number[a]	C18:0	C20:4
Males[b]	F_0	24	10.8 ± 0.5[c]	12.3 ± 0.5
	F_1	27	15.0 ± 0.6	13.1 ± 0.6
	F_2	23	12.6 ± 0.4	16.4 ± 0.8
	F_3	9	15.5 ± 0.5	15.1 ± 0.7
Females[d]	F_0	20	17.3 ± 0.7	13.3 ± 0.9
	F_1	26	23.0 ± 0.6	15.0 ± 0.4
	F_2	24	19.0 ± 0.6	20.4 ± 0.8
	F_3	12	26.5 ± 0.8	19.0 ± 0.7

[a] Number of livers analyzed.
[b] Exclusive of F_3 males fed HL and all generations fed HCS.
[c] Mean ± SEM.
[d] Exclusive of F_0 females fed CS and all generations fed HCS.

Sex related differences

There have been numerous reports dealing with differences in the lipid metabolism of male and female rats, generally with emphasis on cholesterol and essential fatty acid metabolism. This report provides further evidence of sex differences as influenced by fatty acid composition of the diet. The lack of sex differences in the tissues of weanling rats is in agreement with the results reported by Ostwald et al. (1965) on the liver lipids of 4- to 5-wk-old rats and by Aftergood and Alfin-Slater (1967) on the plasma lipids of immature animals.

The lack of sex differences in the fatty acid composition of depot fat with a majority of the fats investigated agrees with the absence of such differences in plasma triglycerides observed by Lyman et al. (1966) with diets containing 10% hydrogenated coconut oil and free of essential fatty acids.

Considerable evidence has been obtained for sex differences in the metabolism of C16:0 and C18:0 resulting in lower levels of C16:0 and higher levels of C18:0 in liver, heart, plasma and erythrocytes of female rats (Okey et al., 1961; Ostwald et al., 1965; Monsen et al., 1962; Pudelkewicz et al., 1968). Christiansen et al. (1969) have reported that the microsomes from livers of female rats have greater ability to elongate C16:0 to C18:0 than those from male rats. This report provides further evidence for highly significant sex differences in the concentration of C18:0 in liver lipids regardless of the kind of fat fed, except when the dietary fat was HCS.

Although there was a tendency for lower concentrations of C16:0 in the livers of females than in those of males, sex differences were small and generally insignificant for any generation. Sex differences reported for this fatty acid have been based chiefly on the concentration of C16:0 in the phospholipid fraction of the liver lipids (Okey et al., 1961; Aftergood and Alfin-Slater, 1965). There appears to be little influence of sex on the concentration of C16:0 in the triglyceride fraction which contains significant proportions of this fatty acid (Okey et al., 1961). Thus, the tendency for only small sex-related differences in the concentration of C16:0 in the total liver lipids may be due to dilution of the phospholipids by the triglycerides.

Regardless of diet or generation, differences between the

concentrations of total saturates in the livers of males and females were highly significant except for the small differences with rats fed HCS. The high concentrations of total saturates in the females observed under the conditions of this investigation, however, were in contrast to the reverse sex differences reported by Pudelkewicz et al. (1968) with fat-free diets supplemented with linoleic or linolenic acid. The fats in the present study all contained more than adequate levels of C18:2 and significant levels of C16:0 and C18:0.

The extent to which the concentrations of C18:1 or C18:2 varied with sex depended upon the concentrations of these fatty acids in the diet. When the dietary fat contained 48–61% C18:1, the levels in the livers of males were significantly higher than in the livers of females regardless of generation; with fats containing 25–30% C18:1, the concentrations of this fatty acid were similar in males and females of the F_0 generation but significantly higher in males than in females of succeeding generations. With CS, which contained the lowest level of C18:1, sex differences were small but consistent, with an overall difference significant at the 5% level. No significant sex differences were observed with HCS.

The concentrations of C18:2 in liver fat of male rats fed diets containing high levels of this fatty acid were significantly higher than in the corresponding livers of females; no significant sex differences were found when the dietary fat contained 14% C18:2 or less. The lack of sex differences in the livers of rats fed low levels of C18:2 and the decrease in the differences observed when rats were fed the lower levels of C18:1 may be related in some way to differences in the positional distribution of these fatty acids. Tove (1961) has reported that both oleic and linoleic acids esterified in the β position are relatively inert when compared with these fatty acids esterified in the α position.

Although Aftergood and Alfin-Slater (1965) have reported higher concentrations of C20:4 in the plasma lipids from female rats than from male rats fed a diet containing 15% cottonseed oil, sex differences in the level of this fatty acid in liver phospholipids were small. Lack of sex differences in the concentration of this fatty acid in the liver phospholipids of rats fed 10% cottonseed oil or coconut oil was also observed by Okey et al. (1961). According to Christiansen et al. (1969), no sex differences were observed in the ability of microsomes from livers of males and females to perform the dehydrogenation and chain elongation steps leading from linoleic to arachidonic acid.

The tendency for higher average levels of C20:4 in the livers of the three generations of females (Table 4) than in the livers of comparable males is due chiefly to increased concentrations of this fatty acid in the livers of the F_2 generation rats (Table 5). Sex differences were small and generally insignificant in the F_0 and F_1 generations. The highest levels in the F_2 generation females were observed in the rats fed CS and HCS; the lowest were observed in rats fed L and HL.

Differences between the concentrations of the fatty acids in livers of young males and females of the F_3 generation were similar to those observed in the older rats except when the dietary fats were HCS and HL. The tendency for small and generally insignificant sex differences in the older rats fed HCS was even more apparent in the livers of the F_3 rats. The concentration of C18:0 in the livers of males was 21.1% and in the livers of females 23.6%. Comparable values for total saturates were 44.7 and 44.2% and for C18:2, 21.8 and 18.8%. In contrast to the sex differences observed with the older rats fed HL, the differences between males and females of the F_3 generation were generally insignificant. The concentration of total saturates in the F_3 males was 42.7 and in the females 45.7%; for C18:1 the corresponding values were 26.7 and 25.5%. Differences between the response to HCS and HL are probably related to the kinds and/or amounts of the byproducts from the heating procedure, possibly due to differences in the relative concentrations of urea non-adducting fraction present in these two heated fats (Poling et al., 1970).

SUMMARY

THE FAT SOLUBLE chlorinated hydrocarbon insecticides in levels 200 times that found in diets in the United States had no significant influence on the fatty acid content of the tissues of four generations of rats fed different commonly consumed food fats. The body apparently exerts considerable control over the fatty acid composition of the tissues even with the high level of fat fed for prolonged periods of time. In most respects, the fatty acid compositions of the carcasses of weanlings and of adult depot fat were similar. The fatty acids in the livers of male and female weanlings were similar to those in the livers of the sire but differed in several respects from those in the livers of the dam. The concentration of oleic and linoleic acids in the tissues consistently reflected dietary intake. Stearic acid levels in the livers were similar regardless of the concentrations of this fatty acid in the diet, and differences in the levels in the carcasses or adult depot fat were also not directly related to intake. Differences between the levels of oleic or linoleic acid in the livers of adult males and females varied with the levels of these fatty acids in the diet. Differences unrelated to the fatty acid composition of the diet were due chiefly to differences in the fatty acids found predominantly in the phospholipids. Consistent generation differences suggest a slow adaptation to high-fat diet resulting in a gradual shift from elevated levels of stearic acid in the F_1 generation to elevated levels of arachidonic in the F_2 generation. Although the results for rats fed heated fats follow a pattern similar in many respects to that observed with other fats, differences in the composition of the livers of males and females tend to be reduced or eliminated, especially with HCS.

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FACTORS AFFECTING THE RELATIVE BIOLOGICAL VALUE OF FOOD GRADE ELEMENTAL IRON POWDERS FOR RATS AND HUMANS

INTRODUCTION

ELEMENTAL IRON POWDER, frequently referred to as reduced iron and ferrum reduction, is the most common iron supplement used in the enrichment of food. In Canada flour and some cereal products are fortified with elemental iron and in the U.S.A. it was estimated by Waddell (1973) that this type of iron source was used in about 50% of the enriched food.

There is increasing evidence that the biological availability of iron from various food grade iron supplements is quite variable and in some cases it has been seriously overestimated (Waddell, 1973). The efficacy of some preparations of elemental iron powders for the prevention of iron-deficiency anemia also has been questioned by several groups of workers (Reduced iron: Freeman and Burrill, 1945; Elwood, 1968; Pla and Fritz, 1971; Amine et al., 1972. Electrolytic and reduced iron: Hinton et al., 1967; Pla and Fritz, 1971; Shah and Belonje, 1973). It has been shown that the low biological value of food grade iron powder may be due at least in part to the presence of a large proportion of coarse particles of iron in commercial preparations which conform to the specifications of Food and Chemical Codex with respect to particle size (Reduced iron: Hoglund and Reizenstein, 1969; Motzok et al., 1972. Electrolytic and reduced iron: Shah and Belonje, 1973; Pla et al., 1973; Pennell, 1974).

On the other hand, recent research has shown that fine iron powder, freshly prepared, possesses high biological availability. Cook et al. (1973) using radioisotopes of iron found that fine reduced iron (particle size 5–10 μm) was utilized by human subjects as well as the iron from ferrous sulphate baked in bread. Our current findings support the latter observations and the present indications are that elemental iron powder containing a high proportion of fine particles may be a satisfactory source of supplemental iron for food enrichment. A comparison was also made of the biological availability of iron from discrete fractions based on particle size of samples of electrolytic iron powder and elemental iron produced by hydrogen reduction.

EXPERIMENTAL

TWO SAMPLES of electrolytic iron and one sample of elemental iron powder produced by hydrogen reduction were separated each into seven fractions based on particle size from less than about 7 μm to larger than 40 μm by elutriation with nitrogen to avoid the possible oxidation of iron by oxygen when air elutriation is used. A particle segregation apparatus, the Infrasizer (Haultain, 1961), was used. The flow of nitrogen was adjusted to 21 in. of water on the differential manometer gauge and the elutriation time was approximately 1.5 hr per 100g of sample. The separated fractions of the iron powders were stored under nitrogen until used.

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Bioassays with rats

In general the procedure described by Pla and Fritz (1971) was employed for measuring the relative biological value of reduced iron. The criterion was the response in hemoglobin in young rats to graded levels of ferrous sulfate (reagent grade $FeSO_4 \cdot 7H_2O$ as the reference standard) and the test supplement. Male rats (Wistar strain) weaned at 19 days of age were housed individually in stainless steel screen-bottom cages and each diet was fed ad libitum to 9–10 animals.

In the curative procedure the animals were made anemic as prescribed in the proposed official procedure (Pla and Fritz, 1971) and the experimental diets were fed to anemic rats for 2 wk. In prophylactic assays the experimental diets were fed to rats from weaning at 19 days of age. The duration of the feeding periods is given in the tables containing the experimental data.

The AOAC basal diet (Pla and Fritz, 1971) containing skim milk powder was used in one assay (Experiment 1, Table 2). In the remaining experiments the basal diet contained casein instead of skim milk powder as the major source of protein. Its composition is given in Table 1. Three levels of ferrous sulfate and three or more levels of each test sample were fed at equal log increments in amounts which gave linear and parallel responses in hemoglobin when plotted against the logarithm of added iron.

Blood samples were obtained from the tail of the rat for hemoglobin determination by the cyanomethemoglobin procedure of Crosby et al. (1954). The colorimetric procedure described by Davies et al. (1972) was employed for the determination of iron in the basal and experimental diets to test the adequacy of diet preparations. The iron content of the basal diets was about 7–10 mg/kg.

Iron absorption by human subjects

In studies on iron absorption by human volunteers the general procedure was similar to that described by Pla and Fritz (1971). A sample

Table 1—Composition of low iron basal diet containing casein

Ingredient	%
Glucose monohydrate	55
Casein (vitamin-free)[a]	20
Yellow corn meal	15
Corn oil	5
Vitamin premix in dextrose[b]	1
Mineral premix (excluding iron) in corn meal[c]	4

[a] Obtained from General Biochemicals, Inc., Chagrin Falls, Ohio
[b] Supplied the following per kg diet: 5 mg thiamine, 10 mg riboflavin, 4 mg pyridoxine, 40 mg calcium pantothenate, 40 mg niacin, 100 mg p-aminobenzoic acid, 0.2 mg biotin, 2 mg folic acid, 5 mcg cobalamin concentrate, 250 mg inositol, 2g choline bitartrate, 250 mg Vitamin A powder (20,000 USP units/g), 1.8 mg calciferol (850,000 I.U. g), 400 mg DL-alpha-tocopherol powder (250 I.U./g), 5 mg menadione (dextrose to make up to 10g).
[c] Supplied the following per kg diet: 20.34g $Ca(H_2PO_4)_2 \cdot H_2O$, 6.92g $CaCO_3$, 1.81g K_2SO_4, 1.74g K_2CO_3, 1.39g $MgCO_3$, 0.824g NaCl, 0.405g Na_2CO_3, 0.154g $MnSO_4 \cdot H_2O$, 0.023g $ZnCO_3$, 9 mg $CuCO_3Cu(OH)_2 \cdot H_2O$, 0.2 mg KI (corn meal to make up to 40g).

of blood (2–3 ml) was obtained from each individual at about 9 a.m. after an overnight fast and the test dose (100 mg iron) of iron supplement was then taken orally in a gelatin capsule; 2-hr later the second sample of blood was obtained. Each subject served as her or his own control. Iron absorption was calculated from the increase in plasma iron after ingestion of the oral dose using an estimated blood volume based on sex, height and weight (Nadler et al., 1962). The amount of iron absorbed following ingestion of 100 mg iron furnished by ferrous sulfate was arbitrarily assigned a value of 100. The absorption of iron after ingestion of 100 mg iron from the test sample was recorded as a percentage of that from the dose of ferrous sulfate. The micro procedure of Caraway (1963) was used for the determination of plasma iron.

Statistical evaluation of data

The statistical procedure of Bliss (1952) for parallel line bioassay was employed to calculate the relative biological values of the test samples for rats and the estimates of the fiducial limits of the assays at the 95% level of probability. A Fortran (Watfiv) computer program was devised and the statistics generated included the test for linearity and slopes of standard, test and combined response curves and a test for parallelism of the standard and test curves.

Experiments with electrolytic iron

In these studies a freshly prepared commercial batch of electrolytic iron, designated as sample No. 1, was used. Rice cereal, containing 50 ppm Fe, was fortified with the elemental iron powder to a level of 1000 ppm total Fe in a pilot plant facility. In Experiment 1 a rat assay was made to determine the relative biological value of the iron in the fortified cereal and of the electrolytic iron added to the assay diets alone and with quantities of the unfortified cereal in amounts equal to those provided by the enriched preparation. Unfortified rice cereal was also added to an extra diet supplemented with 32 ppm Fe/kg as ferrous sulfate, i.e., the upper level of added iron in the standard reference curve.

In Experiment 2, 500g of the electrolytic iron were separated into fractions according to particle size and the relative biological availability of the iron for rats was determined in two of the fractions, designated as 7–10 μm and 27–40 μm.

In both trials the experimental diets were fed to anemic rats according to the AOAC recommended procedure (Pla and Fritz, 1971).

Comparison of electrolytic and hydrogen reduced iron

In Experiments 3–5 fresh samples of electrolytic iron (sample 2) and elemental iron powder produced by hydrogen reduction were separated into fractions based on particle size. Two of the fractions, designated as 7–10 μm and 20–26 μm, from each type of iron powder were assayed with rats using the prophylactic (Experiment 3) and curative (Experiment 4) assay procedures. In Experiment 5 human subjects were used to evaluate the relative availability of the iron from the four preparations by the iron absorption procedure described above.

Scanning electron microscopy (SEM)

Iron particles from the two size-fractions, 7–10 μm and 20–26 μm, of electrolytic iron and hydrogen reduced iron were coated very thinly with gold-palladium, examined in a SEM (Cambridge Stereoscan Mk II A) and photographed on Ilford FP4 120 film. The tilt angle was 45°.

RESULTS & DISCUSSION

THE DATA from Experiment 1 (Table 2) obtained with electrolytic iron powder (sample 1) indicate that the availability of iron after enrichment and processing of rice cereal was the same as that of the elemental iron alone. Furthermore, the addition of unfortified cereal to the assay basal diet supplemented with electrolytic iron or ferrous sulfate had no significant effect on the utilization of the respective iron supplements by anemic rats. Theuer et al. (1971) showed that processing (heat sterilization and lyophilization) of a liquid soya isolate infant formula product supplemented with ferric pyrophosphate or sodium iron pyrophosphate markedly increased the relative biological values (RBV) of iron from these sources. The present findings show that processing of a rice cereal product fortified with electrolytic iron in a pilot plant facility had no beneficial or adverse effect on the relative availability of this type of elemental iron powder for rats.

The biological data from Experiment 2 (Table 3) show that the size of particles influenced the availability of electrolytic iron, the RBV of fine particles (7–10 μm) being significantly higher than that of coarse particles (27–40 μm). Samples of the two fractions were supplied to James C. Fritz, Associate Referee of the AOAC (Washington, D.C.) on measuring iron availability, for the collaborative study conducted in 1973. The data in Table 3 show that estimates of availability of iron from the two fractions obtained in our laboratory were in general agreement with the average values obtained by the collaborators (Fritz et al., 1974). The present data regarding the effect of particle size on the RBV of electrolytic iron are similar to those obtained previously with reduced iron produced by carbon monoxide reduction (Motzok et al., 1975).

The distribution of particle-size fractions of elemental iron powders (Tables 3 and 4) shows that the proportion of fine particles was considerably higher in samples of electrolytic iron than in hydrogen reduced iron. According to Patrick (private communication) the same milling equipment was used in the commercial preparation of the two types of elemental iron powder. Patrick also observed that electrolytic iron appears to be somewhat harder (more brittle) than reduced iron and thus it grinds to a higher subsieve content (greater percentage of fine particles). Furthermore, it is of interest to note that the percentage distribution of the several particle-size fractions of the two types of iron powder achieved by nitrogen elutriation in the present study is similar to the particle size distribution

Table 2–The relative biological value (RBV) of iron from rice cereal enriched with electrolytic iron powder[a] for rats (Exp. 1)

Iron source and treatment	RBV[b] (vs $FeSO_4$ = 100) Mean	Limits[c]
Cereal enriched with electrolytic iron[d]	45	37–53
Electrolytic iron	47	38–56
Electrolytic iron plus unfortified cereal	49	40–61
Ferrous sulfate plus unfortified cereal	108[e]	

[a] Sample 1, gift of Gerber Products Co., Fremont, Mich.
[b] Based on response in hemoglobin of anemic rats fed the experimental diets for 2 wk
[c] Fiducial limits at 95% level of probability
[d] Rice cereal enriched to contain 1000 ppm total iron, courtesy of Dr. George A. Purvis, Gerber Products Co., Fremont, Mich.
[e] Calculated by the procedure described by Pla and Fritz (1971)

Table 3–Particle size distribution of electrolytic iron[a] and its effect on the relative biological value for rats (Exp. 2)

Fraction (μm)	Percent distribution	Relative biological value (vs $FeSO_4$ = 100) Mean	Limits[b]	Coll. study[c]
<7	2.4			
7–10	12.9	66	50–86	63.5 ± 11
11–14	14.8			
15–19	15.9			
20–26	18.3			
27–40	11.4	23	17–29	37.9 ± 12
>40	2.3			

[a] Sample 1, gift of Gerber Products Co., Fremont, Mich.
[b] Fiducial limits 95% level of probability
[c] Data from AOAC collaborative study (Fritz et al., 1974)

in better commercial products determined by roller analysis (Cook et al., 1973).

In addition to the effect of particle size on the relative availability of elemental iron powder, evidence has been obtained which shows that the method of manufacturing of elemental iron may affect the degree to which the product is utilized biologically. Data on the RBV of electrolytic iron and hydrogen reduced iron for rats (Experiments 3 and 4) and humans (Experiment 5) are given in Table 5. From two fraction sizes (7–10 μm and 20–26 μm) the iron powder produced electrolytically was more available for rats than the iron produced by hydrogen reduction, the difference in RBV being statistically significant at the 95% level of probability for the 20–26 μm fractions by both prophylactic and curative procedures. It is of interest to note that in the curative assay procedure there were no significant differences among the RBV estimated from the final hemoglobin values and those based on the change in hemoglobin of anemic rats after the 2-wk feeding period of the experimental diets. Furthermore, there was no marked difference in the variability of the estimates by the two criteria as indicated by the 95% fiducial limits.

The differences in availability of the two types of iron observed in the study on iron absorption by human subjects (Table 5) were similar to those obtained with rats. It is recognized that different experimental procedures and criteria of response were used in studies with rats and human subjects and the absolute values of relative availability are different. However, the ratios of the availability of each fraction-size of the two types of iron were about the same for the two species (Table 6). On this basis, rats appear to be satisfactory as laboratory test animals for estimating the relative availability of elemental iron powders for humans as measured by the procedures employed in this study.

The surface structures of the four samples of elemental iron powders assayed biologically were examined with the aid of a scanning electron microscope and photomicrographs of the iron particles are shown in Figure 1. Patrick (Waddell, 1973) described the shape of electrolytic iron as irregular and dendritic or fernlike from which it receives its high surface factor. This is clearly illustrated by scanning electron microscopic examination which shows that the electrolytic iron (Fig. 1c) is definitely more fragmental with more sharp corners than reduced iron (Fig. 1d). Thus it is likely to possess a greater surface area as well as a defect structure than the annealed structure of elemental iron powder produced by hydrogen reduction.

It appears from a review of the literature on this topic that factors other than particle size and method of reduction of

Table 4—Particle size distribution of electrolytic iron and hydrogen reduced iron[a] (Elutriation with nitrogen)

Fraction (μm)	Percent distribution: Electrolytic iron[b]	Percent distribution: Reduced iron by H_2
<7	13.6	2.5
7–10	11.0	2.5
11–14	20.1	6.2
15–19	23.7	17.0
20–26	26.8	33.0
27–40	3.1	36.8
>40	1.7	2.0

[a] Iron samples kindly provided by Mr. John Patrick Jr., Glidden-Durkee Div. of SCM Corp., Johnstown, Pa.
[b] Designated as Sample 2

Table 6—Ratios of the relative biological values (RBV) for electrolytic and hydrogen reduced iron of each particle size for humans and rats calculated from data in Table 5

Particle size (μm)	Ratio of RBV (Elec./H_2 reduced): Humans	Ratio of RBV (Elec./H_2 reduced): Rats[a]
7–10	1.51	1.40
20–26	2.20	1.92

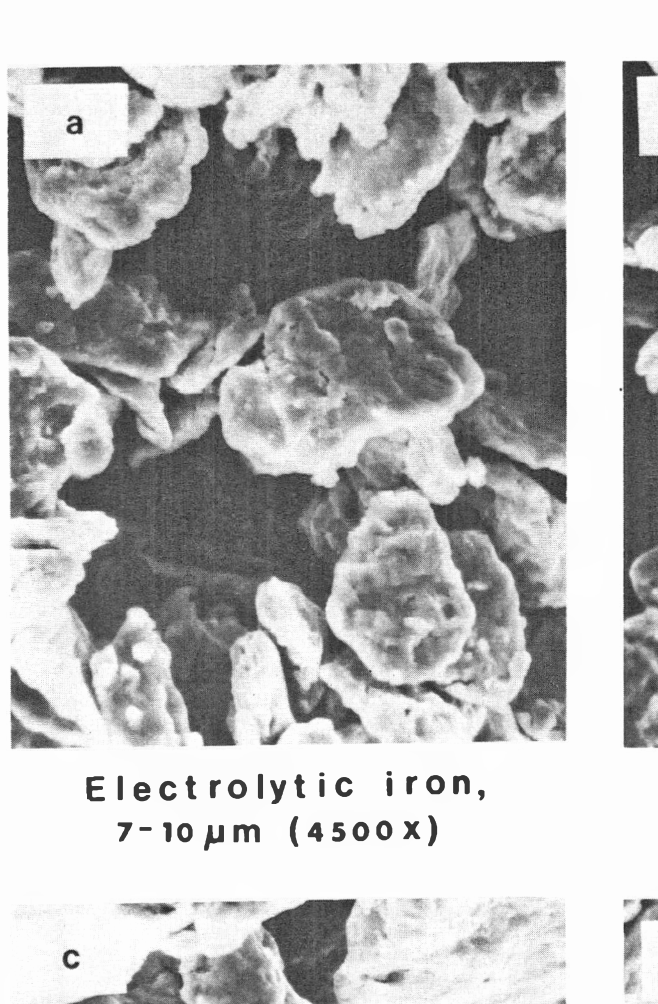
[a] Average of ratios of RBV obtained by the four criteria used in assays with rats (Table 5)

Table 5—Relative biological values of electrolytic[a] and hydrogen (H_2) reduced iron powders for rats and humans (Exp. 3–5)

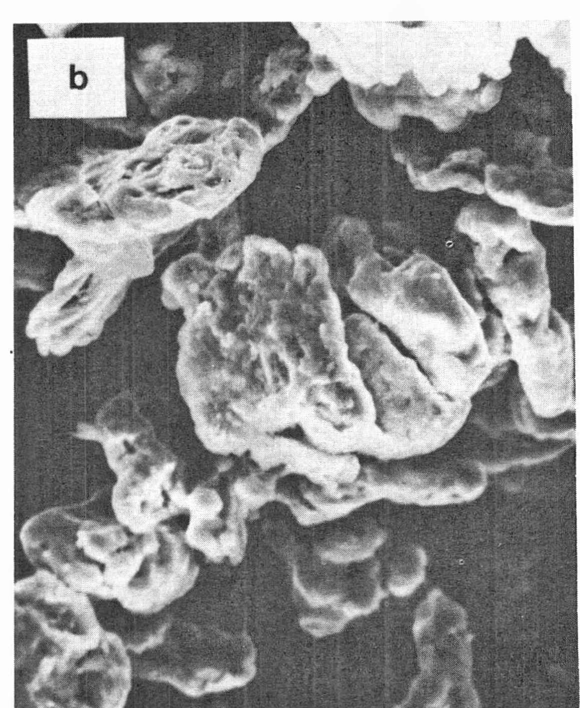
Method of production	Particle size (μm)	Rats, Prophylactic[b]: 3 wk	Rats, Prophylactic[b]: 4 wk	Rats, Curative[c]: Hb[e]	Rats, Curative[c]: ΔHb[f]	Humans (Response in plasma iron)[d]
Electrolytic	7–10	58 (48–69)[g]	64 (52–79)	50 (45–57)	55 (48–62)	74 ± 15.4[h]
H_2 reduced	7–10	38 (27–50)	43 (28–58)	42 (36–50)	40 (33–48)	49 ± 11.7
Electrolytic	20–26	35 (28–43)	41 (33–53)	41 (36–47)	38 (33–43)	22 ± 5.4
H_2 reduced	20–26	21 (17–26)	25 (20–30)	18 (16–22)	18 (15–21)	10 ± 3.1

Relative biological values (vs $FeSO_4$ = 100)

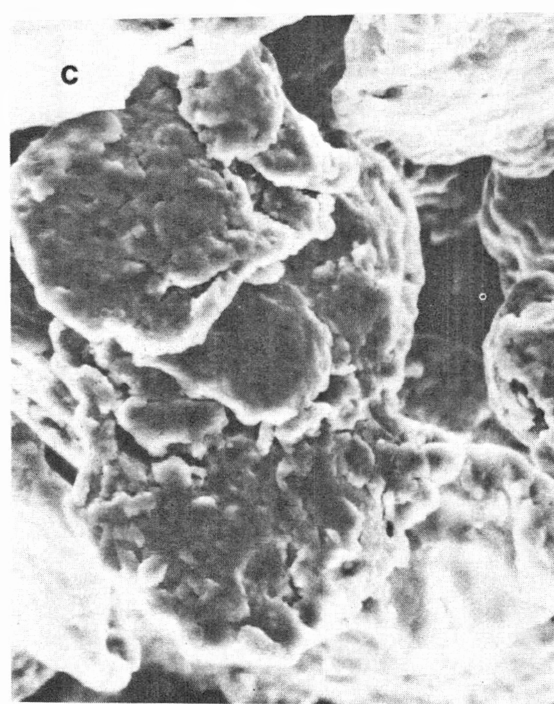
[a] Sample 2
[b] Based on hemoglobin values after feeding of test diets for 3 and 4 wk from weaning at 19 days of age (Exp. 3)
[c] Test diets fed for 2 wk to anemic rats (Exp. 4)
[d] Based on total iron absorption calculated from the increase in plasma iron 2 hr after ingestion of 100 mg iron from ferrous sulfate and test samples after an overnight fast using estimates of blood volume based on sex, height and weight of subjects (Exp. 5)
[e] Based on hemoglobin values at the end of the test period
[f] Based on change in hemoglobin values after 2 wk on test diets
[g] Mean and fiducial limits at 95% level of probability
[h] Mean ± S.E. Particle size: 7–10 μm, 11 subjects (6 females, 5 males); 20–26 μm, 13 subjects (8 females, 5 males).



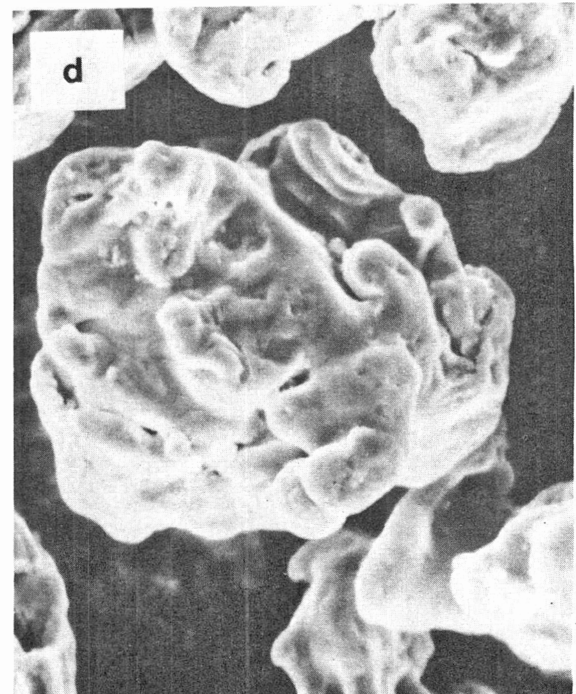
Electrolytic iron,
7-10 μm (4500X)



H_2 Reduced iron,
7-10 μm (4300X)



Electrolytic iron,
20-26 μm (4000X)



H_2 Reduced iron,
20-26 μm (4400X)

Fig. 1–Scanning electron photomicrographs of elemental iron powders (particle sizes, 7–10 μm and 20–26 μm) produced electrolytically (a) (c) and by hydrogen reduction (b) (d).

iron may affect the biological availability of elemental iron powders. Shah and Belonje (1973) reported relative biological values of 21 and 32 for two samples of electrolytic iron which had 94 and 99% of the particles less than 10 μm, respectively. Considerably higher values for the relative availability of iron were obtained for electrolytic iron of comparable particle size with rats and humans in the present study and for hydrogen reduced iron (5–10 μm) with humans in the study by Cook et al. (1973) who used a laboratory preparation containing radioactive iron.

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RESEARCH NOTES

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A Research Note
OBJECTIVE ASSESSMENT OF MEAT JUICINESS

INTRODUCTION

METHODS used to measure the water-holding capacity of meat have been reviewed by Hamm (1960). In many of the methods homogenized meat samples are subjected to arbitrarily defined but standard treatments and the relative changes between samples measured. The press method (Grau and Hamm, 1953; 1957) seemed the most practical although attempts to correlate these water-holding capacity measurements with subjective assessment of juiciness were not very successful (Hamm, 1960). More recently the Akroyd high speed centrifugation method has been used to follow changes in the moisture retention properties of meat in relation to pH and cooking temperature (Bouton et al., 1971; 1972; 1973). No direct comparison, however between subjectively assessed juiciness and either cooking loss (CL) or the amount of centrifugally expressed juice (EJ) has been reported and therefore, the investigation reported here was undertaken.

MATERIALS & METHODS

Experimental material

Following slaughter, the carcasses of 50 beef steers (2–4 yr old) were hung from the Achilles tendon and chilled in a room temperature controlled to 0–5°C for 24 hr before muscles were removed. 28 semimembranosus (SM) muscles, 16 pairs of longissimus dorsi (LD) muscles, and a deep pectoral (DP), semitendinous (ST), gluteus medius (GM) and psoas major (PM) muscle from each of the remaining 6 carcasses were used. One from each pair of the LD muscles was vacuum sealed in a Cryovac bag and aged for 2 wk at 0–1°C before use. With the exception of these 'aged' LD muscles all samples were cooked on the day that they were removed from the carcasses. Any visible external fat and connective tissue were trimmed from the muscles before they were cut into samples weighing 450–500g. Each SM muscle yielded 4 samples. Those from 22 animals were assigned, at random, equally to the cooking treatments of 65, 70, 80 and 90°C for 2 hr. Those from the remaining 6 animals were assigned equally to cooking treatments of 1, 2, 3 and 4 hr at 90°C. Each LD muscle yielded 2 samples which were assigned at random to cooking at 60 or 80°C for 2 hr. A sample from each of the DP, ST, GM and PM muscles was cooked at 90°C for 1½ hr. All raw samples used had pH values in the range 5.4–5.8.

Cooking methods

The cooking methods have been described, in detail, elsewhere (Bouton et al., 1971). The samples were all weighed before and after cooking (once surface moisture had been removed using paper towels) to determine cooking losses. After cooking, drying and weighing each sample was divided into two parts one of which was assigned at random to subjective assessment and the other to objective assessment.

Subjective assessment of juiciness

Subjective assessment of juiciness was determined by a taste panel of 12 members, trained and experienced in meat tasting. Four coded samples of different treatments were presented at each session (e.g., SM cooked at 4 different temperatures) and the order of tasting was balanced among panelists to cancel out any contrast effect on their judgments. Taste sessions were conducted under green light to disguise color differences. Juiciness scores were recorded on a 16-point scale viz. 0 = extremely juicy, 3 = very juicy, 6 = juicy, 9 = slightly dry, 12 = dry and 15 = very dry.

Objective assessment of juiciness

The Akroyd high-speed centrifugation method, as described by Bouton et al. (1972), was used. Quintuplicates of between 3–4g of each muscle sample were weighed to the nearest mg then centrifuged at 36,000 rpm (100,000G) for 1 hr, in stainless steel tubes, using a type 50 rotor in a Model L Beckman Spinco Preparative Centrifuge (Spinco Div. of Beckmans Instruments Inc., Palo Alto, Calif.). After centrifuging the meat samples were removed from the tubes with forceps, carefully dried with paper towels, reweighed and EJ was calculated as the amount of juice expressed per gram of cooked meat. These EJ measurements were converted to a percentage of the original raw weight of the meat sample (EJ*) so that the CL and EJ* measurements were both expressed as a percentage of the original sample weight. Total juice loss (TJL) was the sum of the combined percentage CL and EJ* measurements.

Statistical treatment of results

Regression analyses were carried out to find the lines of best fit to the experimental points and simple correlation coefficients were obtained.

Table 1—Correlation matrix obtained for apparent or subjective juiciness (J), expressed juice (EJ), cooking loss (CL), corrected expressed juice (EJ*) and total juice loss (TJL = CL + EJ*) for n = 200

	J	EJ	CL	EJ*	TJL
J	1.00	−0.87	0.92	−0.90	0.54
EJ		1.00	−0.87	0.98	−0.31
CL			1.00	−0.94	0.66
EJ*				1.00	−0.42
TJL					1.00

RESULTS & DISCUSSION

THE CORRELATION matrix obtained between the various parameters viz. subjective juiciness (J), EJ, CL, EJ* and TJL is shown in Table 1. The EJ, CL and EJ* measurements were all highly correlated with juiciness. CL was highly (r = 0.94) but negatively correlated with EJ*. Correlations for TJL with all other parameters were generally low, albeit significant at the P < 0.01 level.

Measurement of either CL or EJ* appeared to offer objective methods capable for meat of normal pH (5.4–5.8) of explaining 84.2 and 81.5%, respectively, of the total variation in the juiciness determined by a taste panel. The regression equation fitted to the experimental points obtained for subjective juiciness (J) vs CL was J = 0.27 CL – 1.37 and for J vs EJ* was J = 11.4 – 0.35 EJ*. Bouton et al. (1973) showed that, over a wider pH range (5.4–7.2) expressible juice and juiciness were more closely related than CL and juiciness since both of the former changed little with change in pH whereas cooking losses decreased rapidly as pH increased.

Juiciness has been described by Weir (1960) and Cover et al. (1962) as being made up of two effects. One is the impression of moisture released during chewing while the other is related to salivation produced by flavor factors. The first effect appears likely to be related to the moisture which could be squeezed out of the cooked meat by centrifugation. Flavor changes in cooked meat could well increase salivation and hence apparent juiciness. Such flavor changes, and any relationship between them and juiciness, would be highly subjective and probably impossible to assess objectively.

Samples prepared by conventional methods such as grilling or roasting have a temperature distribution problem. Variation in internal temperature causes considerable variation in moisture loss and hence in expressible juice throughout the sample (Bouton and Harris, 1972). Such variation could explain, at least in part, why cooking-loss from grills, roasts and similar cooking methods reported in the literature do not usually show high correlations with juiciness (e.g., Harries et al., 1972).

Expressible juice (EJ or EJ*) measurements would seem to have greater applicability than cooking loss (CL) measurements in assessing juiciness in cooked meat but in many circumstances (provided that the pH of the raw meat was less than 5.8) cooking loss measurements could be more convenient to use. Correlations would be expected to decrease, however, if the cooking methods used produced large variability in cooking loss or EJ throughout individual samples.

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A Research Note
THE ARMOUR TENDEROMETER AS A PREDICTOR OF COOKED MEAT TENDERNESS

INTRODUCTION

PREVIOUS EVALUATIONS of the tenderometer as a predictor of bovine muscle tenderness have led to equivocal results. Huffman (1974) found the Armour Tenderometer (AT) superior to USDA quality grade or marbling score for placing cattle into homogeneous tenderness groups. Based on a highly significant correlation between AT and Warner-Bratzler shear (r = 0.48), Luckett et al. (1972) concluded that the AT was of value in measuring carcass tenderness; and Carpenter et al. (1972) concluded that AT values might be useful to stratify carcasses into different tenderness groups, however imprecisely. Hanson (1972) also reported high correlation coefficients between taste panel tenderness and AT for USDA Choice and Good carcasses. In other studies, however (Parrish et al., 1973; Dikeman et al., 1972; Luckett et al., 1972), much lower relationships were observed between AT and Warner-Bratzler (W-B) shear or taste panel tenderness. These results suggest that the AT values are of limited value as a predictor of cooked meat tenderness.

The AT values may be more related to subjective and objective carcass traits rather than to organoleptic traits. In this respect, Parrish et al. (1973) found a highly significant relationship (r = 0.98) between AT value and muscle firmness score. Attempts to categorize carcasses into tough and tender groups in that study were not possible because even these categories were not very different with respect to tenderness.

The purpose of the present experiment was to assess the predictive value of AT measurements for certain carcass and organoleptic traits for beef carcasses that vary greatly in body composition.

EXPERIMENTAL

DATA for this study were collected on 384 steer carcasses obtained from breeding Hereford and Angus cows to Hereford, Angus, Jersey, South Devon, Limousin, Simmental and Charolais bulls.

After a 24-hr chill, components of the USDA quality grade were assigned. The left sides of the carcasses were transported to Kansas State University where the longissimus muscle of each carcass was probed at the cut surface of the 12th rib with the AT. At the time of probing muscle temperatures were approximately 4°C. Steaks of uniform thickness were removed from the wholesale rib. The longissimus muscle from the 12th rib steak was chemically analyzed for intramuscular fat. The 10th and 11th rib steaks were cooked at 177°C in a preheated rotary oven to an internal temperature of 66°C and subsequently cooled at room temperature for about 30 min prior to core removal and taste panel evaluation. Six 1.27 cm cores were removed from each 11th rib steak and subjected to the W-B shear test. A six-member taste panel (TP) was served cores from the 10th rib steak for evaluation of tenderness, juiciness, flavor and overall acceptability on a 9-point hedonic scale. Correlation coefficients and standard partial regressions (Tables 1 and 2) were either calculated over all sire breed groups and computed and pooled within breed of dam and year subclasses or were pooled within sire breed group, breed of dam and year subclasses.

RESULTS & DISCUSSION

HETEROGENEITY TESTS between correlation coefficients involving AT values among breed of sire subclasses were not statistically significant, indicating that carcasses of different breeds represented were similar in associated variation for the traits measured. Dikeman et al. (1972) studying intact and sex

Table 1—Simple correlations between carcass measurements and tenderness indicators and among tenderness indicators[a]

Item	Skeletal maturity	Lean maturity	Final maturity	Color lean	Firmness lean	Texture lean	Marbling score	Longissimus fat, %	AT tenderometer	W-B shear	TP tenderness
Over all[b]											
AT tenderometer	0.17	0.09	0.15	0.12	−0.32	0.12	0.37	0.43	–	0.07	−0.05
W-B shear	−0.01	0.16	0.01	0.12	0.06	0.14	−0.21	−0.23	0.07	–	−0.63
TP tenderness	0.00	−0.09	−0.02	−0.11	−0.16	−0.10	0.32	0.31	−0.05	−0.63	–
Pooled[c]											
AT tenderometer	0.14	0.10	0.12	0.12	−0.28	0.15	0.28	0.35	–	0.15	−0.11
W-B shear	0.00	0.15	0.00	0.11	−0.02	0.11	−0.13	−0.16	0.15	–	−0.61
TP tenderness	−0.01	−0.09	−0.03	−0.09	−0.10	−0.05	0.26	0.26	−0.11	−0.61	–

[a] Correlations ≥ 0.10, P < 0.05; Correlations ≥ 0.13, P < 0.01
[b] Over all sire breed groups, pooled within breed of dam and year subclasses
[c] Pooled within sire breed group, breed of dam and year subclasses

Table 2–Standard partial regression coefficients for dependent variable taste panel tenderness

Subclass basis	R^2 [a]	Tenderometer	Marbling	Longissimus fat, %	Firmness	Age	Final maturity
		Independent traits and standard partial regression coefficients					
Over all[b]	0.00	–0.05	–	–	–	–	–
	0.10**	–	0.32	–	–	–	–
	0.14**	–0.20	0.39	–	–	–	–
	0.14**	–0.23	–	0.41	–	–	–
	0.00	–0.05	–	–	–	0.00	–
	0.00	–0.05	–	–	–	–	–0.01
	0.04**	–0.12	–	–	–0.20	–	–
	0.10**	–	0.31	–	–0.03	–	–
Pooled[c]	0.01	–0.11	–	–	–	–	–
	0.07**	–	0.26	–	–	–	–
	0.11**	–0.20	0.32	–	–	–	–
	0.11**	–0.23	–	0.34	–	–	–
	0.01	–0.11	–	–	–	–0.01	–
	0.01	–0.11	–	–	–	–	–0.02
	0.03**	–0.15	–	–	–0.15	–	–
	0.07	–	0.26	–	–0.01	–	–

[a] Asterisks indicate level of statistical significance, $P < 0.01$
[b] Standard partials were calculated ignoring sire breed group and computed and pooled within breed of dam and year subclasses
[c] Standard partials were computed and pooled within sire breed group, breed of dam and year subclasses

altered male Hereford and Angus cattle also found no differential response to AT probe.

Simple correlation coefficients for qualitative and organoleptic traits with the three measures of tenderness are shown in Table 1. Over all sire breeds, AT did not show a significant relationship with either TP tenderness or W-B shear (r = –0.05 and r = 0.07, respectively). These data confirm the results of Parrish et al. (1973), Hanson (1972) and Hendrickson et al. (1972) who also found a low relationship between W-B shear and AT values. Parrish et al. (1973) reported no significant relationship between TP tenderness and AT measurements whereas Dikeman et al. (1972) showed a significant relationship, but the magnitude of the correlation was low (r = –0.30). Only Hanson (1972) has indicated a high correlation coefficient value for AT reading and TP tenderness.

The original Armour and Company data (unpublished) indicated that the probe resistance increased with higher degrees of marbling such that USDA Choice should be allowed a greater AT resistance value than USDA Good beef. In the present study, expression of the data by pooling within sire breed rather than over all sire breeds reduced the correlation of AT reading with percentage of longissimus fat and increased its correlation with TP tenderness and W-B shear values. This occurrence is undoubtedly due, in part, to a reduction in amount of variation associated with marbling score and chemical fat. Over all sire breeds the amount of variation in TP tenderness accounted for by the AT value increased to significant ($P < 0.01$) levels when either marbling score or chemical fat were held constant (Table 2). Marbling score alone accounted for 10% of the variation in TP tenderness and the additional variation accounted for by the AT measurement in a multiple regression equation is of questionable practical importance even though, statistically, the usefulness of the AT is significantly increased when used at constant marbling score or in conjunction with marbling.

The correlation between AT value and muscle firmness score was highly significant, but considerably lower in magnitude than that reported by Parrish et al. (1973). Addition of firmness score to an equation already containing marbling score did not improve the predictive value of the multiple regression equation for TP tenderness.

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A Research Note

EFFECT OF SODIUM PYRUVATE ON THE TEXTURE OF FROZEN STORED COD FILLETS

INTRODUCTION

INVESTIGATIONS on the role of free amino groups in the denaturation of fish muscle protein during frozen storage have led to the discovery of the preventive effect of pyruvic acid (Tran, 1974). Addition of sodium pyruvate to fish muscle homogenates prior to frozen storage was shown to prevent protein insolubilization. The presence in vitro of sodium pyruvate in frozen fish homogenates reduced to various extents the protein insolubilizing effects of such chemicals as sodium linoleate, malonaldehyde and formaldehyde. The latter substances were known to cause aggregation or polymerization of fish muscle proteins (King et al., 1962; Buttkus, 1970; Castell et al., 1973). Moreover, it was shown that addition of sodium pyruvate to fish fillets or minced fish muscle greatly improved their hydration capacity upon subsequent frozen storage. Similar so-called antidenaturant properties were also exhibited by sodium α-ketoglutarate and sodium oxaloacetate. These properties could be accounted for by assuming that covalent bondings which involved the α-keto group of the α-keto acids and the free amino groups of fish muscle proteins have occurred.

Since protein solubility, aggregation or polymerization and hydration capacities in raw fish muscle could presumably be related to the texture of cooked fish (Dyer and Dingle, 1961), it seems reasonable to expect the α-keto acids to show a tenderizing effect upon frozen fish muscle. The present report deals specifically with the effect of sodium pyruvate upon frozen cod fillets.

MATERIALS & METHODS

SODIUM PYRUVATE (10%, pH 7.0) was prepared from pyruvic acid (Eastman) and sodium hydroxide. Cod fillets were excised in the laboratory from gutted fish of uniform size (4–5 lb) which had been kept on ice for 4 days. One of the two fillets from each fish was treated with sodium pyruvate and compared to the other untreated fillet (control) in subsequent texture evaluation. The pyruvate treatment was carried out as follows: the fillet was weighed and flooded with 10% sodium pyruvate solution in a shallow pan (7 ml of solution for every 100g of fish muscle) care being taken to moisten both sides of the fillet. After a 2-min standing period, during which time the fillet was overturned once, it was drained and weighed. Both pyruvate-treated and control fillets were frozen individually in plastic pouches at −23°C and stored at this temperature for up to 180 days.

Organoleptic evaluation was carried out at intervals on cooked fish. The frozen pyruvate-treated and control fillets were allowed to thaw at room temperature for 2 hr, cut into approximately 40-g pieces, and cooked over steam in aluminum foil for 15 min. Texture evaluation was effected by 8–10 panelists who were chosen among the laboratory staff, and who had shown some experience in organoleptic evaluation. The panelists were asked to base their rating on a 6-point texture scale (1, extremely tender; 2, very tender; 3, moderately tender; 4, moderately tough; 5, very tough; 6, extremely tough). Tests were carried out in the morning and were repeated once in the following afternoon. The scores which were obtained on a same day were treated as independent ones in statistical analysis (Steel and Torrie, 1960).

RESULTS & DISCUSSION

COD FILLETS were evaluated for texture after 8, 22, 54 and 180 days of storage at −23°C. Results of the analyses are given in Table 1. For untreated (control) fillets, the average texture scores increased steadily from 3.40 after 8 days to 4.37 after 180 days, indicating gradual toughening during the storage period. For pyruvate-treated fillets, the average texture scores decreased from 3.30 after 8 days to 2.67 after 22 days and then to 1.88 after 54 days. It did not change much during the remaining storage period (54–180 days). Analysis of variance showed that after 22 days of storage, pyruvate-treated cod fillets were organoleptically more tender than untreated fillets ($P < 0.01$). After 54 days of storage, the difference in organoleptic texture between treated and untreated fillets were significant at $P < 0.001$.

It might be emphasized that the sodium pyruvate treatment did not only prevent texture toughening but it also showed a tenderizing effect upon the cod fillets. From the weight in-

Table 1—Average scores, standard deviations and Student t-test for texture evaluation of control and sodium pyruvate-treated cod fillets during frozen storage at −23°C

Storage time (days)	Control fillets		Pyruvate-treated fillets		Test of difference			
	Average score	Standard deviation	Average score	Standard deviation	Degrees of freedom	$\bar{d}$	$s_{\bar{d}}$	t
8	3.40	0.88	3.30	0.65	38	0.10	0.244	0.41
22	3.50	0.71	2.67	0.83	30	0.83	0.273	3.04
54	3.88	0.70	1.88	0.60	34	2.00	0.217	9.21
180	4.37	0.71	1.93	0.68	30	2.44	0.246	9.91

crease after the pyruvate treatment (about 3%) the pyruvate/protein ratio in the treated fillets was calculated to be about 0.014. At this level, sodium pyruvate was shown to cause an increase in the hydration capacity of frozen stored fish muscle, but it could only incompletely prevent protein insolubilization in frozen fish muscle homogenates (Tran, 1974). The tenderizing effect of sodium pyruvate appeared therefore to be better correlated to the hydration capacity than to protein solubility in the raw fish muscle. Cowie and Little (1966) found that tenderness of fish fillets stored at −29°C was poorly correlated to protein solubility but it was better correlated to muscle pH, i.e., to hydration capacity. In pyruvate-treated, frozen stored, cod fillets, protein polymerization through disulfide bridging (Buttkus, 1970) or formaldehyde bridging (Frankel-Conrat and Olcott, 1948; Castell et al., 1973) might have taken place which affected protein solubility. But as far as organoleptic texture was concerned, the polymerization effect appeared to be overcome by the increase of protein-water affinity. On the other hand, the tenderizing effect of pyruvate upon frozen cod fillets could also have arisen from the breaking of intermolecular Schiff bases between protein molecules, especially in the collagen fractions, of fish muscle, and from their replacements by protein-keto acids Schiff bases. The existence of intermolecular Schiff bases between protein molecules in muscular tissues were reported by several workers (Mao and Sterling, 1970; Page and Benditt, 1969; Tanzer, 1968; Bailey, 1968).

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A Research Note
PRODUCTION OF CHEDDAR-LIKE CHEESES VIA NONFERMENTATIVE pH MANIPULATION

INTRODUCTION

WHILE LARGE-SCALE systems for the production of cheese have been designed and used for decades, only recently has the continuous production of cheese been commercially successful. This success, however, has not extended to Cheddar-type cheeses but only to Cottage cheese and others of close similarity.

Various equipment manufacturers have concentrated their efforts on the mechanization of traditional Cheddar processes and have been able to put various aspects of it on a continuous basis. Czulak and Sutherland (1972) reported on a semi-continuous system to produce Cheddar curds.

When the age-old methods of cheese production through fermentation and rennet addition are studied, however, the question arises whether mechanization of those processes is logical or if research to by-pass those processes might be more productive. In particular, the research summarized by this paper was conducted as the first stage of process development for the production of products with texture resembling traditional Cheddar and processed Cheddar cheeses without the use of microorganisms or enzyme systems.

Kosikowski (1972) has summarized the processes involved with several Italian cheeses. Ricotta cheeses are made by heating (about 88°C) and acidifying (usually with lactic, acetic or citric acid) whole or part skim-milk. The curds thus produced have occluded air and float to the top of the vat. Impastata and Queso Blanco cheeses are sinking curd cheeses produced in a similar fashion.

The process described in this paper extends the concepts used in the production of the above mentioned cheeses, sodium and calcium caseinates, and milk coprecipitates. Through pH manipulation, as described in the body of this paper, coprecipitates can be modified to achieve textures very like Cheddar and processed Cheddar. The process was designed so that extension to continuous production methods would be straight forward. The dairy industry has not utilized continuous presses but this aspect too can be made continuous through the use of a "serpentine" belt press (Coffelt and Giannini, 1973) or semi-continuous through the use of several batch-type presses operating in parallel.

The process produces extremely bland tasting products. Since cheese normally is expected to have a definite "cheese" flavor, provision has been made for the addition of flavoring materials. There are several possible sources of such materials. Harper and Kristoffersen (1970) and Singh and Kristoffersen (1972) have experimented with cheese flavor production via curd slurries. Sources of concentrated cheese flavor are also available through flavor houses and through manipulations of normal production methods which can produce extremely highly flavored cheeses suitable for blending with those of low flavor.

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Concept

A processing scheme for the continuous production of cheese is depicted in Figure 1. As shown, milk is received, standardized and homogenized in the usual manner (Anderson et al., 1970).

The fat level in the standardized milk is somewhat optional and can be varied to produce corresponding variations in the texture of the final product. The value of 2% fat is representative. Common experience with cheeses made in a normal manner from such milk would suggest that the resulting cheese would be tough and rubbery. In this case, however, the denatured whey proteins are incorporated into the product. In addition, the microstructure of the product is believed to be different from that found in a cheese produced via rennet action. These differences help to overcome the problem of toughness.

Following homogenization, the milk is heated to at least pasteurization temperatures, although a temperature of 88°C will give a better yield of whey proteins in the final product (Nielsen et al., 1973). Hydrochloric acid is added to the hot milk bringing it to the isoelectric point. The resulting curds are separated from the whey and pressed to lower the moisture content to the desired level. The whey can be sent to multiple effect evaporators for concentration prior to lactose crystallization. The lactose harvesting process is simplified because the protein content is reduced through coprecipitation of the heat denatured serum proteins. The opportunity exists to use elevated temperature crystallization methods to speed the process (Jelen and Coulter, 1973).

Following pressing, the cake is comminuted and sufficient NaOH is incorporated with thorough mixing to obtain a pH in

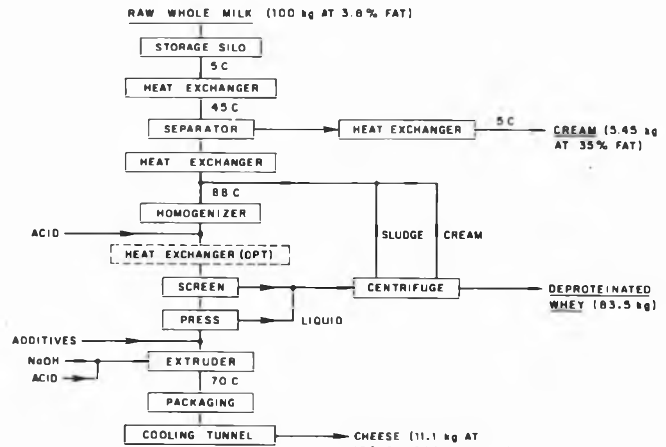

Fig. 1—Process flow chart

the range of 7–9. The temperature at this point is maintained around 70°C. Food colorings and other additives may be incorporated.

Following the NaOH addition, acid is incorporated with continued mixing to again lower the pH to about 5.3. Other additives such as flavoring ingredients may also be incorporated at this point. The hot mixture can be packaged in the same manner as processed Cheddar and cooled. The product may be marketed without aging. It is envisioned that the product would be kept refrigerated; the long term stability at room temperature has not been tested.

It should be noted that no rennet or starter cultures are used in the process.

EXPERIMENTAL

TO INVESTIGATE the feasibility of the process outlined above without overly large expenditures for equipment and raw materials, the following steps were performed.

30-gal batches of homogenized 2% fat milk were heated in a Cherry-Burrell Univat. Upon reaching 88°C, cooling was initiated and 1N HCl added (simultaneously) until a pH of 4.4 was maintained. The curds were collected (the temperature at this point was about 20°C) and pressed overnight at a pressure of 3.4 bar in cheese hoops. Experience with the expression process emphasized the importance of curd pH. If sufficient time (about an hour) is not allowed for the curd to equilibrate with the whey, the curds cannot easily be pressed to the desired dryness.

The press cake was comminuted in a meat grinder and 2 kg portions placed in a Ross mixer. The jacket temperature was maintained at 70°C. Sufficient 10N NaOH was added to raise the pH to the range of 7–9. Following this, 80% lactic acid was used to lower the pH to 5.2. Vacuum was maintained on the mixer with an aspirator to minimize air entrainment into the product. The mass was poured into processed cheese cartons and refrigerated.

RESULTS & DISCUSSION

A MECHANICAL SPECTROMETER (Anon.; Macosko and Starita, 1971) was used during development of the process described above. The device uses eccentric rotating disks to apply dynamic shear strain to thin disks of the test material. The textural properties of the experimental product, as tested by the machine, lie between those of medium Cheddar (Land O'Lakes) and processed Cheddar (Kraft). This is not only true of the storage and loss moduli but also of the breakdown behavior under increasingly larger strain.

Examination of the experimental product by a trained Cheddar cheese judge produced a similar verbal description that the sensory textural properties of the experimental product can be described as midway between those of medium Cheddar and processed Cheddar. It is recognized that evaluation by a single trained judge is not equivalent to a thorough examination through sensory panel methods. Mention is made here simply to point out that the trained judge did not discern gross defects (not measured by the mechanical spectrometer) that would make the product unacceptable.

A preliminary cost estimate for the process based on a new plant, a milk intake of one million pounds per day, and a 20% return on investment showed the product could be produced for 20¢/lb less than the existing wholesale price for Cheddar. In addition, the product is lower in fat (17% vs. 32%) and higher in protein (30% vs. 25%) than Cheddar.

The process and formulation as described are not final. There are many textural modifiers that might be used to further enhance the product. The use of vegetable proteins to further lower the cost while maintaining protein content is an obvious future possibility. Flavors can be added to give product identity and appeal over the bland base obtained by the simple formulation as described.

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A Research Note

IDENTIFICATION AND DETERMINATION OF SUGARS IN SOURSOP, ROSE APPLE, MOUNTAIN APPLE AND SURINAM CHERRY

INTRODUCTION

SUGARS can be directly and conveniently separated, identified, and quantified by gas liquid chromatography (GLC) (Kline et al., 1970; Sweeley et al., 1963; Mason and Slover, 1971; Heatherbell, 1974). Using these methods, the distribution patterns of sugars in various tropical fruit or fruit products have been reported for banana and pineapple by Kline et al. (1970), mountain pawpaw by Heatherbell (1974), papaya, lychee, mango, guava and passion fruit by Chan et al. (1975a, b). Reported herein are the sugar compositions of four tropical fruits: soursop, rose apple, mountain apple and Surinam cherry.

MATERIALS & METHODS

Fruit samples and sugar extraction

Soursop (*Anona muricata*), rose apple (*Eugenia jambos*), mountain apple (*E. malaccensis*), and Surinam cherry (*E. michelli*) were harvested from the Lyon's Arboretum, University of Hawaii Agricultural Experiment Station farm. 100g of the fruits' edible portion were blended with 300 ml of 80% ethanol for 5 min in a Waring Blendor, transferred quantitatively to a 500-ml flask and diluted to volume with 80% ethanol.

Extraction and silylation of sugars

The ethanolic mixtures were further treated for the extraction and isolation, and silylation of sugars by the method of Kline et al. (1970). After standing for 1 hr or more, a portion of the ethanolic extract was filtered. A 10-ml aliquot of the filtrate was transferred to a centrifuged tube to which was added 0.5 ml of saturated lead acetate solution. The tube was capped, shaken thoroughly, and allowed to stand 15 min, then centrifuged at 2000 × G for 10 min. An aliquot, 1.05 ml, of clear supernatant was pipetted into a 7-ml screw-cap vial (Pierce Chemical Co.) containing ca. 0.1g of Celite (Johns Manville Co.). The sample was evaporated to dryness in a vacuum oven at 30°C. To each vial a weighed amount of myo-inositol (Calbiochem Co.), 4–6 mg, was added as an internal standard along with ca. 0.1g of anhydrous $CaSO_4$. The sugars were silylated to their trimethylsilylated (TMS) derivatives with 2 ml of TriSil (Pierce Chemical Co.), sealed with a teflon-lined screw-cap (Pierce Chemical Co.), shaken, and let stand at 45°C for 1/2 hr. After the solids had settled, ca. 0.5–1.0 μl of the clear solution was injected into the gas chromatograph.

Gas chromatography

A Varian Aerograph Model 200 gas chromatograph with a flame ionization detector was used. The carrier gas (nitrogen) flow rate was 25 cm^3 per min, and the hydrogen flow rate was 44 cm^3 per min. The 7 ft × 0.093 in. i.d. stainless steel column was packed with 3% OV-17 on Chromosorb G A/W HMDS 60/80. The column was operated isothermally at 170°C for fructose and glucose assays and 250°C for sucrose assays. The detector and injector temperatures were 270°C and 260°C, respectively.

A standard regression curve was prepared for each of the sugars. Peak area ratios of sugar/internal standard were plotted against their corresponding weight ratios.

In quantifying sugar values in six or more replicate fruit samples, the peak area ratios of sugar/internal standard were determined from the chromatogram and converted (by use of the regression curve) to the corresponding weight ratios. These values were then multiplied by the weight of the internal standard in the sample (McNair and Bonelli, 1966).

Thin-layer chromatography (TLC)

The ethanolic sugar extracts (2 μl) were applied directly to precoated cellulose sheets (Polygram Cellulose MN 300, Brinkmann Instruments, Inc.). The chromatograms were developed in ascending direction with FMBW (formic acid-methyl ethyl ketone-tert-butanol-water) 15:30:40:15 (v/v) (Vomhof and Tucker, 1965), dried and developed again in the same direction with the same solvent. The spots were visualized with diphenylamine-p-anisidine spray reagent (Bailey, 1962). Rg values as well as the characteristic colors (fructose, yellow; glucose, bluish-gray; sucrose, grayish-yellow) were used to identify the sugars.

RESULTS & DISCUSSION

WHEN THE TMS SUGARS of soursop, rose apple, mountain apple and Surinam cherry were chromatographed on 3% OV-17 at 170°C, the relative retentions of their peaks matched those of fructose, α-glucose and β-glucose (0.36, 0.56 and 0.77, respectively). At 250°C, all samples except mountain apple yielded a peak which had a relative retention of 4.25 and corresponded to TMS sucrose.

The quantitative data obtained by GLC are shown in Table 1. Glucose is expressed as the sum of the α- and β-anomers. In soursop, sucrose was the predominant sugar, 61.5%, followed by glucose, 21.5%, and fructose, 17.0%. In rose apple, glucose was the major sugar, 44.3%, followed by fructose, 29.0%, and sucrose, 26.7%. In mountain apple, glucose, which constituted 58.0% of the sugars, was present in only slightly greater quantities than fructose, 42.0%; sucrose was not detected. In Surinam cherry, the distribution was nearly even: fructose, 28.0%, glucose, 35.9%, and sucrose, 36.1%.

The identities of the sugars were further confirmed by TLC. Chromatographs of sugar extracts from soursop, rose apple and Surinam cherry showed the presence of three sugars whose

Table 1—Quantitative determination of sugars in tropical fruits by GLC

Sugar content (%)	Soursop	Rose apple	Mountain apple	Surinam cherry
Fructose	1.80 ± 0.10	1.96 ± 0.26	1.49 ± 0.12	1.07 ± 0.03
D-Glucose	2.27 ± 0.08	3.00 ± 0.10	2.06 ± 0.16	1.37 ± 0.02
Sucrose	6.57 ± 0.74	1.81 ± 0.57	–	1.38 ± 0.26
Total	10.58	6.77	3.55	3.82

Rg values and characteristic color development of diphenylamine-p-anisidine corresponded to those for fructose, glucose, and sucrose (1.22, 1.00 and 0.74, respectively). For mountain apple only two sugars were present, fructose and glucose.

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A Research Note

DETERMINATION OF TOTAL ASCORBIC ACID IN VEGETABLES FROM ALCOHOL SLURRIES

INTRODUCTION

EXTRACTANTS for ascorbic acid determination are almost as numerous as the methods for the assay of the vitamin. Meta-phosphoric acid (Fujita and Iwatake, 1935) oxalic acid (Ponting, 1943) meta-phosphoric acid and EDTA (Jager, 1948) trichloroacetic acid and EDTA (Freebairn, 1959) and acetic acid (Gawron and Berg, 1944) are among those that are used. The suitability of the extractant is determined by the material to be analyzed, the method of analysis to be used, and the conditions to be met. In our laboratory, it was desirable to prepare a single slurry of frozen vegetable tissues from which analyses of solids, nitrogen, fiber, ash, metallic ions and five vitamins could be performed. The object was to determine these constituents in various plant materials at time of harvest in a study on the effect of pollutants on plants. This report presents the results of the analyses of total ascorbic acid from ethanol slurry of 10 frozen vegetables, and compares them with those obtained from a standard meta-phosphoric acid slurry.

MATERIALS & METHODS

CORN, GREEN BEANS, spinach and broccoli were purchased from local markets as frozen 9, 10 or 12 oz. cut or chopped packaged vegetables. Fresh celery, eggplant and bell peppers from local markets were frozen in liquid nitrogen at the laboratory. Fresh carrots, lettuce and cabbage were frozen in liquid nitrogen at the time of harvest. The liquid nitrogen frozen samples were crushed or coarse blended before analysis.

A sample of 450–600g of a frozen vegetable was mixed in a polyethylene bag, 8 × 4 × 18 Py 9H (Zellerbach Co.), while the bag was in contact with dry ice. Two portions, of approximately 200g each, were slurried, one with ethanol and the other with meta-phosphoric acid.

Extraction using an alcohol slurry

Equal weights, measured to the nearest 0.1g, of frozen sample and absolute ethanol were blended in a rheostat-controlled blender, initially at low speed until uniform pieces were obtained, then at full speed for 1 min. The blender was returned to low speed to keep the slurry mixed; an approximately 20-g aliquot was drawn into a 25 ml pipet (tip removed), and weighed to the nearest 0.1g in a tared volumetric flask.

A volume of 10% meta-phosphoric acid, equal to the weight of slurry, was added to adjust the concentration of the meta-phosphoric acid in the slurry to 5%, and the mixture was brought to volume with 5% meta-phosphoric acid.

Meta phosphoric acid extract

In the same manner, equal weights of 10% meta-phosphoric acid, 5% meta-phosphoric acid and frozen sample were blended to prepare the 5% meta-phosphoric acid slurry. An approximately 30-g sample was pipetted into a tared 100 ml volumetric flask, weighed and diluted to volume with 5% meta-phosphoric acid.

Table 1—Comparison of absorbance values of pure ascorbic acid 2,4-dinitrophenylhydrazine derivatives formed from ethanol-water-5% meta-phosphoric acid and from aqueous 5% meta-phosphoric acid

	I Ethanol-water-5% HPO_3		II Aqueous 5% HPO_3		
Ascorbic acid (μg)	Mean absorbance[a]	Std error of mean	Mean absorbance[a]	Std error of mean	Student's t-test I vs II T[b]
20	0.175	0.003	0.174	0.003	0.423
40	0.336	0.005	0.337	0.005	0.409
80	0.644	0.010	0.641	0.010	0.535

[a] Six replications carried out over a period of a year
[b] T at a probability of 0.05 = 2.57.

Table 2—Comparison of 50% ethanol slurry and 5% meta-phosphoric acid slurry for total ascorbic acid content in vegetables

	mg Ascorbic acid per 100g vegetable		
Vegetable	Lot no.	50% Ethanol	5% Meta-phosphoric acid
Celery	1	4.7	4.9
Celery	2	5.4	5.8
Celery	3	5.8	5.7
Eggplant	1	8.2	8.0
Eggplant	2	6.8	6.9
Eggplant	3	6.9	6.5
Corn	1	8.0	8.4
Corn	2	9.0	8.5
Corn	3	7.9	8.4
Carrots	1	10.0	9.6
Carrots	2	11.3	11.7
Carrots	3	12.9	12.8
Lettuce	1	13.7	14.0
Lettuce	2	13.8	14.5
Lettuce	3	13.5	14.5
Green beans	1	15.7	14.6
Green beans	2	14.1	14.1
Green beans	3	13.4	14.4
Spinach	1	33	31
Spinach	2	31	32
Spinach	3	33	30
Cabbage	1	50	50
Cabbage	2	52	52
Cabbage	3	48	48
Bell peppers	1	96	100
Bell peppers	2	111	110
Bell peppers	3	95	100
Broccoli	1	79	80
Broccoli	1	81	78
Broccoli	1	78	77

Assay procedure

Total ascorbic acid was determined according to Freed, 1966. Curves were prepared from oxidized standard containing comparable ethanol concentration, as contained in the samples, along with the meta-phosphoric acid standard curve. Samples were read in 19 mm Bausch and Lomb colorimetric tubes at 540 nm in a Coleman Jr IIA spectrophotometer. The formation of the osazone was carried out at 38°C in a Fisher Isotemp Oven, Junior Model.

RESULTS & DISCUSSION

ABSORBANCE VALUES obtained from the oxidized ascorbic acid standard containing ethanol, agreed within 1% with the absorbance values of the ascorbic acid standard, prepared as described (Freed, 1966) in 14 of the 18 paired determinations. The remaining values reflected variations up to 5%. Average absorbance values of the two ascorbic acid standards at three levels did not differ significantly (Table 1). Total ascorbic acid determinations were made on alcohol slurries and meta-phosphoric acid slurries from three samples of the same lot of broccoli, and from three separate lots of each of the nine other vegetables (Table 2). Of the 30 paired determinations, which ranged from 4.7–111 mg, 80% of the results showed a difference of 1 mg, or less, of ascorbic acid per 100g vegetables, reflecting a variation no greater than 7%. The remainder, with differences greater than 1 mg, exhibited variations of 4–10%. The results with the ethanol slurries were less in 47%, equal in 13%, and greater in 40% than those with the meta-phosphoric acid slurries. The comparative results with the 50% ethanol slurry and the 5% meta-phosphoric acid slurry fall within the 10% variation permitted by the precision of the described method. Hence, ethanol is suitable as a slurrying medium from which total ascorbic acid may be determined in frozen vegetables.

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A Research Note
A SIMPLE METHOD FOR EMULSION END-POINT DETERMINATIONS

INTRODUCTION

THE RELATIVE ABILITY of a protein to emulsify fat is considered to be a measure of the value of that protein for use in emulsified food products. To predict the emulsification capacity of a specific protein or protein additive, an accurate method must be available for detecting the inversion point in a model emulsion system.

Several researchers (Swift et al., 1961; Pearson et al., 1965; Hegarty et al., 1963; Inklaar and Fortuin, 1969) have utilized the sudden drop in viscosity which occurs at emulsion collapse as an indication of the inversion point or emulsion end-point. This method requires an experienced operator and is not applicable to emulsions of low viscosity (Swift et al., 1961). Smith et al. (1973) and Crenwelge et al. (1974) employed a variable autotransformer and a microammeter to measure the change in amperage required to drive the blender motor used for forming the emulsion. In the latter studies, amperage was visually monitored and a sudden decrease in amperage requirement was used as an indicator of the emulsion end-point. This method for identifying the end-point is dependent on the viscosity changes that occur at the point of emulsion collapse and is of little use with either higher or low viscosity systems (Smith et al., 1973; Crenwelge et al., 1974).

The continuous (water/protein) phase of a meat emulsion has a high conductivity when compared to that of the discontinuous (lipid) phase (Webb et al., 1970). Becher (1965) demonstrated that the conductivity of an emulsion is proportional to the conductivity of the continuous phase. Webb et al. (1970) monitored the resistance between two electrodes, separated by a fixed distance, in a forming emulsion and was able to detect the inversion point of low viscosity emulsions. However, emulsions of low viscosity exhibit electrophoretic properties which distort direct electrical current resistance determinations of the emulsion end-point (Haq et al., 1973). Also, emulsions of high viscosity tend to coat the electrodes and increase resistance (Haq et al., 1973). The use of alternating current, in a system similar to that used by Haq et al. (1973), eliminates some of the problems associated with the use of direct current evaluations of emulsion end-points.

The technique evaluated in the present study utilizes the increased visibility of colored oil droplets to facilitate visual identification of the point of emulsion collapse. This procedure was designed to provide an alternative method for determining emulsion end-points and to increase the precision of such measurements.

EXPERIMENTAL

PROTEIN SLURRIES were prepared from the longissimus muscle of young bullock carcasses, soy protein isolate (92% protein), soy protein concentrate (60% protein), soy flour (50% protein) and blood serum protein (80% protein) by the method of Swift et al. (1961). By using varying quantities of longissimus muscle slurry and holding the total volume of the emulsion before oil addition constant (50 ml), emulsions of three viscosities were produced (very low, 2g of slurry; medium, 10g of slurry; and high, 18g of slurry). The soy products and blood serum protein were rehydrated by combining 50g of the dry product with 100 ml of cold (4°C) distilled water prior to slurry production. The protein additives were allowed to rehydrate for 20 min at 4°C, after which slurries were formed with 50g of rehydrated soy and blood protein and 200 ml of 1M NaCl. Emulsions of soy product were prepared by combining 12.5g of slurry with 37.5 ml of cold (4°C) 1M NaCl solution. Blood protein solutions were prepared by combining 6.25g of slurry with 43.75 ml of cold (4°C) 1M NaCl solution.

Wesson vegetable oil was used for determinations of emulsification capacity. Colored vegetable oil was prepared by adding 0.3g of the biological stain, Oil-Red-O, to 1 liter of oil and mixing for 60 min by use of a magnetic stirrer operating at low speed. The colored oil did not require further mixing prior to use since Oil-Red-O is highly soluble in most lipids and will not sediment. Both colored oil and noncolored oil were chilled to 4°C and maintained at that temperature during end-point determinations.

Emulsions were formed in a blender jar that had blades modified to accommodate small volumes of liquid. Muscle, blood and soy protein slurries were emulsified with colored and noncolored oil. The blender was operated at full speed and the oil was delivered to the system at a constant rate of 0.5 ml/sec through a 6 mm hole bored in the lid of the blender jar. Delivery of oil was accomplished by means of Tygon tubing and a variable-speed Sage Tubing Pump (Model 375-A) operating at 77.3% of maximum rate. The duration of oil flow required to reach the point of emulsion collapse was determined by six experienced observers who repeated each run five times. The time in seconds required to reach the end-point was converted to ml of oil for both colored and noncolored oil. Mean values were subjected to analysis of variance and standard deviations were tested by use of variance-ratio F-test (Steel and Torrie, 1960).

RESULTS & DISCUSSION

PRELIMINARY ATTEMPTS to determine emulsifying capacity by use of a gravity-flow system for oil addition necessitated the incorporation of the Sage Tubing Pump into the model system. Use of the pump removed the inaccuracies of rate and volume of flow inherent to the gravity-flow system and simplified oil delivery. The Sage Tubing Pump was used for all of the emulsion end-point determinations in the present study.

Comparisons of end-point determinations made with colored and noncolored oil are presented in Tables 1 and 2. The use of colored oil was associated with significant ($P < 0.05$) reductions in the volume of oil required to produce emulsion collapse for all three muscle protein emulsions and emulsions of soy protein isolate, soy flour and blood serum protein. This difference was undoubtedly the result of the increased visibility of the coalesced droplets of colored oil at the point of emulsion collapse.

Since it is important that a system for emulsification capacity determinations perform equally well with high and low viscosity emulsions, data were collected for emulsions of three viscosities. The differences between mean volumes of colored and noncolored oil increased as the emulsion viscosity increased (Table 1), thus the accuracy of end-point determi-

nations may be enhanced by the use of colored oil as viscosity increases, due to greater visibility of the coalescing oil droplets in more concentrated solutions. In addition to a need for satisfactory performance over a range of viscosities using a single protein, it would be advantageous if such a technique would also enhance the accuracy of end-point determinations for nonmeat protein additives. The data in Table 2 indicate that the use of colored oil reduced the quantity of oil required to reach a visual emulsion end-point for three of four protein additives. End-point determinations for emulsions formed with soy protein concentrate (Table 2) were not significantly ($P < 0.05$) different when the colored oil technique was employed. This lack of improvement in end-point precision may have resulted from the darker appearance of such emulsions. A dark background would provide an improved view of emulsion collapse because of increased visibility.

The standard deviations at each emulsion viscosity for emulsions of longissimus muscle tissue were significantly ($P < 0.01$) reduced (Table 1) when the colored oil technique was employed. In addition, this technique either significantly lowered or did not alter standard deviations for end-point determinations of four nonmeat protein products ($P < 0.05$) as shown in Table 2. The use of colored oil makes the inversion point of the forming emulsion more definitive and therefore, reduces a part of the error inherent to visual emulsion end-point determinations. The increased repeatability afforded by the use of the colored oil technique does not seem to be affected by factors such as increased viscosity which distorts other systems designed to evaluate the same property.

The colored oil technique described here has been utilized in this laboratory to aid in determination of emulsifying capacity for soy protein concentrates, isolates and flours and for blood serum protein. In all instances, the technique has either enhanced or has not decreased the precision of emulsion end-point determinations.

Table 1—Means and standard deviations for volumes of colored and noncolored oil required to reach emulsion end-points for muscle protein emulsions of three viscosities

	Milliliters of oil at emulsion end-point			
	Mean[b]		Standard deviation[b]	
Emulsion viscosity	Colored oil	Noncolored oil	Colored oil	Noncolored oil
Very low (2g of slurry)[a]	35.3[c]	36.7[d]	0.8[e]	1.9[f]
Medium (10g of slurry)[a]	54.9[c]	57.4[d]	0.9[e]	1.5[f]
High (18g of slurry)[a]	76.2[c]	80.9[d]	0.5[e]	1.6[f]

[a] Slurries were brought to a volume of 50 ml with 1M NaCl before oil addition.
[b] Each mean value and each standard deviation is based on 30 observations (5 observations by each of 6 observers).
[c,d] Means on the same line bearing different superscripts are significantly different ($P < 0.01$).
[e,f] Standard deviations on the same line bearing different superscripts are significantly different ($P < 0.01$).

Table 2—Means and standard deviations for volumes of colored and noncolored oil required to reach emulsion end-points for emulsions of four protein additives

	Milliliters of oil at emulsion end-point			
	Mean[a]		Standard deviation[a]	
Protein additive	Colored oil	Noncolored oil	Colored oil	Noncolored oil
Soy protein isolate	38.0[b]	40.4[c]	0.8[d]	1.8[e]
Soy protein concentrate	28.1[b]	27.9[b]	1.5[d]	1.2[d]
Soy flour	36.4[b]	46.7[c]	0.5[d]	2.0[e]
Blood serum protein	98.7[b]	100.0[c]	0.5[d]	0.7[e]

[a] Each mean value and each standard deviation is based on 30 observations (5 observations by each of 6 observers).
[b,c] Means on the same line bearing different superscripts are significantly different ($P < 0.05$).
[d,e] Standard deviations on the same line bearing different superscripts are significantly different ($P < 0.05$).

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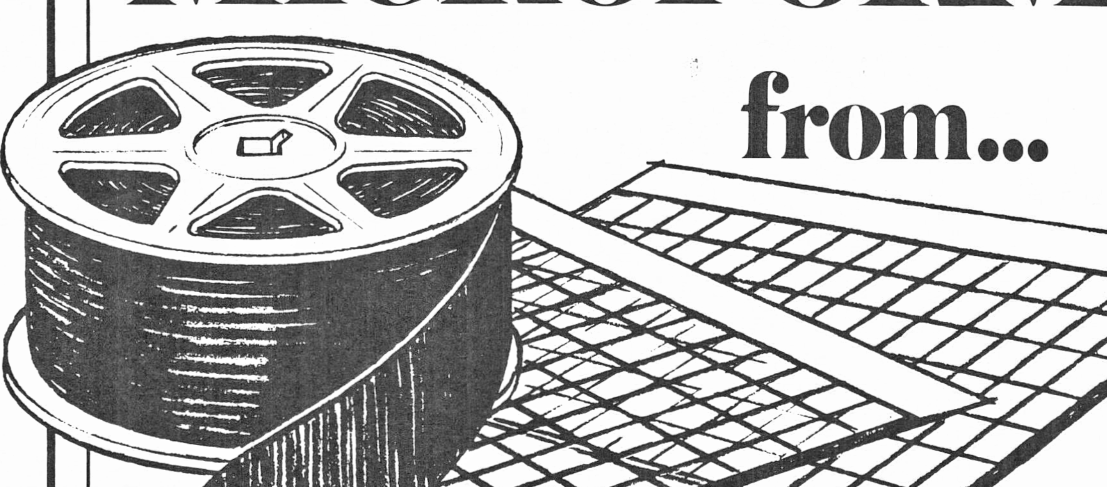

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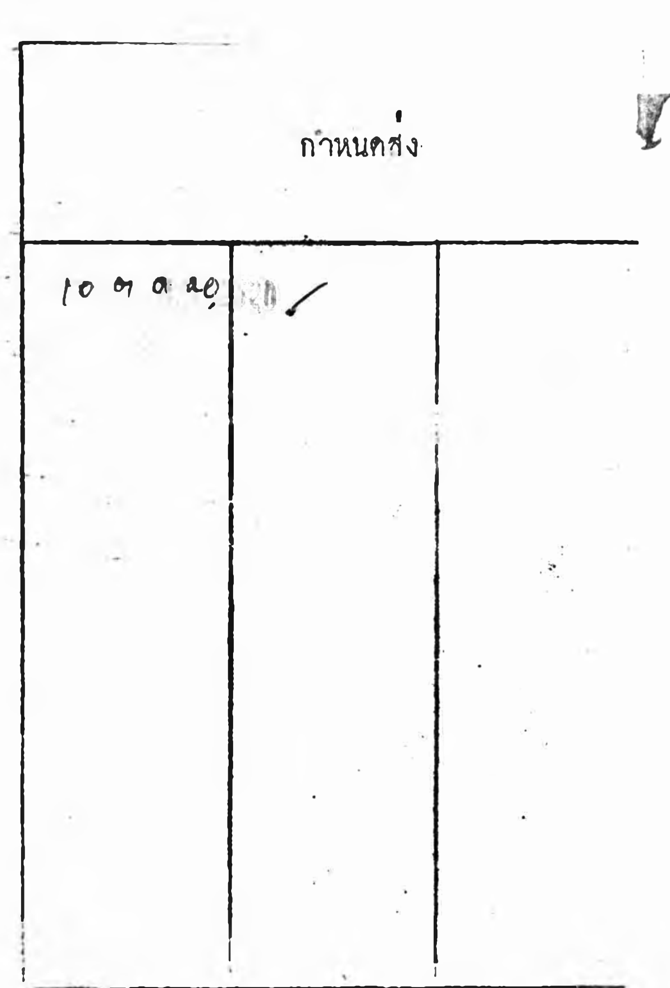

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