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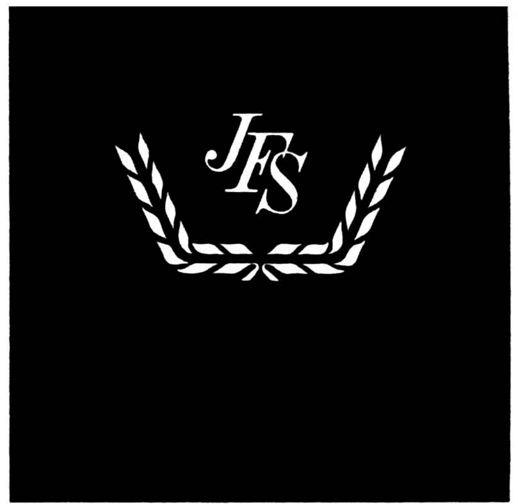

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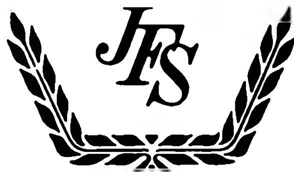

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ABSTRACTS:
IN THIS ISSUE

CONDITIONS NECESSARY FOR STERILITY TESTING OF HEAT PROCESSED CANNED FOODS. G.M. EVANCHO, D.H. ASHTON & E.J. BRISKEY. *J. Food Sci.* 38, 185–188 (1973)–The conditions necessary to prevent the occurrence of positive subcultures due to contamination during sterility testing of heat processed canned foods were investigated. The sterility of 4,740 cans of pasta-containing products was tested by subculturing particulate matter and serum. The investigators and the working area were found to be important sources of contamination. Only heat sensitive organisms were subculture contaminants when the sterility testing was conducted in an isolated, positive pressure room with filtered, irradiated air. Contamination was virtually eliminated when the investigators also wore face masks. These results demonstrate that contamination occurs to a greater extent than expected unless extra precautions are taken.

LEVELS OF DDT ISOMERS IN TURNIP GREENS AFTER BLANCHING AND THERMAL PROCESSING. J.G. FAIR, J.L. COLLINS, M.R. JOHNSTON & D.L. COFFEY. *J. Food Sci.* 38, 189–191 (1973)–Turnip greens were sprayed with DDT, harvested, washed and blanched by steam or by water in kettles with and without enamel linings. The greens were processed at 120°C for 45 min in plain tinplate and enamel-lined cans. Samples of greens were taken according to the following schedule: prior to the first application of DDT, after application of DDT but prior to washing, and immediately following each process treatment. Samples were extracted and cleaned up by the Mills, Onley and Gaither procedure and the residues were analyzed by gas-liquid chromatography. Blanching method and length of time did not produce significantly different residue levels. Enamel lining in the kettles reduced DDE and o,p'-DDT contents but the type of cans had no effect. Washing and blanching reduced the level of all isomers of fresh greens. Thermal processing caused a further reduction with the exception of DDD which was increased.

IN-PLANT, CONTINUOUS HOT-GAS BLANCHING OF SPINACH. J.W. RALLS, H.J. MAAGDENBERG, N.L. YACOUB, D. HOMNICK, M. ZINNECKER & W.A. MERCER. *J. Food Sci.* 38, 192–194 (1973)–A new blanching system for vegetables (hot-gas blanching) which showed promise in pilot plant studies was tested in a commercial spinach cannery. The hot-gas blancher was operated at 93–121°C with a spinach residence time of 108 sec. Four tons of washed spinach was hot-gas blanched and returned to the commercial filling line; the volume of wastewater produced was 1% of that from the commercial blancher (per ton blanched). A reduction of 96% in COD was achieved by substituting hot-gas blanching for hot-water blanching. Differences were found in the flavor, headspace gas composition and ascorbic acid content of hot-gas blanched spinach compared to commercial canned samples.

HEAT PROCESSING EFFECTS ON PHYSICAL AND CHEMICAL CHARACTERISTICS OF ACIDIFIED CANNED TOMATOES. D.R. SCHOENEMANN & A. LOPEZ. *J. Food Sci.* 38 195–201 (1973)–Pilot plant and commercial canned tomato packs of several different varieties were treated with several acid and salt additives, heat processed at 99°C, and stored at 24°C for 6 months. Determinations were made of pH, total and alcohol soluble acidities, amino nitrogen, reducing sugars, nonvolatile acids, refractive index, can vacuum and drained weight. Overall pH differences were nonsignificant and the processing time-additive interaction on pH was small. It is indicated that mitochondrial activity ceased within 15 min of heat processing at 99°C. Overall results show that citric, malic, fumaric and phosphoric acids effectively maintain a desirable reduced tomato pH without accelerating physical or chemical changes during heat processing. Pyrrolidone carboxylic acid concentration increased significantly when processing time was increased from 15 to 45 min.

USE OF AMMONIUM COMPOUNDS FOR CHLOROPHYLL RETENTION IN FROZEN GREEN VEGETABLES. M.S. EHEART & D. ODLAND. *J. Food Sci.* 38, 202–205 (1973)–NH_4OH was used in the microwave blanching of frozen broccoli and NH_4HCO_3 in the conventional blanching and/or cooking of frozen green beans. Excessive chlorophyll losses, previously reported during microwave blanching of broccoli in baggies, were prevented by the use of NH_4OH. Addition of NH_4HCO_3 to both blanching and cooking water doubled the retention of chlorophylls in frozen-stored cooked green beans. Losses of reduced ascorbic acid were not increased by either ammonium compound. A trained taste panel rated all NH_4HCO_3-treated green bean samples higher than controls for color, texture, flavor and acceptability.

SURVEY OF AEROBIC MESOPHILIC BACTERIA IN DEHYDRATED ONION PRODUCTS. J.M. SHENEMAN. *J. Food Sci.* 38, 206–209 (1973)–Normal aerobic bacterial microflora of well-processed dehydrated onion products consists almost entirely of spore-forming bacteria of the genus *Bacillus*. These have little health hazard significance but are important only in that they are responsible for high total plate counts in the finished product. Specially prepared raw materials allow reductions in counts of some finished products, and milling and sizing operations segregate high count material in finer piece size fractions. Onions processed on perforated metal-belt equipment have very low populations of nonspore-forming bacteria, due to the pasteurization effect of the process and are of little importance in onion products. No *Salmonella* or *Escherichia coli* were found in onion products during a survey for these types of bacteria.

EFFECT OF BRINING ON OBJECTIVE TEXTURE PROFILES OF CUCUMBER VARIETIES. W.M. BREENE, D.W. DAVIS & H-E. CHOU. *J. Food Sci.* 38, 210–214 (1973)–Textural comparison of raw and brined fruit of 24 cucumber varieties via Instron Texture Profile Analysis (TPA) showed the following correlations: brittleness, $r = 0.27$; hardness, $r = 0.86$; elasticity, $r = 0.76$; "first bite" work of compression, $r = 0.61$; "second bite" work, $r = 0.87$; cohesiveness, $r = 0.52$; gumminess, $r = 0.80$; and chewiness, $r = 0.81$. Brining reduced mean values for all parameters in all varieties but not equally for either varieties or parameters. Brittleness disappeared to a considerable degree. Retention of texture was highest in the variety "Model." Results indicate that textural quality of brined cucumbers of most varieties can be predicted by TPA of the raw fruit.

EFFECT OF DIFFERENT TREATMENTS ON PHYTATE AND SOLUBLE SUGARS IN CALIFORNIA SMALL WHITE BEANS (Phaseolus vulgaris). S. KON, A.C. OLSON, D.P. FREDERICK, S.B. EGGLING & J.R. WAGNER. *J. Food Sci.* 38, 215–217 (1973)–Incubating raw beans whole or ground at pH 5.2 at 55°C for 20 hr results in the formation of large amounts of free galactose, inositol and inorganic phosphate. These data are interpreted in terms of a reduction in the amount of raffinose family of oligosaccharides and of phytate in the beans. Reducing the amount of these substances normally found in beans may be important in improving the nutritional value and digestibility of beans and in reducing discomfort from flatus formation.

CONDITIONING AND DISPOSAL OF SOLIDS FROM POTATO WASTEWATER TREATMENT. G.A. RICHTER, K.L. SIRRINE & C.I. TOLLEFSON. *J. Food Sci.* 38, 218–224 (1973)–Aerobic biological secondary treatment of primary clarified potato processing wastewater at the R.T. French Co. in Shelley, Idaho yields biological solids in excess of that needed to operate the treatment system. In the past, these diluted biological solids were discharged into the Snake River or dewatered along

with silt water (at a high chemical cost) and used as landfill. A study at the R.T. French Co. was conducted by CH2M/HILL during the 1970–71 potato processing season to investigate alternative methods of waste biological solids conditioning and disposal. A pilot scale spray field and pilot scale equipment, including a basket centrifuge and aerobic digester, were used during the study. Conclusions from the study are: (1) Operation of a waste biological solids spray irrigation system at reasonable application rates should not produce objectionable odors; successful operation will require prescreening and special sprinkler heads for freezing weather application. (2) Basket centrifuges are effective for concentrating waste biological solids from aerobic biological treatment systems with sludge volume indexes up to and over 250. (3) 15 days aerobic digestion substantially improves the dewaterability of the waste biological solids at 16°C.

BIOCHEMICAL CHANGES IN CITRUS FRUITS DURING CONTROLLED-ATMOSPHERE STORAGE. P.L. DAVIS, B. ROE & J.H. BRUEMMER. *J. Food Sci.* 38, 225–229 (1973)–Marsh grapefruit and Pineapple and Valencia oranges were stored in atmospheres of controlled O_2 and CO_2 concentrations. Rind pitting of grapefruit was reduced by the presence of CO_2, but prolonged exposure resulted in other rind damage. Citrate and malate decreased in all stored fruit. Malate showed least change in Pineapple oranges and Marsh grapefruit stored in atmospheres containing 10% CO_2. Ethanol concentration was consistently higher in low O_2 and high CO_2 atmospheres. The ratio of acetaldehyde to ethanol decreased in Pineapple oranges and Marsh grapefruit under all treatments. The decrease was greater in waxed than unwaxed fruit. The redox ratio of $NAD/NADH_2$ showed little change in unwaxed fruit stored in 21% O_2 and in 21% O_2 + 5% CO_2, but it increased in waxed fruit under these same treatments.

DAMAGING STRESSES TO FRESH AND IRRADIATED CITRUS FRUITS. E.M. AHMED, F.G. MARTIN & R.C. FLUCK. *J. Food Sci.* 38, 230–233 (1973)–Forces required to rupture, puncture, shear or rupture an oil gland were lower for the irradiated citrus fruits than for the field run or waxed fruits. High force values were associated with Persian limes and Bearss lemons, low values were found for Dancy tangerines and Navel and Temple oranges, and intermediate forces were exhibited by Valencia oranges and Duncan grapefruit. To minimize fruit damage, Dancy tangerines and Navel and Temple oranges should be exposed to forces in the order of 1/10 to 1/4 of those of other citrus fruits during harvesting, washing, waxing, packaging and marketing.

A CALORIMETER FOR MEASURING HEAT OF RESPIRATION OF SMALL SAMPLES OF BIOLOGICAL MATERIALS. D.A. SUTER & B.L. CLARY. *J. Food Sci.* 38, 234–238 (1973)–The design of an 1800 ml capacity calorimeter for measuring heat of respiration of biological materials is presented. The calorimeter was used to measure heat of respiration of high moisture Spanish peanuts. Adiabatic condition is maintained to minimize energy transfer through the walls of the calorimeter flask. The respiring material is aerated to prevent an excessive concentration of carbon dioxide in the intermass air which might otherwise inhibit respiration. The effluent air removed from the flask during aeration may be examined to determine carbon dioxide concentration.

EFFECT OF POLYPHOSPHATES ON THE FUNCTIONAL PROPERTIES OF SPRAY DRIED EGG ALBUMEN. P.K. CHANG. *J. Food Sci.* 38, 239–241 (1973)–Portions of desugared liquid egg albumen were treated with sodium polyphosphate, spray dried, and in-package pasteurized. The dry products were used to prepare whipped foams and angel food cakes in order to evaluate functional characteristics. All of the albumen samples treated with polyphosphates produced angel food cakes with higher specific volumes than dried albumen without added phosphate. Cake volumes increased with increasing chain length of the polyphosphate up to at least an average value of $\bar{n}$=10. By contrast, phosphate treatment is, at best, equivalent to no treatment when the dried albumen is evaluated in foam. Polyphosphates were also found to be effective in overcoming deleterious effects of yolk-contamination in both liquid and spray dried egg albumen (SDEA) in angel food cake. As shown by disc gel electrophoresis, lysozyme, the component in albumen that possibly interacts with yolk lipids to form a harmful complex during yolk contamination, also interacts with long-chain polyphosphates.

PARAMETERS OF TEXTURAL CHANGE IN FROZEN-STORED COOKED LOBSTER (Homarus americanus) TAIL MUSCLE. B. DAGBJARTSSON & M. SOLBERG. *J. Food Sci.* 38, 242–245 (1973)-Several physicochemical parameters which may be related to the toughening of cooked lobster (Homarus americanus) meat during frozen storage were measured. Live lobsters were cooked for 10 min in boiling 2.5% NaCl brine. The tail muscles were chilled, removed from the shells, deveined, quick-frozen and stored at −12°C or −27°C. The Warner-Bratzler shear (W-B) was used to evaluate texture of the lobster meat. The pH, water-holding capacity (WHC), solubility of proteins in 0.6M KCl, 0.22M KCl and sodium dodecyl sulfate, and amount of free sulfhydryl groups were measured after different storage time intervals. The Warner-Bratzler shear and pH of samples increased during storage at −12°C and −27°C, while water-holding capacity declined during storage at −12°C and −27°C. Protein solubility and free sulfhydryl groups did not change significantly during frozen storage. The relationship between pH, WHC, and W-B shear remains obscure.

PILOT PLANT PRODUCTION OF AND LARGE SCALE ACCEPTANCE TRIALS WITH QUICK-SALTED FISH CAKES. F.R. DEL VALLE, M. PADILLA, A. RUZ & R. RODRIGUEZ. *J. Food Sci.* 38, 246–250 (1973)–A pilot plant for production of quick-salted fish cakes has been set up in Progreso, Yucatan, Mexico. It has been found that good cakes can be made from "trash" species as well as trimmings and rejects from filleting plants. In determination of yields and operating conditions, it has been calculated that pressing removes approximately 50%, while sun-drying removes an additional 25% of the water originally present in fish flesh. Yields of dry cakes with respect to flesh are of the order of 1:1. The dry cakes contain approximately 25% protein, 58% salt and 17% water. In large scale acceptance trials conducted among Mayan adults and children, acceptance of the product after desalting has been found to be excellent. Total investment in a plant for making 200 kilos of dry cakes in 8 hr is approximately $12,000, while cost of the dry cakes varies from $0.14–0.56 per kilo.

OXIDATIVE RANCIDITY IN DRY-CURED HAMS: EFFECT OF LOW PRO-OXIDANT AND ANTIOXIDANT SALT FORMULATIONS. D.G. OLSON & R.E. RUST. *J. Food Sci.* 38, 251–253 (1973)–Three lots of nine hams each were dry-cured as country style hams by using a curing mixture of salt, sugar and $NaNO_3$ a salt low in heavy metal ion content; and Lot III, a proprietary salt mixture containing BHA, BHT, citric acid and propylene glycol as antioxidants. After aging, hams were chemically analyzed and a taste panel scored each lot for flavor, saltiness, tenderness and overall satisfaction. Lot III showed a significant reduction of rancidity as measured by TBA values for fat and a greater flavor preference. However, no correlation between flavor preference and rancidity could be established because of the design of this experiment.

EFFECTS OF FREEZING AND PACKAGING METHODS ON SHRINKAGE OF HAMS IN FROZEN STORAGE. B.H. ASHBY, G.M. JAMES & A. KRAMER. *J. Food Sci.* 38, 254–257 (1973)–27 pallet lots of hams were shipped 2 days after slaughter for frozen storage. The hams were frozen in still air, circulating air and a blast freezer. The entire pallet lots were then polybagged, spray glazed or spray glazed and polybagged. Individual hams were weighed and examined for freezer burn after 2, 4 and 6 months' storage at −5°F. The greatest weight loss occurred during slow still air freezing. There was some gain in weight during storage which was maintained upon thawing for the first 4 months of storage, but not for longer periods. Glazing improved weight retention over polybagging, but glazing plus polybagging resulted in the least weight loss. Hams in the center of the pallet lost more weight than those at the edges.

EFFECTS OF FREEZING AND PACKAGING METHODS ON FREEZER BURN OF HAMS IN FROZEN STORAGE. B.H. ASHBY, G.M. JAMES & A. KRAMER. *J. Food Sci.* 38, 258–260 (1973)–Data were collected to compare the effects of various freezing and packaging methods on the amount of freezer burn that forms on hams stored for 4 and 6 months in a refrigerated warehouse. The amount of freezer burn more than doubled between the 4- and 6-month storage periods. Hams frozen in a forced-air room sustained significantly more freezer burn and freezer burn trim loss than those frozen in a still-air room or blast freezer.

ABSTRACTS:
IN THIS ISSUE

Polybagging entire pallet loads of either glazed or unglazed hams reduced the amount of freezer burn that forms between the 4- and 6-month storage periods. Significant differences were found between the amount of freezer burn on hams stacked at different locations on the pallet. A significant ($P < 0.05$) correlation ($r=0.30$) was found between the amount of freezer burn and the amount of freezer burn trim loss. No correlation ($r=0.05$) was found between the amount of freezer burn and the amount of total shrinkage.

REDUCTION OF BACTERIA ON PORK CARCASSES. G.W. BIEMULLER, J.A. CARPENTER & A.E. REYNOLDS. *J. Food Sci.* **38**, 261–263 (1973)–Use of acetic acid, stannous chloride, hydrogen peroxide and steam on hog carcasses inoculated with test cultures of *S. enteriditis* indicated the effectiveness of these treatments in reducing both total aerobic bacterial population and the incidence of salmonellae. Acetic acid, selected as the most acceptable choice of treatments, also proved effective in reducing total bacterial population and incidence of salmonellae on 500 pork carcasses sampled in a local plant with pH 2.0 and pH 2.5 being more effective than pH 3.0. It is suggested that control of bacteria and reduction of salmonellae from hog carcasses can be achieved by strict sanitation procedures during processing followed by spraying the carcass with acetic acid pH 2.0.

EFFECT OF CARCASS SUSPENSION METHOD ON SENSORY PANEL SCORES FOR SOME MAJOR BOVINE MUSCLES. R.L. HOSTETLER, B.A. LINK, W.A. LANDMANN & H.A. FITZHUGH JR. *J. Food Sci.* **38**, 264–267 (1973)–Five methods of suspending sides of bovine carcasses were compared for their effect on sensory tenderness scores of nine muscles. The methods were: conventional suspension by the pelvic limb (vertical); laying the side on a table, bone side down, with limbs perpendicular to the vertebrae (horizontal); suspending from cervical vertebrae with limbs tied together to bring the pelvic limb perpendicular to the vertebrae (neck-tied); suspending from the obturator foramen with limbs hanging free (hip-free); and, suspending from the obturator foramen with the limbs tied together to bring the thoracic limb perpendicular to the vertebral column (hip-tied). The panel gave clear evidence of being able to detect improvements in the tenderness of five of nine muscles from sides of animals given the experimental treatments. Only the semitendinosus and biceps femoris did not respond to the treatments. The psoas major and the triceps brachii were less tender by several experimental methods but the tenderness of both remained acceptable by the hip-free method. The hip-free method is practical and the desirability of its use is indicated.

UTILIZATION OF BEEF AND PORK SKIN HYDROLYZATES AS A BINDER OR EXTENDER IN SAUSAGE EMULSIONS. L.D. SATTERLEE, N.Y. ZACHARIAH & E. LEVIN. *J. Food Sci.* **38**, 268–270 (1973)–A powder obtained from the enzymatic hydrolysis of either beef or pork skin was shown to have possible use as a binder or extender in meat emulsions. The hydrolyzates had a slightly lower emulsion capacity, when compared to nonfat dry milk (NFDM) on a ml oil emulsified/100 mg protein basis, but a much larger emulsion capacity when compared on a per g sample basis because of their high peptide content. The pancreatin and pepsin digests of both beef and pork skin showed that a slightly better emulsion stability could be obtained when they were used to replace NFDM in a sausage formulation. The increase in emulsion stability could be a result of the higher protein (peptide) content of the skin hydrolyzates, when compared to NFDM.

EFFECT OF COMPOSITION ON THE STABILITY OF SAUSAGE-TYPE EMULSIONS. A. HAQ, N.B. WEBB, J.K.WHITFIELD & F.J. IVEY. *J. Food Sci.* **38**, 271–274 (1973)–Sausage-type emulsions were prepared with varying lipid levels and two different meat:water ratios (1.5:1.0 and 2.0:1.0) using a prototype system. Emulsions were evaluated by cook stability, physical properties, DC resistance, AC resistivity and viscosity methods. A noteworthy result of this study was the failure to obtain a highly stable emulsion at a 15% lipid level. Stable emulsions were obtained at a 25% total lipid level, near the level of commercial sausage emulsions, but the cook stability was lower than at higher lipid levels (50–65%). The physical properties rating of the cooked emulsions and the AC resistivity of the uncooked emulsion agreed closely with the cook stability results. It was concluded that stable sausage emulsions could be prepared with higher lipid levels by increasing the added water level while maintaining a constant protein level. The emulsion system studied broke between the 70–75% total lipid levels, which was apparently caused by using up the continuous phase. When the emulsions were inverted (75% lipid levels), the DC resistance and AC resistivity sharply increased, whereas the measured viscosity sharply decreased.

QUALITY CHANGES IN PRERIGOR POULTRY AT –3°C. J.R. BEHNKE, O. FENNEMA & R.W. HALLER. *J. Food Sci.* **38**, 275–278 (1973)–18-month old chickens were frozen immediately following slaughter and selected muscles were excised prerigor and held at –3°C for various periods. Tenderization of thawed-cooked muscles was achieved equally well by holding prerigor samples for identical times at either –3°C or 0°C. Similar results were obtained with intact carcasses of 18-month old chickens that were slaughtered and dressed under commercial conditions. Based on these findings, it appears possible, without impairing tenderness, to eliminate the conventional prerigor chilling operation, and replace it with a –3°C holding treatment of approximately equal duration.

IMPROVING THE QUALITY OF MECHANICALLY DEBONED FOWL MEAT BY CENTRIFUGATION. G.W. FRONING & F. JOHNSON, *J. Food Sci.* **38**, 279–281 (1973)–Replicate samples of regular and dwarf-size S.C. White Leghorn fowl were processed. The following treatments of each fowl type were utilized: (1) whole carcasses were mechanically deboned (BX-66 Beehive deboner); (2) whole carcasses were mechanically deboned and centrifuged to remove excess fat and heme pigments; and (3) hand-deboned meat run through the mechanical deboner without bones. Centrifugation of both fowl sources significantly ($P < 0.05$) increased protein content, significantly ($P < 0.05$) decreased fat content, significantly ($P < 0.05$) improved emulsifying characteristics, significantly decreased the rate of TBA increase and significantly lowered the TBA values of mechanically deboned meat (MDM) from regular-sized fowl. Total heme pigments and Gardner color values indicated that MDM gained significant ($P < 0.05$) quantities of heme pigments from the bone marrow. Regular-size fowl meat deboned immediately after processing showed greater changes in TBA values after frozen storage (0, 4 and 8 wk) than machine-deboned dwarf fowl meat. Delayed mechanical deboning (after 2 months of frozen storage) significantly ($P < 0.05$) increased the rate of rancidity development during frozen storage of deboned product.

TEXTURE STABILITY DURING STORAGE OF FREEZE-DRIED BEEF AT LOW AND INTERMEDIATE MOISTURE CONTENTS. D.R. HELDMAN, G.A. REIDY & M.P. PALNITKAR. *J. Food Sci.* **38**, 282–285

(1973)–The optimum storage water activities for freeze-dried beef and similar food products depends on changes in product texture during storage as well as other reactions causing deterioration. The selection of an optimum storage environment must account for texture changes along with changes in color and flavor. The influence of two storage temperatures (39 and 100°F) and four water activities (0, 0.25, 0.5 and 0.75) on the texture of freeze-dried beef was investigated. Two parameters (hardness and chewiness) of the texture profile analysis were measured at monthly intervals for a period of six months. The results revealed that both hardness and chewiness parameters increased significantly after 4 months of storage at 100°F and water activities of 0.5 and 0.75. All product stored at 39°F maintained texture stability throughout the 6 month storage period. The observed influence of storage duration on product texture was confirmed by statistical analysis using data collected at monthly intervals followed by an overall analysis. The analysis revealed that the chewiness parameter was more sensitive to product texture changes than the hardness parameter.

SYSTEMATIC VARIATION IN TOUGHNESS WITHIN THE BEEF LONGISSIMUS DORSI AND SOME OF ITS IMPLICATIONS. L.J. HANSEN. *J. Food Sci.* 38, 286–288 (1973)–The structural variability within a muscle is shown to have an effect on the consistency of Warner-Bratzler shear determinations. The need is shown for recognizing this in deciding on the number of replicate determinations to make, and in interpreting the results from shear tests.

CONVERSION OF BOVINE MYOGLOBIN INTO MULTIPLE, CHARGE-HETEROGENEOUS SUBFRACTIONS. J.R. QUINN. *J. Food Sci.* 38, 289–293 (1973)–Resolution of the multiple myoglobins of beef muscle and the determination of their pI values by polyacrylamide gel electrofocusing is described. The rate of conversion of the main myoglobin fraction to charge-heterogeneous, more acidic components increases with increasing temperature and pH, and is affected by type of buffer ion, ionic strength and the presence of urea. It is postulated that the conversions result from deamidation reactions, that these reactions are influenced by protein conformational changes, and that they also occur in vivo.

PRE-RIGOR PRESSURIZATION OF MUSCLE: EFFECTS ON pH, SHEAR VALUE AND TASTE PANEL ASSESSMENT. J.J. MACFARLANE. *J. Food Sci.* 38, 294–298 (1973)–Pressures of the order of 100 meganewtons/m^2 (MNm^{-2}) applied for periods of 1 min or more to many pre-rigor ox and sheep muscles held at about 30°C were found to greatly influence the texture of the cooked post-rigor muscle. Only one of the muscles investigated (sheep semitendinosus) did not give consistently lower shear values than the corresponding "standard" muscle removed from the other side of the carcass post-rigor. Taste panel evaluations also showed that pressurized muscles were more tender, though less juicy, than the corresponding standard samples. A rapid fall in the pH of muscle as a result of pressurization indicated that glycolysis was greatly accelerated.

THE EFFECT OF CURING AGENTS, pH AND TEMPERATURE ON THE ACTIVITY OF PORCINE MUSCLE CATHEPSINS. J.C. DENG & D.A. LILLARD. *J. Food Sci.* 38, 299–302 (1973)–The proteolytic enzymes, cathepsins, of porcine muscle were optimally active at pH 4.0 and 9.0 using urea-denatured hemoglobin substrate but only active at pH 9.0 with sodium chloride soluble muscle protein substrate. Optimum temperature of the enzymes was 47.5°C, and the enzymes were stable at temperatures under 37°C. Although their activity was reduced at NaCl concentrations above 0.5M, low cathepsin activity still existed at NaCl concentrations as high as 1.5M. The activity of the enzymes was not affected with pure sucrose at low concentrations and was decreased greatly at concentrations above 0.3M. The cathepsin activity was not affected either with potassium chloride or potassium nitrate concentrations lower than 0.001M.

PROPERTIES OF GRAM NEGATIVE AEROBES ISOLATED FROM MEATS. C.M. DAVIDSON, M.J. DOWDELL & R.G. BOARD. *J. Food Sci.* 38, 303–305 (1973)–Organisms from a wide range of meat products were examined with the object of establishing a simple scheme for the identification of *Pseudomonas* and organisms which formerly would have been assigned to *Achromobacter*. Whether exact identification of an organism is likely to contribute to a better understanding of its role in bringing about spoilage is questionable. The study indicates that from a routine control standpoint, the gram negative aerobic bacteria developing on chilled meat can be assigned to major goups on the basis of the following tests: gram stain, shape, motility, position of flagella (if any), oxygen requirements, oxidase test and action on gluconate.

UTILIZATION OF HIGH PROTEIN TISSUE POWDERS AS A BINDER/EXTENDER IN MEAT EMULSIONS. L.D. SATTERLEE, B. FREE & E. LEVIN. *J. Food Sci.* 38, 306–309 (1973)–Desiccated, defatted powders obtained from certain by-product tissues of beef and pork were found to be high in protein and to possess emulsifying properties. The following powders, ranked from highest to lowest in emulsion capacity, were studied: blood, brain, lung, stomach, duodenum, liver, spleen, heart and kidney. Histological analysis of emulsions formed from each tissue powder showed that the high capacity emulsion (those from blood, brain, lung and stomach) possessed lipo-protein globules which were small and closely packed, whereas the low capacity emulsions (those from duodenum, kidney, liver, spleen and heart) possessed globules which were large and oftentimes partially disrupted. Some, but not all, of the powders which possessed high emulsion capacity possessed a high emulsion stability. This study indicates that certain tissue powders (blood, lung, stomach) excel all others in their emulsifying characteristics and should be considered as a possible binder or extender in meat emulsions.

FORMATION OF LACTIC ACID, VOLATILE FATTY ACIDS AND NEUTRAL, VOLATILE MONOCARBONYL COMPOUNDS IN SWEDISH FERMENTED SAUSAGE. H. HALVARSON. *J. Food Sci.* 38, 310–312 (1973)–The effect of two process conditions on the formation of some flavor components in Swedish fermented sausage was studied. During fermentation the concentrations of lactic acid, volatile fatty acids (C_1–C_6) and neutral, volatile monocarbonyl compounds (C_1–C_8) were followed in one batch of smoked and stored and in one batch of drying sausages. The results showed that the smoking temperature accelerated formation of lactic acid, formic acid and acetic acid in the smoked sausage compared with the initial development in the drying sausage. After ripening for 3 wk the levels of n-hexanal, n-octanal and some 2-alkenals were higher in the dried sausages compared with the smoked and stored ones. Possible mechanisms connected to the results found are also briefly discussed.

POSTMORTEM CHANGES IN DARK TURKEY MUSCLES. K.J. WISKUS, P.B. ADDIS & R. T-I. MA. *J. Food Sci.* 38, 313–315 (1973)–This study was designed to identify some patterns of postmortem changes in dark turkey muscle. Sarcomere length, response to electrical stimulation and time-course of rigor mortis were determined on biceps femoris muscles obtained from 17 tom turkeys 5 min postexsanguination. Additional samples were obtained at 1, 2, 3 and 24 hr postmortem for determination of sarcomere length and from the opposite biceps muscle for shear determination. Significant correlations ($P < 0.05$) were calculated among threshold of response, duration of response and rigor time course. Similar interrelationships were noted in peroneus longus muscle from 13 turkeys. Shear value was correlated to ultimate sarcomere length ($r = -0.45$, $P < 0.05$; biceps and $r = -0.68$, $P < 0.01$; peroneus), suggesting that degree of contration may be related to tenderness. Mean values for time course of rigor mortis for muscles (biceps femoris = 163 ± 79 min; peroneus longus = 241 ± 85) suggest that in certain commercial processes these muscles from some birds may be chilled or frozen in a prerigor state. To determine whether, under commercial conditions, thaw rigor or cold shortening could toughen intact muscle, three dark, superficial muscles were segregated as to degree of rigor development on the evisceration line. Results demonstrated that cold treatment or freezing tended to shorten sarcomeres and toughen intact muscle in the peroneus longus and gastrocnemius muscles.

ABSTRACTS:

IN THIS ISSUE

SORPTION HYSTERESIS AND CHEMICAL REACTIVITY: LIPID OXIDATION. H-E. CHOU, K.M. ACOTT & T.P. LABUZA. *J. Food Sci.* **38,** 316–319 (1973)–Methyl linoleate was oxidized in model gels of either cellulose or amylopectin in systems which showed sorption hysteresis. Amylopectin, which swells, exhibits a larger hysteresis loop than the nonswelling cellulose system. In the range of intermediate moisture foods, water accelerates oxidation when the trace metal content is low or is bound to the solid support. In addition, when prepared by a desorption technique the oxidation rate was much larger than the same system prepared by adsorption to a similar water activity. Most of the effect can be attributed to the diffusion limitation in the aqueous phase which affects catalyst mobility and swelling of the polymeric matrix which exposes new catalyst sites.

FATE OF WATER SOLUBLE SOY PROTEIN DURING THERMOPLASTIC EXTRUSION. D.B. CUMMING, D.W. STANLEY & J.M. DeMAN. *J. Food Sci.* **38,** 320–323 (1973)–Polyacrylamide disc gel electrophoresis was employed to evaluate the changes in water soluble soy protein resulting from thermoplastic extrusion. As temperature increased and other processing parameters were held constant the general electrophoresis pattern was altered considerably and much of the protein became insoluble. Six major fractions were designated and tentatively identified. As a result of processing, the intact soy proteins yielded breakdown products consisting of multiples of a 28,000 MW subunit. For good texturization it is apparently necessary that a significant proportion of the water soluble protein becomes insoluble as a result of thermal processing.

FUNCTIONAL CHARACTERISTICS OF WHEY PROTEIN CONCENTRATES. C.V. MORR, P.E. SWENSON & R.L. RICHTER. *J. Food Sci.* **38,** 324–330 (1973)–17 different whey protein concentrates (WPC) prepared by electrodialysis, metaphosphate complex, ultrafiltration, Sephadex gel filtration, dialysis, CMC complex and iron complex were studied for chemical and elemental composition, available lysine, zonal electrophoresis and several important functional properties. Wide differences were obtained in protein, ash and lactose content of the different WPC. Protein content of the WPC examined ranged from about 33–66%. Discrepancies were observed for elemental analysis of metaphosphate-containing WPC. Available lysine content varied from 4.5–11.5 mg per 100 mg protein and was dependent upon the lactose content, which apparently interferred with the determination. Initial pH of WPC dispersed in water, ranged from 3.2 to 8.2. Most WPC exhibited a dip in their solubility curve at pH 4.5–5.0, except metaphosphate complex, iron complex and CMC complex WPC, which had 0–30% protein solubility below pH 4. Apparent denaturation, studied by polyacrylamide gel electrophoresis and insolubility at pH 4.6, ranged from 12–40% for five different electrodialysis WPC studied. α-lactalbumin was the most susceptible whey protein to denaturation during WPC preparation. Emulsion capacities (EC) were similar for all WPC (32–42g corn oil per 100 ml dispersion), except CMC complex WPC, which had a EC value of 64. Metaphosphate complex WPC failed to form a stable foam, but the other WPC studied ranged from 450–680% mean overrun compared to a value of 1120% overrun for sodium caseinate. CMC complex WPC was extremely viscous and formed the most stable foam. Whipped topping mix with WPC as protein source produced lower overrun than the same mix containing nonfat dry milk solids as protein source. The buffer capacities (BC) over the pH 3–8 range varied greatly for the different WPC preparations depending upon the extent of demineralization, alkali neutralization and residual CMC and metaphosphate protein precipitants.

CHEMICAL NATURE OF STONE CELLS FROM PEAR FRUIT. A.S. RANADIVE & N.F. HAARD. *J. Food Sci.* 38, 331–333 (1973)–Stone cells (sclereids) were isolated from the pulp of mature pear fruit and shown to be lignocellulosic by chemical analyses and infrared absorption spectra. The lignin fraction was estimated to comprise approximately 18% of the dry weight and contained syringaldehyde and vanillin as the principle oxidation products. The major sugars in the carbohydrate fraction were glucose and xylose.

TEXTURE OF CUCUMBERS: CORRELATION OF INSTRUMENTAL AND SENSORY MEASUREMENTS. I.J. JEON, W.M. BREENE & S.T. MUNSON. *J. Food Sci.* 38, 334–337 (1973)–This study compared sensory evaluation of firmness and crispness with Instron texture profile analysis (TPA) values of brittleness, hardness, and total work of compression as well as with the Magness-Taylor fruit pressure test (FPT) of firmness using 1-in. diam. raw fruit of four firm and two soft cucumber cultivars. In addition, FPT firmness values were compared with the TPA values. For Instron evaluation, two different methods of sample preparation were used: (1) compression of cross-sectional slices including skin (SKIN) and (2) compression of slices with skin removed (NO SKIN). All comparisons showed good correlations ($r > 0.9$). However, the highest correlation coefficient (0.988) was obtained between NO SKIN total work and sensory crispness. Correlation of TPA values with sensory tests was slightly higher for NO SKIN samples than for SKIN samples. FPT firmness showed good correlations with sensory scores as well as with TPA parameters. In addition, variability was higher in TPA values of NO SKIN than in those of SKIN but they showed similar trends for their textural parameters.

MODIFIED METHOD FOR ANALYSIS OF SWEET POTATO α-AMYLASE. V.C. HASLING, E.A. CATALANO & H.J. DEOBALD. *J. Food Sci.* **38,** 338–339 (1973)–A modified method for the determination of α-amylase content in sweet potatoes, suitable for in-plant control, is presented. The method includes new procedures for obtaining the crude juice, a rapid method of clarification and a simplified analysis using equipment commonly found in control laboratories. The standard and modified methods for determining α-amylase activity were compared. Their relationship shows a definite pattern, highly comparable in the 0.1–1.5 SDU range and in practical agreement throughout. Therefore, amylase activity determined by the modified method is recommended for prediction of processing characteristics by sweet potato processors.

METHOD FOR DETERMINING NARINGIN CONTENT IN GRAPEFRUIT JUICE. J.H. TATUM & R.E. BERRY. *J. Food Sci.* 38, 340–341 (1973)–A simplified method for determining naringin in grapefruit juice is presented. Whole juice is applied directly to commercially available thin-layer chromatographic plates and naringin is separated from its tasteless isomer, 7-β-rutinoside of naringenin. A modified Davis test, for colorimetric analysis of the eluted spots uses an inexpensive colorimeter found in most citrus plant quality control laboratories.

A NEW METHOD FOR THE DETERMINATION OF CAPSAICIN IN CAPSICUM FRUITS. A. TREJO-GONZÁLEZ & C. WILD-ALTAMIRANO. *J. Food Sci.* 38, 342–344 (1973)–The content of capsaicin in isopropanol extracts of green Serrano chili peppers was measured spectrophotometrically at a wavelength of 281 nm. The charcoal clean-up efficiently separated other compounds which could interfere with the capsaicin measurements. Further chromatographic clean-up of the extracts was not necessary. The average capsaicin content of 10 green chili extracts was 0.45% on a dry weight basis with a standard deviation of 0.06.

ISOLATION AND IDENTIFICATION OF VOLATILE COMPOUNDS FROM POTATO CHIPS. R.E. DECK, J. POKORNY & S.S. CHANG. *J. Food Sci.* **38,** 345–349 (1973)–A sample of potato chips manufactured from Kennebec potatoes by frying in a mixture of corn and cottonseed oils was selected by a trained panel as having a desirable flavor. The volatile compounds in this sample were isolated by vacuum steam distillation of a water slurry of the potato chips. The isolated volatile compounds were fractionated by repeated gas chromatography, and the separated components were identified by a combination of infrared and mass spectrometry. The identification was confirmed by the infrared and mass spectra, and the retention times of the authentic compounds. A total of 53 compounds was identified; these included eight nitrogen compounds, two sulfur compounds, 14 hydrocarbons, 13 aldehydes, two ketones, one alcohol, one phenol, three esters, one ether and eight acids. Among the compounds identified, the alkyl-substituted pyrazines and the 2,4-dienals were of great importance to the flavor of potato chips.

AN IMPROVED METHOD FOR THE DISCRIMINATION BETWEEN BIOGENIC AND SYNTHETIC ACETIC ACID WITH A LIQUID SCINTILLATION COUNTER. T. KANEKO, S. OHMORI & H. MASAI. *J. Food Sci.* **38,** 350–353 (1973)–Acetic acid samples were prepared from genuine fermentation products and their radioactivities were determined in a liquid scintillation counter. They contained about 20.3 dpm of carbon-14 per g carbon, and the deviation between samples was found to be less than a few percent of the average. After considerations on the experimental errors, it was concluded that the improved method will be useful for the estimation of biogenic acid in vinegars.

RAPID ANALYSIS OF MOISTURE IN MEAT BY REFRACTIOMETRY. P.B. ADDIS & A.S. CHUDGAR. *J. Food Sci.* **38,** 354 (1973)–Accurate monitoring of meat moisture is an essential feature in quality control and insures compliance with governmental regulations. Development of rapid product manufacturing processes has increased the need for rapid, accurate proximate analyses. The method consists of extracting water from meat by homogenization with anhydrous isopropanol. Extract is filtered and equilibrated to the specific temperature of the determination. An Abbe Refractometer (sodium D light, λ = 5892.62Å in air) was used to measure refractive indices of extracts which were compared to known isopropanol-water mixtures. The method is fast (< 10 min) and as accurate as conventional methods (oven drying and toluene distillation). The precision is lower than conventional methods but is sufficient for quality control.

RAPID ANALYSIS OF MOISTURE IN MEAT BY GAS-LIQUID CHROMATOGRAPHY G.A. REINECCIUS & P.B. ADDIS. *J. Food Sci.* **38,** 355 (1973)–The method developed utlizes thermal conductivity gas-chromatography of methanol extracts of meat and products. Meat samples (5g) are homogenized with anhydrous methanol (20 ml) followed by the determination of water content of the methanol extract using a Hewlett-Packard gas chromatograph (Model 7620) and electronic integrator (Model 3370B). Separation of methanol-water mixtures was accomplished on a 1m × 0.32 cm OD Poropak Q column operated isothermally at 110°C. Results demonstrated that the moisture content of a wide variety of meats and meat products could be determined accurately within 10 min.

MODIFICATION OF COCKTAIL SHRIMP TEXTURE. E.M. AHMED, V.T. MENDENHALL & J.A. KOBURGER. *J. Food Sci.* **38,** 356–357 (1973)–Enzymatic and chemical means were used to reduce the toughening effect observed during storage of cocktail shrimp. Treatment with 0.005% Protease G solution maintained at 25°C resulted in decreased toughness of the shrimp. Shrimp treated with 5% sodium tripolyphosphate and 2.4% sodium chloride solution at 25°C were more tender and more preferred organoleptically than either the enzyme treated or the nontreated muscles. The use of water and polyphosphate dip at 65°C resulted in increased toughness of the shrimp.

CONTINUOUS CONVERSION OF STARCH TO GLUCOSE BY AN AMYLOGLUCOSIDASE-RESIN COMPLEX. Y.K. PARK & D.C. LIMA *J. Food Sci.* **38,** 358–359 (1973)–Amyloglucosidase obtained by submerged fermentation with Aspergillus niger NRRL 3122, was insolubilized by binding with an anion exhange resin (Amberlite IR-45). Conversion of liquefied starch into glucose by the enzyme-resin complex column is described.

SOME NEWLY FOUND ORANGE ESSENCE COMPONENTS INCLUDING TRANS-2-PENTENAL. M.G. MOSHONAS & P.E. SHAW. *J. Food Sci.* **38,** 360–361 (1973)–Aqueous orange essence was extracted with both methylene chloride and ethyl chloride and the extracts analyzed by GLC to afford 37 identified components including seven newly found orange essence components. One of these, trans-2-pentenal, is a new citrus constituent with a fruity aroma. Its flavor threshold in water was determined to be 100–150 ppb using a synthetically prepared sample. Its quantity in aqueous essence was estimated to be approximately five times its threshold level. Use of the lower-boiling solvent, ethyl chloride, was not advantageous in retaining more of the volatile components when compared to methylene chloride.

CRYOGENIC FREEZING OF TOMATO SLICES. R. HOEFT, R.P. BATES & E.M. AHMED. *J. Food Sci.* **38,** 362 (1973)–Freezing of 6 mm thick tomato slices was evaluated using liquid nitrogen and slow freezing, four tomato breeding lines and calcium pretreatment. All treatments resulted in poorer texture than unfrozen controls, but slices frozen in liquid nitrogen were firmer and more acceptable than those frozen at −34°C. Application of 150 ppm calcium produced no significant improvement in slice firmness. There were no significant differences among the four breeding lines examined. Improved cultivars and methodology will be required in order to approach the quality of fresh tomato slices.

STORAGE STABILITY OF DEHYDRATED POTATO GRANULES PACKAGED IN CANS AND CARTONS. G. LISBERG & T.-S. CHEN. J. Food Sci. **38,** 363–364 (1973)–Dehydrated potato granules were stored in nitrogen packed cans and air packed foil-lined cartons at 75°F and 50% RH for 24 wk. The changes in moisture content, concentrations of hexanal, BHT and sulfite, color, aroma and flavor during storage were determined. The carton was as effective as the can in protecting the potato granules from absorbing moisture and against nonenzymatic browning. However, rancidity was detected in carton-packaged samples due to the presence of air.

EXAMINATION OF FROZEN VEGETABLES BY TWO SAMPLE PREPARATION PROCEDURES. M.P. REEVES. *J. Food Sci.* **38,** 365–366 (1973)–A comparison of two methods for frozen food sample preparations was investigated: blending as recommended by AOAC; and shaking, manual vs. mechanical. Eight varieties of frozen vegetables were examined for total aerobic plate counts, coliforms, *Escherichia coli* and coagulase positive *Staphylococci*. Individual subsamples were analyzed in duplicate. One series was blended, the other shaken. Results clearly indicated the superiority of the blending procedure. Aerobic plate counts, coliforms and *E. coli* levels showed 10-fold, 6-fold and 33-fold increases, respectively, when the blending procedure was employed. Little variation in recovery was observed between the two methods of shaking.

CARRAGEENAN. A Scientific Status Summary. IFT EXPERT PANEL ON FOOD SAFETY & NUTRITION. *J. Food Sci.* **38,** 367–368 (1973)–Carrageenan, a naturally occurring hydrocolloid obtained commercially from red algae seaweed, is widely used in the food industry as a stabilizer and gelling agent; however, its safety was recently questioned and food-grade carrageenan was tested as part of the GRAS review. Results indicate no evidence of human hazard exists at present levels of usage of food-grade natural carrageenan. (Chemically hydrolyzed carrageenan, C-16, has never been used as a food additive and should not be confused with the natural form.) FDA discontinued carrageenan's GRAS status, strengthening the Food Additive Regulation for carrageenan to ensure continued use of only the natural, unhydrolyzed form in food. FDA concluded an adequate margin of safety exists to continue safe use of food-grade carrageenan at any present or anticipated use levels in food.

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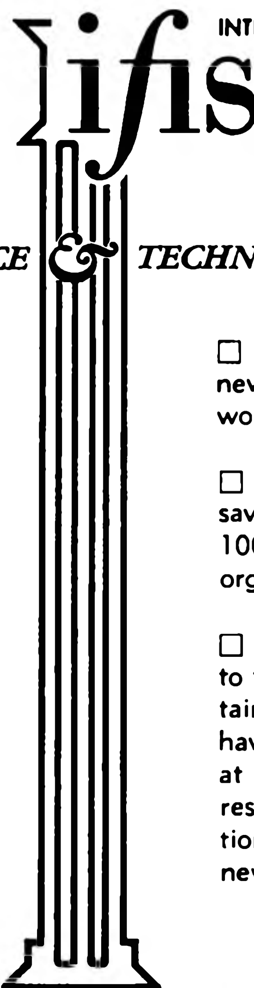

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CONDITIONS NECESSARY FOR STERILITY TESTING OF HEAT PROCESSED CANNED FOODS

INTRODUCTION

STERILITY TESTING is used to assist in determining the efficacy of a heat process and in the assessment of the soundness of containers. Thus, it is extremely important to establish a reliable technique for excluding contamination from any source during the subculturing operation. Meaningful conclusions regarding the process and the containers can be made only if contamination has been precluded.

The occurrence of contamination during sterility testing of foods has been recognized and documented. In an early study, Williams and Clark (1942) stressed the importance of multiple controls in sterility testing of canned salmon. Denny (1970) found, in a collaborative study of sterile product by nine laboratories, that 7.1% of the samples were contaminated during the process of subculturing. In a subsequent study, Denny (1972) reported a 20% contamination rate when only a minimum of precaution was taken.

The conditions reported to be necessary to eliminate contamination, however, have been confusing and even conflicting. The results of one study indicate that testing can be done in a laboratory (Denny, 1970); others stress the use of laminar air-flow equipment (Favero and Berquist, 1968). Lips of tubes can be flamed (Daugherty and Lamberti, 1954) or flaming can be eliminated (Brunker and Fernandez, 1972). A method of "sterilizing" in common practice is the use of the alcohol flaming technique, yet evidence for suspecting flaming as a source of contamination has been presented (Doyle and Ernst, 1969). Even the media commonly used in sterility testing have fallen suspect, not of causing contamination, but of failing to detect contamination if it does occur (Hibbert and Spencer, 1970).

Barbeito et al. (1967) have shown that beards retained microorganisms despite washing with soap and water. Denny (1972) recognized the importance of hair as a source of contamination and restricted participants in a collaborative study to those not having beards, mustaches or sideburns. Disposable operating room caps which completely covered the hair were also used to reduce contamination from this source. Hair, particularly long hair, has been incriminated in staphylococcal infections in hospitals (Medical World News, 1971).

Denny (1972), reporting on the results of a collaborative study involving nine laboratories showed contamination during subculturing was eliminated by the use of hair coverings and washing the hands and face with soap followed by a detergent sanitizer solution. This conclusion, however, was based on results of the analysis of only four cans by each laboratory.

The present study was undertaken to examine some of the phases of sterility testing and to isolate and identify factors which contribute to contamination. A technique which would sharply reduce, or completely eliminate contamination, if possible, was sought. The reliability of any chosen technique to eliminate contamination was to be borne out by large numbers of analyses.

EXPERIMENTAL

Products tested

Samples of eleven different canned, pasta-containing products were obtained from production facilities located on the East and West Coasts and in the Mid-West. All cans were flat and appeared to have normal vacuum. In most cases, product from at least two, and occasionally as many as seven production lots, was analyzed. A total of 4,740 cans of product were sterility tested in this study.

Subculture media

Cooked Meat Medium (CMM) (Both Difco and BBL) was used as the anaerobic subculture medium and Brom Cresol Purple Broth (BCP) (16g of Difco Purple Broth Base, 5g glucose, 1,000 ml distilled water) was used as the aerobic subculture medium. All lots of media used in this study were tested and found to support the growth of microorganisms. Media were dispensed in 18 x 150 mm tubes, 10 ml per tube. CMM tubes were steamed for 15 min and cooled immediately prior to inoculation. After subculturing, 4 ml of 2% agar were used to overlay CMM tubes to maintain anaerobiosis and reveal production of gas. Incubation of subculture tubes was at 35°C for 7 days (in experiment 1, incubation was for 14 days).

Subculture procedure

Labels were removed from the cans, the cans were examined for external defects, and the can descriptions recorded. The cans were numbered with a permanent waterproof marking pen for identification. Counter tops were wiped with chlorine solution (100 ppm or greater) immediately before using. In experiment 1, the noncoded end of the can was immersed in alcohol, and held over a large, lighted Meker burner until all condensation was evaporated. In experiments 2, 3 and 4, the can was subsequently placed on the counter top with the heated end up and the burner inverted on the can until the end was slightly domed. The can opener (Bacti-Disc Cutter) was dipped in alcohol and flamed sufficiently to destroy all microorganisms and used to make a hole approximately 2 in. in diam in the noncoded (can manufacturer's end), heated end of the can. Approximately 1/3 of the contents of the can was poured off (the product may have received enough heat during sterilization of the surface of the can to destroy nonspore forming organisms if present), making certain that no splashing occurred to contaminate the remaining contents of the can. The contents were subcultured immediately. In all experiments, pasta was removed with sterile forceps (alcohol flamed) and inoculated into one tube of CMM and one tube of BCP broth, approximately 4g per tube. In several experiments, serum also was subcultured into both media using a sterile wide mouth glass pipet, 2 ml per tube. The lips of

media tubes were flamed after addition of the food. The odor and appearance of the product in the can were noted and a heat-fixed smear of the food was examined microscopically (the smear was stained using crystal violet). The pH of the product was measured using a pH meter and reference buffer near the pH of the food. The cans were washed with hot soapy water, dried and saved until incubation of subcultures was completed. If a positive subculture was obtained, the corresponding can was vacuum tested for leaks, torn down, and examined for container defects. The types of organisms recovered from subculture tubes also were determined.

Exposure plates (Plate Count Agar) were used to detect atmospheric contamination levels during subculturing. Two plates were placed on the bench top directly in front of each investigator and the third exposed some distance away. The plates nearest the investigators detected contamination contributed by the workers while the third detected contamination levels in the atmosphere.

Pipetes were autoclaved and were known to be sterile; however, the same forceps were used repeatedly to subculture pasta forms, being sterilized before subculturing each can by alcohol flaming in a burner. To check on the efficiency of this sterilization technique, after every fifth sample subcultured, the forceps were flamed and one tube of each medium was inoculated by touching the forceps in the media. The tubes were handled as all other subcultures.

Experimental design

Five experiments were conducted in this study.

Experiment 1. Cans of product were subcultured in a bacteriology laboratory by two teams (two members each) of investigators using the techniques outlined. Teams and team composition remained unchanged throughout the study. Pasta forms were subcultured from nine different types of product. Rountine work other than sterility testing, (the work was not part of this study) involving both aerobic and anaerobic spore formers, also was being done in the laboratory. A total of 981 cans was examined.

Experiment 2. Cans of product were subcultured in a positive pressure room equipped with ultraviolet treated, filtered air. Pasta forms were again subcultured using the same techniques as in experiment 1. A total of 431 cans was examined.

Experiment 3. Results obtained in experiments 1 and 2 dictated the conditions employed in this experiment. Three teams (two members each) of investigators participated. Subculturing was done both in a laboratory and a positive pressure inoculating room studying the four variables listed below:

(a) Use of sterile head covers, face masks and lab jackets;
(b) Use of sterile face masks;
(c) Use of sterile head covers and lab jackets; and
(d) No special precautions taken.

During subculturing in the laboratory, all work other than sterility testing was suspended. Prior to subculturing, hands were washed, dried and swabbed. Swabs were placed in CMM and incubated at 35°C for 7 days. Types of organisms recovered from the medium were identified. Both pasta forms and serum were subcultured. A total of 1,296 cans was examined.

Table 1–Results from products subcultured in a bacteriology laboratory using currently accepted techniques

		Team 1				Team 2			
			Number of positive subcultures				Number of positive subcultures		
Product	pH	Total cans subcultured	PA[a]	B[b]	C[c]	Total cans subcultured	PA[a]	B[b]	C[c]
1	6.1	117	6	1	7	205	0	7	5
2	5.5	48	0	0	0	48	0	2	0
3	4.6	24	0	0	0	–	–	–	–
4	4.9	96	2	9	7	12	0	0	0
5	4.6	72	0	2	4	–	–	–	–
6	6.0	287	2	10	18	–	–	–	–
7	6.2	48	0	0	1	–	–	–	–
8	5.4	–	–	–	–	24	0	0	0
Total		692	10	22	37	289	0	9	5
Recovery rate		69/692 = 9.97%				14/289 = 4.84%			

[a]PA = putrefactive anaerobe.
[b]B = *Bacillus.*
[c]C = contaminant type organisms (nonheat resistant forms such as cocci, nonsporeforming rods and molds).

Table 2–Results from products subcultured in a positive pressure inoculating room using currently accepted techniques

		Team 1				Team 2			
			Number of positive subcultures				Number of positive subcultures		
Product	pH	Total cans subcultured	PA[a]	B[b]	C[c]	Total cans subcultured	PA[a]	B[b]	C[c]
1	6.1	120	0	0	4	141	0	0	2
3	4.6	24	0	0	0	16	0	0	0
4	4.9	–	–	–	–	61	0	0	1
5	4.6	24	0	0	0	22	0	0	2
9	6.3	–	–	–	–	23	0	0	0
Total		168	0	0	4	263	0	0	5
Recovery rate		4/168 = 2.38%				5/263 = 1.90%			

[a]PA = putrefactive anaerobe.
[b]B = *Bacillus.*
[c]C = contaminant type organisms (nonheat resistant forms such as cocci, nonsporeforming rods and molds).

Experiment 4. After settling upon the optimum conditions for subculturing as determined in experiment 3, cans of product were examined using these techniques. Subculturing was done in a positive pressure inoculating room, using sterile head covers, face masks and lab jackets. Hands also were swabbed. A total of 1,456 cans was examined.

Experiment 5. Three cases of each product were incubated at 35°C for 6 months to encourage the growth of any viable organisms which might be present in the cans. After incubation, cans were examined for evidence of spoilage. Cans of each product were randomized and two of the three cases of each product were tested for sterility using the subculturing techniques used in experiment 4. A total of 576 cans was examined.

RESULTS

INITIAL RESULTS obtained in experiment 1 (Table 1) indicated the possible recovery of heat resistant organisms, both putrefactive anaerobes (PA) and *Bacillus* sp., from several of the products tested. All cans examined were flat, appeared to have good vacuum, and had no external defects. No leaks were evident upon vacuum testing and no obvious defects could be detected during container breakdown. The product had normal appearance, odor and pH. In almost all cases of positive subcultures, either the CMM tube or the BCP tube showed growth, but seldom were both from the same sample positive. In light of these results, and because of the recovery of some nonheat resistant organisms (e.g. cocci), a close examination was made of the techniques of subculture used.

Because some of the organisms recovered from subculture media were identical to organisms being used in the laboratory, subculturing in experiment 2 was done in a positive pressure room. Incubation was reduced from 14 days (in experiment 1) to 7 days because all positive tubes showed growth within 7 days.

Table 3—Results from products subcultured in a positive pressure inoculating room and a laboratory under a variety of conditions

Subculture conditions	Total no. of cans subcultured by three teams of investigators	Total positive subcultures					
		Serum			Pasta		
		PA[a]	B[b]	C[c]	PA[a]	B[b]	C[c]
Positive pressure room							
Face mask, head cover, lab jacket	288	0	0	0	0	0	1
Face mask	144	0	0	0	0	0	0
Head cover, lab jacket	144	0	0	0	0	1	11
None of the above	144	0	0	0	0	1	4
Laboratory							
Face mask, head cover, lab jacket	144	0	0	0	0	0	0
Face mask	144	0	0	0	0	0	0
Head cover, lab jacket	144	0	0	2	0	0	5
None of the above	144	0	0	1	0	1	0
Total	1,296	0	0	3	0	3	21

[a]PA = putrefactive anaerobe.
[b]B = *Bacillus.*
[c]C = contaminant type organisms (nonheat resistant forms such as cocci, nonsporeforming rods and molds).

Table 2 indicates that a marked reduction occurred in positive recovery rates (from 9.97% to 2.38% for team 1 and from 4.84% to 1.90% for team 2), as well as the complete elimination of organisms being studied in the laboratory (PA and *Bacillus*).

The only organisms recovered in experiment 2 were cocci and since cocci are common inhabitants of human beings, attempts were made to identify some of the areas contributing to this contamination.

The results of experiment 3, presented in Table 3 indicate that the single, most important precaution to be taken, is the wearing of a face mask during subculturing. With one exception, all positive subcultures were eliminated by use of a face mask, indicating the naso-pharyngeal passages or facial skin as the most likely source of contamination. The one positive recovery obtained while using a face mask was a coccus and occurred in only one of the two subculture tubes. The results also indicate that contamination during subculturing in a laboratory can be reduced if it is done at a time when other organisms are not being used or introduced in the area.

Hand swabs revealed the presence of cocci, a known inhabitant of the skin. *Bacillus* sp. also were demonstrated frequently on the hands, and on four occasions, PA organisms were isolated.

Subsequent work using an antiseptic soap has shown that cocci can be eliminated from the hands by washing; however, *Bacillus* and PA have been isolated from hand swabs after as many as three washings with antiseptic soap.

Exposure plates were examined to help determine atmospheric contamination levels. Plates exposed up to ½ hr in the positive pressure room when no investigators were present showed no colonies. Plates exposed in the room when the investigators were subculturing showed the presence of organisms. Those plates exposed for ½ hr near each investigator showed cocci, *Bacillus*, gram positive rods, yeast and molds. As many as 24 colonies per plate were present. Plates exposed some distance from the investigators showed a maximum of nine colonies; generally no more than three. Cocci and an occasional gram positive nonsporeforming rod were the only organisms isolated. It appeared that most of the organisms in the room atmosphere were contributed by the investigators.

Therefore, it seemed that contamination could be eliminated, or at least dramatically reduced, if subculturing were done in a positive air pressure room using sterile head covers, face masks and lab jackets. To test the validity of this, 1,456 cans representing nine different products were sterility tested under the above conditions. Of these, only one can showed a positive subculture in CMM, a contamination rate of 0.07%. No growth was detected in the parallel BCP tube from this sample. The organism isolated was a *Bacillus.* Vacuum testing and can examination showed no obvious defects. The unopened can was flat, appeared to have good vacuum, and product looked and smelled normal. The product smear showed no organisms present. The exposure plate assessing room contamination showed no colonies; however, the plate exposed near the investigator subculturing the pasta showed four colonies, all *Bacillus.* The data indicated contamination during subculturing as the most likely source of the *Bacillus* in the CMM tube.

Flaming of forceps proved to be adequate for sterilization because all control tubes remained sterile. It is highly improbable that the forceps were the source of contamination when positive subcultures were encountered from product.

All cans incubated at 35°C for 6 months remained visibly flat and showed no visible evidence of spoilage. No growth was detected in any of the subcultures from the 576 cans tested for sterility, after the cans had been incubated for 6 months at 35°C.

DISCUSSION

ALL OF THE currently acceptable techniques present in a bacteriology laboratory are not suitable for sterility testing of canned food products. When sterility testing is being carried on concurrently with other work in the laboratory (in the case of experiment 1, examination of swollen cans from inoculated packs was being done in the same laboratory), chance contamination can be as high as 10%.

Elimination of the above source of contamination by conducting sterility testing in a positive air pressure room, shows that the single most important source of contamination remaining is the investigator himself. Precautions must be taken to eliminate this source by covering or cleansing as much of the exposed surface of the investigator as possible. The precaution which appears most important is the wearing of a face mask. Elimination of this source (face and nasal passages), virtually eliminates contamination.

The occurrence of one positive in 2,032 cans examined, using the rigid precautions outlined, emphasizes the importance of including the proper controls in the sterility testing procedure, for although chance contamination can be severely reduced, it can probably never be completely eliminated. Product smear, pH, odor and appearance, and condition of the container must be considered before the data can be intelligently evaluated and the occurrence of a positive tube be attributed to organisms isolated from the product or organisms introduced through chance contamination. The inoculation of two tubes per sample along with the use of exposure plates to evaluate levels of contaimination should be practiced routinely.

The occurrence of nonheat resistant organisms (e.g. cocci) in subculture tubes is generally attributed to contamination, since it is felt that these organisms would not survive any commercial heat process.

Only if a container defect can be demonstrated are these organisms suspected of being isolated from the product. The data presented indicate that in addition to cocci, heat resistant forms and particularly PA organisms can also be involved in contamination. Heat resistant forms have been isolated from subculture tubes and have been demonstrated to occur on the hands of the investigators on several occasions. Washing the hands with antiseptic soap does not eliminate these organisms.

Improper analysis of canned foods can give rise to erroneous recoveries of microorganisms. For meaningful interpretations of subculture recoveries, it is absolutely essential that all testing be carried out under sterile conditions, avoiding the use of microbiology laboratories where microorganisms are routinely exposed to the atmosphere and to working surfaces, and eliminating contamination contributed by the investigators.

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LEVELS OF DDT ISOMERS IN TURNIP GREENS AFTER BLANCHING AND THERMAL PROCESSING

INTRODUCTION

USE OF PESTICIDES has been an important factor in increasing the yield of crops throughout the world. Unfortunately, residues of pesticides sometimes remain on the products and such persistence has received considerable attention. Research has been directed toward ascertaining the effects of processing on pesticide levels of foods. Tressler (1947) determined that 1,1,1-trichloro-2,2-bis(p-chlorophenyl)-ethane (DDT) was stable to heat in water and to acid buffers in pyrex containers, but was slightly decomposed in these media when heated in tinplate containers. DDT was decomposed to an even greater extent when heated with various food products. Ott and Gunther (1965) observed that DDT was decomposed to 1,1-dichloro-2,2-bis(p-chlorophenyl)ethane (DDD) during the process of analyzing the pesticide residue on a gas chromatograph. They theorized that the reaction was a reductive dechlorination of DDT as a result of contact with the iron present in the stainless steel chromatographic columns. Langlois et al. (1964) and Fleck and Haller (1944, 1946) indicated that iron and certain other metals decomposed DDT.

Several investigators demonstrated that processing and storage decomposed DDT as well as certain other pesticides. Hemphill et al. (1967) observed that the degradative loss of DDT could not be explained on a time-temperature basis. They concluded that the composition of the container walls might be a factor in the loss of DDT. This was consistent with findings which resulted from a study on snap beans that were heat processed or frozen (Carlin et al., 1966). After 11 months of storage, the DDT content was reduced to zero in canned beans and by more than one-half in frozen beans, as compared with the unwashed product.

Farrow et al. (1966) and Lamb et al. (1968) detected the conversion of DDT to DDD during thermal processing of spinach in plain tinplate cans, in enamel lined cans and in glass test tubes. Hollowell (1968) reported this same conversion in turnip greens and indicated that DDT-fortified samples showed evidence of "feathering" of the can enamel. Thus, he theorized that the discoloration of the enamel was due to the presence of DDT which could have extracted chlorophyll from the greens and deposited the pigment along with the DDT on the can enamel.

Moss (1970) initiated a study to investigate the phenomenon suggested by Hollowell (1968) and reported that the can lining of epoxy-phenolic enamel did have the ability to sorb p,p′-DDT and to catalyze its decomposition further to o,p′-DDT. Moss reported that the enamel did not sorb DDD or 1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene (DDE) during the thermal processing of turnip greens.

This study was conducted to determine the effects of epoxy-phenolic enamel in steam-jacketed kettles during blanching and in cans during thermal processing on removal of DDE, DDD, o,p′-DDT from turnip greens.

EXPERIMENTAL

THE STUDY was conducted with Purple Top White Globe Turnips (*Brassica rapa*) which were hand-planted on August 9, 1971, at the University of Tennessee Plant Science Farm, Knoxville. The experiment consisted of three plots 9.8m x 12.2m and each plot was handled as a replication. The seedlings were sprinkle irrigated once with lake water.

On September 7, a 100-g sample was selected at random from each plot. The sample (no DDT spray applied) was washed in approximately 18.9 liters of water (26°C) for 1 min and allowed to drain for 3 min. Each sample was chopped, mixed thoroughly, and three, 25-g portions were selected and placed into individual 0.35 liter screw-cap jars. The jars were sealed and the material was stored at −27°C.

After samples were collected on September 7, a 50% wettable powder commercial formulation of DDT was applied to the plant surfaces at the rate of 0.68 kg active ingredient in 113.5 liters of water per 0.405 hectare (acre). The spraying procedure was repeated on September 14 and 24, and on October 1.

On October 4, a 100-g sample was selected at random from each plot. Each sample (DDT previously applied) was washed for 2.5 min in 18.9 liters of water at 35°C followed by a similar wash at 20°C. The greens were drained 3 min after which they were chopped, mixed and three, 25-g portions were selected and each portion was stored in a jar at −27°C.

On October 5, greens from plot 1 were harvested by hand and divided into four, 3.2 kg sublots. Each sublot was washed 2.5 min in 68 liters of water at 35°C followed by a similar wash at 20°C. The greens were chopped and mixed thoroughly.

Sublot 1 was further divided into two, 1.6 kg portions. The first portion was blanched by steam in an atmospheric blancher for 3 min, cooled by a spray of tap water, and allowed to drain for 3 min. A 100-g sample was selected from the blanched greens, from which three, 25-g portions were selected and stored separately in sealed jars at −27°C. From the remaining greens, three, 330-g portions were selected and each portion was placed into a No. 303 x 407 (7.7 cm x 10.3 cm) plain tinplate can to be thermally processed. The second portion of sublot 1 was treated in a manner similar to the first portion, except that the three, 330-g portions of greens were placed in enamel-lined cans to be thermally processed. A 100-g sample was not selected.

Sublot 2 was subdivided similarly to sublot 1, but the greens were blanched in water at approximately 100°C for 3 min in a steam-jacketed aluminum-lined kettle (used throughout study) containing 7.6 liters of water. Sampling was similar to that in sublot 1 with the same number being prepared.

Sublot 3 was treated similarly to sublot 2, except the kettle was lined with a section of epoxy-phenolic enameled tinplate with the enamel directed toward the inside. Sampling was similar to sublot 2. The kettles were scrubbed thoroughly with detergent and water after each operation and the enamel liners were replaced after use once with unused ones.

Sublot 4 was treated similarly to sublot 2, but with the following exceptions: the blanching time was 5 min; the first 1.6 kg portion of greens was blanched in an unlined kettle but the second 1.6 kg portion was blanched in an enamel-lined kettle. From each 1.6 kg portion, one 100-g of greens were taken, from which three, 25-g portions were selected and each was stored in sealed jars at −27°C. No samples of greens were thermally processed.

After all cans were filled with greens, 2g of table salt and 100 ml of boiling water were added to each can. The cans were sealed immediately, (initial temperature at least 60°C) placed in a retort, and the greens were processed at 120°C for 45 min (National Canners Association recommendation). After processing, the contents of the cans were cooled by allowing tap water to flow over the can for 20 min. The contents of each can were blended separately for 60 sec in an Osterizer blender from which a 25-g sample was selected, sealed in a jar and stored at -27°C. This 25-g sample from each can was utilized later for analysis of pesticide residues.

Greens from plots 2 and 3 were harvested

and processed on October 6 and 7, respectively. Processing was performed according to procedures given for the first harvest. All 25-g samples were stored at −27°C until analysis for pesticide residues.

The Mills, Onley and Gaither procedure for extraction and cleanup of nonfatty vegetables was used to prepare the samples for analysis by gas-liquid chromatography (GLC) (Mills et al., 1963). A single elutant was used which consisted of six percent ethyl ether in petroleum ether. After the elutant was evaporated to dryness, the residue was redissolved in 10 ml of n-hexane and transferred to a 15 ml flint glass bottle for storage at 4°C.

GLC was carried out with a Micro-Tek Model MT-220 gas chromatograph with a Nickel-63 electron capture detector. Pyrex glass columns (U-shaped, 1.83m long, 4 mm ID) were packed with 3% OV17 on Gas Chrom Q, 100/200 mesh. The operating conditions were as follows: nitrogen carrier gas flow rate, 50 ml/min with an inlet flow of 2.81 kg/sq cm (40 psi); column temperature, 235°C; inlet temperature, 230°C; and detector temperature, 300°C. The aliquot for injection was 0.4 μl for samples taken immediately prior to harvest, and 1 μl for all other samples. The samples collected prior to the first application of DDT were not diluted before injection, but all other samples were diluted to one-tenth.

Standard solutions of p,p(-DDT, o,p'-DDT, DDD and DDE were obtained from Analabs, Inc. (Hamden, Conn.). The standards were obtained in concentrations of 100 ppm and were diluted with n-hexane to working standards of 5, 1, 0.1, 0.01 and 0.001 ppm. The concentrations of the injected known isomers were quantified by calculating the product of peak height x width of peak at half height. A standard curve was developed from which concentrations of isomers in test samples were\determined.

Samples for residue analysis consisted of three taken prior to spraying; three taken after spraying but prior to harvesting; nine each from sublots 1, 2 and 3; and six from sublot 4. Thus, the number of samples per replication was 39 with a total of 117. One GLC reading was made per sample.

The data of each isomer were handled separately. Analysis of variance procedures were used to test the significance of specific orthogonal contrasts (these contrasts were determined a priori). The design was a randomized complete block. The 0.10 level of probability was used (Sanders, 1971).

RESULTS & DISCUSSION

WHEN THE PRESPRAYED greens were analyzed, two peaks were produced on the chromatogram which indicated that o,p'-DDT (0.8 ppm) and p,p'-DDT (1.7 ppm) were present. Since DDT had not been applied to the land for at least 10 yr, it seems unlikely that the peaks were due to DDT. It has been established that polychlorinated biphenyl (PCB) compounds give rise to chromatographic peaks similar to those of DDT isomers with the use of certain types of columns and electron capture (Reynolds, 1969). Recently differentiation of PCB's from DDT has been accomplished by separation on silica gel (Snyder and Reinert, 1971) and by carbon-skeleton chromatography (Asai et al., 1971). Since the greens had been sprinkle irrigated with lake water, it is reasonable to suspect that the peaks might have been caused by PCB's. Other crops grown on the same land in previous drought periods were irrigated in a similar manner. The source of water was the Tennessee River Embankment (Tennessee Valley Authority) and the point of intake was about 6 km downstream from the city.

At the time of harvest the residue level of DDT observed in the greens was excessively high. The high concentration, which was intentional, is attributed to the fact that more applications of pesticide material were made than were necessary to control insects and that greens were harvested before the once recommended waiting period after the final application of DDT (21 days for most greens, but no tolerance or time given for turnip greens) (USDA, 1971).

The mean concentration (ppm) of pesticide residues present in the turnip greens after being blanched are presented in Table 1. There were no significant differences in the residue levels of greens when blanching was by steam or by water, in unlined or in enamel-lined kettles, or for 3 or 5 min.

Greens which were steam blanched and subsequently thermally processed in plain tinplate or enameled cans did not contain significantly different levels of residue (Table 2). Greens that were water blanched 3 min in an unlined kettle and subsequently thermally processed resulted in a significantly higher level of DDE in greens from enameled cans than from

Table 1–Concentration (ppm) of pesticide residues in turnip greens after being subjected to blanching[a]

Process treatment	DDE	DDD	o,p'-DDT	p,p'-DDT
Blanching method				
Steam-atmospheric pressure[b]	2.3a	0.8d	6.7g	11.1j
Water – 100°C[c]	2.2a	0.7d	6.3g	9.5j
Kettle lining[d]				
Unlined	2.2b	0.8e	6.1h	9.0k
Lined with enamel	2.2b	0.6e	6.4h	10.1k
Time (min)[d]				
3	2.2c	0.6f	6.1i	9.3m
5	2.3c	0.8f	6.4i	9.7m

[a]Pairs of means within each column are not significantly different (P < 0.1).
[b]Mean of 9 observations
[c]Mean of 36 observations
[d]Mean of 18 observations

Table 2–Concentration (ppm) of DDT isomers in turnip greens after being subjected to blanching and thermal processing[a]

Process treatment	DDE	DDD	o,p'-DDT	p,p'-DDT
Steam blanched - atmospheric pressure[b]				
Plain can	1.5a	1.3h	6.2m	1.6t
Enameled can	1.4a	1.3h	6.1m	1.8t
Water blanched in unlined kettle - 3 min[b]				
Plain can	1.3c	1.1i	6.0n	1.5u
Enameled can	1.7b	1.4i	5.2n	1.1u
Water blanched in enamel-lined kettle - 3 min[b]				
Plain can	1.0d	1.2j	4.2o	1.0v
Enameled can	1.3d	1.0j	4.4o	1.0v
Blanching method				
Steam[c]	1.4e	1.3k	6.1p	1.7w
Water[d]	1.3e	1.2k	5.0q	1.1x
Kettle lining - min[c]				
Unlined	1.5f	1.3l	5.6r	1.3y
Lined with enamel	1.1g	1.1l	4.3s	1.0y

[a]Pairs of means within each column not followed by the same letter are significantly different at the (P < 0.1).
[b]Mean of 9 observations
[c]Mean of 18 observations
[d]Mean of 36 observations

Table 3—Concentration (ppm) of DDT isomers in turnip greens before and after processing

	DDE	DDD	o,p'-DDT	p,p'-DDT
Before processing - fresh[a]	4.4	1.0	15.6	25.2
After washing and blanching[b]	2.2	0.7	6.3	9.8
After thermal processing[c]	1.3	1.2	5.3	1.3

[a]Mean of 9 observations
[b]Mean of 45 observations
[c]Mean of 54 observations

tinplate cans. Thus, it is concluded that the enamel of the cans did not decrease the total residue level over that of tinplate as originally theorized. The level of other isomers was not different for greens processed in the two types of cans.

The level of residues which remained in greens that had been water blanched 3 min in an enamel-lined kettle and subsequently processed in the two types of cans was not significantly different.

Following thermal processing, the levels of o,p'-DDT and p,p'-DDT were significantly less in greens that had been water blanched compared to greens that were steam blanched.

Levels of DDE and o,p'-DDT were significantly less on greens that had been water blanched 3 min in an enamel-lined kettle and processed compared to greens that had been water blanched 3 min in an unlined kettle and processed. The kettle lining was an important factor in reducing DDE and o,p'-DDT from the greens. However, the can enamel did not reduce the level of isomers over that of tinplate.

Both treatments, washing and blanching and thermal processing, decreased the levels of all isomers except DDD in the fresh greens (Table 3). DDD was increased slightly by thermal processing, resulting from the reductive dechlorination of p,p'-DDT. The data, although not analyzed statistically, show the initial levels of pesticide residues present in the greens and the over-all effects of treatments on the levels of residues. The o,p'-DDT content of fresh greens represented 34% of the total isomers, whereas, the level in commercial DDT is about 22%; the reason for this is not clear.

Results of this study indicate that the epoxy-phenolic enamel of the kettle during blanching did exhibit a sorptive capacity for two of the DDT metabolites. However, the enamel of the cans did not produce a similar effect. Thus, the results of this study seem to refute, at least partially, those of Moss (1970) and Hollowell (1968).

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IN-PLANT, CONTINUOUS HOT-GAS BLANCHING OF SPINACH

INTRODUCTION

CONCERTED EFFORTS to abate pollution will force drastic changes in food processing during the next decade. Many operations which produce large volumes of waste materials will be supplanted by new processing systems. A substantial research effort is required to insure that the new processing systems will produce food which is sanitary, wholesome, nutritious and safe, and at the same time have practical production rates and costs.

Vegetable blanching prior to preservation by freezing, canning or dehydration is an operation which generates large volumes of strong liquid wastes. For the seven vegetable commodities processed in largest quantities, an average of 40% of the total BOD generated during processing results from the steam or hot-water blanching step (Anonymous, 1971). The conventional blanching methods leach out components, especially water soluble vitamins and minerals, which reduce the nutritional value of the final product.

Two relatively recent innovations in vegetable blanching namely fluidized bed blanching (Mitchell et al., 1968) and IQB (Lazar et al., 1971) have been demonstrated to reduce the wastewater volume; however, neither of these systems has the potential for almost complete elimination of wastewater.

A pilot plant study of four blanching systems (microwave, hot-gas, steam and hot-water) demonstrated exceptional promise for hot-gas blanching as an effective blanching method which generated almost no liquid waste (Ralls et al., 1972). This present report describes a study of hot-gas blanching of washed spinach using a side stream of commercial material in a cannery.

EXPERIMENTAL

THE HOT-GAS BLANCHER used in these studies is shown in outline drawings in Figure 1. The washed spinach was conveyed through a heated zone boxed around the conveyor. The combustion products from a 125,000 BTU/hr natural gas burner were forced through the bed of spinach by a 3800 standard cu ft/min blower. Steam could be injected into the blower discharge duct. Part of the combustion gas mixture was released through two damped vent pipes and the remaining gas mixture recirculated through the blower.

Washed spinach was taken from the commercial line of the cannery just before it went to a paddle-wheel spreader and entered the steam and hot-water blancher. The washed spinach was transferred by hand from a wheeled cart to a moving conveyor belt. The washed spinach was delivered to the feed conveyor of the hot-gas blancher and was spread by hand to get uniform loading on each flight and to avoid overloading. After passing through the hot-gas blancher, the spinach dropped on a moving conveyor belt and was delivered to the commercial conveyor system which took all blanched spinach to the container filling lines.

Small samples of hot-gas blanched spinach were taken during short duration runs, filled into cans, brined and thermally processed. The sample cans were subjected to the established quality control tests used by the cannery. The cannery management was satisfied with the results of the inspection and allowed all of the hot-gas blanched spinach to go into commercial production during the 4 days when the hot-gas blancher was operated for periods of 4 hr or longer.

Wastewater samples were collected hourly from the steam condensate line of the hot-gas blancher. The volume was measured and portions were analyzed for COD and SS (Anonymous, 1969).

The wastewater from the commercial blancher was collected at two points (steam condensate overflow and hot-water overflow) and analyzed for COD and SS.

Peroxidase levels were determined for washed spinach and blanched spinach (Dietrich et al., 1970).

Canned samples of spinach from the commercial blanched material and the hot-gas blanched material were analyzed for vitamin and mineral content (Horwitz, 1970), headspace gas composition, vacuum, drained weight, flavor difference and preference (National Canners Association, 1968).

Six 303 × 406 cans of spinach coded D23W7/SLAG6 were placed in a 30°C incubator for 4 days. The cans were then cooled at room temperature for 4 hr and at 2°C for 4 hr.

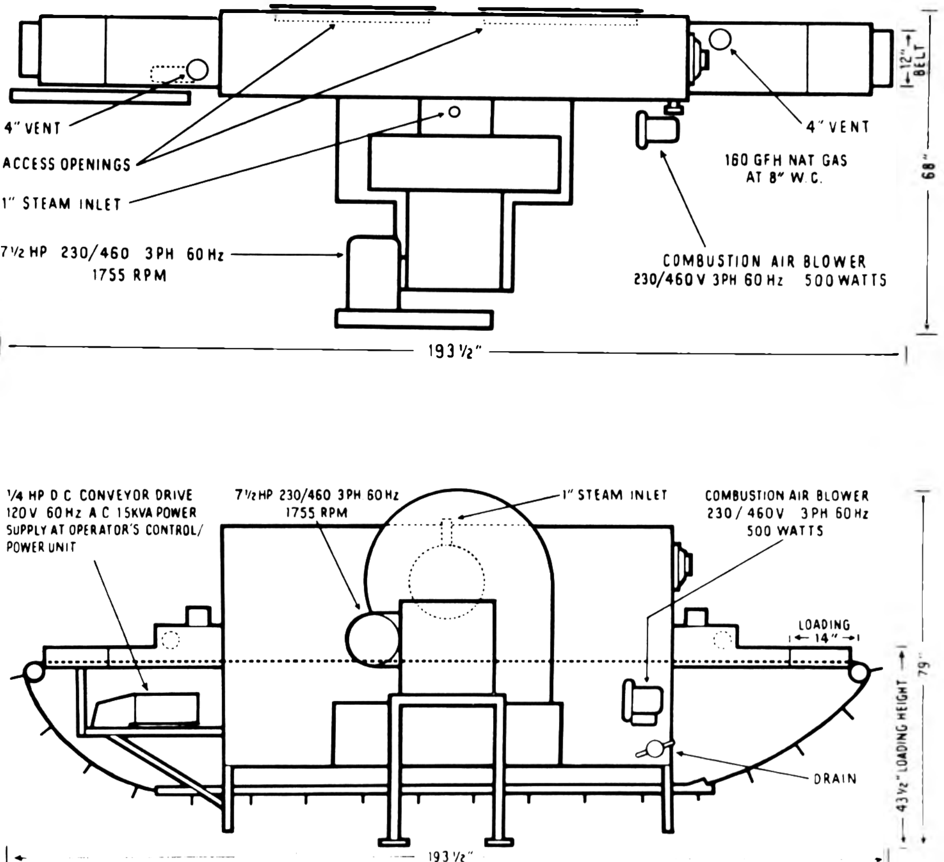

Fig. 1—Outline drawing of hot-gas blancher: (top) top view; (bottom) side view.

This temperature cycle was chosen to promote slow crystal growth and formation of large crystals if any were to form.

The six cans were weighed, a puncture vacuum reading made, and drained weights determined. The drained liquid contents were filtered through a tared filter paper. The filter papers were dried overnight at room temperature and weighed. The dried filter papers were examined under a dissection microscope (800 × magnification) for presence of crystalline material.

RESULTS & DISCUSSION

THREE RUNS of 6, 6.5 and 7 hr duration were completed with wastewater monitoring. The weight of washed spinach blanched during these runs was 2637, 1724 and 3448 lb, respectively. The residence time of spinach in the heated zone of the hot-gas blancher was 108 sec for all three runs at operating temperatures of 104–118, 99–121 and 93–110°C, respectively. Under these conditions the peroxidase activity in the blanched spinach was reduced to 1–3% of that in the raw, washed spinach.

The blanched spinach was wilted sufficiently for a trouble-free filling operation. No determination of weight loss based on measurements of solids content of washed and hot-gas blanched spinach was attempted.

The volume of wastewater collected from the hot-gas blancher during the three runs was 0.44, 0.18 and 0.097 gal, respectively. The volume weighted averages for COD were 50,000, 130,000 and 158,000 mg/l, respectively. The volume weighted averages for SS were 6,100, 2,700 and 5,000, respectively. The wastewater discharged from the commercial blancher was measured at two points: (1) steam condensate overflow which had a volume of 1800 gph and (2) hot-water make-up overflow which had a volume of 3600 gph. The average COD of these wastewaters were 4100 and 300 mg/l, respectively. The average SS were 110 and 80 mg/l, respectively.

The data comparing the wastewater generation and pollutional loading from hot-gas and commercial steam and hot-water blanching of washed spinach are summarized in Table 1. The volume of wastewater (steam condensate per ton for hot-gas blanching was lower for runs number 3-28 and 3-29 since steam injection was used for run number 3-27 only. The water held on leaf depressions of the washed spinach was converted to steam within the heated section of the hot-gas blancher obviating the need of steam injection to humidify the recirculating hot gases.

Numerous tests of hot-gas blanched spinach were performed by quality control specialists employed by Tillie Lewis Foods, Inc. to convince the company management that all quality and processing specifications would be met in the event that cans from commercial production contained only hot-gas blanched spinach. In a few early experiments on March 22 and 23, the use of hot-gas blanched spinach gave cans having slightly low total weights. By lowering the operating temperature the total weight, net weight and drained weight were within normal ranges.

Technical persons from the canning industry pointed out two concerns about hot-gas blanching of spinach. The first was that hot-gas blanching would not remove occluded gases (especially oxygen) from spinach tissue. Samples of hot-gas and commercial blanched spinach in 603 × 700 cans packed on March 29 were analyzed for composition of headspace gases on March 31. The results of the analysis are tabulated in Table 2. The data show that the percentage oxygen content of the headspace gases in hot-gas blanched samples of canned spinach is lower than that in the headspace gas from commercially (hot-water) blanched samples.

The higher content of carbon dioxide found in hot-gas blanched samples of canned spinach suggested that oxygen in the tissues was being displaced by carbon dioxide during the exposure to combustion gases in the hot-gas blancher.

The second concern expressed was the possibility of lower rates of removal of oxalic acid during hot-gas blanching of spinach as compared to hot-water blanching. Brine from six cans of spinach were filtered to collect particulate material.

When viewed through a microscope, filter papers from samples 1, 2 and 3 (hot-gas blanched) showed more crystal formation (including some clusters of crystals) than filter papers from samples 4, 5 and 6 (hot-water blanched) which also showed crystals but fewer crystal clusters. The average weight of solids collected was 0.075 for samples 1, 2 and 3 and 0.052 for samples 4, 5 and 6.

It was concluded by the co-authors that the amount of crystalline material formed in cans of hot-gas blanched spinach would be too small to be detected by a consumer visually or by mouthfeel.

The hot-gas samples show lower vacuum, lower gross weight and higher drained weight. The average drained weight of 11.3 oz approaches the maximum drained weight of 11.7 oz established by NCA for thermal processing considerations. Data on hot-gas blanched spinach samples collected by Tillie Lewis Foods, Inc. show a similar trend of lower vacuum and net weights and higher drained weights compared to commercial samples (private communication). These results can be explained from the fact that the hot-gas blanched spinach has a lower moisture content, higher solids content and higher carbon dioxide content. The rehydration of the spinach in the closed can leads to the higher drained weights and the release of carbon dioxide from the spinach tissue produces a lower headspace vacuum. These differences between hot-gas and hot-water blanched spinach do not appear to be large enough to cause any concern about adequacy of process or for difficulty in meeting quality standards. It would be necessary to change check weight limits and brine addition weights for commercial filling of cans in an operation using all hot-gas blanched spinach to more frequently hit the middle of the target ranges.

Taste panel results

A triangular set of commercially blanched and hot-gas blanched spinach was presented to a panel after the canned spinach had been stored 10 wk at room temperature. In a total of 57 individual judgements obtained in four sessions (using a randomized presentation of the samples) 29 were correct (significant at the $p = 0.01$ level). Therefore, there is a significant difference in canned spinach prepared by commercial hot-water blanching or hot-gas blanching. A ranking test of flavor preference using three commercial canned samples and the hot-gas blanched spinach (canned under commercial conditions) was made. Statistical evaluation of the results of the ranking test

Table 1—Comparison of hot-gas and commercial hot water blanching of spinach

Run no.	Wastewater gal/raw ton		COD produced lb/raw ton		SS produced lb/raw ton	
	Hot-gas	Commercial	Hot-gas	Commercial	Hot-gas	Commercial
3-27	0.33	740	0.15	9.7	0.013	0.44
3-28	0.20	630	0.33	8.3	0.012	0.53
3-29	0.06	680	0.050	9.2	0.002	0.62

Table 2—Canned spinach headspace gas analysis

Blanching unit	Percent of			
	N_2	CO_2	Argon + O_2	H_2
Hot-gas	87.6	10.7	1.7	0
Commercial (hot water)	88.5	4.3	5.3	1.9

(Kramer, 1963) showed no significant difference among the four samples. It was concluded from the results of these taste panel evaluations that hot-gas blanched canned spinach had a different flavor than hot-water blanched canned spinach and that this flavor would be accepted by consumers.

Vitamin and mineral content

Analysis of two cans of hot-water blanched spinach gave an average carotene value of 5.4 mg/100g total can contents. The average carotene content of three cans of hot-gas blanched spinach was 3.9 mg/100g total can contents. The average contents of riboflavin from analysis of two cans each of hot-water and hot-gas blanched spinach were 0.12 and 0.10 mg/100g total can contents, respectively. These limited results were not sufficient to establish a difference in vitamin retention in canned spinach due to the method of blanching used. Because of can-to-can variation in vitamin content and the lack of precision in analytical methods used, a study beyond the scope of this present project would be required to rigorously establish if hot-water blanching and hot-gas blanching produced canned products with different retentions of carotene and riboflavin.

Due to less time consuming analysis for ascorbic acid and since initial analyses showed substantial differences due to the blanching condition used, a more detailed study of the content of this vitamin in canned spinach was made. The averages of duplicate determination of six cans each of hot-water blanched and hot-gas blanched spinach for ascorbic acid content were 20.8 and 34.2 mg/100g total can contents. Analysis of the individual results by Student's t-test showed that the difference between the two blanching treatments was significant at the 95% confidence level.

Single analyses of two cans each of hot-water and hot-gas blanched spinach showed no major differences in calcium, magnesium or phosphorus content.

It can be concluded from this study that the new process for hot-gas blanching produces a commercially acceptable canned spinach. The higher percentage retention of ascorbic acid compared to hot-water blanching retention suggests that hot-gas blanching of spinach may yield a nutritionally improved canned product with respect to certain water-soluble vitamins. The most significant finding in the study was the reduction of wastewater volume by 99% and pounds of COD/ton by 96% when hot-gas blanching was substituted for hot-water blanching. Hot-gas blanching may offer relief from sewer surcharges in a food processing operation discharging liquid waste to an overtaxed treatment plant.

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HEAT PROCESSING EFFECTS ON PHYSICAL AND CHEMICAL CHARACTERISTICS OF ACIDIFIED CANNED TOMATOES

INTRODUCTION

IN FOOD CANNING, one of the most important chemical characteristics of the food is its pH. pH is an important factor influencing the activity and heat resistance of enzymes and microorganisms in foods.

Unless pH is 4.3 or lower, *Bacillus coagulans* may survive heat processing in canned tomatoes and cause flat-sour spoilage. Since 1959, pack failures caused by microbial growth have been associated with new tomato varieties having a relatively high pH. Furthermore, mechanical harvesting of tomatoes causes substantial contamination with soil-borne thermoduric microorganisms, and a pH lower than 4.3 is likewise desirable to prevent spoilage. A pH higher than 4.6 creates conditions favorable for growth of *Clostridium botulinum* and toxin production.

The use of acidulants for adjusting pH values of tomatoes to a desired range is of practical importance. The 1966 amendment to the FDA Standards of Identity for canned tomatoes authorized the addition of edible organic acids to canned tomatoes for acidification purposes. However, questions arise as to the degree of effectiveness of various acidulants and possible reactions that may take place between them and the product.

Cameron and Esty (1940) classified foods having a pH ranging from 4.5–3.7 as acid foods and included the tomato in this group. The heat processing of acid foods in boiling water is considered adequate since *C. botulinum*, which survives long processing at this temperature, has not been grown experimentally at pH values of 4.6 or lower (Townsend et al., 1954). However, flat-sour spoilage by *Bacillus coagulans* frequently occurs near pH 4.5. Work by Rice and Pederson (1954b) has shown that a pH as low as 4.3 is advisable to prevent spoilage in tomato products by *B. coagulans*.

Surveys made during 1959 and 1961 (Farrow, 1963) indicated that some then popular canning tomato varieties exhibited a high frequency of pH values above 4.5 from all regions of the United States. A concurrent increase in product spoilage has been observed and associated with the higher pH trend (Adams, 1961; and Lopez et al., 1968).

Synergism between *C. botulinum* and other microorganisms confounds the problem. In 1933, three cases of botulism poisoning by home canned tomatoes were reported in Canada (Doleman, 1964) but no data were available on pH values or other contaminants that may have been present. Slocum et al. (1941) studied home canned tomatoes that produced botulism toxin type B. The pH was between 4.0 and 4.2 but there was some doubt that the toxin was actually produced at this pH range since a *Bacillus* sp. and a coccus type of bacterium were also found. A follow-up study failed to reveal that toxin could be produced synergistically.

The pH buffer system of the tomato is complex and variable depending on variety, growing conditions, harvesting method, holding conditions and processing methods (Andreotti and Ceci, 1955; Kattan et al., 1956; Daskalov, 1957; Gould, 1957; Mahdi et al., 1959; Freeman and Woodbridge, 1960; Leonard et al., 1960; Villareal et al., 1960; Hamdy and Gould, 1962; Lamb et al., 1962; Davies, 1966; and El Miladi et al., 1969).

Pectinic substances account for a portion of the acidity of tomatoes (Katchalski, 1954; Hulme, 1970). The observed dissociation constants for pectinic substances range from pKa of 3.0–5.0 depending on the degree of methanolic esterification. The higher pKa value is associated with higher esterification.

Acidity in tomatoes is increased when greater amounts of potassium are supplied through fertilizer (Davies, 1964). Nitrate fertilizers have been found to increase the organic acid content of tomatoes (Clark, 1936). Other workers such as Culvern (1948) and Bradley (1960) found similar trends. Bradley (1960) and Davies (1964) postulated that tomatoes synthesize acids to buffer against high potassium levels.

Anderson (1957) found pH and acidity differences between fruit of the same plant. It has not been feasible to control fruit pH by adjusting field conditions. If pH is to be controlled, product acidification by additives is necessary.

Wall (1940) found increased ammonium and amino nitrogen in potassium deficient tomatoes. This is part of the mechanism of the tomato fruit for maintaining its buffer system. Similar results were obtained by Gulyakin et al. (1965).

The acid fraction in the fresh tomato has been found to be very complex, with citric and malic acids being the most predominant in terms of milli-equivalency. Bradley (1960) found citric, malic, phosphoric, pyrrolidone-carboxylic, galacturonic, acetic, lactic and fumaric acids in that approximate order to account for nearly all the acids in tomato puree samples. Carangal et al. (1954) found a ratio of 10:1 for citric to aconitic acids in raw tomatoes. Rice and Pederson (1954a, c) found no aconitic acid in tomato juice after processing. Hamdy and Gould (1962) studied heat sensitive acids in eight varieties of tomato. They found citric acid to be reduced through processing. Dihydroxytartaric acid was detected in a few varieties but none was found after processing. Pyruvic acid was unstable during extraction, and heat processing caused further loss. Alpha-keto-glutaric acid increased during processing with the increase being attributed to deamination of glutamic acid. The presence of pyrrolidone-carboxylic acid (PCA) in tomatoes has been studied by Mahdi et al. (1959) who found that PCA was produced from glutamine during heat processing and storage of tomato juice. The concentration by weight of PCA becomes second to that of citric acid (Mahdi et al., 1959; and El Miladi et al., 1969).

McElroy and Munsell (1938) measured contents of about 16.4–24.7 mg ascorbic acid per 100g in five varieties of fresh tomatoes. The contribution of ascorbic acid to pH is probably not great since its pKa value is high at 4.17 (White et al., 1959).

Tomato enzymes are involved in chemical changes that take place in the buffer system during heat processing. Most work in this area has involved pectic enzymes. Recently, Luh and Daoud (1971) have shown that the "break temperature" for juice preparation must reach 83°C for 15 sec to inactivate tomato pectin esterase (PE) or 104°C for 15 sec to inactivate tomato polygalacturonase (PG). The preliminary action of PE is to de-esterify pectin before the pectic acid produced can be broken down by PG (Luh et al., 1954). These reactions present the possibility of pH changes during tomato processing operations. Metabolic enzymes may have a complex role during heat processing. Since the amount of mito-

chondrial protein is reduced more rapidly than that of soluble protein during ripening (Padwal-Desai et al., 1969), maturity is a factor in the activity of enzymes in tomatoes.

The application of acidulants for canning tomatoes in the Eastern United States has been investigated (Pray and Powers, 1966; Powers, 1967; Lopez et al., 1968; and Lopez and Schoenemann, 1971). The addition of 350 mg of citric, malic, fumaric, phosphoric, adipic, or succinic acid had little or no adverse effects on flavor and appearance. Although phosphoric acid is not included as an optional ingredient in the FDA Standards of Identity for canned tomatoes, Lopez et al. (1968) recommended that this acid be proposed for use in tomatoes since it is widely used in soft drinks and offers a cost advantage over edible organic acids. The same authors found the approximate cost of fumaric acid to be 62% and phosphoric acid 28% of citric or malic acid on an acid-equivalent weight basis.

The objectives of the research reported here were to determine the effects of heat processing and of various acids on physical and chemical characteristics of acidified canned tomatoes of several varieties, and to determine whether significant changes in pH or in other characteristics take place during product storage.

EXPERIMENTAL

Materials

Acid and salt tablets were commercially prepared as described by Lopez et al. (1968). Each acid tablet contained calcium sulphate, sodium bicarbonate and 350 mg of citric, malic, or fumaric acid. After effervescing, the remaining acidity (functional acidity) was 4.25, 4.00 and 4.81 meq for citric, malic and fumaric acids, respectively. The salt tablets were composed of calcium and sodium chlorides as described by Lopez and Schoenemann (1971). A 5% phosphoric acid solution was prepared from 85% phosphoric acid and 7 ml added to each can. The functional acidity for phosphoric acid was 7.1 meq as HPO_4 + $2H^+$. The additives were used at a rate of one tablet in each can. When phosphoric acid was used, one salt tablet was also added.

All tomatoes canned in this study were packed in #303 × 406, F-enameled tinplate cans.

Tomatoes of uniform size, ripeness, and freedom from blemish were selected to be canned whole. Other tomatoes were quartered, extracted and heated in a steam-jacketed kettle to 66°C for juicing. The whole tomatoes were washed, scalded in boiling water for 1 min, hand-peeled, cored and trimmed. 12 oz of tomatoes were weighed into the cans followed by additives and hot juice to a total fill of 16 oz. The cans were then exhausted in steam to a final juice temperature of 66°C and sealed in a steam-flow closing machine. Duplicate sets of samples were processed at 99°C for 15 and 45 min in a still retort. The cans were then cooled in running tap water for 15 min to 35°C (95°F). Cans were cased and stored at 24°C. 3 wk were allowed for equilibration of solutes before any determinations were made. The samples processed for 15 min were given a lower heat treatment than the 45 min recommended to commercially sterilize the product so that heat effects could be studied at different levels of heat treatments.

The experimental packs were made at VPI& SU using LaBonita, Campbell 28, and STEP 539 varieties of tomatoes grown in South Carolina, and Campbell 17 and Roma varieties grown in the Eastern Shore area of Virginia. Tomatoes of canning red ripeness were used in all packs.

Samples for the first phase of study consisted of LaBonita, Campbell 28 and STEP 539 tomato varieties packed without additives and with malic and fumaric acid tablets. The objective in this phase was to identify major chemical and physical changes associated with the length of heat processing time. Determinations were made of pH, titratable and alcohol soluble acidities, refractive index, reducing sugars, amino nitrogen, drained weight, can vacuum and acid dissociation.

The second phase of this work consisted of Campbell 17 and Roma tomato varieties packed with salt citric, malic and fumaric acid tablets and pho phoric acid solution. The objective of this ph e of the research was to study changes in the nonvolatile acids, lactic, oxalic, fumaric, succinic, malic, citric, pyrrolidone-carboxylic and phosphoric. Water insoluble solids, pH, titratable acidity and refractive index determinations were also made to establish interdependencies among these variables. Cans were stored for 6 months at 24°C before analysis.

Determinations were replicated five times in the first phase of study using 80 cans of tomatoes and two times in the second phase using 40 cans of tomatoes.

Analytical methods

Vacuum was measured in canned tomatoes using a Bourdon-type gauge at 25°C. Percent drained weight was determined according to the procedure described by Lopez (1969).

The entire contents of each can were blended in a Waring Blendor with 10 drops of a thymol-toluene solution. pH and titratable acidity were determined immediately after blending using a Corning glass-electrode pH meter at 25°C and by titrating 20g blended tomato diluted in 30 ml H_2O to pH 8.0 with 0.1N NaOH solution. Refractive index was measured on filtered sera at 20°C by an Abbe refractometer. Tomato blends from LaBonita, Campbell 28, and STEP 539 varieties were extracted with ethanol for further chemical analysis. Blends from Campbell 17 and Roma varieties were frozen for determination of nonvolatile acids.

Alcohol extracts containing solutes from 0.2g tomato per ml of 70% ethanol were used for classification of sample compounds. Alcohol soluble acidity was measured by titration of 20 ml extract with 0.02N NaOH solution to pH 8.0. The alcohol extract was used for determination of reducing sugars by the method of Sumner and Sisler (1944) and for the determination of alpha amino nitrogen by the method of Rosen (1957).

Titration curves of alcohol insoluble tomato acidities were determined on alcohol insoluble solids after reconstitution with water to the original volume. The alcohol insoluble solids were separated from canned tomatoes using the method of Kertesz and McColloch (1950).

Frozen blended Campbell 17 and Roma tomatoes were thawed for ortho-phosphoric acid determination using a procedure developed by Allen (1940) and for gas-liquid chromatography of nonvolatile organic acids. 4g of each blend was weighed into a 50 ml beaker and mixed with 20 ml acetone and 1 ml of wet Amberlite IR 120 (H^+). After filtering, washing the residue, and evaporating the acetone, the solution was allowed to percolate for 30 min through a column containing 15 ml of Dowex 1 × 8 formate. After washing with 200 ml H_2O, the acids were eluted from the column with 100 ml 6N formic acid into a 250 ml vacuum flask. Volatiles were evaporated from the acid mixture on a rotary type evaporator at 50°C (122°F) until the acids were apparently dry.

The isolated acids from 4g tomatoes were scavenged of moisture by 0.5 ml $SOCl_2$ for 15 min at 70°C. 50 ml freshly prepared anhydrous methanol containing 5% HCl was added to the vacuum flask and stoppered with a polyester-wrapped neoprene stopper. The methylating mixture was placed in a drying oven at 70°C for 1 hr, after which the mixture was cooled and evaporated at 0°C. Corrosives were removed by co-distilling with 10 ml of anhydrous methanol. Remaining esters were dissolved in 50 ml freshly prepared anhydrous methanol for sample injection into the gas chromatograph.

Esters of lactic, oxalic, fumaric, succinic, malic, aconitic, tartaric, citric and pyrrolidone-carboxylic (PCA) acids individually and combined were prepared from reagent grade acids by esterification as in the above procedure for tomato acids. Standard curves were prepared by esterifying known quantities of each acid.

The esters were separated and identified by gas-liquid chromatography (GLC) using a Perkin-Elmer Model 900 gas chromatograph with dual column flame ionization detectors. The columns used were 3ft × ¼ in. ID stainless steel, packed with 10% DEGS (diethylene glycol succinate) on 100/200 mesh acid washed chromosorb WAW, prepared by Supelco, Inc., Bellefonte, PA 16823. The injection port and detector temperatures were both 300°C. The column oven temperature was programmed from 90–210°C at 12°C/min and maintained

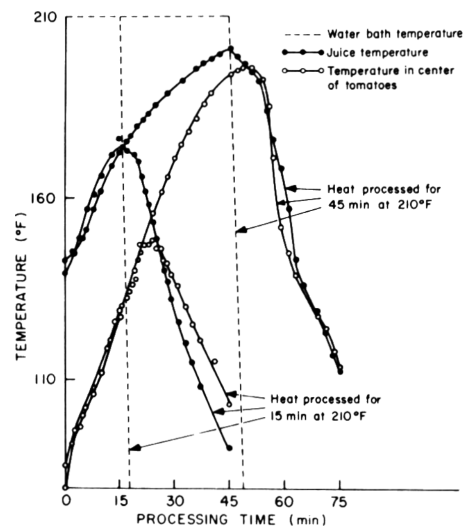


Fig. 1—Heat penetration curves for the liquid and fruit centers of canned tomatoes of unknown variety, heat processed for 15 and 45 min at 99°C in still water bath with subsequent cooling in running tap water for 30 min.

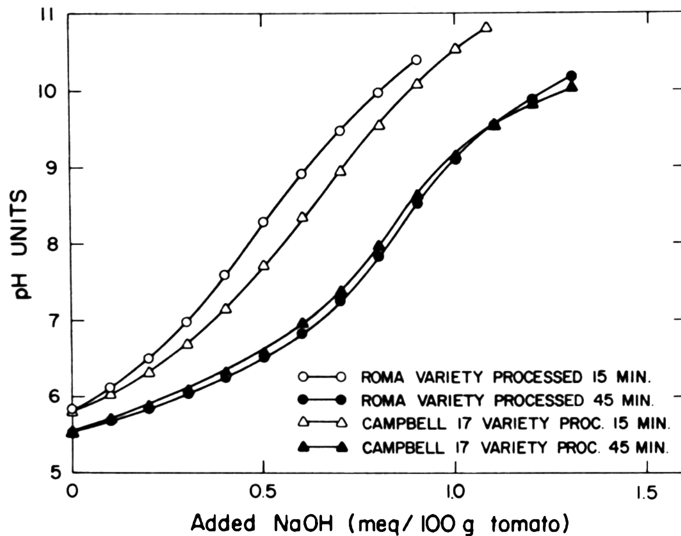


Fig. 2–Acid titration curves of alcohol insoluble solids from Roma and Campbell 17 tomato varieties packed with additives and heat processed for 15 and 45 min at 99°C in #303 × 406 cans.

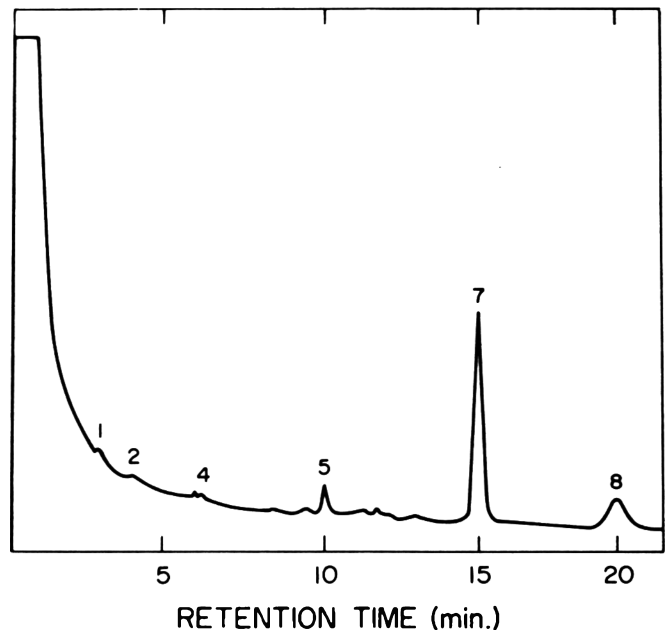


Fig. 3–Typical gas chromatogram of methyl esters of natural organic acids in canned tomatoes.

isothermal at 210°C for 10 min. Gas flow rates were 75, 100 and 35 ml/min for carrier N_2 in each column, air and hydrogen in each detector. The sample size was 1 μl. Peak areas were quantitated by multiplying the height by the width at half-height in mm. Acid concentrations were estimated by computer programming acid dissociation constants, pH, titratable acidity and GLC data with adjustment of acidities by subtracting alcohol insoluble acidities and phosphoric acid contents in individual samples.

Computer programs were written in FORTRAN IV language and compiled by WATFIV compiler (Cress et al. 1969) on IBM 360 or 370 computers at the VPI&SU Computer Center.

Most statistical application to the data was by a packaged program, Statistical Analysis System, written by Barr and Goodnight (1971). The program used options capable of most common designs based on factorial, regression, or mixed model analysis. Partial correlations among dependent variables were calculated after adjustment by independent variables in each problem studied. Significance probabilities were determined in the program for each estimated parameter by variance ratio or student t-testing. Duncan's Multiple Range testing of all means were made at a 5% level of probability.

RESULTS & DISCUSSION

Heat penetration curves

Figure 1 shows heat penetration curves for canned tomatoes packed for this study. A maximum internal fruit temperature of 66°C was obtained during the 15 min process. Kertesz (1939) recommended a temperature of 80°C for 45 sec to inactivate pectin esterase (PE) which indicates that probably this enzyme and, more likely, others such as polygalacturonase (PG), remained active after heat processing for 15 min at 99°C. The internal fruit temperature reached 93°C in the cans processed 45 min at 99°C.

Overall tomato pH differences due to the length of heat processing time were not significant in either phase of study as shown in Table 1. However the total acidities were 5.99 and 6.51 meq/100g versus 6.09 and 6.80 meq/100g for the respective 15 and 45 min heat processes of the two phases of study. Although the differences in total acidities were signifi-

Table 1–Effect of heat processing for 15 and 45 min at 99°C on pH, total acidity, PCA, alcohol soluble acidity, reducing sugars, refractive index, can vacuum and drained weight of acidified canned tomatoes[a]

Variable	Phase I[b] Processing time 15 min	Phase I[b] Processing time 45 min	Phase II[c] Processing time 15 min	Phase II[c] Processing time 45 min
pH	4.28	4.28	4.07	4.08
Total acidity (meq/100g)	5.99	6.09	6.51	6.80
Pyrrolidone-carboxylic acid (meq/100g)			0.50	0.61
Alcohol soluble acidity (meq/100g)	4.26	4.42		
Alcohol insoluble acidity (meq/100g)	1.73	1.67		
Reducing sugars (%)	3.14	3.05		
Refractive index	1.3417	1.3415	1.3398	1.3401
Can vacuum (in. Hg)	10.1	10.9		
Drained weight (%)	65.4	62.3		

[a]Means with the same underscore are not significantly different from one another at a 5% level of probability.

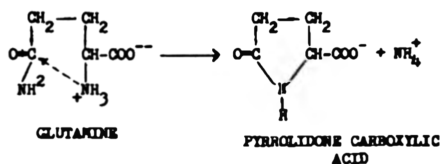
[b]Includes LaBonita, STEP 539, and Campbell 28 varieties

[c]Includes Roma and Campbell 17 varieties

cant ($P < 0.05$), the measured alcohol soluble and insoluble acidities were not found to differ significantly at $P < 0.05$. This was a result of a greater variability in these acid fractions.

Table 1 also shows that the refractive indexes gave reverse heat processing trends between the two phases of study. The differences were small but significant in each case with the mean refractive index being 0.0002 higher and 0.0003 lower for the 15 min compared to the 45 min heat process in the respective first and second phases of study. The mean reducing sugar contents were statistically the same for the two processing times but this was a probable result of a lack of precision in the determinations.

Other means in Table 1 gave indications that the 45 min heat process may have affected small but significantly greater nonenzymatic changes than did the 15 min process. The mean value for pyrrolidone-carboxylic acid (PCA) was 0.11 meq/100g higher in the tomatoes processed 45 min. The drained weight was significantly lower by 3.1% while the can vacuum was 0.8 in. Hg higher. The formation of PCA from glutamine is often found to result from heat processing of fruits and vegetables. No pH changes associated with PCA formation were found or anticipated since the pK values of the products are similar to those of the reactant (glutamine). This reaction would not produce any acidity changes since no ammonium or carboxylate ion concentrations are changed as indicated below:



Higher can vacuums were likely to result from higher oxidation rates during the longer heat process.

Differences in the alcohol insoluble fraction caused by the length of heat processing is further indicated by Figure 2. The acid titration curves of the alcohol insoluble solids from Roma and Campbell 17 tomatoes were similar for both processing times with the buffering capability having been either reduced in the 15 min process or increased in the 45 min process by solubilization of pectin. The former case is the probable cause since the lower heat processing time would permit greater pectinesterase (PE) activity in the early part of processing. The demethylating action of PE on pectin is known to reduce its solubility which removes this important acid (pectinic acid) from the tomato buffering system. This trend would be slightly reversed by the activity of polygalacturonase in digesting pectic acid producing water soluble short chain acid molecules.

Since differences in the mean values of the variables studied were small and often nonsignificant, the calculation of partial correlation coefficients among the variables within each heat processing group affords a better understanding of changes that took place during and after processing.

Partial correlations among variables of the first phase of study are shown in Table 2. The effect of heat processing on changing relationships among these variables is evident for refractive index, acid dissociation, reducing sugar, alcohol soluble and insoluble acidities, amino nitrogen and drained weight values. The acid dissociation showed a negative relationship (−0.738) with the refractive index of the raw tomato samples. This suggests that one variable is affected differently by heat processing from the other. After heat processing positive relationships developed between acid dissociation and reducing sugars (0.301 and 0.319). This indicates that the samples within each processing group have higher sugar contents when the tomato acids are more highly dissociated. Such a positive relationship is not likely to be caused by higher sugar contents accompanied by low acidity since this relationship was actually shown to be positive in the samples processed 15 min (0.379, $P < 0.01$). Opposite trends between samples processed 15 min (−0.620) and those processed 45 min (0.344) are notable between the alcohol insoluble acidity and reducing sugars. These trends support the likelihood that a higher proportion of sugars in the tomatoes processed 15 min is galacturonic acid produced from the pectin fraction through enzymatic action permitted by the lower temperatures achieved during the 15 min heat processing (Fig. 1). The high correlation of 0.732 ($P < 0.01$) between the amino nitrogen and alcohol soluble acidity of tomatoes processed for 45 min raises a probability that while a major portion of the sugar fraction was galacturonic acid in the tomatoes processed 15 min, amino acids composed a significant portion of the acidity of tomatoes processed for 45 min. This was further substantiated by the correlation of −0.562 ($P < 0.07$) between amino nitrogen and acid dissociation of tomatoes processed for 45 min. The can vacuum of tomatoes processed for 45 min was positively correlated with its total acidity (0.303, $P < 0.02$)

Table 2—Effects of heat processing on partial correlation coefficients[a] among physical and chemical characteristics of raw and of acidified canned tomatoes of Phase I of study[b]

	Correlation coefficients (r) with probabilities (P) according to t distribution					
	Raw		15 min processing		45 min processing	
Variables correlated	(r)	(P)	(r)	(P)	(r)	(P)
Refractive index vs.						
Acid dissociation	−0.738	0.02	−0.075	0.64	−0.009	0.94
Acid dissociation vs.						
Reducing sugars	0.000	0.99	0.301	0.05	0.319	0.01
Alcohol soluble acidity vs.						
Reducing sugars	−0.167	0.67	0.379	0.01	−0.015	0.90
Alcohol insoluble acidity vs.						
Reducing sugars	−0.123	0.75	−0.620	0.00	0.344	0.01
Alpha amino nitrogen[c] vs.						
Alcohol soluble acidity			0.095	0.79	0.732	0.01
Acid dissociation			−0.011	0.97	−0.562	0.07
Can vacuum vs.						
Total acidity			0.056	0.72	0.303	0.02
Drained weight vs.						
Refractive index			−0.183	0.24	0.239	0.06

[a]Partial correlations were made after adjustment by packs, additives, and net product weight in heat processed samples or by packs in raw samples.

[b]Includes LaBonita, STEP 539, and Campbell 28 varieties

[c]Amino nitrogen was only determined in Campbell 28 variety

Table 3–Gas-liquid chromatographic retention times and prediction equations derived from flame ionization detector responses for methyl esters of known organic acid standards

Peak no.	Methyl ester	Retention time (min)	Prediction equations[a] (b x 100)	a	S_e	r
1	Lactate	3.2	0.78	0.20	0.07	0.995
2	Oxalate	4.2	1.43	0.11	0.16	0.998
3	Fumarate	5.6	0.22	0.29	0.07	0.988
4	Succinate	6.2	0.17	0.19	0.18	0.969
5	Malate	9.9	0.03	0.25	0.30	0.989
6	Aconitate	11.9	0.18	0.08	0.11	0.989
(T)	Tartrate	13.6	0.43	0.33	0.03	0.996
7	Citrate	14.8	0.26	0.02	0.44	0.992
8	Pyrrolidone-carboxylate	19.4	0.20	0.11	0.20	0.960

[a]Equation: $Y = a + bX$, where X is the FID response in in.^{2} and Y is the weight of the respective acid in µg before methyl ester derivation. The linear correlation coefficients, r, are all significant at 1% level of probability with nine degrees of freedom for error.

Table 4–Effects of heat processing on partial correlation coefficients[a] among physical and chemical characteristics of acidified canned tomatoes of Phase II of study[b]

Variables correlated	15 min processing (r)	15 min processing (P)	45 min processing (r)	45 min processing (P)
Total acidity vs.				
Oxalic acid	0.537	0.09	–0.347	0.30
Phosphoric acid vs.				
Pyrrolidone carboxylic acid	0.524	0.10	–0.033	0.92
Acid dissociation	0.400	0.22	0.666	0.02
Refractive index vs.				
Fumaric acid	0.099	0.77	0.552	0.08
Acid dissociation	0.125	0.71	0.517	0.10
Lactic acid vs.				
Oxalic acid	0.894	0.00	0.861	0.00
Fumaric acid	0.166	0.63	0.794	0.00
Succinic acid	0.662	0.03	0.949	0.00
Malic acid	0.311	0.65	0.803	0.00
Pyrrolidone carboxylic acid	0.151	0.66	–0.625	0.04
Oxalic acid vs.				
Fumaric acid	0.029	0.93	0.653	0.03
Succinic acid	0.687	0.02	0.827	0.00
Malic acid	0.263	0.56	0.539	0.08
Fumaric acid vs.				
Succinic acid	0.228	0.50	0.880	0.00
Malic acid	–0.058	0.86	0.684	0.02
Succinic acid vs.				
Malic acid	–0.346	0.30	0.721	0.01
Pyrrolidone carboxylic acid	–0.184	0.59	–0.550	0.08
Malic acid vs.				
Pyrrolidone carboxylic acid	0.147	0.67	–0.560	0.07
Citric acid vs.				
Pyrrolidone carboxylic acid	–0.916	0.00	–0.686	0.02

[a]Correlations were determined after adjustment for variety and additive.
[b]Includes Roma and Campbell 17 varieties

which suggests that the acid concentration may have accelerated the reaction of the tomatoes with oxygen in the cans.

Figure 3 illustrates a typical gas chromatogram of tomato acids after methyl esterification. The peaks are identified by their retention times shown in Table 3 as esters of lactic, oxalic, succinic, malic, citric and pyrrolidone-carboxylic (PCA) acid. PCA was second in concentration to citric acid as is expected in heat-processed tomatoes. The simple correlation coefficients (r) calculated for each ester and its flame ionization detector response shows that the relationships were all linear and highly significant. Although tartaric acid is not normally found in tomatoes, it was included as a check for the stability of its secondary alcohol groups against the effects of thionyl chloride.

The only acid significantly altered by the length of heat processing was PCA (see Table 1). None of the other acids including citric, malic, fumaric, succinic, lactic and phosphoric acids differed significantly between the two heat processes.

Partial correlation coefficients between each acid and the other variables concurrently determined with them are illustrated in Table 4. Phosphoric acid was correlated with acid dissociation in the samples processed 45 min ($r=0.666$, $P < 0.02$). The correlations among all the acids were more significant in the samples processed 45 min except between citric acid and PCA ($r= -0.916$, $P < 0.00$) in the samples processed 15 min.

All the correlations among the acids of the samples processed 45 min were positive, except those correlated with PCA, and were significant at probability levels ranging from 0.00–0.08. Close correlations among concentrations of individual acids imply that they normally coexist in higher concentrations or they imply that some may be produced from others through enzymatic or nonenzymatic chemical reactions. If the second implication applies to the samples of this study, the reactions would have been nonenzymatic since they were most evident in the samples processed 45 min. The greater can vacuum (10.9 in. Hg) for the samples processed 45 min shows that oxygen may have been used to drive these reactions. Spot tarnishing of the cans was noticeable and indicates that chemical reactions with tin or iron were other chemical reactions that took place during the 6 months in which the canned tomatoes were stored before analysis. The hypothetical scheme in Figure 4 was devised to show reactions among acids that could give correlations that are shown in Table 4.

The role that enzymes play in affecting compositional changes in the Kreb's related acids of tomatoes during heat processing is still not clear. Heat proc-

Table 5–Effect of additive and processing time interaction on pH of canned tomatoes heat processed at 99° C[a]

Additive x processing time	Phase I[b] pH	Phase II[c] pH
Nothing added		
Raw	4.46A	
15 min	4.46A	
45 min	4.45A	
Salts		
15 min		4.24A
45 min		4.25A
Salts with citric acid		
15 min		4.17B
45 min		4.19B
Salts with fumaric acid		
15 min	4.18C	4.10C
45 min	4.16D	4.12C
Salts with malic acid		
15 min	4.21C	4.14BC
45 min	4.22B	4.18B
Salts with phosphoric acid		
15 min		3.70D
45 min		3.64E

[a]Means within each phase of the study and having the same letter attached are not significantly different from one another at a 5% level of probability.

[b]Includes LaBonita, STEP 539, and Campbell 28 varieties

[c]Includes Roma and Campbell 17 varieties

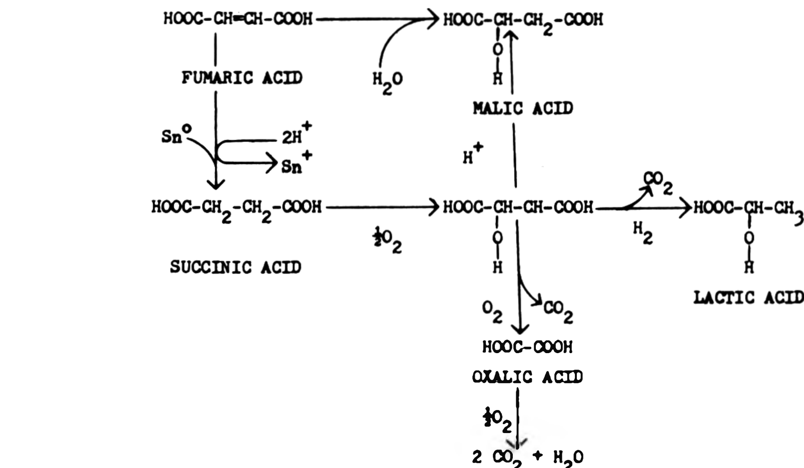


Fig. 4–Hypothetical scheme to show reactions among acids that could give correlations shown in Table 4.

essing at lower temperatures such as that which occurred in the samples processed for 15 min or those processed at slower heat penetration rates will likely cause enzyme activities favoring the more thermoduric enzymes. Hamdy and Gould (1962) using a high-temperature short-time process of 116°C for 2.5 min found an increase in α-ketoglutaric acid and a decrease in citric acid in tomato juice. In later work, El Miladi et al. (1969) using a holding process of 104°C for 20 min to process tomato juice in hermetically closed cans observed opposite trends for the same two acids. The process in hermetic cans could permit a more extended activity of thermoduric enzymes because of the slower penetration of heat into cans, but these investigations did give sufficient evidence that the changes were induced enzymatically. The samples of Hamdy and Gould would more likely be exposed to greater oxidation in the plate-type heat exchanges. Citric acid is subject to oxidation to other compounds and α-ketoglutaric acid is produced by nonenzymatic oxidative deamination of glutamic acid.

The lack of correlations between any of the individual Kreb's acids and other variables of the samples processed 15 min leads to the conclusion that mitochondrial activity had ceased early in the heat processing for both 15 and 45 min times. Some of the variability of the characteristics measured was probably accountable to the stage of ripening in the individual tomato fruit.

The interaction effects of additive and processing time on pH were significant but small, as shown in Table 5. None of the mean pH changes due to heat processing time was greater than 0.06 pH for acidified canned tomatoes.

The quantity of phosphoric acid added to the tomatoes in this study was greater than that which would be required to prevent spoilage. The resultant pH means of 3.70 and 3.64 adversely affected the flavor by lowering the sugar-acid ratio. However this degree of acidification illustrated the capability of the tomato fruit to resist adverse changes during cooking at low pH values.

As a whole, the results of this investigation show that citric, malic, fumaric and phosphoric acids maintain a pH desirably reduced for preservative qualities without accelerating physical or chemical changes during heat processing.

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USE OF AMMONIUM COMPOUNDS FOR CHLOROPHYLL RETENTION IN FROZEN GREEN VEGETABLES

INTRODUCTION

THE USE OF alkaline additives to retard the formation of pheophytins from chlorophylls by hydrogen ions has been studied for a number of years. Numerous patents (Bendix et al., 1952; Blair, 1940a, b; Malecki, 1958, 1959, 1965; Stevenson and Swartz, 1942) have been obtained for addition of alkaline compounds to conventionally heat-processed green vegetables. These patents involve the use of organic and/or inorganic salts of the alkali and alkaline earth metals.

Gupte and Francis (1964) reported that although the use of $MgCO_3$ in combination with HTST (300°F) processing initially improved chlorophyll retention in puréed spinach, the effect was not stable during storage. Other sodium and magnesium compounds tested were less effective than $MgCO_3$.

Clydesdale and Francis (1968) reported that $MgCO_3$, used in conjunction with low temperature (155°F) blanch of spinach, increased the formation of chlorophyllides from chlorophylls and decreased the decomposition of both chlorophylls and chlorophyllides to pheophytins and pheophorbides, respectively. However, chlorophyllides were not found to be more stable than chlorophylls to the action of the H^+ ion. The positive effects of using $MgCO_3$ during blanching were subsequently lost when over 90% of chlorophyll a was destroyed during heat processing.

Gieseker (1949) patented the use of 0.1N NH_4OH in various steps in the thermal processing of peas. The ammonium compound was used since the author recognized that sodium compounds caused undesirable softening, calcium compounds undesirable toughness and magnesium compounds the formation of hard white crystals of $MgNH_4PO_4$.

Few studies have been found on the use of alkaline compounds for improvement in color of frozen green vegetables. Although the problem in these vegetables is not as great as in canned green vegetables, improvement in color retention during cooking and especially during steam table holding is of interest. Sweeney and Martin (1961) improved color retention in frozen green beans by adding a citrate-phosphate buffer (pH 6.6–6.8) to the cooking water but found that texture deteriorated during steam table holding. Similar results were reported by Clydesdale et al. (1971) for frozen green beans, peas and spinach cooked in solutions containing phosphate buffer (pH 7) or $MgCO_3$. In another study, Sweeney (1970) found that the addition of Ca acetate to $MgCO_3$ in a 1:2 ratio improved texture of green beans held on a steam table for 30 min.

The present study was undertaken to determine the feasibility of using ammonium compounds as alkaline additives to prevent chlorophyll losses in cooked frozen green vegetables. The advantages of ammonium compounds were thought to be: (1) their decomposition to NH_3 gas which would neutralize volatile acids to form NH_4^+ salts; (2) the absence of metal ion residues in the vegetable such as is the case with alkali or alkaline earth metal salts; and (3) the safety of the additive, since a number of ammonium compounds are listed by the Food and Drug Administration (1969) as "generally recognized as safe" for use as buffers and neutralizing agents.

EXPERIMENTAL

TWO EXPERIMENTS were conducted. In the first, NH_4OH was used in an attempt to neutralize volatile acids during microwave blanching or cooking broccoli in confined containers. In an earlier study (Eheart, 1970) soaking broccoli in a citrate-phosphate pH 7 buffer solution prior to microwave blanching in baggies was found to be ineffective in preventing the excessive chlorophyll losses due to volatile acids dissolving in the condensed H_2O vapor and dripping back on the broccoli. Blanching in baggies had been found necessary to prevent desiccation by microwaves. In the second experiment, NH_4HCO_3 was used in the blanching and/or cooking of frozen green beans by conventional methods. The salt was thought to be an improvement over NH_4OH since it is not associated with household detergents and as a solid could be handled more easily, especially for home use.

NH_4OH-treated broccoli

Three replications of Coastal variety of broccoli were harvested at Salisbury, Md., transported to the laboratory and stored in air at 36°F (34°–38°F) for 0 and 2 days. At each storage period, 300-g lots of 5 in. radially cut pieces of broccoli were treated as follows: (1) blanched 3 min in boiling water; (2) blanched 2 min in a microwave oven in a loosely-closed baggie containing 50 ml of 0.24M NH_4OH or; (3) cooked 7 min under the same conditions described in (2). The concentration of NH_4OH used was established in a preliminary study. This amount (12 meq) was about twice as much as the average total acids in raw broccoli but was found necessary for effective color improvement. All samples were refrigerator-cooled, packed in freezer bags/waxed cartons and frozen/stored at –15°C for 5 mo. Broccoli which had been H_2O-blanched was conventionally cooked in 150 ml H_2O in a covered pyrex utensil for 11 min. That which had been microwave-blanched was cooked in 40 ml H_2O in a covered pyrex casserole in the microwave oven for 6 min. Microwave-precooked broccoli was reheated in the microwave oven in 40 ml H_2O in a covered casserole for 1½ min, the frozen mass broken apart and heating continued for 1¾ min. The combined pretreatment and cooking methods were found in preliminary work to give cooked broccoli of a similar degree of doneness as judged subjectively. Two samples from each replicate for each storage period were analyzed before and after re-heating or cooking for dry matter, pH, titratable acidity, reduced ascorbic acid and chlorophylls (2x3x2x3x2 = 72 samples).

Dry matter was determined by drying samples to constant weight (72 hr) at 65°C in a forced-draft oven. For titratable acidity, samples of vegetable slurry were titrated with 0.05 N NaOH to a pH of 8.1 using a Beckman Model G pH meter. Methods for pH, reduced ascorbic acid and chlorophylls have been previously described (Eheart and Gott, 1965). Chlorophylls were calculated as the percentage of the total pigment present which had not been converted to pheophytin.

NH_4HCO_3-treated green beans

Field-grown Harvester green beans were washed, stemmed, cut into 1½ in. pieces and mixed. Samples of 300g were taken for the following treatments: (1) H_2O blanched-H_2O cooked; (2) H_2O blanched-NH_4HCO_3 cooked; (3) NH_4HCO_3 blanched-H_2O cooked; and (4) NH_4HCO_3 blanched-NH_4HCO_3 cooked. The NH_4HCO_3 solutions used were 0.1% in all cases, a concentration found in preliminary work to be sufficient to improve color retention but maintain the pH of the vegetable within the critical range of 6–7. The beans were blanched according to Tressler et al., (1968) at 93°C in a thermostatically controlled deep-fat fryer for 4 or 4½ min as predetermined by negative peroxidase test (Masure and Campbell, 1944). Blanched samples were cooled in ice water, packed and frozen as in the experiment on broccoli. After both 5 wk and 6 mo of frozen storage, samples were analyzed before and after cooking (giving six treatments for blanching/cooking) for constituents listed in the first ex-

periment. Samples were cooked in 150 ml H_2O or NH_4HCO_3 solution in a pyrex utensil for time intervals which were subjectively predetermined to give the same degree of doneness. Cooking times for the four treatments listed above were, respectively, 11, 9, 9 and 8 min. Six field replications were studied (6x6x2 = 72 samples).

In addition to chemical analyses, cooked samples (four treatments) were panel-tested after 1 and 5 mo of frozen storage for color, flavor, texture and acceptability. Six replications were studied (6x4x2 = 48 samples).

At each of 12 sessions, a panel of six judges rated four samples (from the four treatments). The beans were served to each judge in coded white styrofoam cups. No physical references were used during the experiment; however, preliminary training of the judges involved use of physical samples to illustrate the terms used on the scorecard. White lights were used throughout the sessions. Color, texture and flavor were evaluated on a descriptive rating scale with seven intervals.

A bipolar scale was used for texture and flavor with optimum at mid-scale. Texture could thus be rated as too firm or too soft and flavor as too bland or having off-flavor. A unipolor scale was used for color. For acceptability a modified 7-point food action scale (FACT) was used which ranged from "would eat very often" to "would never eat" (Schutz, 1965).

Statistical Analyses

Analyses of variance, Duncan's multiple range and correlation coefficients were used to analyze the data. The 5 and 1% levels of significance were used.

RESULTS & DISCUSSION

NH_4OH-treated broccoli

The only significant effects (Table 1) of storage (3°C) of fresh broccoli for 2 days were increases (WB) in total acidity and reduced ascorbic acid. Increases in ascorbic acid during storage of fresh broccoli have been reported previously (Eheart, 1970; Eheart and Odland, 1972).

The use of NH_4OH (Table 1) in microwave blanching was successful in reducing chlorophyll losses. Microwave-NH_4OH blanched samples retained significantly more chlorophyll a and total chlorophyll than the water-blanched whereas in a previous study (Eheart, 1970) microwave-blanched broccoli was significantly lower in chlorophylls than the water-blanched. Even the microwave-NH_4OH pre-cooked broccoli was as high in chlorophylls as the water-blanched. Samples cooked and/or blanched in the alkaline medium were not lower in reduced ascorbic acid than the controls. Moreover, on the wet basis, broccoli precooked in NH_4OH was even higher in ascorbic acid than the control. This was due to the higher dry matter content of these samples. Thus some desiccation occurred in the microwave oven even though baggies and covered casseroles were used. Broccoli blanched in NH_4OH by microwaves was highest in pH and the control was lowest. Total acidity was also lower in the controls than in treated samples, a result which was unexpected and which cannot be explained at present.

Cooking caused a loss of approximately 27% of both reduced ascorbic acid (D.B.) and total chlorophyll. The cooked broccoli contained 79.1 mg of ascorbic acid per 100g (W.B.) and 54.4% unconverted total chlorophyll.

Interactions among the three factor effects, storage, NH_4OH treatment and cooking, were found to be significant only for chlorophyll a, which was reflected in total chlorophyll. Interactions are not given in tabular form. The NH_4OH-microwave precooked samples were the lowest in chlorophyll of the three treatments after freezer storage (uncooked) but the highest after final cooking or reheating. This could be expected since these samples were subjected to more severe heat treatment prior to freezing but to less heat after freezing than the other two treatments. Fresh storage also affected treatment. At 0 days the three treatments did not differ in chlorophyll but after 2 days at 3°C the microwave-blanched samples were higher in chlorophyll than microwave-cooked or water-blanched samples. The reason for this difference is not as yet apparent.

NH_4HCO_3-treated green beans

The pH of both the cooked beans and cooking water (Table 2) increased in the expected order with treatments: H_2O blanched-H_2O cooked, NH_4HCO_3 blanched-H_2O cooked, H_2O blanched-NH_4HCO_3 cooked and NH_4HCO_3 blanched-NH_4HCO_3 cooked. The pH of the beans, however, was lower than 7 in all cases. Titratable acidity varied inversely with pH and the NH_4HCO_3 blanched-NH_4HCO_3 cooked samples contained about one-half the acidity of the control (W.B.). As in broccoli, the alkaline additive did not decrease ascorbic acid content. Although not statistically significant, the samples cooked in NH_4HCO_3 were actually higher in ascorbic acid than corresponding samples cooked in H_2O. That the addition of

Table 1–Effects of fresh storage (3°C), NH_4OH-microwave pretreatment and cooking on the composition of frozen broccoli[a]

Factor	Dry matter (%)	pH	Total acidity[b] (meq/100g) W.B.	Total acidity[b] (meq/100g) D.B.	Reduced ascorbic[b] acid (mg/100g) W.B.	Reduced ascorbic[b] acid (mg/100g) D.B.	Chlorophyll (% not converted to pheophytin) a	Chlorophyll b	Chlorophyll Total
Storage (3°C)[c]									
0 days	12.74	6.73	1.90[b]	14.9	85.5[b*]	683	66.0	61.9	63.6
2 days	13.18	6.73	2.08[a]	15.8	95.3[a]	742	67.0	63.5	64.8
Pretreatment[d]									
Blanched-H_2O-100°C (control)	11.12[c]	6.57[c]	1.50[c]	13.6[b]	80.1[b]	728	64.4[b]	66.7	64.0[b]
Blanched-NH_4OH-MW	13.23[b]	6.85[a]	2.05[b]	15.6[a]	91.4[ab]	706	70.9[a]	62.2	67.2[a]
Cooked-NH_4OH-MW	14.53[a]	6.75[b]	2.42[a]	16.9[a]	99.6[a]	703	64.3[b]	59.2	61.4[b]
Cooking[e]									
Uncooked	12.54[b]	6.71	2.01	15.9	101.6[a]	825[a]	79.5[a]	65.6[a*]	74.1[a]
Cooked	13.39[a]	6.74	1.98	14.7	79.1[b]	600[b]	53.5[b]	59.7[b]	54.4[b]

[a] Means having different superscripts are significantly different ($P < 0.01$) except where noted by *($P < 0.05$).
[b] W.B. = wet basis; D.B. = dry basis.
[c] N = 36 (2 samples x 3 field replications x 3 pretreatments x 2 cookings).
[d] N = 24 (2 samples x 3 field replications x 2 storage periods x 2 cookings).
[e] N = 36 (2 samples x 3 field replications x 2 storage periods x 3 pretreatments).

Table 2–Composition of frozen, stored (–15°C) green beans blanched and/or cooked in NH_4HCO_3 solution [a]

Factor effect	Dry matter (%)	pH		Total acidity[b] (meq/100g)		Reduced ascorbic[b] acid (mg/100g)		Chlorophyll (% not converted to pheophytin)		
		Beans	Cooking H_2O	W.B.	D.B.	W.B.	D.B.	a	b	Total
Blanching/cooking[c]										
H_2O/uncooked	6.94[b]	6.12[d]	–	0.99[a]	14.2[a]	6.2[ab]	90[ab]	59.9[bc]	89.1[a]	65.1[bc]
H_2O/H_2O (control)	8.88[a]	6.11[d]	5.92[d]	1.00[a]	11.2[b]	3.0[c]	34[c]	25.9[d]	56.3[b]	32.7[e]
H_2O/NH_4HCO_3	8.79[a]	6.67[b]	6.92[b]	0.67[bc]	7.5[d]	4.3[abc]	50[c]	50.0[c]	79.6[a]	55.2[d]
NH_4HCO_3/uncooked	7.02[b]	6.44[c]	–	0.79[b]	11.1[b]	6.9[a]	98[a]	76.4[a]	84.7[a]	76.3[a]
NH_4HCO_3/H_2O	8.88[a]	6.42[c]	6.33[c]	0.83[ab]	9.3[c]	3.7[bc]	41[c]	52.6[c]	77.8[a]	57.7[cd]
NH_4HCO_3/NH_4HCO_3	8.78[a]	6.92[a]	7.33[a]	0.54[c]	6.1[d]	5.1[abc]	58[bc]	66.3[b]	80.7[a]	68.7[ab]
Storage (–15°C)[d]										
5 wk	8.00[b*]	6.48[a]	6.75[a]	0.76[b*]	9.6	4.7	62	54.7	87.7[a]	60.1
6 mo	8.43[a]	6.41[b]	6.49[b]	0.85[a]	10.2	5.0	62	55.7	68.4[b]	58.4

[a] Means having different superscripts are significantly different ($P < 0.01$) except where noted by *($P < 0.05$).
[b] W.B. = wet basis; D.B. = dry basis.
[c] N = 12.
[d] N = 36.

alkali did not increase ascorbic acid losses may be explained by control of the pH below 7 and the shorter times necessary for cooking in NH_4HCO_3 solutions. Chlorophyll retention was greatly improved by NH_4HCO_3. Blanching in NH_4HCO_3 increased chlorophyll a retention in the uncooked samples 27% over controls. In cooked samples, the combined use of NH_4HCO_3 for blanching and cooking produced green beans which were more than twice as high in chlorophyll a and total chlorophyll as the controls. Beans blanched or cooked in NH_4HCO_3 solution did not differ in retention of chlorophylls. These samples were considerably higher in chlorophylls than controls but lower than those samples receiving the combined treatment.

Storage of frozen samples for 6 mo at –15°C caused a decrease in pH and an increase in total acids (W.B.), results which confirm an earlier report (Eheart, 1970). Reduced ascorbic acid, chlorophyll a and total chlorophyll not converted to pheophytin did not change during storage. However, there was a significant loss of chlorophyll b. Thus, the improvement in total chlorophyll retention by NH_4HCO_3 blanching held up even after 6 mo of storage.

Interactions between treatment and storage were not significant for any of the constituents studied.

Results for color, as judged by a trained panel (Table 3), were similar to those for chlorophyll retention for the four treatments. Highly significant correlation coefficients were found between color scores and chlorophyll a or total chlorophyll, 0.86 and 0.84 respectively. The mean color score for the combined NH_4HCO_3-treated samples was more than twice as high as that for the control. The single treatments were judged between these extremes with the NH_4HCO_3-blanched being judged greener than the NH_4HCO_3-cooked. The judges were thus able to discern a significant difference between the two single treatments which was not shown by chlorophyll analyses (Table 2). However, the data for these analyses revealed a trend in the same direction. Texture, flavor and total acceptability of all treated samples were judged higher than the controls. There may have been an interrelationship of quality factors involved in scoring samples since white lights were used throughout panel testing and the bias of good color may have affected other scores.

Table 3–Panel test scores of frozen, stored green beans blanched and/or cooked in NH_4HCO_3 solution[a]

Factor effect	Color	Texture	Flavor	Acceptability
Blanching/cooking[b]				
H_2O/H_2O (control)	3.1[d]	5.1[b*]	4.3[c]	4.2[c]
H_2O/NH_4HCO_3	4.8[c]	5.7[a]	4.9[b]	5.2[b]
NH_4HCO_3/H_2O	5.3[b]	5.7[a]	5.3[b]	5.4[ab]
NH_4HCO_3/NH_4HCO_3	6.5[a]	5.7[a]	5.9[a]	5.7[a]
Storage (–15°C)[c]				
1 mo	5.1	5.7[a*]	5.3[a]	5.3[a]
5 mo	4.9	5.4[b]	4.9[b]	4.9[b]

[a] Means having different superscripts are significantly different ($P < 0.01$) except where noted by *($P < 0.05$).
[b] N = 72 judgments on a 7-point scale with 7 being optimum.
[c] N = 144 judgments on same scale as (b).

All quality factors of green beans except color were rated lower by the panel at 5 mo than at 1 mo of freezer storage. Thus color of green beans was maintained during storage as indicated by both panel scores and chemical analysis of total chlorophylls (Table 2).

No significant interactions were found between treatment and storage for any of the quality factors evaluated.

The risk of using NH_4^+ compounds as food additives must be considered. As mentioned earlier, the compounds used in this study were listed by the Food and Drug Administration (1969) as "generally recognized as safe." If the amount of NH_4^+ ion contaminating the vegetable is assumed to be equal to the difference in total acidity of control and treated samples, the broccoli precooked with NH_4OH contained only 0.92 meq/100g of vegetable. Beans which had been both blanched and cooked in NH_4HCO_3 contained even less, 0.46 meq.

The metabolic fate of NH_4^+ compounds in the body has been extensively studied in both man and laboratory animals. Fürst et al. (1969) administered NH_4^+ salts (acetate and chloride) both intravenously and orally to healthy human volunteers in amounts as great as 200 meq per day. These authors concluded that ingested NH_4^+ ion was passed directly to the liver where it was quanti-

tatively changed to urea and excreted. Intravenously infused NH_4^+ ion was transported to tissues where it was used for nonessential nitrogen in protein synthesis. Thus it would seem at the present time that hazardous effects of small quantities of ingested NH_4^+ ions are remote.

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SURVEY OF AEROBIC MESOPHILIC BACTERIA IN DEHYDRATED ONION PRODUCTS

INTRODUCTION

DEHYDRATED onion products are widely used in the formulation of many types of food products. Use levels vary from less than 1% by weight to 10% or more in many types of processed or freshly prepared foods. The patterns of microbial numbers and types characterisitic of the finished onion products are the result of the interaction of various factors involved in their production. These include the high degree of contamination of the raw onions by the soil in which they are grown, the lack of an effective bactericidal heat blanching of the onions, the method of dehydration, and the milling and sizing of the dehydrated product.

Because the aerobic mesophilic bacteria group is most important in measuring the microbiological quality of the product, it is the purpose of this study to define this group qualitatively and quantitatively, and to relate these data to the production methods used and to the microbiological safety of the product.

Vaughn (1951, 1962) investigated various production factors influencing microbiological populations of onions and other dehydrated vegetables processed by the tray-tunnel method. He found that steam blanching of many raw vegetables generally effects a reduction of greater than 99.9% in total plate count. However, after dehydration the unblanched onions generally had high populations, exceeding the counts of all blanched vegetables. In a succeeding report, Vaughn (1970) discussed the incidence and significance of several groups of bacteria in dehydrated onions and garlic. He concluded that it is difficult to select a meaningful quantitative index for microbiological quality control of dehydrated onion products because of the variation of the populations and because of the wide and varied use of the product in food processing.

Vaughn (1951) and other investigators including Prescott (1920a, b), Clague (1936), Clague and Fuller (1936), Haines and Elliot (1944) and Jones (1943) have identified several genera of aerobic bacteria from dehydrated food products. These have included *Bacillus, Pseudomonas, Aerobacter, Escherichia, Achromobacter, Micrococcus, Streptococcus, Lactobacillus* and *Leuconostoc*. However, in spite of serious attempts to isolate health hazard organisms from dehydrated vegetables, no evidence was found of the presence of *Salmonella, Clostridium botulinum* or *Staphylococcus aureus*.

EXPERIMENTAL

DEHYDRATED ONION samples were taken directly from two separate production facilities during the summers of 1970 and 1971. It is believed that these samples were characteristic of typical midsummer production from onions grown in central California. Samples of 10g were mechanically blended for 2–3 min with 180 ml of Butterfield's buffer solution and 10 ml of a 10% filter sterilized solution of sodium sulfite. Anhydrous sodium sulfite was added to the blending medium in a concentration of 0.5% to inactivate the onion bacteriostatic principle as recommended by Wei et al. (1967) to allow the growth of sensitive types at lower plated dilutions in the presence of higher concentrations of onion material.

Plate count agar (Difco) was used for total and spore counts. Pasteurization of the original 1/20 dilutions of blended onion material for the spore counts was accomplished by heating 5 ml quantities in 5 ml sterile glass ampules for 5 min in a water bath at 75°C. Lactic acid bacteria were determined on Ragosa SL Agar (Difco) while the nonspore-forming, nonlactic groups of bacteria were counted using plate count agar with the addition of sterile tylosin lactate (Lilly) in solution to give a concentration of 20 ppm in the medium just prior to pouring the plates. Since the outgrowth of spores and many gram positive organisms are inhibited by this antibiotic, the populations determined on this medium were essentially gram negative rods belonging to *Enterobacteriaceae, Achromobacter*, and *Flavobacterium* groups. *Coliform* populations in production samples were determined using violet red bile agar. All platings prepared in this study were incubated at 35°C for 24 or 48 hr.

Identification of members of the genus *Bacillus* was accomplished using methods described by Smith et al. (1952). Highest dilution platings on plate count agar from 35 randomly selected samples of regular onion powders produced on perforated metal-belt driers were selected for examination. About four colonies of varying morphology from each plating were purified by streaking on the same medium before specific identification procedures were initiated. Thus, the isolates represent those types present in highest numbers and most commonly found in the samples. No nonspore-forming organisms were found among these isolates.

The species identifications of the *Enterobacteriaceae* were done from enrichment fermentations of onion powder in lactose broth rather than in a direct selective medium. This method was chosen in order to overcome the problems presented by sublethally injured cells, well known to those involved in the microbiology of dehydrated foods (Mossel and Vincentie, 1965; Woodbine et al. 1968). 55 samples of 100g of onion powders selected randomly during a 4-wk period were added to 1900 ml quantities of sterile lactose broth, together with 10g of anhydrous sodium sulfite powder in 2 liter wide mouth polypropylene bottles. The sodium sulfite was necessary to allow increase in microbial populations in the presence of the onion bacteriostatic principle. The broths were incubated at 35°C for 24 hr. Surveillance for *Salmonella* was done on all 55 samples by streaking from the lactose broth onto SS agar, HE agar, bismuth sulfite agar and XLBG agar. Also, tetrathionate and selenite-cystine broths were inoculated from the lactose broth for selective enrichment for 18 hr before streaking again on the same differential media.

For the identification of non-*Salmonella* members of *Enterobacteriaceae*, 25 of the lactose enrichment broths were chosen at random for plating on desoxycholate lactose agar. A total of 107 colonies from highest dilution platings were chosen for varying morphology and purified by streaking on Eugonagar (BBL). These colonies were identified using the API system (Analytab Products, Inc., New York) and the Auxotab® system (Wilson Diagnostics, Inc., Glenwood, Ill.) of biochemical identification of enteric bacteria.

The ability of the above procedures to recover and identify members of the *Enterobacteriaceae* was established by inoculated trial runs. Actively growing lactose broth cultures of *Escherichia coli, Enterobacter cloaceae, Procteus vulgaris* and *Salmonella typhimurium* were added to lactose broth sodium sulfite enrichment fermentations in the presence of onion powder. Cultures were diluted so that two low levels of inocula of each species were represented in separate fermentations. The levels were chosen to be equivalent to approximately five and approximately 20 organisms per 100g quantity of onion powder. In all cases, the added organisms were recovered and identified by either the salmonella surveillance or by the other identification systems employed.

RESULTS & DISCUSSION

DIFFERENTIAL bacterial counts for samples of dehydrated onion slices produced by a perforated metal-belt drier and by a tray-tunnel drier are indicated in Table 1 as the percent of samples having less than, or exceeding a given population. The average counts for these fractions of the total numbers of samples are also given. These counts were determined on the products as they were discharged from the dehydration equipment before the milling, screening and packaging operation.

The data indicate that 76% of the slices from the belt drier had total counts less than 100,000 per gram with an average of 27,000, while the remaining 24% of the samples exceeded 100,000, with an average of 240,000. However, the tray-tunnel operation produced only 52% of the samples with less than 100,000 per gram, averaging 34,000, while the remaining 48% of the samples exceeded 100,000 with an average greater than 1 million. Since total counts include all organisms capable of growing on plate count agar, the differential counts listed in the table explain the considerably higher contamination of the 48% of the tray-tunnel samples. The bacterial spore counts were very similar with about 85% of samples from both methods having less than 50,000 spores, with an average of about 12,000. The most significant differences in the products of the two dehydration processes are indicated by the large proportion (40 or 60%) of the samples which had high lactic acid bacteria counts and high nonspore, nonlactic counts (essentially gram negative rods). It was also found that these differences in bacteria type were readily observed by direct microscopic examination of the high count platings of the tray-tunnel dried product. Many more of these colonies were found to be bacteria types other than the large gram positive, spore-forming rods typical of the metal-belt drier product. It was demonstrated that more than 99% of the bacteria colonies examined from the belt drier material were spore-formers, whereas, this type constituted only 40–60% of the colonies from the tray-tunnel product.

These results contrast sharply with the report of Vaughn (1951) who investigated tray-tunnel dehydration of onions. He found that lactic acid bacteria in dehydrated onions comprise at least 90% of the total count and that the majority of the remainder were coliform types. The total counts in that study on 53 samples of tray-tunnel product varied from 12,600 to 7.5 million.

The present study establishes firmly that the primary significant organism type in dehydrated onions is the aerobic spore former. Other organisms from the soil may be present, but their numbers are related more to the pasteurization efficiency of the dehydration system used for processing rather than to the original contamination of the raw materials. Thus, even though raw onions must not be blanched because their enzymatic activity must be preserved, the modern perforated metal-belt onion dehydration process destroys most of the nonspore-forming organisms present and does not allow multiplication during the process time. Therefore, blanching of the raw material is not necessary for control of these organisms.

Table 1—Typical plate counts per gram of regular dehydrated onion slices produced by two methods

Bacteria group counted	No. of samples	% Samples less than % Samples exceeding	Avg counts
Total plate count			
Perforated belt drier	38	76% < 10^5	27 × 10^3
		24% > 10^5	240 × 10^3
Tray-tunnel drier	25	52% < 10^5	34 × 10^3
		48% > 10^5	1,120 × 10^3
Bacterial spore count			
Perforated belt drier	39	84% < 5 × 10^4	11 × 10^3
		16% > 5 × 10^4	130 × 10^3
Tray-tunnel drier	21	86% < 5 × 10^4	13 × 10^3
		14% > 5 × 10^4	97 × 10^3
Lactic acid bacteria count			
Perforated belt drier	39	59% < 400	–
		41% > 400	620
Tray-tunnel drier	23	60% < 400	–
		40% > 400	256 × 10^3
Nonspore, nonlactic count			
Perforated belt drier	39	95% < 400	–
Tray-tunnel drier	23	40% < 400	–
		60% > 400	181 × 10^3

Table 2—Typical plate counts per gram of special rooted and topped onion slices dehydrated on perforated belt drier

Bacteria group counted	No. of samples	% Samples less than % Samples exceeding	Avg counts
Total plate count	28	82% < 10^4	3.2 × 10^3
		18% > 10^4	128 × 10^3
Bacterial spore count	28	82% < 6 × 10^3	1.7 × 10^3
		18% > 6 × 10^3	97 × 10^3
Lactic acid bacteria count	23	79% < 400	–
Nonspore-nonlactic count	28	96% < 30	–

Table 3—Total plate counts per gram of various piece sizes of dehydrated onion products produced on perforated belt drier

Piece size	No. of samples	% Samples less than % Samples exceeding	Avg counts
Powder	35	71% < 800 × 10^3	320 × 10^3
		29% > 800 × 10^3	2,600 × 10^3
Granulated	8	50% < 100 × 10^3	58 × 10^3
		50% > 100 × 10^3	360 × 10^3
Minced	17	59% < 10 × 10^3	5.6 × 10^3
		41% > 10 × 10^3	40 × 10^3
Chopped	9	56% < 5 × 10^3	2.7 × 10^3
		44% > 5 × 10^3	28 × 10^3

Although these investigations were made when both types of driers were used in the industry during the 1970 and 1971 seasons, the older tray-tunnel systems have now been entirely replaced by the more efficient perforated metal-belt dehydrators.

The internal portion of a solid, uninjured, irrigated, California-grown raw onion is essentially sterile. Microbial contamination is associated mainly with the skin and outer scales of the onion, with the outer and inner root crown areas, and with the attached top leafy part of the onion. The complete removal of these areas results in a considerable reduction of bacterial counts in the finished product. Table 2 demonstrates the effective reduction of counts resulting from such procedures. Most of these counts were

Table 4–Spore counts per gram of various piece sizes of dehydrated onion products produced on perforated belt driers

Piece size	No. of samples	% Samples less than / % Samples exceeding	Avg counts
Powder	35	77% < 800 x 10^3	320 x 10^3
		23% > 800 x 10^3	1,400 x 10^3
Granulated	8	88% < 100 x 10^3	31 x 10^3
		12% > 100 x 10^3	240 x 10^3
Minced	17	77% < 10 x 10^3	4.6 x 10^3
		23% > 10 x 10^3	21 x 10^3
Chopped	9	89% < 5 x 10^3	1.5 x 10^3
		11%> 5 x 10^3	16 x 10^3

Table 5–Total plate counts and coliform counts of dehydrated onion products produced on perforated belt driers during a typical month

Product	% Samples less than / % Samples exceeding	Avg Counts
Powder (96 samples)		
Total plate count	56% < 250 x 10^3	200 x 10^3
	44% > 250 x 10^3	330 x 10^3
Coliform count	48% < 1000	480
	52% > 1000	1780
Granulated (97 samples)		
Total plate count	64% < 200 x 10^3	138 x 10^3
	36% > 200 x 10^3	260 x 10^3
Coliform count	82% < 1000	240
	18% > 1000	1770

Table 6–Identification of 140 isolates of aerobic mesophilic spore-forming bacteria from 35 samples of dehydrated onion powders

Species	No. of isolates	% of Total
Bacillus subtilis	35	25.0%
B. licheniformis	32	22.9%
B. cereus	26	18.8%
B. cereus var mycoides	9	6.4%
B. pumilis	14	10.0%
B. firmis	7	5.0%
B. coagulans	6	4.3%
B. megaterium	4	2.9%
B. species	7	5.0%

Table 7–Identification of 107 isolates of Enterobacteriaceae from 25 samples of dehydrated onion powders

Species	No. of Isolates	% of Total
Enterobacter aerogenes	60	56.1%
Enterobacter cloacae	12	11.2%
Enterobacter harniae	4	3.7%
Proteus vulgaris	4	3.7%
Proteus mirabilis	4	3.7%
Proteus morganii	1	0.9%
Serratia marcescens	2	1.9%
Citrobacter	19	17.8%

less than 10,000 compared to the much higher counts for regular onions indicated in Table 1. In these specially prepared onions, as in the regular slices, the total counts consisted almost entirely of spore-formers. The reason that the spore count was invariably somewhat lower than the total count is that spores may vary widely in their resistance to the pasteurization treatment used. Thus, in a heterogeneous population, some portion of the spores may be eliminated by the pasteurization of the sample.

Tables 3 and 4 demonstrate the relationships between piece size and total and spore counts in various dehydrated onion products. Onion powders are consistently higher in bacteria populations (essentially spores) than are the larger piece sizes. The sizing and milling operations on the regular dehydrated pieces from the perforated belt drier (Table 1) tend to concentrate the higher count materials in the smaller piece fractions. However, it should be emphasized that the counts presented here were determined on freshly produced materials. Vaughn (1962) demonstrated that counts almost invariably decrease by 50% or more during storage within 3–6 months. The mechanism of this reduction may be related to sublethal injuries of bacterial cells resulting from the dehydration process. These lesions may become lethal with time.

Table 5 shows the typical significant microbiological characteristics of well-processed regular onion powders and granules as they are produced from perforated metal-belt dehydrators before the natural reduction of counts which occurs during storage. The total count consists essentially of spores, and the coliform count is entirely of nonfecal origin. Here again, the effect of piece size is demonstrated.

Table 6 indicates the identification and frequency of isolation of 140 isolates of aerobic mesophilic spore-formers from onion powders. All isolates belonged to the Group I subdivision of genus *Bacillus*. All species listed are known to be spore formers and are found in soils. None have any health hazard significance except *B. cereus* and *B. cereus* va. *mycoides*. Although outbreaks of food poisoning have been associated with this organism, especially in Europe, they have always been attributed to grossly improper preparation and handling of finished food products at the point of consumption rather than to the mere presence of the organism in the food or food ingredient. Birzu et al. (1968) found that foods consumed during a large institutional outbreak of *B. cereus* food poisoning contained 10^7 to 10^8 organisms per gram of food. Also, Strukova (1967) demonstrated that 250 million cells of *B. cereus* injected intraperitoneally into 10–12g mice caused death in 80% of the animals, while 125 million cells resulted in very few deaths. It is quite obvious that very large numbers of organisms are required to produce significant morbidity in man or animals. However, this organism may consistently be demonstrated in relatively low populations in many raw vegetables, fruits, spices, dried foods and nuts. The primary importance of the aerobic spore-formers in dehydrated onion products is that they are responsible for the entire total plate count. Because they exist as spores in the soil and are essentially unchanged by the processing, they become the typical bacterial microflora of a well-processed onion product.

Table 7 lists the members of family *Enterobacteriaceae* identified from samples of onion powders. The most significant fact of this study is the absence of *Escherichia coli* in 25 samples and the lack of *Salmonella* in any of 55 samples evaluated. Thus, a total of 2,500g of randomly chosen powders yielded no *E. coli* and 5500g produced no *Salmonella*. All of the organism types identified are typically present in soils, dusts and in natural products. They have no health hazard sig-

nificance except that some species may contribute to the standard coliform count. Numerically, they are present only in low populations in metal-belt dehydrator products, normally not exceeding 0.1–0.5% of the total count. The common coliform, *Enterobacter aerogenes*, was represented by more than half of the isolates. *Citrobacter*, frequently confused with *E. Coli* on EMB agar, was second in frequency of isolation. Other genera of aerobic bacteria which have been encountered in dehydrated onion products include *Pediococcus, Lactobacillus, Leuconostoc, Streptococcus* of both lactic and enterococcus types, *Flavobacterium* and *Achromobacter*. Their populations and frequencies of occurrence have not been determined. When they are present in the metal-belt processed products, they must exist as a very small fraction of the total count.

Oval-shaped *Streptocci* similar to the enterococcus have been demonstrated to exist in 9 out of 10 randomly chosen onion powder samples. Other investigators, including Mundt (1961, 1963) have found these organisms to be frequently associated with wild plants not contaminated with fecal materials. The other genera listed have no health hazard significance and are only natural contaminants from the environment.

The results of these studies have shown that well processed perforated metal belt dehydrated onion products may be microbiologically characterized as possessing normal bacterial spore populations ranging from a few thousand per gram in large pieces to several hundred thousand per gram in powders. Coliform counts will generally run in powder from about 200–2,000, with a maximum range from about 100–7,000. Larger piece sizes will usually be much lower in coliforms. However, these types are present only as a tiny fraction of the total count. All products appear to be quite free from aerobic microbiological health hazards, even when they exhibit high total counts. Further investigation will be made of anaerobic and other types of organisms present in onion products.

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EFFECT OF BRINING ON OBJECTIVE TEXTURE PROFILES OF CUCUMBER VARIETIES

INTRODUCTION

TEXTURE is an extremely important criterion of quality in cucumber pickles. The critical test of any new pickling cucumber variety is its inherent capacity to produce a firm, crisp-textured, semi-processed, cured or fermented product termed salt stock or brine stock. Breene et al. (1972) found rather wide differences in textural quality of raw fruit of 24 genetically diverse cucumber varieties. They devised a procedure for objectively determining Texture Profile Analysis (TPA) parameters for cucumbers using the Instron Universal Testing Machine. Cucumber breeders and picklers routinely evaluate textural quality in brined fruit of standard and new varieties by sensory methods and objectively with the Magness-Taylor (MT) and other hand-operated fruit pressure testers which provide only a single overall value. While the Magness-Taylor provides only a single value which is very useful, we felt that there is a need to research other objective methods providing analysis of the components of texture.

Although the literature is replete with references to MT firmness testing of raw as well as brined cucumber fruit, efforts to predict quality of brine stock by evaluating raw cucumbers by this method have been only minimal or peripheral. Sneed and Bowers (1970) undertook to predict quality of the brined product from certain characteristics of raw cucumbers. They studied the relationships of carpel separation, MT firmness and skin toughness as measured by the Chatillion Puncture Test, in raw cucumber fruit (No. 3 and No. 4 size grades) to balloon bloating, lens bloating, skin toughness and firmness in brined fruit. Their analyses, pooled across four unidentified varieties, showed a highly significant positive relationship between carpel separation and balloon bloating. Highly significant inverse relationships were shown between either firmness or skin toughness and balloon bloating. MT firmness measurement of raw fruit gave some indication of the firmness and skin toughness to be expected in salt stock.

The data from a 3-yr study of 15 varieties (Ware et al., 1953) show almost identical MT values in raw and brined stock. Model was highest in MT firmness of No. 1 and No. 2 size raw and brined fruit; brining reduced firmness in Model only from 17.4 to 17.3 lb and from 20.6 to 19.8 lb in these sizes, respectively. Seven varieties were eliminated after the first year because of defects in texture (MT firmness), shape and appearance. It is possible that these seven might have been eliminated by texture measurements of the raw stock alone. Apparently, (Bourne, 1968) textural quality is too complex to be analyzed definitively by an objective procedure providing only a single value. Certainly, cucumber texture is affected by brining.

Nicholas and Pflug (1962) found no varietal differences in MT firmness of No. 2 size raw versus brined fruit in a study of three unidentified cucumber varieties. However, No. 3 size raw fruit were significantly less firm in one variety. Highly significant varietal differences were shown among fresh pack whole and spear pickles made from three varieties. Pickle firmness was measured with a different instrument—a "mechanical recording pressure tester;" results could not be directly compared with those for raw fruit. The authors concluded that "total pressure loss between raw product and finished products is not known."

Jones and Etchells (1950) conducted sensory and MT firmness studies on No. 2 size (up to 1½ in. diam) fruit of 13 varieties in the raw, brined, fresh pack and sweet pickled states. MT firmness of raw fruit ranged from 18.6 lb (Double Yield) to 21.6 lb (New York). Firmness of brine stock ranged from 12.5 lb in one lot of Mincu to 20.6 lb in one lot of Model. Statistical analysis of MT firmness values indicated significant differences among varieties within both raw and brined lots.

The great influence of variety on firmness also was shown by Fellers and Pflug (1965) who compared MT firmness of raw and fresh pack No. 2 size fruit in four varieties (SMR 15, SMR 58, Spartan Dawn, Spartan 27); however, an especially firm cucumber variety did not necessarily make the firmest fresh whole pickle. SMR 58 and Spartan Dawn were firmest as raw stock, but in fresh pack SMR 58 remained significantly firmer than the other three varieties through storage (approximately 300 days).

Jones et al. (1954) evaluated 19 varieties and strains of cucumbers by MT firmness testing of brine stock over a 3-yr period. They organized the varietal MT firmness means into three groups; the means differed ($P < 0.05$) between but not within groups. Mean firmness ranged from 12.75 lb (Mincu) to 17.35 lb (Ohio MR 17). The authors concluded, from the standpoint of texture, that (1) Model, Packer, Earliest of All and Ohio MR 17 were the most satisfactory varieties for brine stock production and (2) Producer, Mincu and Robin 40 were unsuitable for brining because of low firmness and high susceptibility to balloon bloating. They did not measure raw fruit firmness.

Our objectives were (1) to determine the relationship between Instron texture profile parameters of raw and brined cucumber fruit and (2) to determine whether textural quality of raw fruit within a variety can be used to predict textural quality in the brined product of that variety. This research is needed to elucidate further the nature of textural change from raw to brined product and

Table 1—Range of coefficients of variability for the means of 24 varieties of raw and brined cucumbers within each TPA parameter

	BTL	HDN	ELA	A_1	A_2	COH	GUM	CHEW
Raw cucumbers								
Low	10	19	18	17	21	18	20	25
High	25	27	24	24	38	27	37	47
Brined cucumbers								
Low	10	13	7	9	18	17	25	27
High	73	27	17	24	37	33	56	67

also to illustrate to the pickling industry the differing textural behavior of numerous cucumber varieties upon processing.

MATERIALS & METHODS

Cucumber varieties

Samples of 1-in. diam fruit of 24 field grown cucumber varieties were hand harvested two to three times weekly over 24 consecutive harvests in 1970 to obtain data on raw fruit. Reasoning for the choice of varieties was given previously by Breene et al. (1972). It is important to remember that the reason for including some nonpickling varieties in the study was to expand the diversity of the group of varieties used in a basic analysis of texture.

Approximately midway in the harvest season, two 5-lb lots of 1-in. diam cucumbers of each variety were placed in mesh bags and brined in a commercial vat at the M.A. Gedney Co., Chaska, Minn.; no abnormalities were observed in the fermentation. We have since shown evidence (Breene et al., 1972) that although the textural parameters of raw stock vary considerably among varieties, they can be expected to remain relatively constant within a variety through the harvest season.

Sample preparation

Raw stock samples were prepared for TPA as described previously (Breene et al. 1972); 48 1-cm slices were tested for each variety. Brined samples were carefully transferred along with cover brine from the commercial vat to 5-gal polyethylene snap-lid containers in March, 1971, and stored at 60–70°F until testing was completed about 3 wk later. Ten (in two varieties, 15) brined fruit of each variety (20 or 30 1-cm slices) were removed as needed for TPA; individual pickles with obvious internal defects, e.g., bloaters were avoided. Slices were cut, handled and compressed with skin on exactly as described for raw stock (Breene et al., 1972).

Determination of textural parameters

TPA parameters–brittleness (BTL), hardness (HDN), elasticity (ELA), cohesiveness (COH), gumminess (GUM) and chewiness (CHW)–were determined for green stock and brine stock with the Instron TM-M, as described previously (Breene et al., 1972). Samples were cross-sectional slices, one from each side of the midlength point of each cucumber or pickle; they were compressed to 0.25 cm at a crosshead speed of 0.5 cm/min with a chart speed of 5 cm/min. Full scale was set at 100 kg for raw fruit and 50 kg for brined fruit. Areas under the "first bite" and "second bite" compression curves, i.e., A_1 and A_2, respectively, were recorded. Each replicate value for BTL, HDN, A_1 and A_2 was adjusted to correspond to a 1-sq in. sample area.

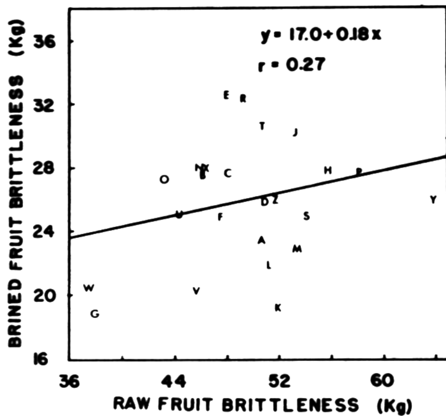

Fig. 1–Scatter diagram, regression equation and correlation coefficient for mean brittleness of brined cucumber fruit vs. mean brittleness of raw cucumber fruit.

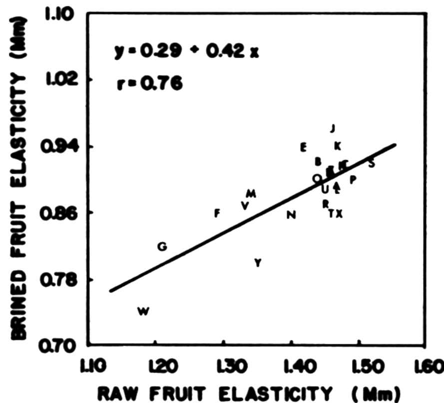

Fig. 3–Scatter diagram, regression equation and correlation coefficient for mean elasticity of brined cucumber fruit vs. mean elasticity of raw cucumber fruit.

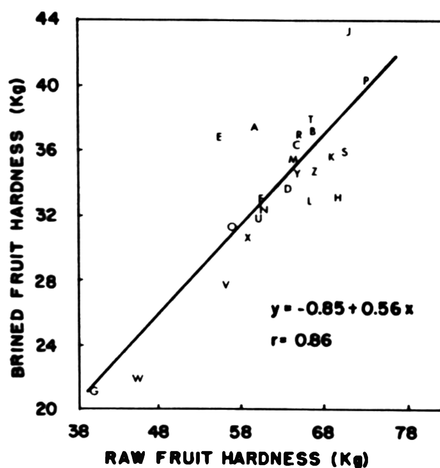

Fig. 2–Scatter diagram, regression equation and correlation coefficient for mean hardness of brined cucumber fruit vs. mean hardness of raw cucumber fruit.

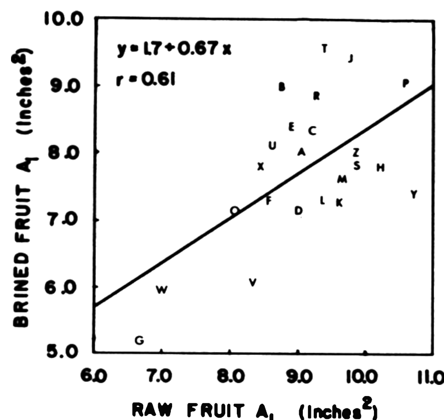

Fig. 4–Scatter diagram, regression equation and correlation coefficient for mean A_1 of brined cucumber fruit vs. mean A_1 of raw cucumber fruit.

RESULTS & DISCUSSION

IN EACH OF Figures 1 through 8, varietal means for one of the eight respective textural parameters measured for raw cucumbers are plotted on the abscissas; those for brined cucumbers are plotted on the ordinates. In addition, the range of coefficients of variability for raw and brined fruit within each TPA parameter is given in Table 1. Varieties may be identified in the figures by reference to Table 2. Regression equations and correlation coefficients are given in each figure. In each of these figures, the location of varieties G and W strongly influences the value of the correlation coefficients. Although removal of these two varieties from the study would alter these values, we would expect that any truly genetically diverse group of varieties would give equal representation to high and low extremes. In retrospect, the sample of varieties selected was somewhat biased in the direction of good texture; extremely poor-textured varieties normally are eliminated early in plant breeding programs and are generally unavailable for research usage.

Brittleness has been equated with crispness by Szczesniak and Smith (1969); it has been termed "fracturability" by Schmidt and Ahmed (1971) and by Ahmed and Dennison (1971). In Figure 1, TPA brittleness values for raw fruit are very distinct. However, brittleness peaks were very poorly defined or nonexistent in compression curves for brined fruit. Fracture peaks observed in brined fruit might be related to skin rupture. When peaks were absent, we did not record a brittleness value. Means were based on the averages of those samples exhibiting a peak which could be construed as brittleness.

The extreme scatter of the brittleness data points and the absence of a significant correlation (r = 0.27) between raw and brined samples led us to conclude that brittleness or fracturability of cucumber tissue essentially disappears dur-

ing brining, probably due to loss of selective permeability of cell membranes. This conclusion is supported further by higher coefficients of variability in varietal mean values for brine stock (up to 73%) as compared with raw fruit (up to 25%). In research on brined cauliflower, Saxton and Jewell (1969) also observed that brittleness rapidly diminished during fermentation.

The high correlation (r = 0.86) between varietal hardness of raw fruit and varietal hardness of brined product (Fig. 2) suggests measurement of this parameter in raw fruit as one means of predicting brining quality of a given variety from a textural standpoint. The inherently soft varieties, Green F, MSU 364 G and Mincu, produced relatively soft brine stock while the inherently harder varieties, Chipper and Explorer, produced relatively hard brine stock.

A good correlation (r = 0.76) was shown between elasticity of raw and brined fruit (Fig. 3) even though the range of variation in this parameter was quite narrow in both.

The area under the "first bite" compression curve is a measure of the total work expended in compressing a sample and thus is a measure of firmness or resistance to pressure. Figure 4 shows the positive relationships between A_1 of raw and brined fruit. The correlation, r = 0.61, was significant at $P < 0.01$ even though the data points showed considerable scatter.

In the objective texture profile analysis method, as devised by Szczesniak et al. (1963) and adapted to the Instron by Bourne (1968), the area under the "second bite" compression curve (A_2) has its principal use in calculation of cohesiveness. It is interesting (Fig. 5) that mean A_2 values correlated as well (r = 0.87; significant at $P < 0.01$) between raw and brined fruit as any of the other parameters measured. There is relatively little scatter to the A_2 data. Perhaps this expression of overall resistance of the cucumber tissue to succumb to one "punch" is more meaningful than was previously believed. At any rate, it appears that A_2 measurement of raw fruit could provide reliable predictions of brining (textural) quality.

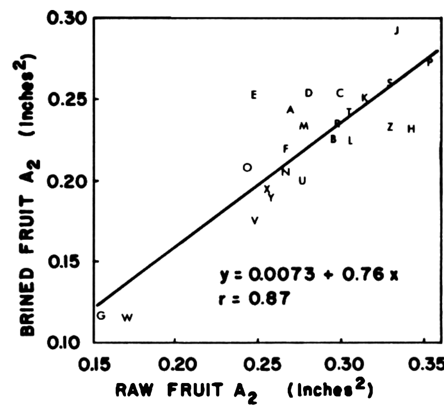

Fig. 5–Scatter diagram, regression equation and correlation coefficient for mean A_2 of brined cucumber fruit vs. mean A_2 of raw cucumber fruit.

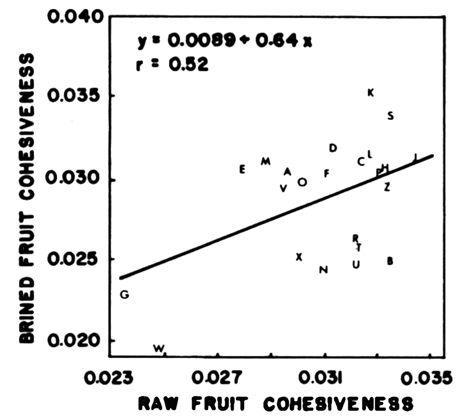

Fig. 6–Scatter diagram, regression equation and correlation coefficient for mean cohesiveness of brined cucumber fruit vs. mean cohesiveness of raw cucumber fruit.

Table 2–Percent retention (% R) of textural characters in brine stock and ranking of varieties with respect to overall % R, overall raw fruit texture, and overall brined fruit texture

		Percent retention (% R) of texture in brine stock[a]								Mean	Ranking of means		
Code	Variety	BTL	HDN	ELA	A_1	A_2	COH	GUM	CHEW	% R	% R	Raw	Brined
A	Pixie	46.2	59.8	60.7	44.3	45.3	102.9	61.9	37.7	57.4	2	15	9
B	Ohio MR 17	59.8	55.9	63.9	51.2	38.1	74.7	41.8	26.9	51.5	18	12	8
C	SMR 58	56.9	56.1	62.1	45.3	42.3	95.5	52.8	32.7	55.5	7	11	6
D	Poinsett	50.8	53.1	61.7	39.4	40.0	102.0	53.7	32.9	54.2	8	14	14
E	Model	67.8	66.8	65.8	47.1	51.1	109.9	72.9	47.6	66.1	1	20	4
F	Ashley	52.0	55.0	66.1	42.8	41.2	97.9	53.9	35.4	55.5	6	18	16
G	Green F	49.2	52.4	67.9	38.8	37.6	97.5	50.7	34.6	53.6	9	24	24
H	Levo	49.7	47.7	62.0	38.1	34.0	91.8	43.8	27.6	49.3	23	2	12
J	Chipper	56.2	61.4	65.6	48.3	43.7	90.6	55.8	36.3	57.2	3	1	1
K	GY 3	37.0	52.1	64.0	37.9	40.0	108.0	55.0	34.4	53.6	10	5	11
L	MSU 713-5	42.7	50.5	60.5	38.9	36.7	96.2	47.9	28.8	50.3	21	8	17
M	SC 10	42.7	55.3	65.9	39.6	43.2	108.4	59.0	38.7	56.6	5	13	13
N	SMR 18	60.5	54.0	60.9	48.8	38.8	79.0	44.2	27.4	52.3	15	17	15
O	Male NK 805	62.9	55.1	62.7	44.1	42.8	109.9	53.8	33.4	56.7	4	22	18
P	Explorer	47.0	55.5	60.2	47.8	38.9	91.5	50.9	30.4	52.8	13	1	2
R	Ranger	65.1	57.1	59.9	47.9	38.8	82.0	47.0	27.6	53.2	11	9	5
S	Southern Cross	45.8	51.0	60.0	39.6	39.2	101.1	51.2	30.1	52.2	16	4	7
T	Pioneer	60.2	57.4	58.7	50.6	39.6	80.1	46.2	27.5	52.6	14	7	3
U	NK 805	56.2	53.0	61.5	47.0	36.1	76.7	41.2	25.6	49.7	22	16	19
V	Mincu	44.2	49.5	65.4	36.2	35.4	99.9	49.6	32.5	51.6	17	21	22
W	MSU 364 G	54.6	48.3	62.4	42.6	33.5	78.5	39.5	25.8	48.2	24	23	23
X	MSU 35 G	60.5	52.3	58.7	46.1	38.0	83.6	43.6	25.5	51.0	18	14	20
Y	MSU 6902 G	40.5	53.8	59.1	34.7	37.0	108.0	58.0	34.1	53.2	16	10	21
Z	Faison	50.5	52.3	61.5	40.8	35.5	88.5	46.8	28.5	50.6	20	6	10
	Mean % R	52.5	54.4	62.4	43.2	39.4	93.5	50.9	31.8	53.5			

[a] % R for each parameter (except A_1 and A_2) = brined fruit mean ÷ raw fruit mean × 100.
% R for A_1 and A_2 = (brined fruit mean ÷ raw fruit mean × 100) ÷ 2.

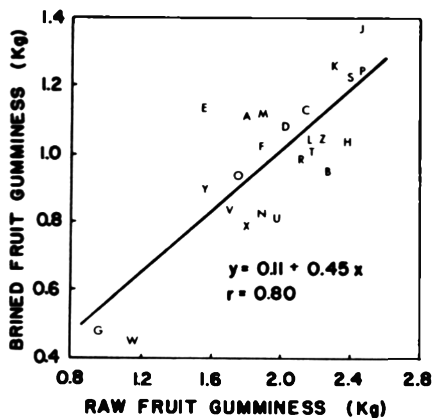

Fig. 7–Scatter diagram, regression equation and correlation coefficient for mean gumminess of brined cucumber fruit vs. mean gumminess of raw cucumber fruit.

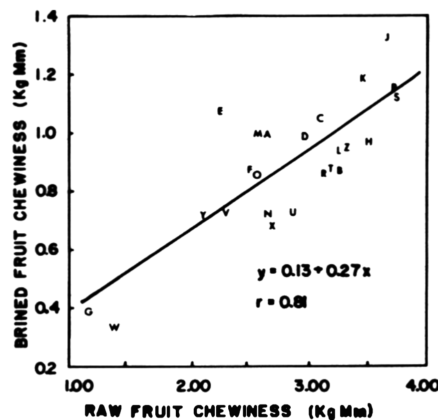

Fig. 8–Scatter diagram, regression equation and correlation coefficient for mean chewiness of brined cucumber fruit vs. mean chewiness of raw cucumber fruit.

Cohesiveness (Fig. 6), while showing a significant ($P < 0.01$) positive relationship between raw and brined fruit, also showed considerable scatter and a smaller correlation coefficient ($r = 0.52$) than any other parameter except brittleness.

Figures 7 and 8 suggest that gumminess ($r = 0.80$) and chewiness ($r = 0.81$) are about equal as predictors of raw cucumber (varietal) brining quality. Both correlations were significant at $P < 0.01$.

It is evident from the data points in Figures 1 through 8 that certain cucumber varieties retained the fresh product textural qualities to a greater extent during brining than did others. For example, Model (E) ranked relatively low in textural values in the raw product; after brining, it ranked relatively high. Chipper (J) ranked relatively high in both the raw and processed forms. Therefore, we calculated for each variety the degree to which raw cucumber textural quality was retained in the brining process. Table 2 lists these percent retention (% R) values for each parameter by variety. Since the full scale setting for brine stock tests was only half that used in raw fruit tests, calculated % R values for A_1 and A_2 were halved.

If we assume that the textural parameters in general parallel each other (Breene et al. 1972), the ranking of mean % R values for all parameters within a variety is a ranking of varieties according to their relative brining (textural) quality. This, however, does not take into consideration differences in absolute or quantitative textural quality. Table 2 also includes rankings as to composite textural quality of raw and brined fruit. A composite value for texture was determined for each variety as follows. Each parameter mean was made a 4-digit whole number by shifting the decimal point to the right, e.g., (BTL) 23.38 = 2338 , (ELA) 1.467 = 1467, (A_2) 0.2695 = 2695, (COH) 0.02347 = 2347, etc. Summation of all eight mean values gave a value for each variety which provided a more meaningful comparison of textural quality among varieties than rank numbers alone.

Table 2 shows that Model, Pixie and Chipper ranked first, second and third, respectively, in mean percent retention of texture across all parameters. Chipper appeared to be the best variety overall; it ranked first in both raw and brined fruit and third in % R. MSU 364 G, one of the lowest ranking varieties as a raw product, ranked lowest in % R. Certain anomalies were noted. Green F, another soft variety, was intermediate in overall texture retention; it also retained its elasticity best among those studied. MSU 6902G, the brittlest raw variety, was poorest in retention of A_1. Levo ranked second in overall raw fruit texture, but dropped to twelfth in overall brine stock texture and was next to last in % R. Model, which was mediocre as a raw fruit, ranked fourth overall in brine stock and was first in % R.

Mean percent retention values for each parameter as composites across varieties are given in the bottom row of Table 2. They show some interesting trends. Brittleness was approximately halved during brining. It should be remembered, however, that retention of brittleness is difficult to interpret because of the poorly defined fracture peaks and extreme variability of data for brine stock brittleness. Hardness decreased by a little less than half and elasticity by one-third. A_1 and A_2 were both reduced by over one-half. There was very little, if any, reduction in cohesiveness. Gumminess dropped to about one-half its original value. Chewiness decreased most of all to about one-third its original value.

It is interesting to compare these results with those reported by others. Saxton and Jewell (1969) observed changes in TPA parameters of cauliflower stems during brining. From their data, one can

Table 3–Composite textural values of 24 raw and brined cucumber varieties relative to a value of 1.00 for Green F

Raw fruit		Brined fruit – Including BTL		Brined fruit – Excluding BTL	
Explorer	1.76	Chipper	1.85	Chipper	1.89
Levo	1.70	Explorer	1.74	Explorer	1.79
Chipper	1.68	Pioneer	1.69	Pioneer	1.70
Southern Cross	1.65	Model	1.69	Model	1.68
GY 3	1.63	Ranger	1.64	SMR 58	1.67
Faison	1.63	SMR 58	1.64	Southern Cross	1.66
Pioneer	1.58	Southern Cross	1.62	GY 3	1.63
MSU 713-5	1.58	Ohio MR 17	1.61	Ranger	1.63
Ranger	1.55	Pixie	1.57	Ohio MR 17	1.63
MSU 6902 G	1.55	Faison	1.56	Pixie	1.61
SMR 58	1.55	GY 3	1.56	Faison	1.59
Ohio MR 17	1.54	Levo	1.55	SC 10	1.58
Poinsett	1.52	SC 10	1.53	Levo	1.56
SC 10	1.51	Poinsett	1.50	Poinsett	1.52
Pixie	1.46	SMR 58	1.50	MSU 713-5	1.52
NK 805	1.44	Ashley	1.48	Ashley	1.50
SMR 58	1.43	MSU 713-5	1.47	SMR 58	1.50
Ashley	1.42	Male NK 805	1.45	NK 805	1.47
MSU 35 G	1.41	NK 805	1.45	Male NK 805	1.45
Model	1.37	MSU 35 G	1.43	MSU 6902 G	1.44
Mincu	1.35	MSU 6902 G	1.43	MSU 35 G	1.42
Male NK 805	1.35	Mincu	1.26	Mincu	1.29
MSU 364 G	1.07	MSU 364 G	1.04	MSU 364 G	1.03
Green F	1.00	Green F	1.00	Green F	1.00

calculate that hardness, elasticity and cohesiveness of the cauliflower had dropped after 7 wk in brine to approximately 20, 55 and 95% of original values, respectively. Cohesiveness of strawberries was found to increase as a result of freezing and thawing, while hardness decreased (Szczesniak and Smith, 1969).

The composite textural values obtained by summation of parameter values for each variety were used to compare varieties with respect to overall texture in Table 3. Each value in Table 3 was calculated by dividing each varietal composite value by the composite value for Green F, the softest variety in both raw and brined states. Since it is questionable whether or not brittleness was present in the brined products, the comparisons were also made with brittleness excluded from each composite value.

Relative brining (textural) quality of individual cucumber varieties, as determined by our procedures, agrees well with results obtained by others using sensory and MT firmness procedures to test samples of these same varieties. However, the TPA method produces more information about texture. Our findings with respect to Model, Ohio MR 17 and Mincu corroborate those of Jones et al. (1954). We found both the raw and brined fruit of Explorer and Chipper to be consistently high in all textural parameters; Pioneer and Ranger also were superior. Bowers et al. (1971), in evaluating brine stock of 50 different varieties and breeding lines, found that Explorer and Chipper rated consistently high in firmness as measured by MT and by sensory methods. They also rated these two varieties high in general acceptability. They did not evaluate raw fruit firmness. Earlier, Bowers (1968) had found brine stock of Chipper to be considerably the firmest (MT) of the six most promising varieties screened from 26 entries.

Jones and Etchells' (1950) studies included two of the varieties we studied, namely, Model and Mincu. They found Model to be second highest in raw fruit MT firmness of 13 varieties tested; it was highest in brine stock MT firmness in one lot and second highest in the other. They found Mincu intermediate in raw fruit firmness but lowest in one lot of brined fruit and second lowest in the other. Model retained its MT firmness well (92% R) while Mincu was the poorest variety they tested (66% R). Mincu was judged not satisfactory for pickling. Model was criticized for having skin not as tender as desirable. Skin toughness probably contributed to the inordinately high % R which we observed in Model; it should be studied for its contribution to texture as measured both by TPA and MT procedures.

Our findings support the conclusion of Sneed and Bowers (1970) that firmness measurement on green fruit gives a good indication of firmness that can be expected in salt stock. We found this to be particularly true with varieties having textural properties in the extremes of the range. The conclusion of Fellers and Pflug (1965), that an especially firm cucumber will not necessarily make the firmest fresh pack pickles, applies equally to the manufacture of brine stock. However, varieties rating high in raw fruit textural quality usually produced brined fruit of high textural quality. Deviations from this general relationship are probably due to unique anatomical or histological varietal characteristics. We intend to investigate these aberrant varieties further as to relationships between textural properties and anatomical macro- and micro-structure as well as chemical composition.

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EFFECT OF DIFFERENT TREATMENTS ON PHYTATE AND SOLUBLE SUGARS IN CALIFORNIA SMALL WHITE BEANS (Phaseolus vulgaris)

INTRODUCTION

THE MATURE BEAN seed (*Phaseolus vulgaris*), like many other seeds, contains relatively large amounts of phytate (inositol hexaphosphate) (Makower, 1969). This material serves as a ready source of phosphate (Mayer, 1956) and inositol (Darbre and Norris, 1957) during germination. Its decomposition may also release certain metals which are essential to the developing plant. Nutritionally, phytate is probably unavailable to humans (McCance and Widdowson, 1935) due to the lack of an endogenous enzyme system (Rapoport et al., 1941) that can catalyze the hydrolysis of the molecule to its moieties. The presence of undegraded phytate in the intestines may render less available for absorption some essential di- and tri-valent cations such as Ca^{++}, Mg^{++}, Fe^{+++}, etc.

Dry bean seeds contain several low molecular weight soluble oligosaccharides which are metabolized during the germination of the seeds. However, about 60% of the total are in the form of stachyose and raffinose (Lee et al., 1970) which are largely unavailable for human nutrition due to a lack of specific enzymes (α-galactosidases and β-D-fructosidases) in human digestive juices (Kuriyama and Mendel, 1917; Gitzelmann and Auricchio, 1965).

Following ingestion, phytate and those oligosaccharides which are not hydrolyzed by gastrointestinal secretions and hence not readily absorbed, continue down the intestine into the presence of a variety of microorganisms common to the colon which have the capability of hydrolyzing phytates and α-galactosidosucroses and fermenting them with the production of gases, (Bergey, 1957; Speck et al., 1970) i.e., flatus, commonly associated with the ingestion of cooked legume seeds.

The present exploratory study was undertaken to establish optimum conditions for initiating and maximizing the hydrolysis of phytate and α-galactosidosucroses by the enzymes of the bean which normally perform these functions at slow rates during germination. Conceivably conditions resulting in sufficiently rapid hydrolysis of these seed constituents could be incorporated into useful processes for the production of improved dry bean food products.

MATERIALS & METHODS

DRY CALIFORNIA small white beans (*Phaseolus vulgaris*) were used in all the experiments. The treatments were combinations of heat treatment, grinding and incubating with and without added phytase enzyme, according to the schedule in Table 1. All samples were made as 10% beans in 0.1M acetate buffer at pH 5.2 except samples 9 and 10 in Table 1 which were at pH 3.5. The incubation was for 20 hr at 55°C. At the end of incubation all samples were cooked. Those that were cooked for 2 hr before incubation as part of the treatment received only a 20 min cook while the others had a 2 hr and 20 min cook. Following completion of the schedule in Table 1, all samples were centrifuged and the clear supernatant assayed for dry weight, nitrogen, total sugars, total phosphorus, inorganic phosphorus and various sugars including inositol (Tables 2 and 3).

Whenever external phytase was used, 1.2% by weight of an active wheat germ preparation was added. The enzyme was obtained from Sigma Chemical Co., #P-1259 Lot 10C-8050-1. The enzyme assayed for 0.018 units/mg, where one unit liberated 1.0 μmole inorganic phosphorus from 1.5 μmole phytate per minute at pH 5.2 and 55°C.

Assay procedure

Dry weight. This was determined on freeze-dried aliquots of the supernatant. As reported in Table 2, values were corrected for the contribution of the sodium acetate buffer.

Phosphorus. This was determined essentially by the phosphomolybdate procedure of Allen (1940). Total phosphorus following perchloric acid hydrolysis and inorganic phosphorus were determined. The results are reported in Tables 2 and 4 and are for mg % phosphorus in whole beans.

Sugars. Total sugars were determined by treating aliquots with invertase and then determining reducing sugars by the atomic absorption method of Potter et al. (1968).

Identification of oligosaccharides was done by paper chromatography following the procedure of McCready and Goodwin (1966).

Mono- and di-saccharides as well as inositol were determined and their quantities estimated by gas liquid chromatography of trimethylsilyl derivatives. These were prepared with Tri-Sil reagent (Pierce Chemical Co. # 48997A). They were run on a Varian Aerograph 1520 C gas liquid chromatography unit with flame ionization detector. The unit was equipped with stainless steel columns 5 or 10 ft long, packed with 3% OV 17 on 100/120 Varaport 30 and temperature programmed for 4°/min rise between 150°C and 240°C. Sugars were identified by comparison of retention times of peaks with those of known sugars run under the same conditions.

Table 1—Sequence of treatments

Sample #	pH during treatment	Treatment[a]			
1	5.2	I	C	B	
2	5.2	E	I	C	B
3	5.2	C	B	I	
4	5.2	C	B	E	I
5	5.2	B	C	I	
6	5.2	B	C	E	I
7	5.2	B	I	C	
8	5.2	B	E	I	C
9	3.5	B	I	C	
10	3.5	B	E	I	C

[a] I = incubate at 55°C for 20 hr; B = blend; C = cook for 2 hr; E = add 1.2% wheat germ phytase.

Table 2—Composition of supernatant fraction as % whole beans

Sample #	Dry wt (%)	Total sugar (%)	Nitrogen (mg%)	Total P (mg%)	Inorganic P (mg%)
1	16.5	4.2	740	280	200
2	16.2	3.9	740	300	230
3	15.6	3.7	640	250	60
4	16.7	3.6	530	270	150
5	18.7	3.2	620	240	70
6	37.8	12.5	780	290	180
7	15.1	3.9	740	240	180
8	15.6	4.4	740	270	250
9	19.0	4.4	880	230	130
10	17.0	4.4	870	260	190

Other analysis and materials. All nitrogen determinations were done by the Kjeldahl method. All chemicals used were reagent grade.

RESULTS

Soluble dry matter

All the treatments with the exception of #6 resulted in very similar percentages of dry weight in soluble form (15–19%) (Table 2). The large increase in sample #6 is due to sugars which will be discussed later. Dry weights of extracts in Table 2 which contained added enzyme have been corrected for the amount of added solids.

Nitrogen

The amount of nitrogen present in the extracts reported in Table 2 show very little differences due to the various treatments. The extracted nitrogen was about 24% of the total bean nitrogen and about double the nonprotein nitrogen of California small white beans (unpublished data). Under the conditions of the experiments as described there was very little evidence of proteolytic activity with or without added enzyme. About 16% more nitrogen was extracted from samples 1 and 7 which were not cooked prior to incubation than from samples 3 and 5 which were cooked prior to incubation.

Sugars

With the exception of treatment #6 (Table 1) there was no significant difference in the amount of sugars present in the extracts and the amount approximates the original sugar content of the beans. There was a large increase in sugar in sample #6. This was due to the presence of α-amylase enzyme in the commercial wheat germ phytase preparation. On subsequent tests with this enzyme preparation under similar incubation conditions, about 21% of a commercial soluble starch was hydrolyzed after 20 hr. From the results in Table 2 it would seem that 17% of the cooked bean starch was hydrolyzed. This probably is a low figure because in the method of analysis used for the bean samples, maltose units would be counted as if they were glucose units, thus decreasing the apparent amount of hydrolyzed material. α-Amylase is only slightly active against crystalline starch, therefore no amylolitic activity could be demonstrated in samples 2, 8 and 10. As found by Kon et al. (1971) little amylase activity can be detected in in vitro digestion experiments when the bean cells are not broken. This is shown by sample #4 in which the beans were cooked before blending which minimizes cell breakage (Kon et al., 1971).

Larger differences between the treatments are found in comparing the individual sugar composition of the samples by gas chromatography of trimethylsilyl derivatives. Thus, 11.1% of the original bean weight in sample #6 is present as maltose. The presence of large amounts of maltose is additional substantiation of α-amylase activity in the wheat phytase preparation. There was no evidence of maltose in any of the other extracts. Presumably only a small amount was produced, as explained above, and this was hydrolyzed further to glucose.

Galactose, glucose, inositol and sucrose were identified in the various samples in having the same column retention times as corresponding standards.

Sucrose was found in the samples in amounts ranging from 2.5–3.5% of the bean dry weight. Samples 1 and 2, which were incubated whole, seem to have more sucrose (Table 3). At the present time we have no explanation for this.

Glucose seems to be affected by the treatments in a different manner. The wheat germ phytase preparation increases the amount of glucose that can be detected. This is due to the presence of α-amylase in the preparation. Starch having different sensitivities to attack by amylase, depending on the pre-treatment, produced progressively more glucose, with the least being sample #2, incubated raw whole, followed by #8, incubated raw blended, then #10, incubated raw blended at pH 3.5, then #4, incubated cooked blended, and finally #6, incubated blended cooked, which has all the cells broken and the starch completely gelatinized having a very large amount of glucose. From the samples without added phytase, the incubation at pH 3.5 by itself produces some hydrolysis with an increase in glucose.

The results for galactose are in agreement with our previous data indicating that there is very little free galactose in untreated California small white beans. There is a large increase in free galactose content in all six samples that were incubated raw. Table 3 shows that all formed about 1% of the bean weight as galactose.

The results for galactose correspond quite well to the relative amounts of stachyose and raffinose identified in these extracts by paper chromatography (Table 5). The spots representing stachyose are much darker for samples #3, 4, 5 and 6 than for the other six samples. Extracts from samples #9 and 10 do not have any detectable stachyose. Spots representing raffinose are missing completely in chromatograms for samples #7, 8, 9 and 10 and are very faint in those for samples #1 and 2. Our interpretation of these findings is that bean α-galactosidase has been activated during the incubation period to hydrolyze both raffinose and stachyose. The activation is the same at pH 5.2 and 3.5, which is in agreement with the findings of Cooper and Greenshields (1961). However, these authors reported that in the intact germinating seed, free galactose did not accumulate as it did under our experimental conditions and presumably was metabolized as rapidly as released. The physical condition of the cell, whole beans compared to blended slurry, apparently does not affect this enzyme activity.

Phytate

The extent to which phytate was degraded by native and added phytase was followed by examining the increase in inositol and inorganic phosphorus follow-

Table 3–Sugar composition of supernatant fraction as mg% whole beans

Sample #	Galactose	Glucose	Inositol	Sucrose
1	900	10	130	3500
2	1000	300	120	3500
3	Trace	100	40	2600
4	Trace	900	30	2100
5	Trace	90	40	2600
6	Trace	5300	40	2400
7	900	300	110	2700
8	1000	600	160	2700
9	1100	600	70	2600
10	1100	700	60	2800

Table 4–Increase in phosphorus and inositol as mg% of whole beans

Sample #	Inositol	Inorganic P
1	90	140
2	80	170
3	None	None
4	None	90
5	None	None
6	None	120
7	70	120
8	120	190
9	30	70
10	20	130

Table 5–Relative amounts of bean oligosaccharides identified by paper chromatography[a]

Sample #	Stachyose	Raffinose
1	Trace	Trace
2	Trace	Trace
3	++	+
4	++	+
5	++	+
6	++	+
7	Trace	ND
8	Trace	ND
9	ND[b]	ND
10	ND	ND

[a]Order of increasing spot intensity not detectable < trace < + < ++.
[b]ND = not detectable.

ing each treatment. There is very little difference between the total phosphorus contents of the extracts. On the average, about 65% of the total phosphorus present in the bean can be extracted under the conditions of the experiment (Table 2). The amounts of inorganic phosphorus and free inositol present varied with treatment. Table 4 shows the increases in inorganic phosphorus and inositol resulting from the individual treatments, using samples 3 and 5 as controls.

The wheat germ phytase used does not hydrolyze all six phosphates from phytate but only five to give inositol monophosphate. This can be seen from the results in Table 4. In samples 4 and 6 which contained added wheat enzyme but were incubated after cooking, free inositol did not increase but inorganic phosphorus did. Sample 6, with its broken cell walls and consequently easier excess of the added enzyme to the phytate present, shows higher values for inorganic phosphorus than sample 4 with its intact cells.

In uncooked whole beans (samples 1 and 2) the addition of wheat phytase produced more inorganic phosphorus but not more inositol. This is not the case in the uncooked blended samples (samples 7 and 8). The more intimate contact of the cell constituents when the cells are broken allows better action of bean phosphatases on the inositol monophosphate produced by the wheat enzyme.

Incubation of uncooked whole or blended beans under the conditions of the experiment reduces phytate present in the beans as measured by the increase in inositol and inorganic phosphorus. The addition of wheat germ phytase enzyme increases this hydrolysis by at least 15%.

Holding foods under conditions that allow the food enzymes to effect changes in the food to make it more acceptable for eating has been practiced by man for centuries although we have not and do not now understand all of the reasons or mechanisms involved in such procedures. Ripening is an excellent example in that desirable changes in sugar levels occur. Sweet potatoes provide another example wherein amylytic enzymes slowly change constituents of the commodity during the conventional curing and storage treatments for the fresh product to increase the sugar content. In recent years, a technique has been developed to rapidly activate the saccharifying enzymes of sweet potatoes during the manufacture of dehydrated flakes independently of curing and storage treatments (Hoover and Harmon, 1967).

The results in this paper suggest the possibility of a schedule of treatments that would transform undesirable bean components into nutritionally available forms and yield a more acceptable product. The problem remaining is quite formidable, requiring extensive data on the influence of a number of variables on the activities of the different enzymes involved, plus assurance that the data can be applied to the preparation of wholesome and acceptable food products. We have been sufficiently encouraged by the results reported here to continue and expand our efforts to obtain the basic data and subsequent applications to product development.

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CONDITIONING AND DISPOSAL OF SOLIDS FROM POTATO WASTEWATER TREATMENT

INTRODUCTION

LARGE QUANTITIES of solid and liquid waste material are generated in the R.T. French Co.'s dehydrated potato processing operation at Shelley, Idaho.

Solid wastes include potato trimmings, floor sweepings and silt material. The trimmings and sweepings are dried and disposed of as cattle feed. The silt is thickened in a clarifier-thickener, dewatered on a vacuum filter and disposed of as landfill material.

Liquid wastewater receives primary and secondary treatment. Primary treatment involves separation of settleable solids from the rest of the wastewater in primary clarifiers. The separated solids are vacuum filtered and sold as cattle feed. The primary clarified wastewater then receives aerobic biological secondary treatment (complete mix activated sludge) to convert soluble organic materials to biological solids (cells) which are separated in a secondary clarifier (Fig. 1). Secondary treatment effluent flows to the Snake River.

The secondary treatment process yields biological solids in excess of that needed to operate the treatment system. In the past, these diluted biological solids were discharged to the Snake River or dewatered along with silt water (at high chemical costs) and used as land fill.

Because waste biological solids (secondary sludge) disposal is difficult and expensive, the Potato Processors of Idaho Assn. retained the consulting engineering firm of CH2M/HILL to investigate alternative methods of waste biological solids conditioning and disposal during the 1970–71 potato processing season. These methods included:

1. Pilot scale spray irrigation (1 acre).
2. Pilot scale aerobic digestion, followed by vacuum filtration and basket centrifugation, with and without chemicals.
3. Pilot scale basket centrifugation, with and without chemicals, followed by vacuum filtration (with and without chemicals), drum drying and vacuum filtration after mixing with primary solids (settleable solids removed in primary clarifiers).

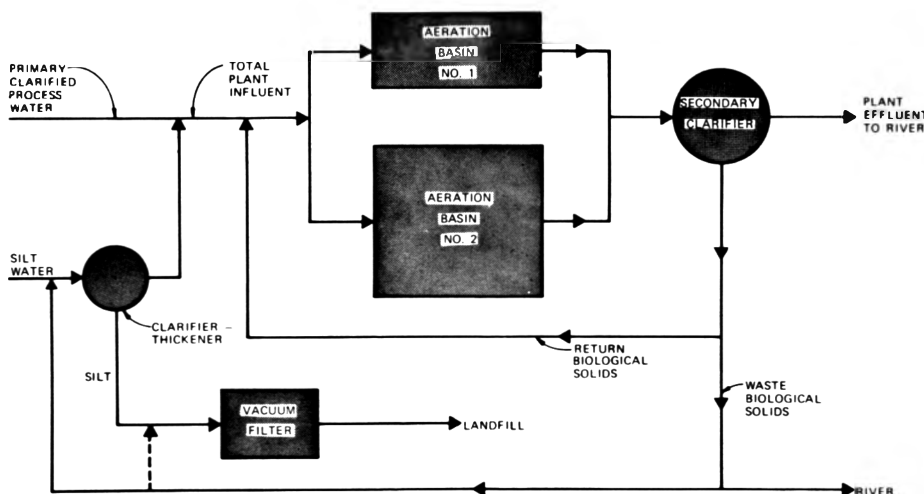

Fig. 1—Simplified flow diagram of secondary waste treatment facility.

EXPERIMENTAL

Description of pilot units

Spray irrigation system. A pilot spray field was located adjacent to the secondary waste treatment facility (Fig. 2). The spray irrigation system consisted of a buried header and laterals with three risers and sprinkler heads. The sprinkler heads were Buckner No. 630 Turf King Oversize Heads with 3/8-in. main nozzles. The composite area of the three spray coverage circles was 1.06 acres at a discharge pressure of 55 psi and a flow of 30 gpm.

Basket centrifuge system. The centrifuge basket (14 in. diam × 6 in. deep) had variable-speed drive up to 3250 ppm (2100 G's). The unit is shown in Figure 3. The pilot centrifuge was fed with a variable speed Moyno pump (0–10 gpm) and the solids cake was removed with a manually controlled skimmer. The centrifuge was operated in the secondary waste treatment facility control building.

Aerobic digestion system. The aerobic digester was a plastic-lined 55-gal drum (Fig. 4) housed in the secondary waste treatment facility control building. Air was supplied with a laboratory vacuum-pressure pump to provide a completely mixed system.

Vacuum filtration testing. A vacuum filter leaf test apparatus was used to determine the filterability of various biological solids concentrations, with and without chemical additions. The equipment consisted of a vacuum filter leaf, various filter media and a vacuum pump.

Vibrating screen testing. Tests were conducted to determine how well a vibrating screen would concentrate various biological solids concentrations. The test screen, 24 in. diam with 50-mesh media (Fig. 5), was evaluated in the secondary waste treatment facility control building.

Drum dryer testing. The drum dryer, with a drum 22 in. diam × 22 in. long (Fig. 6), was operated in the primary waste treatment section of the processing plant. Drum speed and temperature were adjustable.

Waste biological solids characteristics

The characteristics of the waste biological solids from 24 Nov. 1970 through 8 Jan. 1971 are listed in Table 1.

Sampling and analytical techniques

Analytical tests were performed according to APHA (1965), except the suspended solids tests used glass-fiber filter discs and a Millipore® filter holder instead of asbestos mats and gooch crucibles.

All samples were refrigerated until tested.

Spray irrigation

Spray irrigation of waste biological solids was studied to determine potential problem areas related to this type of solids disposal.

Basket centrifugation

A basket centrifuge was tested to determine its ability to dewater waste biological solids.

Aerobic digestion

Aerobic digestion of waste biological solids was evaluated to determine its effect on solids dewaterability.

RESULTS

Spray irrigation

Operation of the pilot spray irrigation system through part of a winter season (4 months) has provided information on potential problems of winter irrigation.

The grease lubricated, Buckner 630 sprinkler head rotates by unbalanced centrifugal action of a vibrator disc in the reaction nozzle arm. The vibrator disc became stuck between cycles when the temperature dropped below 15°F (plus wind) and the sprinkler heads would not rotate by themselves until the reaction disc was removed and cleaned (Fig. 7). This is thought to have been caused by a combination of the characteristics of the waste biological solids and the low air temperature. Private communication with Rogers Brothers personnel indicates that they have encountered similar experiences with this particular sprinkler head at their Rexburg, Idaho operation.

Fig. 2—Spray irrigation system.

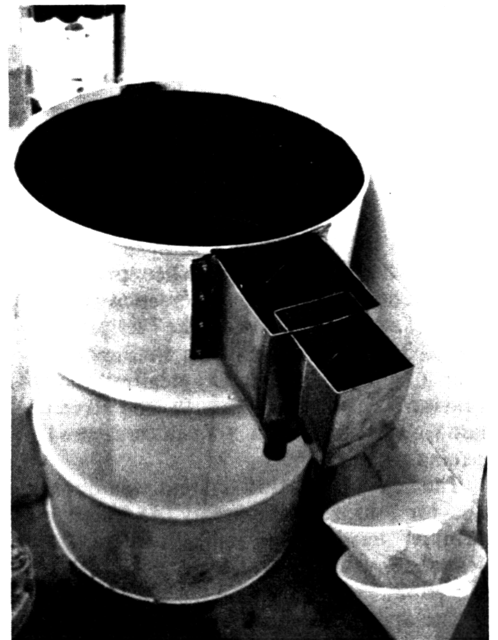

Fig. 4—Aerobic digester.

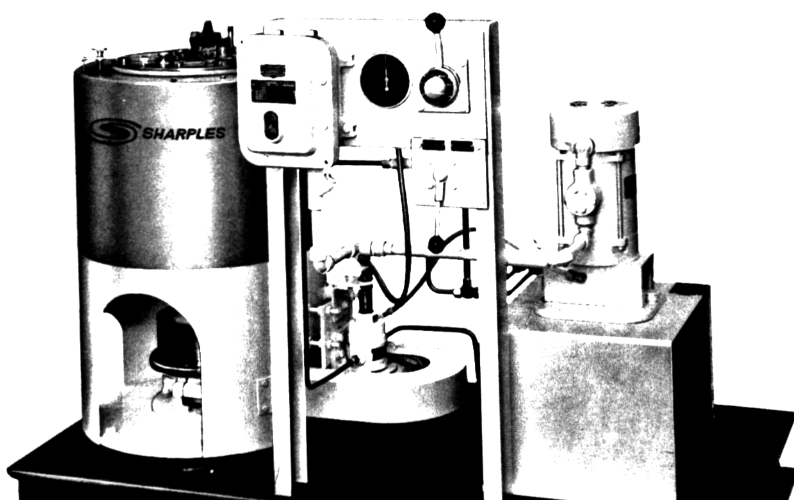

Fig. 3—Basket centrifuge.

Fig. 5—Vibrating screen.

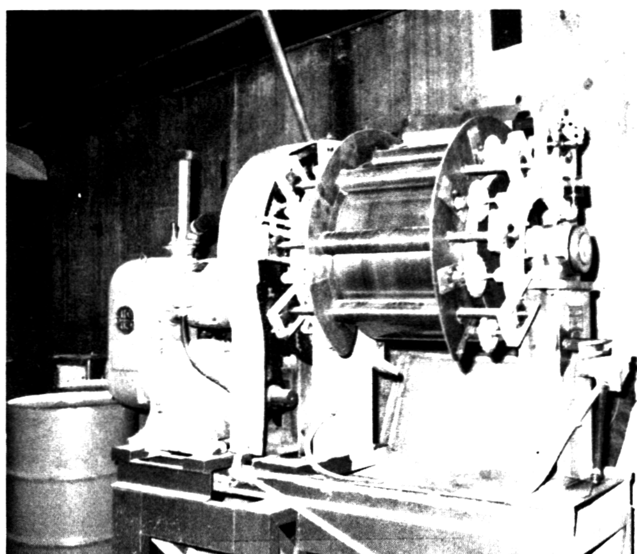

Fig. 6–Drum dryer.

Fig. 7–Nonrotating sprinkler head.

Potato solids, carried over in the waste biological solids, plugged the sprinkler nozzles before an in-line screen was installed upstream from the irrigation pump. This screen is shown in Figure 8 after a typical daily run; typical retained solids are shown in Figure 9. The screen has 3/32-in. holes on 3/32-in. staggered spacing.

The spray irrigation system was operated for 2 hr 15 min per day (45 min per sprinkler head). The in-line screen was cleaned (by necessity) after each day's operation.

The application rate to the pilot spray field was 0.14 in. per day and the precipitation rate was 0.19 in. per hr. The organic loadings are listed in Table 2.

Sprayed waste biological solids dried readily (Fig. 10) and did not produce objectionable odors at operating loading rates.

Complete soil analyses were conducted before and after the spray irrigation testing. Sodium concentrations in the soil increased by about 65% during the 4 months of spray irrigation; however, the ratio of sodium to other cations and the total salt content of the soil were still well within acceptable levels.

Application of results. Pilot-scale spray irrigation during the winter months demonstrated the need for: (1) screening waste biological solids before spraying to prevent sprinkler head clogging; and (2) special sprinkler head design to prevent freezing and sticking of the heads.

Pilot irrigation of waste biological solids demonstrated that average organic loadings of 70 lb of BOD per acre per day and 180 lb of suspended solids per acre per day should be acceptable for full-scale spray irrigation without producing objectionable odors.

It is anticipated that the dried solids will have to be worked into the soil occasionally to avoid excessive matting over the soil. The dried solids will act as a good soil conditioner.

Basket centrifugation

Waste biological solids were fed to a pilot scale basket centrifuge at flow rates of 1–4 gal per min, suspended solids concentrations of 0.5–0.9% and SVI (sludge volume index) levels of 277–444. (The SVI is a measure of the settleability of the solids suspended in the aeration basin liquor. By definition, the SVI is the volume in ml occupied by 1g of activated sludge after 30 min of quiescent settling in a 1,000 ml graduated cylinder. The lower the SVI, the better is the settling quality of the sludge. Sludge with a SVI of 100 or less is considered a good settling sludge.)

Results varied from a 6.0% solids cake and 94% solids recovery at 1 gpm feed rate to 3.7% solids cake and 80% solids recovery at 4 gpm feed rate. Figure 11 shows samples collected during a 1 gpm test. The sample bottles contain, from the left: waste biological solids; centrate at 3.5, 6, 9, 14, 17 and 20 min; centrate at 24 min (breakthrough); liquid skim; and solids skim (centrifuge cake). The centrate suspended solids varied from 385

Table 1–Waste biological solids characteristics

Item	Range	Average
Temperature	8–16°C	12°C
pH	7.0–8.2	7.5
Alkalinity	800–2670 mg/l $CaCO_3$	1360 mg/l $CaCO_3$
Total suspended solids	3050–12,875 mg/l	7450 mg/l
Volatile suspended solids	800–5300 mg/l	2270 mg/l
Dissolved oxygen	0.2–0.8 mg/l	0.5 mg/l

Fig. 8–Irrigation system screen.

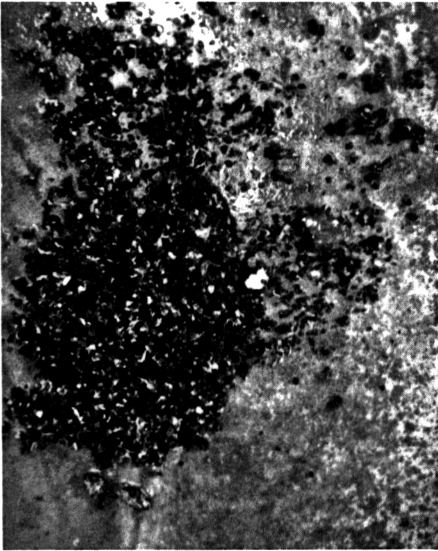

Fig. 9—Irrigation system screenings.

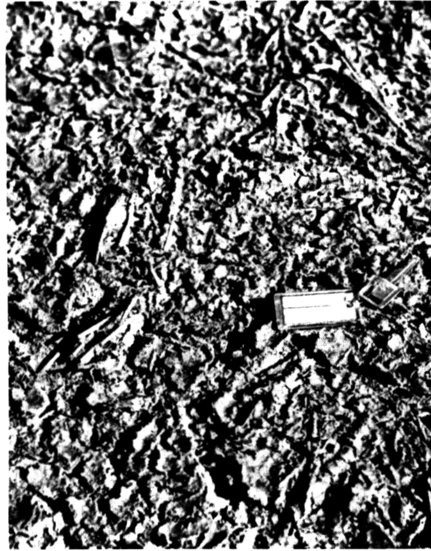

Fig. 10—Dried waste biological solids

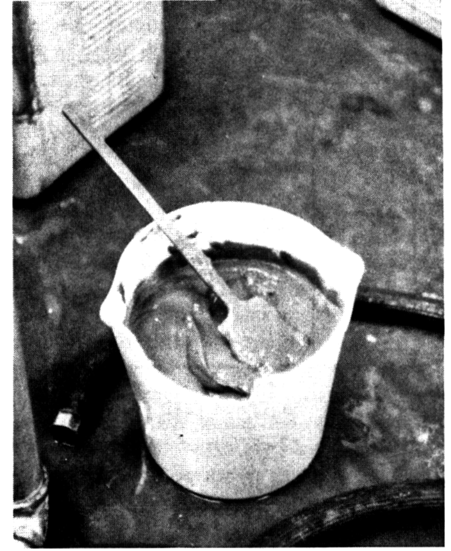

Fig. 12—Concentrated waste biological solids.

mg/l at 3.5 min to 1685 mg/l at breakthrough. The solids cake was 6% (Fig. 12), would not flow by gravity (can be considered dewatered), and had the appearance of chocolate pudding.

Sharples Centrifuges (Sharples-Stokes Div. of Pennwalt Corp.) scaled up the pilot-scale data to provide the estimated full-scale design and operating parameters shown in Figures 13 and 14 for their Fletcher SST-1600 centrifuge (now called Sludge Pak SP-6500). Solids recovery and centrifuge cake solids would both be expected to increase with decreases in SVI. Centrifuge cakes should concentrate to at least 7% at an SVI level of about 150. Keith (1971) reported that chemical additions should increase solids recovery during high SVI operation (allowing higher feed rates); however, centrifuge cake solids would not be expected to increase noticeably.

Samples of concentrated waste biological solids were further dewatered on a drum dryer to between 80 and 90% dry solids. The solids were concentrated to between 7.0 and 9.5% solids before being applied to the drum dryer at a solids loading rate of 1.8–2.5 lb per hr per sq ft of dryer drum. The dried solids were scraped off the drum in flakes up to 6 in. wide and 2.5 ft long (at the higher loading rate), but crumbled readily (Fig. 15).

Attempts to dewater various concentrations of waste biological solids, with and without chemicals, by vacuum filtration were not successful. Addition of the waste biological solids to primary sludge, with and without chemicals, decreased the filter loading rates below the economical feasibility range.

Waste biological solids samples, with and without chemicals, would not concentrate on a 50-mesh vibrating screen.

Application of results. Pilot scale testing has demonstrated that a full-scale basket centrifuge would be effective for concentrating waste biological solids. With SVI levels over 250, 80% solids recovery and 5.5% centrifuge cake solids can be expected with average feed rates up to 50 gal per min to a Sharples centrifuge costing about $37,000 (1971).

Pilot scale testing by the De Laval Separator Co. had similar results.

Full-scale vacuum filtration of waste activated sludge by itself or mixed with

Table 2—Pilot spray field loading rates

Loading	Range	Average
BOD	45–120 lb/Ac/d	70 lb/Ac/d
Suspended solids	65–235 lb/Ac/d	178 lb/Ac/d
Hydraulic application rate	–	0.14 in/d
Hydraulic precipitation rate	–	0.19 in/hr

Fig. 11—Typical basket centrifuge test results.

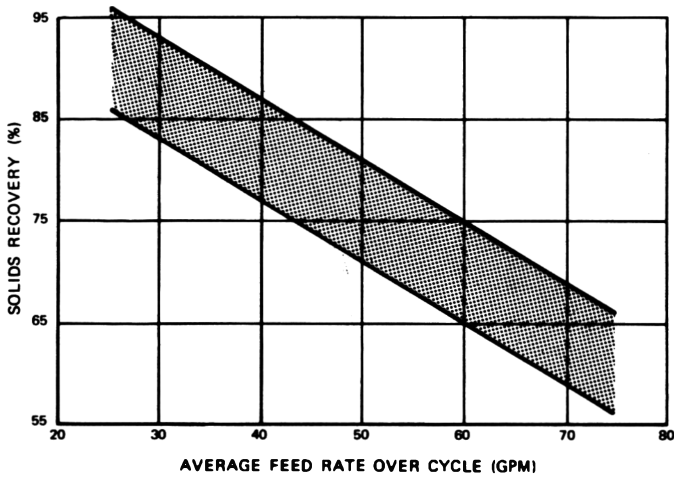

Fig. 13–Effect of feed rate on solids recovery.

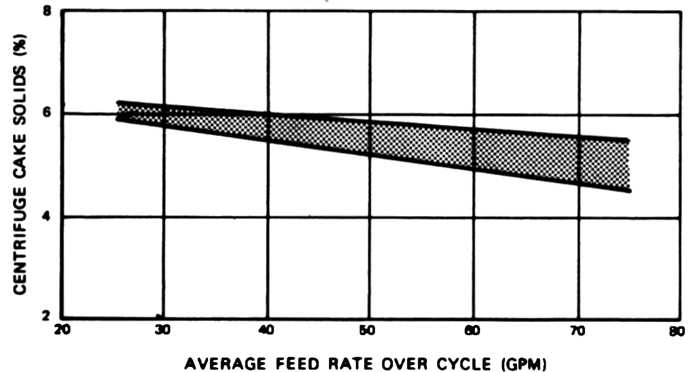

Fig. 14–Effect of feed rate on centrifuge cake solids.

primary sludge does not appear effective (with or without chemical additions).

Aerobic digestion

A pilot scale aerobic digester was operated for about 1½ months to determine its effectiveness for improving waste biological solids dewaterability. The completely mixed digester operated with a uniform influent flow rate of 3 gal per day and a uniform detention time of about 17 days. The average influent and effluent characteristics for 7 wk of operation are listed in Table 3.

About 55% of the volatile suspended solids in the waste biological solids were destroyed in the 17 days, which corresponds to data reported by Loehr (1965), Burd (1968) and Smith (1971).

Tests were conducted with a pilot scale basket centrifuge to demonstrate the effect of aerobic digestion on waste biological solids dewaterability. The results of a centrifuge run with fresh biological solids and a centrifuge run with aerobically digested biological solids are compared in Table 4.

The effect of aerobic digestion of waste biological solids on centrifuge centrate clarity can be seen in Figure 16. The sample on the left is centrifuge centrate of straight waste biological solids, and the sample on the right is centrifuge centrate of aerobically digested waste biological solids. Both samples were collected after 5 min of centrifuge operation with a feed rate of 3 gal per min.

Efforts to concentrate aerobically digested, waste biological solids with a 50-mesh vibrating screen were not successful; however, addition of a small quantity of cationic polymer allowed very effective screening. The results are shown in Figure 17. The captured solids (4%) are shown on the left, the screened liquid (total suspended solids = 20 mg/l) in the middle and the raw sample (total suspended solids = 3640 mg/l) on the right.

Vacuum filtration of the aerobically digested solids resulted in clear filtrate (Fig. 18) and a dry filter cake (16–19%), but the cake was too thin and the filter solids loading was too low (less than 0.1 lb/sq ft/hr). Various filter media were tested without improved results.

Application of results. The findings of the aerobic digestion of waste biological solids are in agreement with data reported by Loehr (1965), Burd (1968) and Smith (1971). Reduction of volatile solids is a function of detention time, with about 15 days being reported usually as optimum. Detention times of 10–15 days have been reported to be optimum for sludge dewaterability.

Full-scale aerobic digestion of waste biological solids could be used in conjunction with basket centrifugation or vibratory screening (with chemicals) to obtain increased solids concentrations.

DISCUSSION

Spray irrigation

Complete soil analyses of waste biological solids spray irrigation disposal fields should be conducted annually to monitor cation balances and total salt buildup. Management practices in the form of occasional leaching with fresh water or chemical amendment additions may be necessary.

The increase in sodium content of the pilot spray irrigation field soil is thought to be the result of the carriage water and not the biological solids. The potato processing plant uses substantial quantities of sodium in their peeling operation, which results in a relatively high concentration of sodium (up to 100–500

Fig. 15–Drum dried waste biological solids.

mg/l) in the secondary waste treatment facility influent. The sodium content of the wastewater is not appreciably affected by biological treatment; therefore, the sodium concentration of the carriage water should be very similar to the concentration in the secondary treatment influent.

The pilot spray irrigation field was not loaded to failure (with resultant odors); therefore, optimum design criteria has not been developed. Optimum design criteria development for a particular soil and locality will provide the best information for an economical system with minimal odors.

Fig. 16—Effect of aerobic digestion on centrifuge centrate.

During severe cold, wastewater or biological solids applied to the irrigation field does not enter the ground or evaporate, but instead becomes stored in "ice beds." The thickness of these beds varies with application rate, temperature and the length of time subfreezing weather is experienced. Ice beds of several feet thickness have been experienced while spray irrigating primary treatment effluent in the Midwest. The presence of these ice beds is in itself not a severe problem to operation of a properly designed system. Major difficulties appear when a spring thaw occurs. A fast thaw can create great difficulty in containing the ice melt, plus the continuously applied wastewater or biological solids. Level or near level land is required in addition to a dike or system of dikes to contain the spring runoff. If permitted to enter the receiving water, the runoff would carry with it large quantities of dissolved and suspended waste solids. The retained wastewater or biological solids deposited during the winter could become septic during the spring and cause odors; however, private communications with American Potato Co. personnel regarding their operation at Moses Lake, Wash. and their pilot spray irrigation project at Blackfoot, Idaho and with Rogers Brothers Co. personnel regarding their operation at Rexburg, Idaho indicate that the ice deposits have not caused objectionable odors. A conventional portable system on other land could be used to relieve the load from the thawing system, allowing it to recover if a problem develops.

Basket centrifugation

Pilot basket centrifugation of waste biological solids provided data which was scaled up to full-scale application rates. The largest basket centrifuge available today for this application appears to have a capacity of only 50 gal per min with an 80–85% solids recovery and a cake with about 5.5% solids concentration. The cost for this machine is estimated to be about $37,000. This estimate is for a sludge from a mixed liquor with an SVI of over 250. Chemical additions or reduced SVI levels would allow a higher feed rate or a longer cycle and possibly a higher solids cake because of better clarification of the centrate.

A disc nozzle centrifuge for the same application would have about the same cost with at least twice the capacity, but would require more operational care and control equipment. The cake from this type of centrifuge during SVI levels over 250 may be only between 3 and 4% and

Table 3—Effect of aerobic digestion on the characteristics of waste biological solids

Item	Influent	Effluent
pH	7.5	8.0
Alkalinity	1360 mg/l, $CaCO_3$	1125 mg/l, $CaCO_3$
Total suspended solids	7450 mg/l	2905 mg/l
Volatile suspended solids	2270 mg/l	1045 mg/l
Dissolved oxygen	0.5 mg/l	1.7 mg/l
BOD	–	250 mg/l

Fig. 17—Effect of screening on aerobically digested waste biological solids.

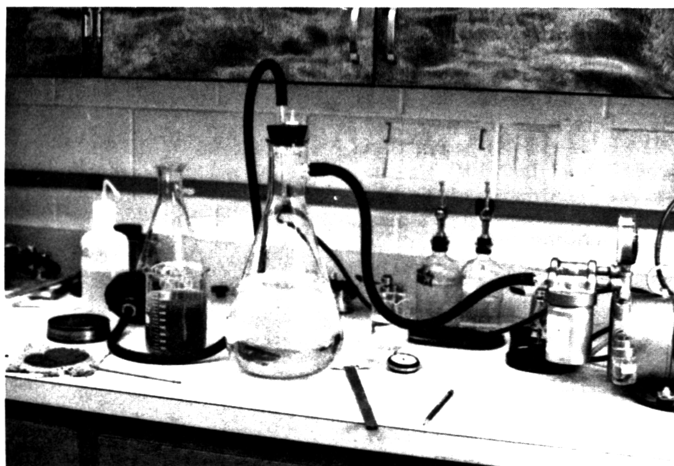

Fig. 18—Effect of vacuum filtration on aerobically digested waste biological solids.

Table 4–Effect of aerobic digestion on waste biological solids dewaterability with a basket centrifuge

Item	Waste biological solids	Aerobically digested waste biological solids
Feed rate	3 gpm	3 gpm
Centrifugal force	1450 G's	1450 G's
Cake solids	4.7%	7.6%
Solids recovery	86%	93%
Time before effluent turned cloudy	5.5 min	17.0 min
Centrate BOD at 9 min	--	120 mg/l

would require substantial prescreening; therefore, the final selection should depend on what cake solids concentration is required for ultimate disposal.

Aerobic digestion

The benefit of aerobic digestion of waste biological solids was demonstrated by improving basket centrifuge cake solids concentrations from 4.7% to 7.6% (62% increase). The digested solids were easily coagulated into a strong floc with minimal polymer additions, allowing excellent solid-liquid separation with a vibratory screen.

Aerobic digestion is very dependent on temperature because all activity is endogenous respiration, which is highly temperature dependent. (Endogenous respiration is the process where microorganisms obtain energy which they need to sustain themselves by consuming the protoplasm of dead cells and, if necessary, some of their own intracellular material. When raw organic waste is limited, the total biological solid mass will decrease.) Low air temperatures experienced in Idaho would probably require sludge detention times of 55–65 days to provide the same amount of conditioning as experienced during the pilot study. A pond of this size probably would not be economical, compared to other available alternatives.

Aerobic digestion would reduce the food value of the biological solids because of the volatile solids destruction. This reduction would be considered a disadvantage if the ultimate disposal method would be animal feed. However, if the ultimate disposal method would be landfill, the volatile solids destruction would reduce the total volume of dewatered solids to be hauled and would then be considered an advantage.

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BIOCHEMICAL CHANGES IN CITRUS FRUITS DURING CONTROLLED-ATMOSPHERE STORAGE

INTRODUCTION

THE OBJECTIVE of controlled-atmosphere (CA) storage of fruits is to prolong storage life through respiratory control. Lowering atmospheric oxygen (O_2) decreased respiration of Valencia oranges (Chace et al., 1967), but after 12 wk in 5 and 10% O_2, the oranges developed distinct off-flavors and high concentrations of ethanol. Anaerobic metabolism produced ethanol in fruits (Wilkinson, 1970), and may also produce off-flavors.

Increasing carbon dioxide (CO_2) concentrations in storage gas has improved quality of many stored fruit, and depression of aerobic and anaerobic respiration of plant tissue by CO_2 has been documented (Smith, 1963). However, CO_2 at 2.5 and 5% did not improve flavor of Valencia oranges stored under 5 and 10% O_2 (Chace et al., 1967), and Young and Biale (1968) reported stimulation of respiration in lemons by 5 and 10% CO_2 with 21% O_2.

Relating various O_2-CO_2 combinations to biochemical changes in citrus fruits would form a rational basis for optimizing conditions for CA storage, and it is toward this end the present work was directed.

MATERIALS & METHODS

ALL FRUIT WERE from commercial groves. Pineapple oranges and Marsh grapefruit were stored 6 wk at 4°C under controlled atmospheres of nitrogen (N_2) with 5, 15 and 21% O_2 and with 21% O_2 + 5% CO_2 and 21% O_2 + 10% CO_2. Valencia oranges were stored 12 wk at 1°C at 10, 15 and 21% O_2 with zero and 2.5% CO_2. Comparable lots of waxed and unwaxed Pineapple oranges and Marsh grapefruit were stored; all lots of Valencia oranges were waxed. Portions of some lots were held 1 wk at 21°C for additional analysis.

Table 1—Condition of Marsh grapefruit and Pineapple oranges on removal from controlled-atmosphere storage after 6 wk at 4°C[a]

	Controlled atmosphere in N_2		Pitting		
Fruit	O_2 (%)	CO_2 (%)	Slight (%)	Severe (%)	CO_2 injury (%)
Grapefruit					
waxed[a]	5	0	13	27	0
	15	0	8	26	0
	21	0	28	17	0
	21	5	2	0	13
	21	10	0	0	21
Unwaxed[a]	5	0	7	70	0
	15	0	7	66	0
	21	0	33	47	0
	21	5	2	4	0
	21	10	0	0	20
Pineapple oranges					
waxed[b]	5	0	1	0	0
	15	0	3	0	0
	21	0	2	1	0
	21	5	2	0	0
	21	10	3	1	0
Unwaxed[b]	5	0	10	0	0
	15	0	5	1	0
	21	0	2	1	0
	21	5	4	0	0
	21	10	1	0	0

[a] 50 grapefruit at each atmosphere
[b] 100 oranges at each atmosphere

Inspection of fruit after storage

Pitting is a physiological disorder resulting in dark, sunken areas of rind. Grapefruit are particularly susceptible to pitting when stored at 4°C and were held at this temperature to determine the effects of atmospheres. The extent of injury was judged "slight" if the total affected area was not more than ¾ in. in diameter and "severe" if greater than ¾ in. in diameter.

Fruit preparation for enzyme and substrate assays

From each treatment group of 100 oranges and 50 grapefruit, 12 oranges or 6 grapefruit were selected randomly for assay of substrate or enzymes. These selected fruit were peeled, and one-half of each fruit was frozen in liquid N_2. The frozen juice vesicles were separated from seeds and coarse section membranes, mixed thoroughly to form a composite sample, and stored in quart Mason jars at −100°C.

Enzyme assays

50g of frozen juice vesicles from the composite sample for each treatment were pulverized in a micro-mill at −196°C at pH 7 with 1.0M Tris. The neutralized powder was thawed after mixing with 10g of presoaked Polyclar AT (25% Polyclar AT in 0.1M potassium phosphate buffer, pH 7.0 containing 5×10^{-3}M dithiothreitol) and strained through cheesecloth. The extract was centrifuged at 5,000 × G for 10 min. The supernatant was saturated with solid $(NH_4)_2SO_4$, equilbrated for 1 hr at 4°C and centrifuged. This residue was suspended in 10 vol of 0.01M Tris buffer, pH 7.4, containing 0.001M EDTA, and dialyzed against distilled water at 4°C for 2 hr. The clear supernatant from the dialysis was assayed for alcohol dehydrogenase (Cossins et al., 1968), pyruvic decarboxylase (Holzer et al., 1965), and malic enzyme (Hsu and Lardy, 1969). Protein content of the supernatant was estimated by the sulfosalicylic acid method of Layne (1957) or by the Potty modification of the Lowry method (Potty, 1969).

Substrate assays

50g of frozen vesicles from the composite sample for each treatment were thawed in an equal volume of 5% perchloric acid, and the deproteinized extract was assayed for citrate (Williamson and Corkey, 1969), malate (Hohorst, 1965), and pyruvate (Neilands, 1955). Composite juice from 10 fruit randomly selected from each treatment was used for analysis of the other substrates. Ethanol and acet-

aldehyde were analyzed by GC analysis of headspace (Davis and Chace, 1969; Davis, 1970). Soluble solids were calculated from refractive indices. Titratable acidity was calculated from the titrated volume of standard NaOH to pH 8.2 and is expressed as % anhydrous citric acid.

Nicotinamide adenine dinucleotide (NAD) assay

30g of frozen juice vesicles from the composite sample for each treatment were pulverized in a micro-mill at −196°C with frozen pellets of 1.0M Tris to raise the pH to 7.0, thawed in 35 ml of 95% ethanol and centrifuged at 5,000 × G for 10 min. A 20-ml aliquot of the ethanol extract was heated to 80°C with 2 ml 2N HCl, immediately cooled to 0°C and neutralized to pH 7. Another 20-ml aliquot was treated similarly with 2N NaOH. The clear supernatants obtained by centrifuging the heat-treated extracts at 5,000 × G for 10 min were used to assay for $NADH_2$ (acid-treated) and NAD (alkali-treated) by a modification of the method of Greenbaum et al. (1965). Into the reaction chamber of the Model 53 Biological Oxygen Monitor were introduced in order: 1.4 ml of a mixture (containing 0.7 ml 0.2M Tris Buffer, pH 7.4; 0.35 ml H_2O; 0.17 ml 95% ethanol; and 0.17 ml 0.01M EDTA); then 0.6 ml 1% phenazine methylsulfate and then 2.0 ml standard NAD solution, juice extract, or H_2O. After allowing 5 min for temperature equilibration and for nonenzymic reduction, 0.04 ml (240U) alcohol dehydrogenase was added. The increase in O_2-uptake rate was recorded for 5 min on a Sargent Model SR recorder. A standard curve for NAD over the range 0.3–10 μg was used to calculate the concentration of NAD and $NADH_2$ in the extracts.

RESULTS & DISCUSSION

Condition of fruit

The total amount and severity of pitting were greater in unwaxed grapefruit than in waxed (Table 1). For example, unwaxed grapefruit held in 21% O_2 had a total of 80% affected fruit, with 47% severely pitted, while waxed grapefruit in 21% O_2 had a total of 45% pitted fruit. Carbon dioxide at 5% decreased the amount of pitting markedly, and at 10% eliminated all pitting, but a different type rind injury developed in CO_2 and affected about 20% of the fruit held in 10% CO_2. This injury was characterized by large areas of soft, gray-brown tissue. Thus, while CO_2 in CA storage of grapefruit is advantageous, caution must be used to avoid injury due to extensive exposure.

Difference in atmospheres had little effect on pitting of Pineapple oranges; the highest incidence, 10% slight pitting, occurred at concentrations of 5% O_2 without CO_2. Valencia oranges were affected even less (data not shown), with no pitting on removal, in any atmosphere, and after 1 wk holding at 21°C only 0.8 and 1.5% pitting in fruit held at 10% O_2 without CO_2 and 10% O_2 with 2.5% CO_2, respectively.

Changes in respiratory substrates during CA storage

Pineapple oranges showed only slight and random changes in Brix and acid (Table 2). Citrate decreased more under 5% O_2 than under 21% O_2 storage, but malate decreased more under 21% O_2 than under any other gas regimen. Pyruvate concentration in citrus juices is near the limit of detectability by the enzyme assay (0.01 μmoles/ml), but highest concentrations were consistently found in Pineapple oranges stored under 5% O_2. Acetaldehyde content increased slightly during CA storage and then increased more rapidly during the 1 wk at 21°C to three and four times the initial level. Ethanol content increased in all stored fruit and was highest in fruit stored at 5% O_2. Ethanol content was consistently higher in fruit stored at 21% O_2 with CO_2 than without. The ethanol content of all samples increased during the additional week at 21°C.

Marsh grapefruit decreased in Brix during CA storage under all regimens (Table 3). The largest decrease was in the fruit stored in 5% O_2. Titratable acidity of fruit in atmospheres containing CO_2 was consistently lower, but otherwise storage did not greatly affect acidity. Citrate decreased during storage consistent with results observed with Pineapple oranges. Both acetaldehyde and ethanol content increased during storage; again, the highest ethanol was found in low O_2 and high CO_2 storage atmospheres.

Valencia oranges showed no change in Brix, but titratable acidity decreased about 20% under all storage conditions

Table 2–Changes in Brix, acidity and substrates of citric acid metabolism in Pineapple oranges during CA storage for 6 wk at 4°C and after 1 wk in air at 21°C[a]

Fruit treatment	Controlled atmosphere in N_2: O_2 (%)	CO_2 (%)	Soluble solids (Brix)	Titratable acidity (%)	Substrate: Citrate (μm/ml)	Malate (μm/ml)	Pyruvate (μm/ml)	Acetaldehyde (μm/ml)	Ethanol (μm/ml)
Waxed									
Initial			11.9	1.06	55.0	7.51	0.02	0.08	17.4
On removal	5	0	11.6	1.04	43.0	6.31	0.04	0.09	103.7
	15	0	11.6	1.02	–	–	–	0.08	59.8
	21	0	12.1	1.01	53.0	6.01	0.02	0.09	50.6
	21	5	11.6	0.94	–	–	–	0.12	62.8
	21	10	11.7	1.00	47.5	6.52	0.02	0.12	80.4
After 1 wk in air at 21°C	5	0	11.6	0.92	–	7.17	0.04	0.26	160.9
	21	0	11.8	0.99	–	6.02	0.02	0.26	97.6
	21	10	11.8	0.93	–	6.67	0.02	0.30	118.5
Unwaxed									
Initial			11.9	1.06	55.0	7.51	0.02	0.08	17.4
On removal	5	0	12.0	0.86	43.2	6.89	0.04	0.17	88.0
	15	0	12.3	1.01	–	–	–	0.16	55.4
	21	0	12.3	1.06	47.5	6.54	0.02	0.10	25.7
	21	5	11.6	1.02	–	–	–	0.13	33.0
	21	10	11.9	0.97	47.5	6.89	0.02	0.13	51.7
After 1 wk in air at 21°C	5	0	12.2	0.97	–	6.71	0.05	0.31	102.2
	21	0	12.9	1.01	–	6.36	0.02	0.21	55.0
	21	10	11.3	0.93	–	6.51	0.02	0.28	71.3

[a] Average of two analyses of composite sample of 10 oranges from 100-fruit lots for solids, acid, acetaldehyde and ethanol; average of two or three analyses of composite sample of 12 oranges from 100-fruit lots for other substrates.

Table 3—Changes in Brix, acidity and substrates of citric acid metabolism in Marsh grapefruit during CA storage 6 wk at 4° C[a]

Fruit treatment	Controlled atmosphere in N_2 O_2 (%)	CO_2 (%)	Soluble solids (Brix)	Titratable acidity (%)	Substrate Citrate (µm/ml)	Malate (µm/ml)	Pyruvate (µm/ml)	Acetaldehyde (µm/ml)	Ethanol (µm/ml)
Waxed									
Initial			9.3	1.40	74	3.73	0.02	0.018	1.5
On removal	5	0	8.2	1.33	65	3.73	0.02	0.050	44.1
	15	0	9.2	1.43	–	–	–	0.063	21.5
	21	0	9.0	1.41	65	3.39	0.02	0.059	15.2
	21	5	8.9	1.34	–	–	–	0.088	23.7
	21	10	8.7	1.24	66	3.73	0.02	0.068	27.0
Unwaxed									
Initial			9.3	1.40	74	3.73	0.02	0.018	1.5
On removal	5	0	8.2	1.40	63	2.95	0.04	0.081	36.3
	15	0	9.1	1.43	–	–	–	0.063	12.0
	21	0	8.7	1.40	65	3.38	0.02	0.054	9.6
	21	5	8.9	1.32	–	–	–	0.084	15.1
	21	10	8.6	1.30	65	3.55	0.02	0.079	20.4

[a]Average of two analyses of composite sample of 10 fruit from 50-fruit lots for solids, acid, acetaldehyde and ethanol; average of two or three analyses of composite sample of six fruit from 50-fruit lots for other substrates.

(Table 4). Citrate decreased, but less than it did in Pineapple oranges. With few exceptions, pyruvate, acetaldehyde and ethanol increased during storage. Ethanol increased with decreasing O_2 concentrations of the atmospheres and was consistently higher in atmospheres containing CO_2.

Relationship between changes in ethanol, Brix and acidity in citrus fruit during CA storage

Ethanol increased and Brix and/or acidity decreased in citrus fruit during CA storage under all conditions. Correlation between these changes was examined statistically because ethanol is a product of sugar and acid metabolism under anaerobic conditions in stored fruits (Smith, 1963). Citrus fruits are capable of metabolizing glucose (Parekh et al., 1971), and malic acid (Clark and Wallace, 1963) to pyruvate and hence to acetaldehyde and ethanol. The equivalent yields of ethanol from sucrose (Brix) and citrate (titratable acidity) were taken to be 4 and 1, respectively. Because all treatments increased the anaerobic metabolism of carbohydrate to ethanol, all treatments were included in the examination of the regression of ethanol increase on decrease in sugar and acid. In grapefruit the increase in ethanol correlates to the decrease in Brix and acid at the 1% level of significance (Table 5). In the Pineapple and Valencia orange experiments, the changes do not correlate.

Correlation between changes in grapefruit and the relatively large decrease in Brix suggest that sugar is the major source of ethanol in grapefruit. However, in the orange, sugar may not be the major source of ethanol. Other compounds, such as amino acids and proteins, may be metabolized and contribute to the ethanol pool. Oranges lost 3–5 µmoles of amino acids per ml during storage. Amino acids are reported to be precursors of ethanol and volatile compounds in ripening fruits (Nursten, 1970), and glutamate from protein breakdown was suggested as a respiratory substrate in lemons (Ramakrishnan et al., 1971).

Changes in enzymes during CA storage

The values for activities of alcohol dehydrogenase (ADH) and CO_2-metabolizing enzymes are maximum velocities with substrate saturation and indicate enzyme levels in the fruit (Table 6). Pineapple oranges had relatively low initial levels of malic enzyme which increased several fold in all storage regimens. Marsh grapefruit and Valencia oranges had comparatively high initial levels which varied randomly during storage.

The relatively low initial levels of pyruvic decarboxylase and ADH in Marsh grapefruit and Pineapple oranges in-

Table 4—Changes in Brix, acidity, and substrates of citric acid metabolism in waxed Valencia oranges during CA storage 12 weeks at 1° C[a]

Controlled atmosphere in N_2 O_2 (%)	CO_2 (%)	Soluble solids (Brix)	Titratable acidity (%)	Substrate Citrate (µm/ml)	Malate (µm/ml)	Pyruvate (µm/ml)	Acetaldehyde (µm/ml)	Ethanol (µm/ml)
Initial		11.9	1.20	56.5	7.0	0.02	0.15	27.0
21	0	11.9	0.96	48.3	7.2	0.02	0.18	25.0
15	0	11.9	1.03	48.6	7.0	0.06	0.32	42.6
10	0	11.9	0.95	45.0	6.8	0.05	0.35	56.6
21	2.5	11.9	0.96	52.0	6.9	0.02	0.35	46.0
15	2.5	11.9	0.93	43.8	6.3	0.06	0.43	84.5
10	2.5	11.9	0.93	50.2	6.5	0.05	0.64	86.2

[a]Average of two or three analyses of composite sample of 10 fruit from 100-fruit lots for solids, acid, acetaldehyde and ethanol; average of two or three analyses of composite sample of 12 fruit from 100-fruit lots for other substrates.

Table 5–Analysis of correlation between changes in ethanol (X_1) and in Brix plus acid (X_2) during CA storage[a]

Fruit and treatment	$\bar{x}_1$ (μm)	Δ-Brix	Δ-%Acid	$\bar{x}_2$ (μm)	r	Significant levels of r
Marsh grapefruit						
Waxed and unwaxed	21.0	0.65	0.04	66.2	0.765	1%=0.765: df=8
Pineapple oranges						
Waxed and unwaxed	44.2	0.03	0.07	6.9	0.616	5%=0.632: df=8
Valencia oranges						
Waxed	10.0	0.00	0.24	12.3	0.457	5%=0.811: df=4

[a]Change in ethanol = Δ-EtOH = X_1 = EtOH after storage minus initial EtOH: $\bar{x}_1$ = mean Δ-EtOh. Change in Brix = Δ-Brix = Initial Brix minus Brix after storage. Change in Acid = Δ-Acid = Initial acid minus acid after storage (titratable acidity). X_2 = 4 times Δ-Brix (μmoles sucrose) plus Δ-Acid (μmoles citric acid): $\bar{x}_2$ = mean X_2. r = correlation coefficient between X_1 and X_2 = $Sx_1x_2/(Sx_1^2 \cdot Sx_2^2)^{1/2}$, where Sx_1X_2, Sx_1^2 and Sx_2^2 are sums of products and squares of deviations from means of X_1 and X_2.

creased several fold during storage which probably contributed to the higher ethanol levels. Valencia oranges had relatively high levels of enzymes, and, in contrast to the others, CA storage had little effect on enzyme levels or their substrates.

Changes in NAD and $NADH_2$ during CA storage

NAD increased in waxed Pineapple oranges, but the $NADH_2$ values changed little, suggesting net synthesis or conversion from a nonextractable form (Table 7). The redox ratios of all waxed fruit increased during storage; the 21% O_2–10% CO_2 treatment showed the least change. The 5% O_2-stored group had the highest redox ratio and in contrast to the other treatments, it did not decrease when the fruit was transferred to air at 21°C for a week.

In unwaxed fruit, NAD decreased, but changes were random and not related to storage conditions. Again, as in waxed fruit, the 5% O_2-stored group had the highest redox ratio, but it declined to the level of the other treatments after 1 wk in air at 21°C. The 21% O_2 and 21% O_2–5% CO_2 treatments showed the least change in the ratios.

If the metabolites of ADH, acetaldehyde and ethanol, are in equilibrium with the dinucleotides, the cytoplasmic NAD/$NADH_2$ ratios should be proportional to the acetaldehyde/ethanol (A/E) ratios according to the equation: NAD/$NADH_2$ = 0.3 A/E × 10^3 (Bruemmer and Roe, 1971). Therefore, if NAD and $NADH_2$ in our assay are representative of free cytoplasmic forms, their ratio should show this same proportionality to A/E. Before storage, the ratio for NAD/$NADH_2$ in Pineapple oranges was about equal to 0.3 × A/E × 10^3 (Table 7). However, the ratio of the nucleotides in waxed fruit increased during storage while the A/E ratio decreased. Only unwaxed oranges stored in 21% O_2 and 21% O_2–5% CO_2 showed a correspondence between nucleotides and substrates for ADH. These treatments also showed the least change in their ratios.

Apparently NAD and $NADH_2$ extracted from stored waxed fruit and from unwaxed fruit stored under 5% O_2, 15% O_2 and 21% O_2–10% CO_2 are not in equilibrium with acetaldehyde and ethanol in the fruit. This might be expected because the concentrations of ethanol in these treated fruit were three to six times the initial concentration (Table 2), while the ADH activity in the fruit increased less than 0.75 times. Therefore, if ethanol in initial juice was near the saturation level for the enzyme to establish equilibrium, then increasing its concentration without a comparable increase in ADH would result in having more ethanol than can react with ADH. This situation creates a metabolic compartment for the ethanol which no longer is in equilibirum with acetaldehyde and ADH. Only in the two treatments where ethanol and ADH increased about the same were the ADH products and reactants in equilibrium.

Table 6–Changes in alcohol dehydrogenase and CO_2 metabolizing enzymes in citrus fruit during CA storage[a]

Fruit treatment	Controlled atmosphere in N_2: O_2 (%)	CO_2 (%)	Enzyme activity Nanomoles per min per mg protein: Malic enzyme	Pyruvic decarboxylase	Alcohol dehydrogenase
Unwaxed Marsh grapefruit, 6 wk at 4°C					
Initial			1660	280	126
On removal	5	0	1040	405	365
	21	0	1615	560	370
	21	10	1330	594	260
Unwaxed Pineapple oranges, 6 wk at 4°C					
Initial			140	280	430
On removal	5	0	460	570	560
	21	0	380	490	750
	21	10	376	454	690
Waxed Valencia oranges, 12 wk at 1°C					
Initial			1270	740	2580
On removal	10	0	1820	1030	3070
	15	0	920	433	4100
	21	0	1310	740	2850
	10	2.5	1250	587	1850
	15	2.5	1600	806	3520
	21	2.5	1210	628	4600

[a]Average of two or three analyses of composite sample of six grapefruit from 50-fruit lots: average of two or three analyses of composite sample of 12 oranges from 100-fruit lots.

CONCLUSIONS

ACCUMULATION of ethanol and acetaldehyde and stimulation of pyruvic dehydrogenase and ADH indicate a shift toward anaerobic respiration in CA-stored citrus. This shift is reflected in decline of the acetaldehyde/ethanol ratio in Pineapple oranges as O_2 in CA decreased and CO_2 increased. Because oxygenated compounds account for most citrus flavor (Stanley, 1958; Wolford et al., 1962), a

Table 7–Changes in NAD and $NADH_2$ concentrations in Pineapple oranges during CA storage for 6 wk at 4°C and after 1 wk in air at 21°C[a]

Fruit treatment	Controlled atmosphere in N_2 O_2 (%)	CO_2 (%)	NAD (μm/10g)	$NADH_2$ (μm/10g)	NAD / $NADH_2$	0.3 × Acetaldehyde × 10^3 / ethanol
Waxed						
Initial			32.0	24.6	1.3	1.4
On removal	5	0	65.6	16.0	4.1	0.3
	15	0	61.3	22.6	2.7	0.4
	21	0	62.8	25.7	2.4	0.5
	21	5	46.2	23.1	2.0	0.6
	21	10	32.1	21.9	1.5	0.5
After 1 wk in air at 21°C	5	0	42.0	10.1	4.2	0.5
	15	0	35.0	16.9	2.1	–
	21	0	26.3	30.4	0.9	0.8
	21	5	26.7	29.2	0.9	–
	21	10	20.7	25.9	0.8	0.8
Unwaxed						
Initial			34.3	27.0	1.3	1.4
On removal	5	0	27.2	9.7	2.8	0.6
	15	0	16.4	35.1	0.5	0.9
	21	0	23.3	18.9	1.2	1.2
	21	5	30.0	21.7	1.4	1.2
	21	10	15.0	24.8	0.6	0.8
After 1 wk in air at 21°C	5	0	18.7	25.1	0.8	0.9
	15	0	27.5	18.9	1.4	–
	21	0	26.6	22.4	1.2	1.2
	21	5	26.4	15.3	1.7	–
	21	10	27.6	35.4	0.7	1.2

[a]Average of two or three analyses of composite sample of 12 oranges from 100-fruit lots.

shift in equilibirum toward reduced forms may drop their concentrations to a lower flavor level and increase the level of more reduced components.

The greater anaerobic states of waxed fruit under all CA regimens show the modifying action of waxing on the O_2-potential of the atmosphere and suggest need for more data on the role of surface coatings in CA storage of citrus.

Further work is indicated to determine whether short-term exposure of grapefuit to CO_2 would decrease pitting without producing other injury. Also, the effects of different coatings on ethanol accumulation and enzyme activities during storage warrant further attention.

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DAMAGING STRESSES TO FRESH AND IRRADIATED CITRUS FRUITS

INTRODUCTION

CAREFUL HANDLING of fresh citrus fruits during harvesting and transportation to the processing plant or to the fresh fruit market is of importance to the producer and the processor. Rough handling results in physical damage and subsequent microbial decay of the fruits. Manually harvested citrus fruits are subject primarily to 'plugging' damage (tearing off a portion of the peel around the fruit stem-end). Mechanically harvested fruits are subjected to damages such as splits, punctures and bruises (Coppock and Grierson, 1970). 'Plugging' in mechanically harvested fruits occurs to much lesser extents than that occurring in manual harvesting (Rockham and Grierson, 1971). Mechanical harvesting of Hamlin, Pineapple and Valencia oranges, and Marsh and Duncan grapefruit resulted in high percentages of fruit with attached stems over 1.3 cm long (Coppock and Grierson, 1970; Grierson, 1968b; Rockham and Grierson, 1971). These stems puncture other fruits during handling and interfere with the efficient use of the in-line FMC juice extractors, resulting in lower juice yields. Chemical treatments of the fruits have been suggested to minimize the adhesion of the stems either as abscission-inducer sprays prior to harvesting the fruits (Wilson, 1969) or treatment of the harvested fruit with ethylene (Coppock and Grierson, 1970). Decay organisms enter the fruits through the existing wounds resulting in additional losses. The use of fungicides and processing the fruit within 36 hr after harvest were suggested to reduce the decay indices (Coppock and Grierson, 1970; Rockham and Grierson, 1971).

External forces required to rupture oil glands present in the peel of citrus fruits vary according to the degree of turgidity of the fruits. Turgid fruits are susceptible to oil gland rupture, while flaccid fruits require larger rupturing forces. The ruptured oil causes unacceptable discolored blemishes on the fruit surfaces. This phenomenon is known as oleocellosis and it was found to occur in lemons (Cahoon et al., 1964; Grierson, 1968a; Oberbacher, 1965), limes (Eaks, 1955), Navel and Valencia oranges and Marsh grapefruit (Turrell et al., 1964). The Magness-Taylor pressure tester and similar instruments with puncturing tips ranging from 0.318–0.953 cm in diameter were used to measure the pressures required to rupture the rind oil glands (Cahoon et al., 1964; Oberbacher, 1965; Turrell et al., 1964). The diameters of these puncturing tips were large enough to include oil glands and surrounding epidermal tissue cells.

Rough handling induced stylar end breakdown of Persian limes (Grierson and Pantastico, 1968). Impact forces applied to the mammiform tip at the stylar end determined the extent of the fruit injury. Turgid fruits were more susceptible to this type of injury than the flaccid ones.

Forces required to rupture whole citrus fruits or to puncture or shear the peels are not reported in the literature. The knowledge of such forces may be of value in adopting better harvesting and handling methods for the production of better quality fruits for the fresh market and processing. The objectives of the present study were to measure the resistance of citrus fruits to forces as influenced by commercial processing and irradiation.

EXPERIMENTAL

Fruits

Navel, Temple and Valencia oranges, Duncan grapefruit and Dancy tangerines were obtained from the G. & S. packing house, Weirsdale, Fla. Bearss lemons were donated by the Foods Div., Coca Cola Co., Plymouth, Fla., and Persian limes were donated by Kendall Packing Co., Goulds, Fla. All fruits were manually harvested.

Sample preparation

Sound fruits free from cuts, bruises, splits or decay were selected for use. Field run samples consisted of fruits as harvested from the trees, gently washed and air dried at the laboratory. Commercially processed fruits (waxed) were washed, waxed and polished by the common practices used in commercial packinghouses. Waxed fruits were also used for the irradiation treatment. Fruits were exposed to ^{137}Cs gamma rays under continuous air flow and at 13°C. Irradiation treatment was limited to a 300 Krad dose. Times required to expose the fruits to these doses were calculated from the minimum dose rates. Variations in minimum dose rates were due to differences in size and shape of the fruits introduced to the radiation field. The intensity of gamma rays in the radiation field is not uniform throughout. The ratio of maximum to minimum intensities is expressed as the uniformity ratio. Irradiation conditions are shown in Table 1.

Texture measurements

The Instron Universal Testing Instrument model TM equipped with load cells CCTM or CB was used for all texture measurements. All tests were performed on individual fruits. There were 20 fruits per replicate and three replicates for each citrus type-treatment combination.

A plastic plate containing the fruit was placed on the CCTM load cell. A flat plate 5.50 cm in diameter was used to compress the fruit until point of rupture. The splitting of fruit peel was considered as rupture point. The crosshead and recorder chart speeds were 2.0 cm/min each. Data are presented as the maximum force (kg) required to rupture the fruit.

A cylindrical probe 0.258 cm in diameter was used to puncture Navel and Valencia oranges, Dancy tangerines, Bearss lemons and Persian limes and a probe 0.264 cm in diameter for Duncan grapefruit and Temple oranges. These probes were selected to approximate the sizes of the fruit stems. A rubber ring was placed on the CB load cell surrounding the fruit to prevent its movement during the test. The probe was permitted to penetrate the fruit until maximum force was reached, then was withdrawn from the fruit. Results are presented as maximum force (kg) needed to puncture the fruit.

Punch shear was carried out on the peel (flavedo and albedo) of citrus fruits. A cork borer was used to obtain a peel plug 1.905 cm in diameter. A sample holder was constructed to shear the peel plugs. The holder consisted of two separate stainless steel circular plates 7.5 cm in diameter and 1.3 cm thick. A hole 0.667 cm in diameter was drilled in the center of each plate. The peel sample was centrally located between the two plates; the plates were aligned and held together by two pins. The sample holder was placed on the CCTM load cell. A circular punch 0.667 cm in diameter was attached to the Instron-crosshead bar and was used to punch shear the peel sample. The crosshead and the recorder chart speeds were 1.0 and 10.0 cm/min, respectively. The sample thicknesses were less than the thickness of the lower plate of the sample holder so that the sheared portion of the plug was not compressed

Table 1—Irradiation conditions for citrus fruits

Fruit type	Min dose rate (Krad/hr)	Uniformity ratio
Oranges		
Navel	196	1.3
Temple	212	1.3
Valencia	203	1.2
Grapefruit	194	1.3
Lemon	193	1.3
Lime	193	1.3
Tangerine	196	1.3

against the load cell. Sample thickness was measured by the Glogau vernier caliper No. 12. The downward movement of the crosshead bar was stopped as soon as the punch penetrated through the peel sample. Shearing stress of the sample was calculated from the formula

$$S = F/\pi DT$$

where S = shearing stress (kg/cm²); F = maximum force (kg); π = 3.1416; D = punch diameter (cm); and T = sample thickness (cm).

A blank drill bit 0.051 cm in diameter was used to puncture the peel oil glands. Oil glands are present at different depths in the outer cell layers of the peel and vary in color from dark to light. Oil glands which were intermediate in color were selected for the puncture test. The fruit was placed on the CB load cell, with a rubber ring under and surrounding the fruit to prevent its movement during the test. An illuminated magnifier was used to select the oil gland and position the punch just above the gland. The Instron was set automatically to penetrate to a 0.20 cm depth. Crosshead and recorder chart were set at 0.5 and 20.0 cm/min, respectively. A puncture test was considered successful if oil exudation was visually sighted during the test. Data are presented as the maximum force (g) required to puncture the oil gland.

Histological examination

Peel segments 4–5 mm wide were randomly obtained from the fruit equator. The segments were fixed in Karnovsky solution (Karnovsky, 1965) and evacuated to remove any air bubbles present. The fixed segments were clarified by soaking for 24 hr each in the following sequence of solutions: 5% acetic acid-glycerol, 50% glycerol and 70% ethanol. A cryostat was used to section the segments into sections 15–20 μ thick. The sections were stained with a saturated solution of safranin-0 in ethyl alcohol and mounted in 50% glycerol. The anatomical preparations were examined microscopically and photographed at the magnification of 35×.

RESULTS & DISCUSSION

FORCES NEEDED to induce stress damages to citrus fruits ranged from small values for Dancy tangerines, and Navel and Temple oranges; to intermediate values for Valencia oranges and to large values for the Persian limes and Bearss lemons (Table 2). Duncan grapefruit showed greater resistances to rupturing and puncturing forces as compared with the Dancy tangerines and Navel and Temple oranges, but similar forces to their shear strength and peel oil gland rupture. Larger forces were required to rupture or to shear citrus fruits than those needed for puncture or peel oil gland rupture. The outer skin of apple fruits plays an important role in their "stiffness" (Abott et al., 1968a, b; Sherman, 1972). The magnitude of rupturing or shearing forces of citrus fruits may be due to the "stiffness" of the peel. The grapefruit peel may possess greater "stiffness" than orange or tangerine peel. Somers (1965) suggested that the structure of cell walls and the turgor of the cells influence the viscoelastic behavior of plant tissues. Differences in these characteristics of plant tissues may be responsible for differential responses of citrus fruits to rupturing and shearing forces.

Table 2—Failure forces and peel shear stress of citrus fruits as influenced by waxing and irradiation; main effect means

	Rupture (kg)	Puncture (kg)	Shearing stress (kg/cm²)	Peel oil gland rupture (g)
		Citrus type		
Tangerine	0.8	0.05	4.1	48.8
Navel	2.1	0.11	4.5	54.7
Temple	9.4	0.66	4.6	46.8
Duncan	28.9	0.87	4.5	57.6
Valencia	20.4	1.20	8.9	73.6
Lime	36.4	4.00	23.4	180.2
Lemon	37.9	3.10	13.7	172.9
		Treatment		
Field run	19.8	1.70	10.0	106.7
Waxed	23.3	1.70	11.3	93.0
Irradiated	15.0	0.83	5.70	72.3

Table 3—Failure forces and peel shear stress of citrus fruits as influenced by waxing and irradiation; interaction means

Citrus type	Treatment[a]	Rupture (kg)	Puncture (kg)	Shearing stress (kg/cm²)	Peel oil gland rupture (g)
Tangerine	F	0.93	0.06	4.7	62.3
	W	0.91	0.05	4.7	49.7
	I	0.57	0.04	2.9	34.3
Navel	F	2.3	0.13	4.6	55.5
	W	2.6	0.13	5.7	56.3
	I	1.5	0.08	3.3	52.2
Temple	F	11.6	0.75	4.9	50.2
	W	9.6	0.71	5.1	55.1
	I	6.9	0.53	3.7	35.2
Duncan	F	28.8	0.98	4.8	67.3
	W	35.4	1.0	4.8	61.3
	I	22.4	0.58	4.0	44.2
Valencia	F	24.2	0.98	9.5	89.6
	W	22.7	1.55	11.7	71.0
	I	14.2	0.96	5.4	60.2
Lime	F	41.1	5.0	27.7	221.9
	W	46.8	4.9	31.2	178.7
	I	21.2	1.9	11.3	140.0
Lemon	F	50.0	3.9	14.0	200.0
	W	45.2	3.6	16.1	178.6
	I	18.5	1.7	9.2	140.2

[a] F = field run; W = waxed; I = irradiated.

Weakening of the structural strength of the irradiated citrus fruits was evident with their decreased resistances to applied forces in comparison with either the field run or waxed fruits (Tables 2 and 3). This irradiation influence was similar to that found for mangoes, strawberries, peaches and tomatoes (Ahmed and Dennison, 1971; Ahmed et al., 1972a, b, c). The influence of commercial processing on the textural properties of citrus fruits varied with the method of stress application and with the type of citrus fruits (Table 3). Increased shearing stress of the peel and decreased peel oil gland rupturing forces were generally found for the commercially processed fruits. Waxed Navel oranges, Duncan grapefruit and Persian limes exhibited greater resistances to rupture and shearing stress as compared with the field run fruits. Commercially processed Temple and Valencia oranges and Bearss lemons showed decreased resistance to rupturing forces. With the exception of Valencia oranges, small differences in puncturing forces were ob-

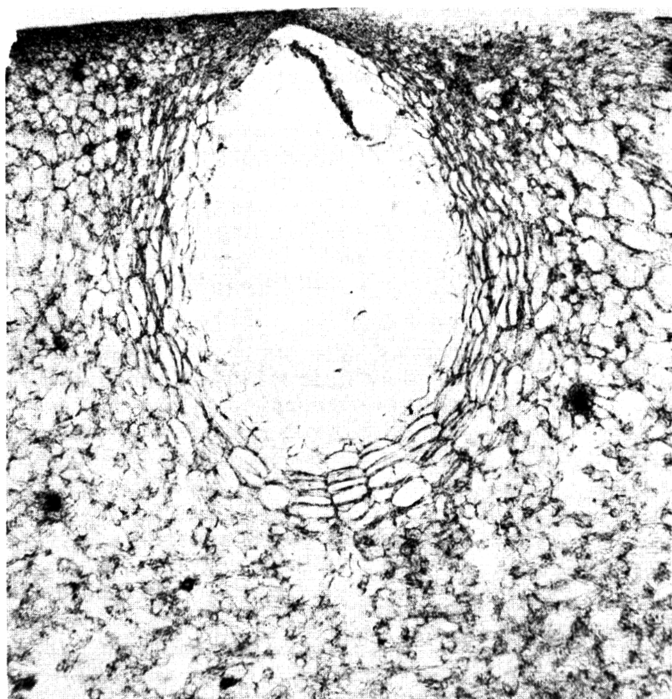

Fig. 1—Cross section of Duncan grapefruit peel irradiated with 300 Krad, showing the tear drop-shaped oil gland. 35X.

Table 4—Punch shear variables of citrus fruits as influenced by waxing and irradiation

Citrus type	Treatment[a]	Peel thickness (cm)	Force at peel thickness depth (kg)	Max force (kg)	Distance at max force (cm)
Tangerine	F	0.19	1.1	1.8	0.12
	W	0.21	1.1	2.1	0.13
	I	0.20	0.52	1.2	0.10
Navel	F	0.41	1.0	3.9	0.27
	W	0.38	1.3	4.5	0.24
	I	0.36	0.92	2.5	0.22
Temple	F	0.26	0.72	2.6	0.14
	W	0.23	0.73	2.4	0.12
	I	0.20	0.55	1.5	0.11
Duncan	F	0.38	1.2	3.8	0.24
	W	0.38	1.4	3.9	0.26
	I	0.37	1.1	3.1	0.25
Valencia	F	0.30	4.1	6.0	0.25
	W	0.26	4.3	6.5	0.22
	I	0.28	2.3	3.1	0.24
Lime	F	0.18	8.4	10.5	0.18
	W	0.21	6.4	13.6	0.19
	I	0.21	3.5	5.1	0.21
Lemon	F	0.47	3.8	13.7	0.29
	W	0.41	6.2	13.8	0.30
	I	0.30	4.0	6.6	0.25

[a]F = field run; W = waxed; I = irradiated.

served between the field run and commercially processed citrus fruits. Waxed Valencia oranges required larger puncturing forces than the field run fruits. Generally, commercial processing resulted in lowering the forces required to rupture the peel oil glands (Tables 2 and 3). Mechanical handling of the fruit during commercial processing may result in either weakening or rupturing of their cuticles or the outermost layers of cell tissues which may result in decreased resistance of the fruits to peel oil gland rupturing forces. The degree of turgidity of the fruits influences the extent of these rupturing forces, with turgid fruits being more susceptible to oil gland rupture (Grierson, 1968a; Oberbacker, 1965). The relatively small forces needed to rupture these glands in tangerines, oranges and grapefruits indicate the need for the gentle handling of these fruits. Rupturing forces for oil glands of lemons and limes were about three to four times those needed for other citrus fruits. Oil glands are spherical structures with diameters ranging from 0.3–1.1 mm and are located close to the flavedo (Fig. 1). No gross histological differences in the oil gland structures were observed among the field run, waxed or irradiated citrus fruits. The force-deformation curves for rupturing these gland are shown in Figure 2. The Instron's crosshead bar was stopped at the moment of visual sighting of the extruded oil (arrows). Differences in the distances where sighting occurred were due primarily to differences in the position of contact areas between the probe and the oil glands and to differences in the depths of the oil glands. Rupturing forces obtained in this test represent the combined effects of rupturing the oil gland walls and any flavedo tissue layers present above these glands. However, since visual sighting of the extruded oil always occurred after the applications of a maximum force, it was considered that these maximum forces represent the rupturing forces of the oil glands. Results obtained in the present study are supported by those found by Turrell et al. (1964), where lemon fruits required larger forces to rupture the oil glands than oranges and grapefruit. Oil gland rupturing forces for the California grown Eureka lemons were lower than those obtained for the Florida grown Bearss lemons. Turrell et al. (1964) reported values ranging from 55 to 80 kg/cm^2 for Eureka lemons while the corresponding values obtained in the present study for Bearss lemons ranged from 66 to 95 kg/cm^2. However, these values do not represent the actual forces required to rupture an individual oil gland. The oil glands averaged about 0.067 cm in diameter, thus probes with smaller diameters

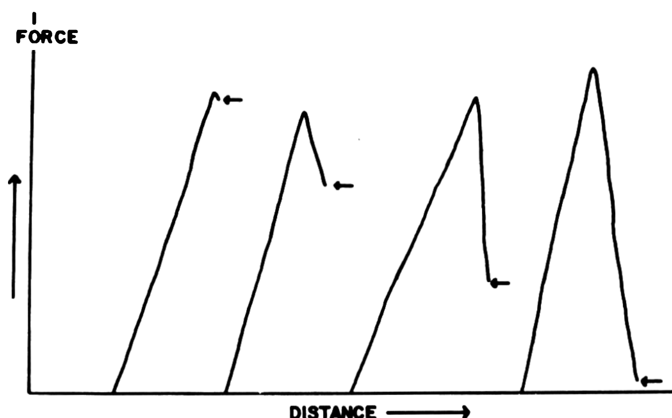

Fig. 2—Force-deformation curves of peel oil gland rupture. Arrows indicate visual sighting of extruded oil.

should be used for their puncture. The diameters of the probes used by Turrell et al. (1964) and Oberbacher (1965) were 0.317 and 0.952 cm, respectively.

Statistical analysis of the experimental data indicated that, in all cases considered the effects of citrus type, treatment and the interaction citrus type × treatment were significant. Some general conclusions could be drawn from the statistical analysis: (a) regardless of which citrus type was used, the lowest value for each stress was obtained by the irradiated fruits; (b) the reduction in the irradiated fruit resistance to applied forces was not always significant; (c) no significant differences were found between field run and commercially processed fruit; and (d) high values of the responses were associated with limes and lemons while low values were found for tangerines and Navel and Temple oranges.

Maximum forces needed to punch shear citrus fruit peel were obtained at depths shallower than the thickness of peel samples (Table 4). Maximum forces occurred at about 60% of peel thickness for Dancy tangerines, Navel and Temple oranges and Duncan grapefruit. The corresponding values for Valencia oranges and Persian limes were about 85 and 95%, respectively. The peel thickness for Bearss lemons varied from 0.304–0.471 cm and maximum forces occurred at depths of 60–80% of the peel thickness. Although similar maximum forces were observed for shearing lemon and lime peel samples (Table 4), the shearing stress of lemon peel was less than that for lime samples (Table 2). This was probably due to the differences in the peel thickness of the two fruit types and to the differences in the proportions of flavedo and albedo in the peel sample of each fruit type. Grierson (1964) stated that the albedo is a soft shock absorbing layer and pressures transmitted through the albedo are conveyed to the fruit segments and their juice vesicles. However, shearing damages to citrus fruit peels only occur when forces are equal or exceed the maximum forces shown in Table 4.

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A CALORIMETER FOR MEASURING HEAT OF RESPIRATION OF SMALL SAMPLES OF BIOLOGICAL MATERIALS

INTRODUCTION

THE METHOD of adiabatic measurements in microcalorimetry was first introduced by T. W. Richards (Swietoslawski, 1946). Adiabatic heating of respiring biological materials may be examined with use of the adiabatic calorimeter described in this paper.

Ostrovskii et al. (1968) developed a universal, double, differential, automatic microcalorimeter using resistance thermometers. Toledo et al. (1969) used a calorimeter to make a direct calorimetric determination of the heat of respiration of some products in controlled atmospheres. An adiabatic specific heat calorimeter was used by Suter et al. (1972) to measure the specific heat of peanut pods, hulls and kernels of Spanish peanuts. Bailey (1940), Kidd (1915), Milner and Geddes (1945, 1946), Milner et al. (1947), Taylor (1942), Todd (1958) and Todd et al. (1969) developed various microcalorimeters for use in respiration studies.

Calvert and Prat (1963) refer to the walls of the calorimeter container as the "internal boundary" and the walls of the surrounding cavity as the "external boundary." They point out that the best types of adiabatic calorimeter are those in which the external boundary temperature is continuously adjusted to match identically that of the internal boundary. The net transfer between the two boundaries would then be zero.

The most important problem in microcalorimetry is to perform the measurements in such a way as to eliminate systematic or accidental errors (Swietoslawski, 1946). The method of comparative microcalorimetric measurements is used to estimate errors involving the calorimeter equipment. Two successive measurements may be made in one calorimeter to produce the same thermal effect by an electrical current as that developed by the object being studied.

EXPERIMENTAL

THE HEAT of respiration calorimeter essentially consisted of (a) two 1800 ml Dewar flasks, located inside an air bath chamber; (b) environment chamber; (c) a system to control and measure the temperature, relative humidity, flow rate and CO_2 content of the air flowing through the flasks; and (d) temperature recording equipment. Test samples in one of the flasks were used for measuring specific heat of respiring material at various temperatures.

An air flow diagram is shown in Figure 1. Location of a portion of the thermocouples used in monitoring and controlling the air temperature is also shown.

Air temperature control and recording equipment

The environment chamber maintained an air temperature surrounding the air bath chamber

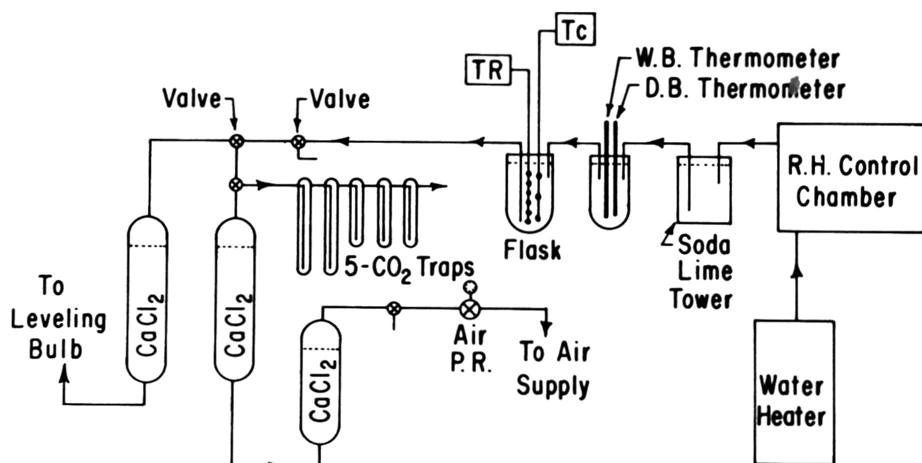

Fig. 1—Air flow diagram of respiration calorimeter.

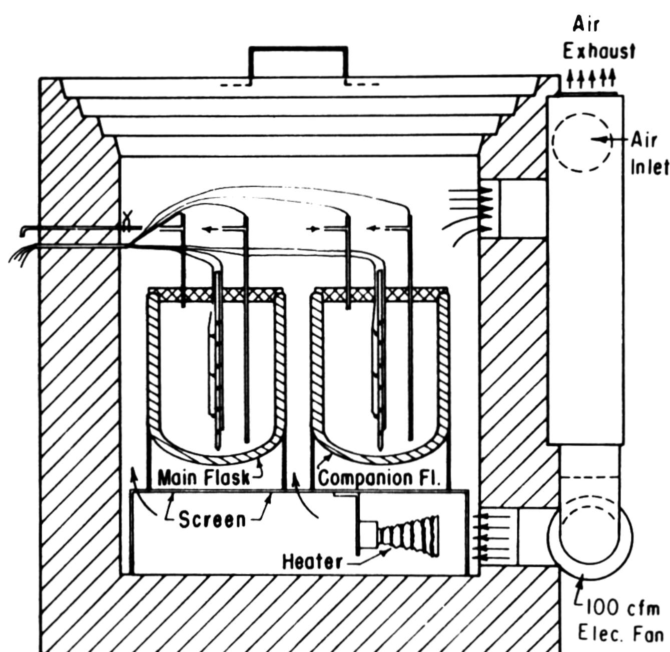

Fig. 2—Cross section of air bath chamber, front view.

at an accuracy of ± 0.5°C within a range of 4–40°C. Temperature fluctuations in excess of ± 1°C would not permit adequate temperature control inside the air bath chamber.

The air bath chamber was located inside the environment chamber to control the environment around the main flask. A cross section of the air bath chamber, constructed of Polyurethane foam, is shown in Figure 2.

A plexiglass shield placed between the air bath chamber and the environment chamber door prevented drastic changes in the calorimeter air temperature when the door was opened. If such drastic change should suddently occur in the environment chamber, the environment in the air bath chamber would be changed so fast that the temperature control system could not adequately perform its function.

Control of temperature of the air surrounding the main flask to insure that it is the same as that inside the flask, is maintained by the use of a Honeywell Cascade control system. It is necessary to maintain identical air temperatures both inside and outside the main flask to prevent heat transfer through the flask walls.

The cascade control system utilized two MV/I transmitters, with an accuracy of ± 0.1 percent of span, with operative limits of 4–49°C temperatures and 5–90% relative humidity. One of the MV/I transmitters serves the set point and the second the process variable. The accuracy of the process variable is ± 1% full scale, and the set point accuracy of ± 0.5% of full scale. The accuracy of the MV/I transmitters is ± 0.1% of full span of –15°C to 40°C. Three copper-constantan thermocouples, connected in parallel to the set point MV/I transmitter, are spaced equidistance vertically in the main flask. The three process variable thermocouples are spaced equidistance around the top of the flask. The vertical scale temperature indicator and electronic controller has an accuracy on the process variable of ± 1% of full scale and an accuracy on the set point of ± 0.5% of full scale.

A 100w electrical resistance heater is located inside the air bath chamber, under the base of the two flasks, to provide the quantity of heat inside the chamber required to raise the temperature of the air bath chamber circulating around the exterior surfaces of the main flask to be exactly the same as that of the air inside the flask.

The temperature at various places in the calorimeter is recorded by a 24 point potentiometer. Six thermocouples are used to monitor the temperature along the vertical center line of the main flask (Fig. 3). The mean value of the temperature of the above six thermocouples, is used as an estimate of the temperature of the peanuts at time, θ. Six thermocouples are placed inside the companion flask for monitoring temperature of samples to be used for periodic specific heat determination (Fig. 2). Temperature measurements were recorded with nine thermocouples uniformly spaced on the outside surface of the main flask to insure that temperature gradients did not exist. Turbulent air velocities were maintained outside the main flask.

Table 1—Data used in determining heat capacity constant of respiration calorimeter main flask

Test no.	Wt hot water (g)	Wt cold water (g)	Initial temp cold water (°C)	Initial temp hot water (°C)	Equilibrium temp (°C)	ΔT_h[a] (°C)	ΔT_c[b] (°C)
1	502.7	404.2	2.9	35.2	21.5	13.7	18.6
2	502.9	400.1	0.8	35.8	21.1	14.7	20.3
3	502.3	401.1	1.0	35.5	21.0	14.5	20.0
4	503.1	406.1	1.8	35.3	21.1	14.2	19.3
5	504.0	401.8	1.8	36.0	21.6	14.4	19.8
6	505.1	391.6	1.6	35.8	21.6	14.2	20.0

[a]Difference between initial hot water and equilibrium temperatures
[b]Difference between initial cold water and equilibrium temperatures

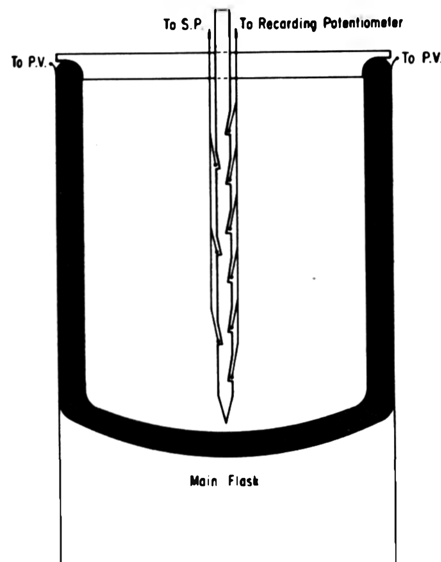

Fig. 3—Location of main flask thermocouples.

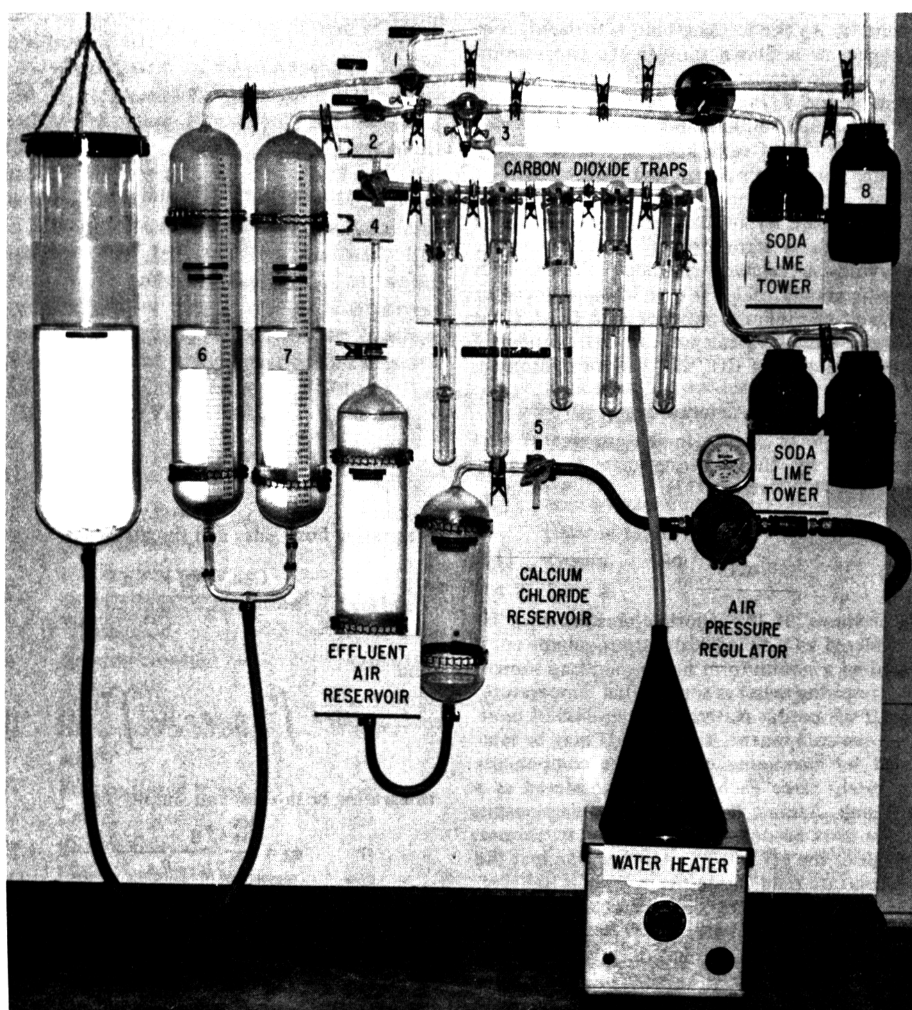

Fig. 4—Aeration and air quality control and analysis equipment.

Table 2–Determination of heat capacity constant, H_C, of main flask of respiration calorimeter

Test no.	H (cal/°C)	Diff. from mean (cal/°C)	Percent difference
1	51.5	0.2	3.9
2	51.3	0.1	1.9
3	50.9	0.3	5.9
4	51.1	0.1	1.9
5	50.9	0.3	5.9
6	51.5	0.3	5.9
Mean	51.2		4.2

Table 3–Respiration calorimeter validation tests' data[a]

Test no.	Volts	Duration of test (sec)	Temp rise in calorimeter (°C)	Calc thermal energy input (cal)	Measured thermal energy input (cal)	Difference between thermal and electrical energy (cal)	Percent difference
1	8.1	39.30	16.7	2282.4	2213.7	68.7	–3.2
2	8.2	35.70	16.7	2099.1	2213.7	114.6	+5.3
3	8.2	36.45	16.7	2143.0	2213.7	70.7	+3.2
4	8.2	32.45	13.9	1913.0	1846.2	66.8	–3.6
5	8.2	30.63	13.9	1800.7	1846.2	45.5	+2.5
6	8.2	30.41	13.9	1787.8	1846.2	58.4	–3.2

[a]The amperes of each test was 0.3. The energy stored in the calorimeter flask and heating apparatus was measured to be 132.82 Cal/°C.

Aeration and air quality control equipment

Air entering the main flask is conditioned to remove CO_2 and raise the relative humidity to equilibrium moisture content. Equipment for aeration and conditioning the air is shown in Figure 4. Wet bulb and dry bulb temperatures (item 8, Fig. 4) are used in determining conditioned air relative humidity.

Aeration rate is controlled by a pumping (lowering) mechanism regulating the position of the leveling bulb, shown at the extreme left of Figure 4. As the leveling bulb is lowered, conditioned air is drawn through the two spirometers of the aeration system at a constant rate (items 6 and 7, Fig. 4). Aeration rates are possible from 500–3000 ml per 24 hr.

At the end of each 12-hr period, the pumping mechanism is stopped and CO_2 content of the air used in aerating the peanuts is measured (Suter, 1972). By regulating various valves, the effluent air is transferred to the effluent air reservoir. A calcium chloride solution with specific gravity of 1.4 is used in the effluent air reservoir to minimize mixture with CO_2. Traps are used to collect calcium carbonate produced in the reaction of CO_2 and barium hydroxide.

Heat of respiration determination

The rate of change in the amount of heat generated by the respiring mass is given by equation [1] (Suter, 1972).

$$\frac{dq_T}{dT} = C_p \rho V \quad [1]$$

Contents of the calorimeter flask may be considered as a cylindrical porous volume composed of a nonuniform heat generating source, q_T, (respiring mass) at some initial temperature, T_{ic}. If the porous material is composed of more than one component, then dq_T/dT may be evaluated by examining each of the components separately since each may be considered as a heat sink. Assume that the average temperature of the mass inside the volume, T_{ic}, is the same as that of the volume T_∞. Also, assume that the temperature field inside the volume is uniform.

Consider transient heating due to respiration of a mass in a Dewar flask with heat energy being stored also in the intermass air and flask walls (Fig. 5). Assume a temperature curve as follows with T_A and T_B representing the mean value of the temperatures of the six thermocouples in the main flask at two times, θ_A and θ_B (Fig. 6). An energy balance equation of the control volume, Dewar flask, would be:

$$Q_{generated} = Q_{stored} \text{ or } Q_g = Q_S \quad [2]$$

The heat stored in each heat sink, i.e., the mass (Q_m), the flask (Q_f) and the air (Q_a) would be

$$Q_s = Q_m + Q_f + Q_a \quad [3]$$

If q_m = rate at which heat is stored in the respiring mass in cal/sec, then

$$Q_m = \int_{\theta_A}^{\theta_B} q_m d\theta = (C_pW)_{mass} \frac{g_c}{g} \int_{T_A}^{T_B} dT \quad [4]$$

After integrating both sides and simplifying

$$q_m = \frac{C_pWg_c(T_B - T_A)}{g(\theta_B - \theta_A)} \quad [5]$$

Similarly,

$$Q_a = \int_{\theta_A}^{\theta_B} q_a \, d\theta = (C_p\rho V)_{air} \int_{T_A}^{T_B} dt \quad [6]$$

Integrating both sides and simplifying

$$q_a = \frac{C_p\rho V(T_B - T_A)}{\theta_B - \theta_A} \quad [7]$$

And

$$Q_f = \int_{\theta_A}^{\theta_B} q_f d\theta = H_c \int_{T_A}^{T_B} dT \quad [8]$$

Integrating both sides and simplifying

$$q_f = \frac{H_c (T_B - T_A)}{\theta_B - \theta_A} \quad [9]$$

Combining terms and solving for q_g, the heat of respiration becomes

$$q_g = \frac{[(C_pW)_m(g_c/g) + (C_p\rho V)_a + H_c] \, [T_B - T_A]}{\theta_B - \theta_A} \quad [10]$$

RESULTS

Heat capacity constant of calorimeter flask

The calorimeter flask was calibrated to determine the flask heat capacity constant, H_c, a constant which defines the amount of heat stored by the flask and attached auxiliary equipment. The value of H_c was measured to be 51.2 cal/°C. Data and results of the calibration tests are summarized in Tables 1 and 2 respectively.

Validation tests of calorimeter

Measurement of the rate of heat generated by the peanuts in the respiration

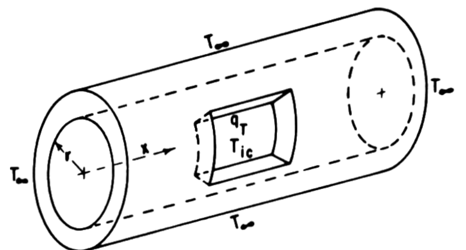

Fig. 5–Schematic representation of calorimeter main flask.

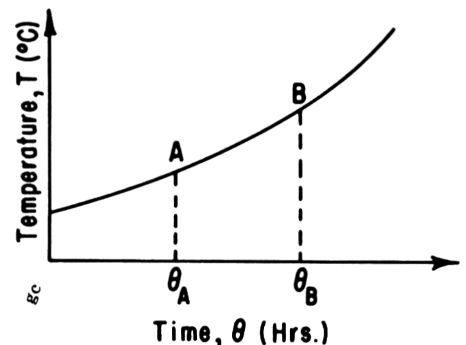

Fig. 6–Typical time temperature curve of respiration tests.

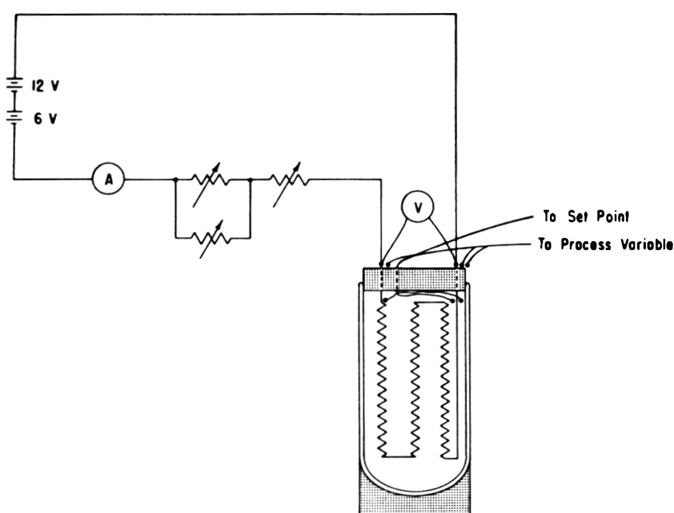

Fig. 7–Wiring circuit used in the validation test.

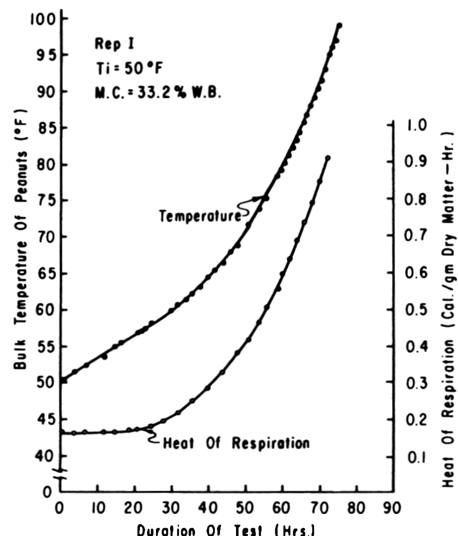

Fig. 8–Representative temperature and respiration data obtained from high moisture Spanish peanuts (from Suter et al., 1972).

calorimeter was validated by measuring the rate of heating in the respiration calorimeter flask with three heaters of known electrical source. The three heaters consisted of 1½ in. OD x 5¼ in. thin-wall pipe with 1/16 wall thickness, wrapped with 26 gauge nichrome wire. The heaters were connected in series to insure uniform heating.

Leads to the power source and oscilloscope were two number 12 copper wires. Since the thermal conductivity of copper is high, it was essential to minimize any temperature differences between the two sides of the Polyurethane foam flask top. Therefore, three set point thermocouples were placed adjacent to the power leads and within 1/16 in. of the flask cover top surface. Three process variable thermocouples were similarly located inside the flask.

The wiring circuit used in the validation test is shown in Figure 7. The variable resistors were set to maintain a 8.1v potential with 0.3 amps current.

Results of the validation test are summarized in Table 3. Percent difference between the calculated and measured values of the generated heat from the electrical resistance heaters ranged from 2.5–5.3 with a mean value of 3.5%.

Largest expected experimental measurement error was calculated using log derivative technique. Possible error in measuring H_c, specific heat of respiring mass, and heat of respiration was calculated as 6.2%, 2.7% and 0.2% respectively.

Heat of respiration from high moisture Spanish peanuts is presented in ASAE Paper No. 72–329 (Suter and Clary, 1972). Typical curves, generated using the calorimeter described in this report, are shown in Figure 8. The temperature time curve represents raw data taken from the recording potentiometer. Heat of respiration, q_g, is calculated from equation [10].

Deterioration of food products due to heat of respiration may be more effectively controlled if respiration heat rates are known. The calorimeter described in this paper measures the heat of the respiring mass.

The unit is designed to reproduce and monitor the temperature history of a product en masse beginning at some specified initial condition. This makes it possible to closely approximate the history of the heat generation of the interior of large masses of biological materials.

SYMBOLS

	Quantity	Units
C_p	Specific heat at constant pressure	cal/(g–°C)
g	Gravitational acceleration standard	cm/sec²
g_c	Newton constant	(cm-g)/(dyne-sec²)
H_c	Heat capacity constant	cal/°C
ϕ	Angle	—
Q_a	Energy stored in air	cal
Q_f	Energy stored in flask	cal
Q_g	Energy generated	cal
Q_m	Energy stored in respiring mass	cal
Q_s	Total energy stored	cal
q_a	Rate of energy stored in air	cal/sec
q_f	Rate of energy stored in flask	cal/sec
q_g	Rate of energy generated	cal/sec
q_m	Rate of energy stored in respiring mass	cal/sec
q_s	Rate of total energy stored	cal/sec
q_T	Rate of internal heat generated, function of temperature	cal/sec
q_T	Rate of internal heat generated per unit volume, a function of temperature	cal/(sec-cm³)
r	Radius	cm
ρ	Density	g/cm³
T	Temperature	°C
T_{ic}	Initial temperature	°C
T_A	Bulk mass temperature at time A	°C
T_B	Bulk mass temperature at time B	°C
T_∞	Free stream temperature	°C
θ	Hour of test	hr
θ_A	Time A	hr
θ_B	Time B	hr
V	Volume	cm³
x	Distance parallel to flask axis	cm
W	Weight	g

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EFFECT OF POLYPHOSPHATES ON THE FUNCTIONAL PROPERTIES OF SPRAY DRIED EGG ALBUMEN

INTRODUCTION

BROWN AND ZABIK (1967) reported that exposure of liquid egg albumen to spray drying causes adverse effects on the functional performance of the dried albumen. The research reported here was initiated to determine the effects of adding polyphosphates to egg albumen prior to drying. The functional performance of both yolk-free and yolk-contaminated egg albumen so treated was evaluated in angel food cakes. Special attention was focused on the type of polyphosphate added to the albumen prior to drying (e.g., chain length). The effect of the polyphosphate on the albumen lysozyme patterns was examined electrophoretically.

EXPERIMENTAL

THE EXPERIMENTAL polyphosphates were added to enzymatically desugared egg albumen (Fermco, 1969) prepared from 1-wk old eggs at 0.5% w/w of liquid egg albumen. For the yolk contamination studies, yolk (0.05–0.2% w/w of liquid albumen) was mixed into the desugared liquid albumen before polyphosphate treatment. The pH of the polyphosphate-treated albumen samples was adjusted to 6.8–7.0 with 7.5% citric acid solution prior to drying. Spray drying of yolk-free albumen was done commercially with a Bowen spray drier (BE-235) equipped with a centrifugal type atomizer. For the yolk-contaminated samples, the Bowen spray drier, fitted with a 2-fluid-type nozzle was used. The inlet and outlet temperatures were 450°F and 175°F, respectively. The spray dried egg albumen (SDEA) samples were in-package pasteurized for 7 days at 130°F following the procedure of Bergquist (1961).

The functional performance of SDEA in foam and in angel food cake was evaluated according to a procedure described by Kohl (1971).

A disc gel electrophoresis procedure described by Davis (1964) was used to separate albumen proteins.

RESULTS & DISCUSSION

IN THE DISCUSSION of the experiments which follow, each experiment should be treated as a single batch study. Neither the batches of albumen nor the spray drying preparations were duplicated. However, in order to determine the statistical significance of data presented in each batch, an analysis of experimental error was conducted. 15 liquid egg albumen samples from one batch of eggs were whipped into foams and baked into angel food cakes. The standard deviations for specific volume (ml/g) of the cake and the % drainage of the foam are 0.1 and 1.1, respectively.

Yolk-free albumen

The foams prepared from all the phosphate-treated albumen samples with the exception of the one treated with SPP ($\bar{n}$=10) reconstituted to pH 6.7, were as stable, as measured by % drainage as the corresponding control (SDEA) (Fig. 1). Samples treated with a SPP ($\bar{n}$=10) produced a foam less stable than SDEA without phosphate. It was observed that foam stability was greater for albumen at pH 6.7 than at 8.9. This latter observation is in agreement with Kline et al. (1965) who reported the optimal pH value for beating egg albumen, in the presence of sugar and cream of tartar, to be between 6.5 and 7.0.

When SDEA samples containing phosphates of varying chain lengths were used in the preparation of angel food cakes, cakes with improved specific volume over those prepared with the untreated SDEA control were produced (Fig. 2). The improvement in specific volume of cake seems to correlate well with the chain length of the tested polyphosphates. It is interesting to note the inverse relationship which was shown to exist between cake volume and foam stability (Fig. 1) for SDEA containing SPP ($\bar{n}$=10). Apparently, cake ingredients and temperature

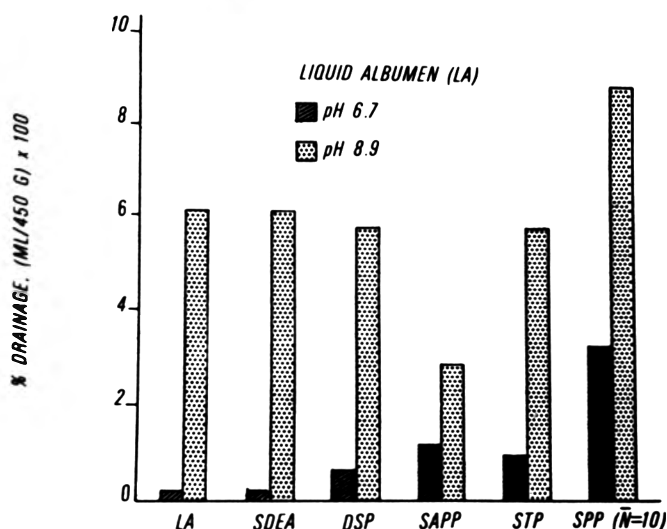

Fig. 1—Foam characteristics of phosphate-modified spray dried egg albumen (P-SDEA). Phosphates were added at 0.5% (w/w of liquid albumen); disodium phosphate (DSP); sodium acid pyrophosphate (SAPP); sodium tripolyphosphate (STP); sodium polyphosphate with average chain length of 10 (SPP, $\bar{n}$=10).

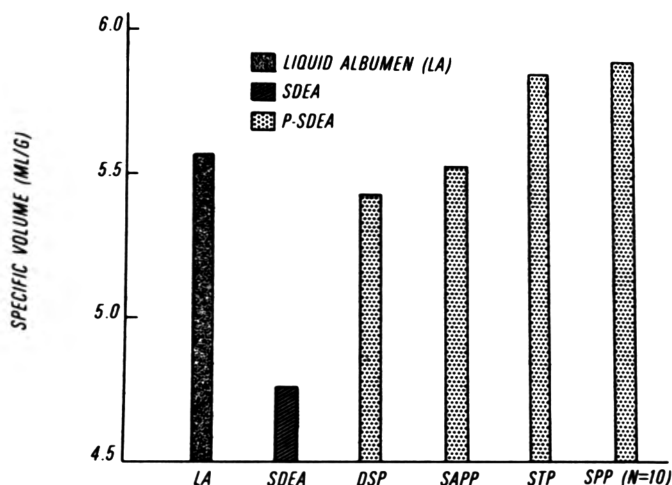

Fig. 2—Influence of phosphate-modified spray dried egg albumen (P-SDEA) on specific volume of angel food cake. Phosphates were added at 0.5% (w/w of liquid albumen).

of baking had a greater effect on this particular sample than on the other tested.

Yolk-contaminated albumen

Table 1 compares the effects on native liquid albumen of yolk contamination as well as the effect of polyphosphate on yolk-contaminated albumen. Albumen with no yolk contamination produced a high specific volume cake. With the addition of 0.1% yolk (w/w), angel food cakes of poor quality with respect to volume were obtained, indicating the harmful effect of yolk contamination. The addition of 0.5% (w/w) polyphosphates with different chain lengths to yolk-contaminated albumen caused a restoration of quality of angel food cake. Of all the polyphosphates tested, those with chain lengths less than or equal to 18 were effective in restoring functional performance of yolk-contaminated albumen, including cake volume. Cakes made with albumens containing polyphosphates of chain length greater than 10 were fragile in texture and in some instances, collapsed upon cooling.

The ability of polyphosphate ion to overcome the adverse effect of yolk contamination in SDEA was also investigated. As shown in the same table (Table 1), SDEA containing 0.2% yolk produced an angel food cake with a volume comparable to that produced by liquid albumen containing 0.1% yolk. The addition of polyphosphate improved the cake volume only slightly; the cake so produced was still unacceptable. As the level of yolk contamination dropped from 0.2 to 0.05% (w/w), the beneficial effects of polyphosphate in restoring cake volume was demonstrated.

Table 1–Influence of polyphosphate on functional performance of yolk contaminated liquid and spray-dried egg albumen (SDEA)

Sample	Angel Food Cake Specific vol (ml/g)
Liquid albumen	
No yolk	5.0
0.1% yolk	
No polyphosphate	3.1
0.5% STP	4.9
0.5% SPP ($\bar{n}$=10)	5.2
0.5% SPP ($\bar{n}$=18)	5.5
0.5% SPP ($\bar{n}$=25)	Cake fell from pan
0.5% SPP ($\bar{n}$=37)	Cake fell from pan
SDEA	
0.2% yolk	
No polyphosphate	3.0
0.5% STP	4.3
0.5% SPP ($\bar{n}$=10)	4.0
0.05% yolk	
No polyphosphate	4.6
0.50% STP	4.9
0.50% SPP ($\bar{n}$=10)	5.3

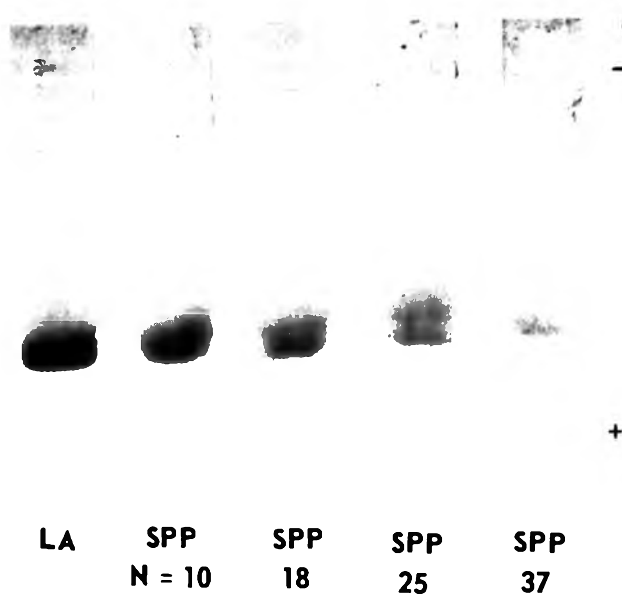

Fig. 3–Effect of sodium polyphosphate with various average chain length (SPP, $\bar{n}$=10–37) on electrophoretic patterns of yolk contaminated (0.1%, w/w) liquid albumen (LA).

Changes in electrophoretic patterns of lysozyme in yolk contaminated albumen

In an attempt to explain the phenomena involved when yolk contaminates albumen, Cunningham and Cotterill (1971) reported that yolk contamination did not change the paper electrophoretic patterns of lysozyme, but did change its chromatographic profile. Using ion exchange chromatography, they found that three lysozyme components were present in yolk-free albumen but only two were found in yolk-contaminated samples. Cunningham and Cotterill, therefore, believe that the harmful effects of yolk in liquid albumen are caused by complexes formed between yolk lipids and albumen lysozyme. The harmful complexes become dissociated upon heat treatment (Cotterill et al., 1965).

Lysozyme, a basic protein in egg albumen with an isoelectric point of 10.5 would be very likely to interact with polyphosphate ion. The effect of polyphosphate ion on the electrophoretic pattern of lysozyme of liquid albumen is shown in Figure 3. In the presence of polyphosphate ion, the electrophoretic pattern of lysozyme is changed owing to the interaction of the two species. The electrophoretic studies further show that the degree of interaction of polyphosphate with lysozyme in yolk-contaminated albumen is directly related to the chain length of the polyphosphate. The intensity of the lysozyme band appearing on the electrophoretogram is an indication of the degree of interaction. The denser bands indicate a diminished degree of interaction while the lighter bands indicate a higher degree of interaction. The resulting complexes between lysozyme and polyphosphate ion may be electrostatically stable and remain on the top of the gel.

Under the influence of polyphosphate ion, the electrostatic complexes of lysozyme and egg yolk lipid, as postualted by Cunningham and Cotterill (1971), may

have become dissociated. This dissociation may have aided in the restoration of functional performance of yolk-contaminated albumen.

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PARAMETERS OF TEXTURAL CHANGE IN FROZEN-STORED COOKED LOBSTER (Homarus americanus) TAIL MUSCLE

INTRODUCTION

TEXTURAL CHANGES occur in muscle tissue of mammals (Love, 1966; Luyet, 1966), fish (Love, 1966; Connell, 1968; Dyer, 1969) and crustaceans (Dagbjartsson and Solberg, 1972b) during frozen storage. The molecular reactions which cause toughening of muscle tissue during frozen storage are not well defined. Molecular aggregation and cross-linking of protein chains (Dyer, 1969), combination of fatty acids with myofibrillar proteins (King, 1966; Anderson and Ravesi, 1970) and freeze thaw damage of lysosomes or other cellular organelles releasing protein insolubilizing enzymes (Dyer, 1969; Olley et al., 1969) have been suggested as factors in the textural alteration. Some support was given to the aggregation and cross linking hypothesis by the observation that myosin molecules, frozen in model solutions, polymerize in a side-to-side fashion (Buttkus, 1970).

Very little has been published concerning changes in cooked muscle during freezing and frozen storage. Khan and Van den Berg (1965) reported reduced protein solubility, decreased sulfhydryl group concentration and lessened enzymatic activity in precooked chicken during frozen storage. Huber and Stadelman (1970) and Huber et al. (1970) investigated precooked chicken and turkey and found more soluble sarcoplasmic protein in slowly frozen than in cryogenically frozen muscle but no difference in the extractability of myofibrillar protein due to different freezing rates.

Hamm (1966) reviewed the biochemical changes that occur in muscle proteins during heating, stating that proteins are not completely denatured at 85°C during a short heating period. Therefore, some of the reactions which occur in raw tissue during frozen storage may also take place in heated muscle.

This study evaluated some of the biochemical and physicochemical parameters which may be related to the textural changes occurring in precooked lobster meat during frozen storage. The parameters selected for study were all related to texture toughening in the scientific literature.

EXPERIMENTAL

LIVE 625–825g lobsters were purchased from a local fishmarket on special order from a single source in Portland, Me. The lobsters were cooked in groups of six by boiling for 10 min in 25 gal of 2.5% NaCl brine in a steam-jacketed kettle thus bringing the internal temperature of the lobster tail to 80–85°C. After cooking, each lot was cooled with running water (10–12°C) for 30 min, bringing the internal temperature of the lobster tail to 16–22°C. The tail was then separated from the rest of the lobster; the tail muscle was removed from the shell and the digestive tract removed from the muscle. Groups of four tail muscles were packed in 3 mil thick mylar/saran/polyethylene pouches and heat sealed. Freezing was accomplished in a rapid airblast freezer (Associated Laboratories, Wayne, N.J., Model SK-3105). Frozen lobster tails were stored at −12° ± 1°C or −27° ± 1°C. One bag (4 lobster tails) constituted a sample for each testing date.

The Warner-Bratzler (W-B) shear was used for the texture evaluation. One-half of a longitudinally split lobster tail was cut into ½-in. cubes which were sheared perpendicular to the direction of the fibers. pH measurements were made on the cut surface of the tail half with a surface electrode and an Expandomatic pH meter (Beckman Instrument Co., Fullerton, Calif.).

From the other half of each lobster tail, 35g of frozen muscle were cut into small pieces, mixed with 100–150g of dry ice powder and cold milled in a precooled stainless steel Waring Blendor cup at maximum speed for several 10-sec periods. This muscle powder served as the raw material for the other biochemical and physicochemical analyses which were carried out in duplicate.

The water-holding capacity (WHC) was determined by a centrifugal method developed for this purpose (Dagbjartsson and Solberg, 1972a). This is a modification of the centrifugal methods of Wierbicki et al. (1957) and Miller et al. (1968) in which minced or pulverized muscle is subjected to certain centrifugal force and the amount of expressed juices measured.

To extract salt-soluble proteins, 30g of the cold milled muscle powder were mixed with 150 ml of ice cold $KCl/NaHCO_3$ buffer (0.6M KCl + 0.01M $NaHCO_3$; pH = 7.2) in a Waring Blendor at low speed for 3 min. The solution was allowed to stand at 0–4°C overnight (16–20 hr) and then reblended for two 5-sec periods at low speed before centrifugation at 2°C and 8,000 × G for 20 min. The protein content in the clear supernatant was determined by the biuret method (Bailey, 1967) and was called "total extractable proteins" (TEP), expressed in % of sample weight.

A 10-ml aliquot of the supernatant was diluted with 27 ml of cold distilled water in a 40 ml screwcap centrifuge tube, allowed to stand for another 16–20 hr (at 0–4°C) and then centrifuged at 8,000 × G for 30 min at 2°C. The protein content in this supernatant was called "actomyosin free extract" (AFE), expressed in % of sample weight.

5g of the residue from the first extraction (insoluble in 0.6M KCl) were washed with cold 0.6M KCl, filtered until dry on a coarse glass suction filter, dissolved in 50 ml 2.5% sodium dodecyl sulfate (SDS) by slow stirring at room temperature for 16–20 hr and then centrifuged at approximately 2,500 × G for 10 min (25°C). The protein content of this supernatant (diluted when necessary) was determined by the biuret method and was called "SDS-soluble proteins" (SDS) expressed as % of KCl-insoluble matter (Connell, 1965; Mao and Sterling, 1970).

Free sulfhydryl groups were determined by reacting muscle powder with N-ethylmaleimide (NEM) according to a method developed by Hamm and Hofmann (1966), with the modification that the reaction mixture was filtered through fine paper (Whatman No. 42) after addition of 2 ml of 20% trichloroacetic acid instead of centrifugation at 15,000 × G as in the original method. Preliminary experiments showed no difference between filtration and centrifugation in samples prepared from cooked muscles.

Sulfhydryl groups were expressed as moles/5×10^5g of tissue which contained an average of 22.0% protein with a range from 20.2–24.9% protein determined by Nitrogen Analyzer (Coleman II). Selected samples were analyzed for protein.

All methods used for statistical analyses were adopted from Steel and Torrie (1960). Analysis of covariance was carried out with the aid of a Computer (IBM-360/67) using the Biomedical computer programs, X-series (Dixon, 1969).

RESULTS & DISCUSSION

THE WARNER-BRATZLER shear force increased significantly during all storage experiments. Figure 1 demonstrates the relative change in shear force as a function of storage time and temperature. The open and closed circles are data from Dagbjartsson and Solberg (1972b), and the open and closed triangles are results from studies initiated 1 yr later. The

reproduceability of the data is evident. The more recent data demonstrated less variation between lobsters, and between measurements within the same lobster tail. Increased standardization of experimental procedures may account for the reduced variability which resulted in a smaller standard deviation and increased statistical significance. The W-B shear measurement was selected as the standard criterion in these experiments since it was reported to be highly correlated to a panel evaluation for cooked lobster meat (Dagbjartsson and Solberg, 1972b).

In all experimental groups a slight increase from a prestorage pH of 6.8 was observed as a function of storage time (Fig. 2). Although the magnitude of the pH changes was not very great, the differences were statistically significant. Changes in pH during storage after freezing may be caused by several factors. Van den Berg (1968) suggested that interactions of proteins with ionic substances, intrinsic enzymatic activity, changes in buffering capacity, changes in solute concentration, and precipitation of salts may be related to pH changes in frozen stored foods. Cooked lobster muscle has a high initial pH and a high concentration of polyvalent anions. Van den Berg (1968) postulated that food with high initial pH and high levels of phosphate, carbonate and other polyvalent anions such as cod and prerigor meat would usually show a slight drop in pH immediately after freezing and a subsequent increase due to precipitation of acid potassium salts. In cooked muscle, the salt precipitation might be enhanced because of the coagulation of proteins and reduced buffering capacity. Two of the three test groups

Table 1—Water-holding capacity (WHC) and moisture content of raw and treated lobster (Homarus americanus) tail muscle

	Raw	Cooked	Cooked frozen thawed	Frozen[a] stored 9 wk	Frozen[a] stored 15 wk
WHC (%)	62.6	44.5	42.4	34.3	32.3
Moisture content (%)	78.8	76.9	75.7	75.2	76.0

[a]Storage at −12°C

showed an initial decrease in pH which was not statistically significant.

The water-holding capacity (WHC) dropped significantly due to the freezing operation itself, as may be seen in Table 1. The WHC changes during frozen storage at −12°C or −27°C are shown in Figure 3. At −12°C the WHC decreased rapidly during the first 8 or 9 wk of storage and then seemed to approach an equilibrium value at about 30–32% moisture retained by the muscle after centrifugation at 12,500 x G for 10 min. At −27°C, the changes in WHC were much slower and the equilibrium value may not have been reached even after 30 wk of storage. Changes in WHC due to post freezing storage at −12°C were highly significant whereas the WHC decrease at −27°C was slightly significant. The 1 wk storage determination of WHC for the sample stored at −27°C was probably an error due to unfamiliarity with the measuring technique.

Water-protein interactions in tissue are poorly understood and highly speculative. Any attempt to relate the relatively subjective measurement of water-holding capacity to conformation and structure must be regarded as a low probability hypothesis at best. Attachment of water to the polar groups of protein side chains (Grau and Hamm, 1953; Miller et al., 1968) or "iceberg" structures of water around nonpolar groups (Klotz, 1958; Karmas and DiMarco, 1970) or enclosure of water into the framework of the native protein structure (Hamm, 1966; Anderson and Ravesi, 1970) have all been proposed as explanations for water binding. Denaturation generally causes loss of water-holding capacity. Freezing and frozen storage of fish causes loss of fluid or "drip loss" upon thawing (Love, 1966). Karmas and DiMarco (1970) found that heating of proteins to 82–83°C causes a sudden increase in heat absorption which they proposed was due to the collapse of the "icebergs" around the nonpolar side chains. In both cases the loss of native structure reduces the ability of the proteins to retain water.

Cooking of the lobster meat causes severe protein denaturation. The loss of WHC due to cooking, determined by the centrifugal method, is evident from Table 1. The total moisture content (MC) of the

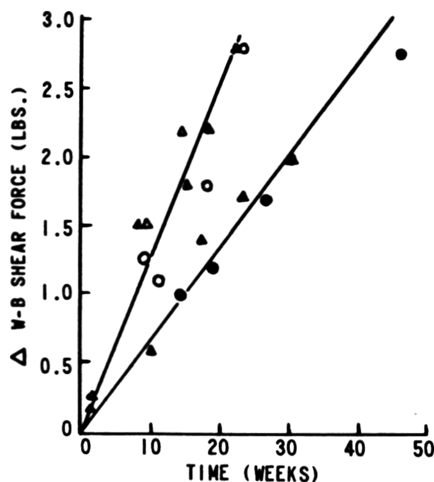

Fig. 1—Textural change in cooked lobster tail muscle measured by Warner-Bratzler shear force expressed as difference from prestorage shear value, after various periods of frozen storage. [○–○, −12°C (1970); △–△, −12°C (1971); ●–●, −27°C (1970); ▲–▲, −27°C (1971)]

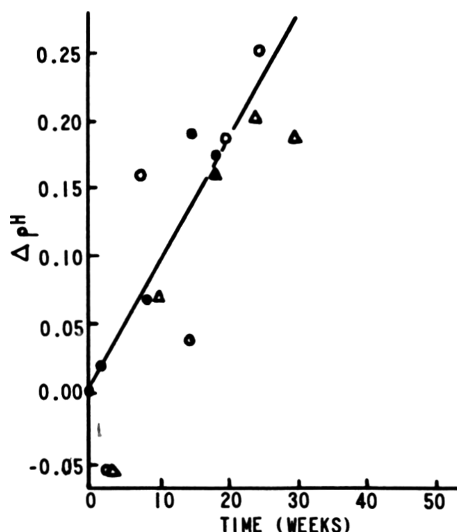

Fig. 2—Change in pH of cooked lobster tail muscle during frozen storage. [○–○, −12°C (Exp. 1); ●–●, −12°C (Exp. 2); △–△, −27°C]

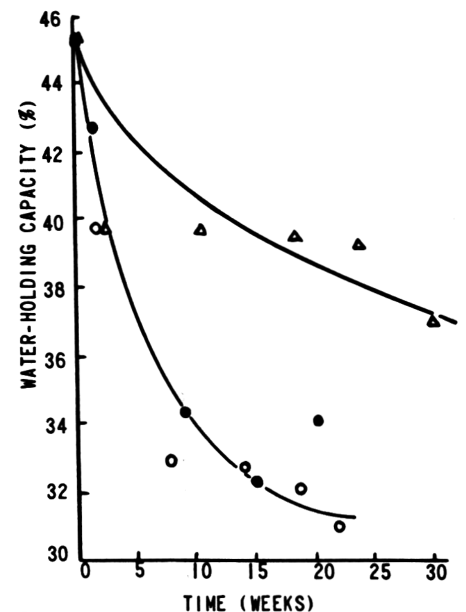

Fig. 3—Change in water-holding capacity of cooked lobster tail muscle during frozen storage. [○–○, −12°C (Exp. 1); ●–●, −12°C (Exp. 2); △–△, −27°C]

muscle was reduced only slightly by this treatment and then remained unchanged throughout the frozen storage.

The results in Table 1 show that most of the moisture is retained in the lobster muscle but does not remain as tightly bound. The heat treatment applied to the muscle may "melt" the "iceberg" to a certain extent, but some of the water molecules may remain "entrapped" in the protein structure or attached to ionic compounds through electronic forces. Freezing and frozen storage may then collect the water into ice crystals. The muscle proteins, having lost their native structure, may no longer be able to reabsorb the water into its original structure. Some capillary entrapment may still hold the water loosely and prevent it from "dripping" from the system (drip loss measured on samples stored for 20 wk at $-12^\circ C$ and 30 wk at $-27^\circ C$ was only 6–9%). Such capillary or adhesive forces would probably not be great enough to counter the centrifugal forces used to determine the WHC.

Figures 4A and B show that little change occurred in protein solubility as a result of frozen storage. The actomyosin free extract (AFE) decreased significantly due to the freezing operation itself (Fig. 4B) but differences between storage times were not statistically significant. No statistically significant differences were observed in total extractable proteins (TEP) either as a result of freezing or during storage (Fig. 4A). Heating of proteins greatly reduces their salt solubility. Prior to cooking, the TEP for lobster muscle was 12.3–13.2%. After cooking the lobster muscle, only 1.5–2.0% of the muscle weight was soluble in 0.6M KCl. This approximates the results of Huber et al. (1970) and Huber and Stadelman (1970) who found more than a 90% reduction in solubility of chicken and turkey muscles after heating to $85^\circ C$. The small soluble fraction might play a secondary role in the texture toughening during frozen storage but the absence of any significant change during 30 wk of storage rules out any primary function with respect to textural change.

Figure 4C presents the solubility of KCl-insoluble proteins in 2.5% sodium dodecyl sulfate (SDS). SDS is an effective agent for the cleavage of secondary and tertiary noncovalent protein bonds. Decreased SDS solubility of a protein should be indicative of covalent bond formation. The absence of change in SDS solubility does not mean that molecular aggregation could not be taking place. Apparent molecular weight determinations are needed to confirm any conclusion. Although there seems to be a trend toward decreased SDS-soluble protein as a function of storage time in Figure 4C, the high variability of the measuring technique makes it difficult to interpret the significance. Decreased protein solubility in uncooked muscle is generally attributed to molecular aggregation, which is also related to toughening (Dyer, 1951; Connell, 1964; Love, 1966; Anderson and Ravesi, 1970). Luyet (1966) stated that cooked muscle proteins resist further structural disturbance by freezing. The results presented in Figures 4A, 4B and 4C support the previous statement and indicate that protein aggregation is not involved in the progressive textural toughening of cooked lobster tail muscle during frozen storage.

The quantity of free sulfhydryl groups did not change significantly during frozen storage (Figure 4D). Several authors have indicated that the formation of disulfide bonds may be one of the major cross-links formed in protein denaturation (Connell, 1964; Khan and Van den Berg, 1965; Hamm, 1966). Buttkus (1970), who did not find any change in free-SH-groups in his studies on the polymerization of myosin in vitro, offered an interesting hypothesis on how cross-links via –S–S– bonds can proceed without change in free –SH– groups as a result of transformation of intermolecular –S–S– bonds into intramolecular –S–S– cross-links. The results of Figure 4D neither prove nor disprove the hypothesis. Figure 4D indicates that if –S–S– bond formation is one of the reasons for the toughening of cooked lobster meat, during frozen storage, it is not reflected in a net reduction of the number of free –SH– groups as measured by the NEM-method.

Actomyosin formation, actomyosin denaturation and cross-links via –S–S– bonds, all of which have been related to texture toughening of frozen meat products, do not seem to occur in cooked and frozen, stored lobster meat.

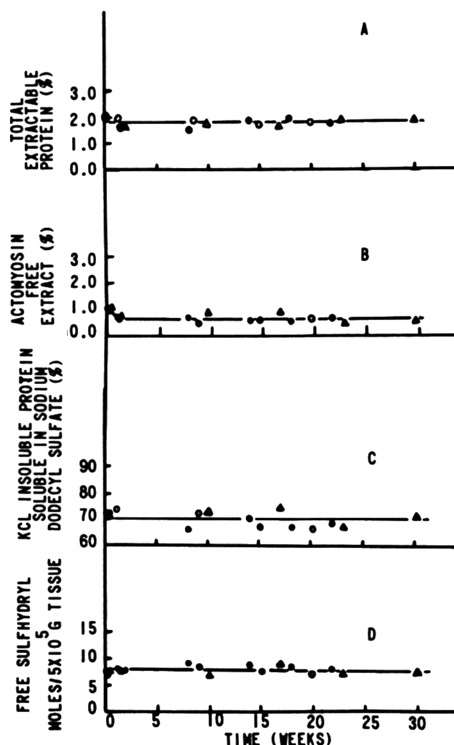

Fig. 4–The effect of frozen storage upon various physicochemical and biochemical parameters of cooked lobster tail muscle as a function of storage time. [o–o, $-12^\circ C$ (Exp. 1); •–•, $-12^\circ C$ (Exp. 2); △–△, $-27^\circ C$]

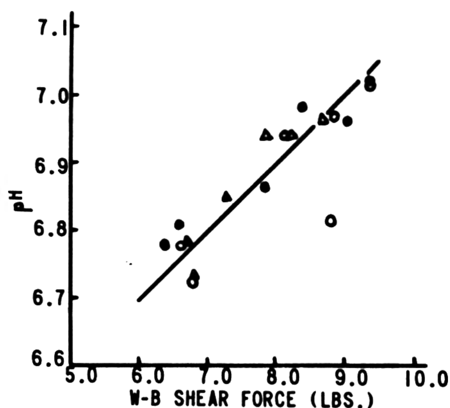

Fig. 5–The relationship between pH and Warner-Bratzler shear force for cooked frozen lobster tail muscle as a function of storage time. [o–o, $-12^\circ C$ (Exp. 1); •–•, $-12^\circ C$ (Exp. 2); △–△, $-27^\circ C$]

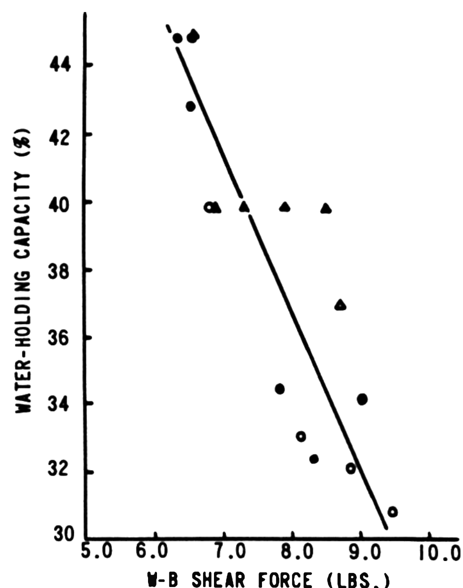

Fig. 6–The relationship between water-holding capacity and Warner-Bratzler shear force for cooked frozen lobster tail muscle as a function of storage time. [o–o, $-12^\circ C$ (Exp. 1); •–•, $-12^\circ C$ (Exp. 2); △–△, $-27^\circ C$]

Figures 5 and 6 demonstrate that the changes in pH and WHC, both of which are physicochemical parameters, correlated fairly well with W-B shear force ($r = 0.82$ and 0.91 respectively). Since the pH of the stored lobster muscle increased and moved further from the isoelectric point of most myofibrillar muscle proteins there is little chance that the WHC is related to the pH change in any way. The pH change may be involved in textural alteration but its involvement must be indirect since it was independent of storage temperature. It is possible that changes in pH are necessary for the activation of the temperature-dependent reactions which are responsible for textural change. There may be some common factor or factors which result in the observed change in pH, WHC and W-B shear force, but these remain obscure.

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PILOT PLANT PRODUCTION OF AND LARGE SCALE ACCEPTANCE TRIALS WITH QUICK-SALTED FISH CAKES

INTRODUCTION

A QUICK-SALTING process for fish has been previously described (Del Valle and Nickerson, 1968; Del Valle, 1972). Briefly speaking, the process consists of grinding pieces of fish flesh with simultaneous addition of salt, pressing of the ground salted meat to form cakes and sun drying the cakes. Simultaneous grinding and salting assures rapid and uniform preservation of the flesh, while pressing of the ground salted meat expels a maximum amount of water mechanically, leaving the remainder to be removed by evaporation in the sun-drying step. Amounts of salt added to the fish flesh are those necessary to coagulate its proteins, and vary from 25–100% of the weight of the wet flesh. Pressures applied in the pressing operation are of the order of 120–150 kg/cm^2 (1700–2100 psi). The dry salted fish cakes have been found to be indefinitely stable without refrigeration, since their high salt and low water contents render them resistant to attack by microbes, insects, rodents and the like. Before consumption, the cakes are desalted by leaching twice in boiling water. The cakes do not lose their shape when they are so treated, and the resulting desalted fish meat may be cooked as desired. Protein losses in the press liquor as well as in the desalting water have been found to be negligible (Del Valle and Gonzales-Iñigo, 1968).

The main advantages of the process are: (1) Rapid and uniform preservation of fish flesh, accomplished by intimate mixing of ground meat and salt. In contrast, in the normal salting process salting times can vary from 2 wk to as much as 2 months, depending upon such factors as temperature and thickness of the fillets. (2) Obtainment of a product which is indefinitely stable without refrigeration. This is of especial value in developing countries with warm climates, since it makes available an inexpensive high protein food for the undernourished population.

EXPERIMENTAL

A PILOT PLANT was set up in Progreso, Yucatan, Mexico with the purpose of studying the quick-salting process in a pilot scale. The flow diagram for the plant is shown in Figure 1. Design capacity of the plant was 250 kilos of dry salted cakes in 8 hr. Basic equipment consisted of a Hobart-Dayton Model 4612 meat grinder equipped with a ½ HP motor and stainless steel parts, a specially designed hydraulic press (sketched in Fig. 2) and sun-drying racks. The press was equipped with a 5 gpm, 2500 psi (maximum pressure) hydraulic pump and an 8 HP, 1750 RPM motor. All parts of the press which came in contact with ground salted material were made of type 316 stainless steel. Piston diameter, which was also equal to cake diameter, was 6 in.

The height of the pressed cakes was approximately ½-in. Each dried cake weighed approximately 450g.

Research with the plant was directed to studying applicability of the process to different raw materials, including trimmings and rejects from filleting plants as well as "trash" species. It should be noted that "trimmings" refers to pieces of fresh flesh rejected from filleting operations; "rejects" refers to slightly spoiled whole fish and fish fillets; and "trash species" refers to species of little or no commercial value which are normally caught with commercially valuable ones. Table 1 lists and classifies those species which were studied. It should also be mentioned that trimmings and rejects were the most abundant raw material for the plant and consisted of red snapper and grouper.

Data obtained in the different runs were concerned with yields and operating conditions for the different steps of the process. As will be seen in a subsequent section of the paper, "yields" refers to weights of product obtained per unit weight of raw material, while "operating conditions" refers to production rates, salt doses, pressing times and pressures, and sun-drying times. Other pertinent data obtained in the work were calculated analyses of the dry and wet cakes. These determinations were made in order to obtain an approximate idea of the protein, salt and moisture contents of the cakes. Calculations were made assuming literature values for raw fish flesh and considering salt doses employed, as well as all pertinent yields in going from whole fish to dry salted cakes.

Taste tests were also conducted among adults and children from Yucatan. The cakes were desalted and cooked according to a popular local recipe for fish described in Table 2. The resulting product, somewhat resembling "chile con carne," was served to tasters in tortillas in the form of tacos. Tests with adults were conducted in five different inland villages. It should be noted that only inland locations were considered because it was desired to test acceptance of the product among people who do not normally eat fish. An average of 65 persons from each village participated in the tests. Results of the tests were expressed in two ways: (1) subjective scores for attributes of color, flavor and texture; and (2) a hedonic rating (Table 3).

Tests with children were conducted within breakfast and lunch programs of the Instituto de Proteccion a la Infancia de Yucatan (a Government-sponsored institution). All test participants were of preschool age (2–5 yr). Cakes were desalted and cooked in the manner previously described. The cooked product was served to tasters in the form of sandwiches. Approximately 25 sandwiches were prepared from each 450-g cake. Acceptance was measured by simply noting if a child ate or rejected the sample. Four tests were conducted, consisting of two breakfasts and two lunches. An average of 50 children participated in each test. Cakes used in all of these tests were made from a mixture of 50% trimmings + 50% rejects.

The last part of the work involved a preliminary economic evaluation of the process. This consisted of listing of costs for the pilot plant, and calculation of production costs for the dry

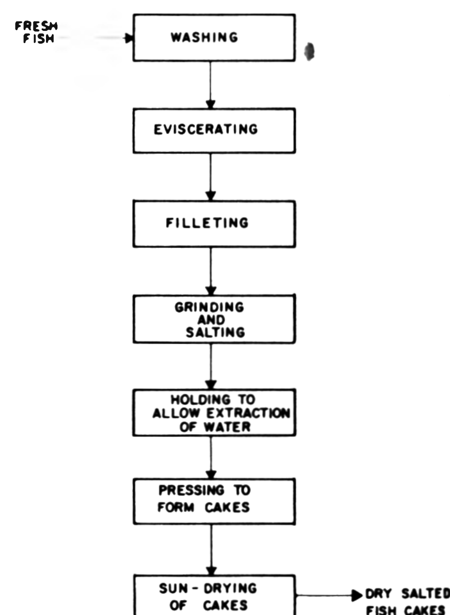

Fig. 1—Flow diagram for quick-salting process.

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Table 1–Names of species studied

Spanish name	Scientific name	English name
Rubia	Paranthias furcifer	Red gurnard
Mojarra	Eucinostomus gula diapterus plumieri	
Mero	Epinephelus morio	Grouper
Huachinango	Lutianus guttatus	Red snapper
Chac-chic	Aemulum espurus	
Sardina	Anchoa mychilli	Sardine

salted cakes. Pilot plant costs, reported in Table 4, included the following items: inventories, building, equipment, and installation and organization costs. Table 5 shows a breakdown of equipment required for the pilot plant. Production costs were calculated in the usual manner and included the following concepts: raw materials, indirect and direct labor, manufacturing costs (electricity, etc.), and amortization-depreciation. Table 6 reports the above costs for cakes made from the different species.

RESULTS & DISCUSSION

TABLE 7 reports data on the filleting operation necessary to produce pieces of flesh for grinding and salting. Filleting rates were determined by noting times required by two skilled persons, working simultaneously, to fillet approximately 10 kg of whole fish. Depending upon species, filleting times varied from 5–15 min. Yields were determined by dividing weight of fillets obtained by weight of whole fish (approximately 10 kg). All species of fish were filleted by the same two persons. It may be seen that filleting rates depended upon species of fish, being high for large species and low for small ones. It is generally considered that a skilled person can produce roughly 250g of fillets per minute. Yield of fillets to whole fish was also found to vary with species, depending upon such factors as relative weight of heads, bones and viscera with respect to flesh. A fillet yield of 40% or over was considered to be very good.

Table 8 shows data on salting and grinding. Rates were determined by noting times required to pass exactly 10 kg of fillets, with the corresponding amount of salt, through the meat grinder. Depending upon species, salting-grinding times varied from 2–5 min except in the case of sardine, where they were equal to 20 min. Yields were determined by dividing weight of ground-salted material obtained (in kilos) by 10. All runs were performed by the same person. It may be seen that fairly high salt doses were employed in order to obtain rapid and thorough coagulation of the flesh proteins. Production rates were found to depend upon ease of grinding of the flesh. In the case of sardines which were ground

Table 2–Recipe for preparation of quick-salted fish cakes for taste trials

Ingredients	
200g tomatoes	3 cloves garlic
50g onions	1 sour orange
20g "achiote" (a local condiment)	1 fish cake
¼ of a sweet pepper	¾ cup vegetable oil

Procedure

Desalt cake by boiling 10 min in 2 liters of water. Throw away desalting water. Repeat if necessary, according to taste. Fry garlic in vegetable oil. Fry onion in same oil until it becomes transparent. Add cut tomatoes and green pepper to oil, frying mixture for about 10 min. Add shredded desalted fish cake, followed by suspension of achiote paste in juice from sour orange. Simmer gently for 30 min. Serve in tacos or sandwiches.

Table 3–Results of taste trials with adults: Hedonic ratings

	Small mero (%)	Trimmings (%)	Rejects (%)	Rejects + Trimmings (%)
Like extremely	41	28	23	21
Like very much	52	60	63	63
Like a little	6	11	9	12
Neither like nor dislike			3	2
Dislike a little				1
Dislike very much				
Dislike extremely	1	1	2	1
Total no. of tasters	286	304	336	320

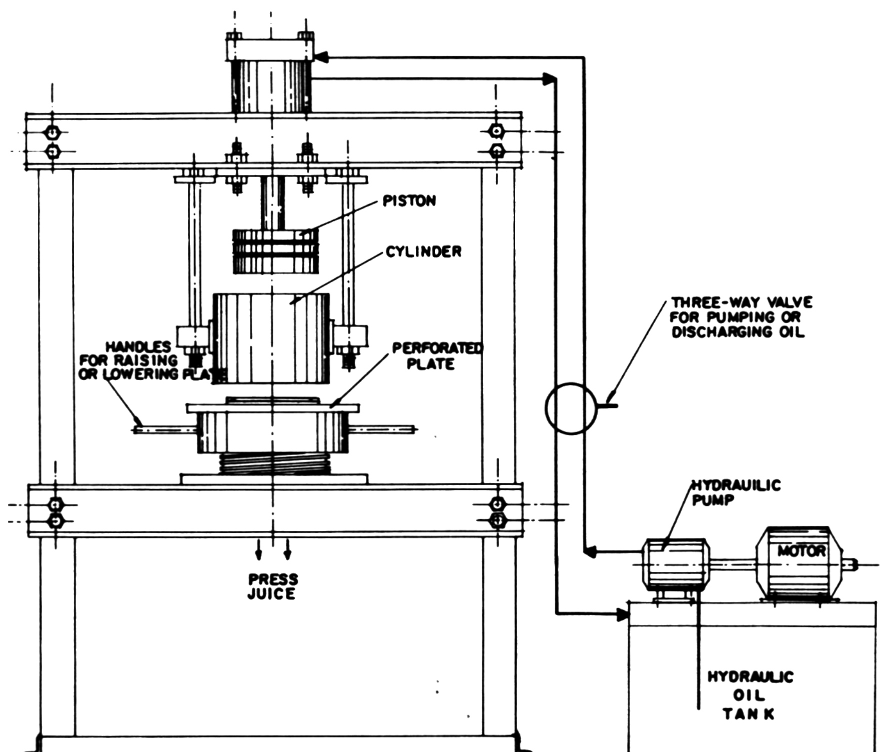

Fig. 2–Sketch of hydraulic press used for making quick-salted fish cakes.

whole, trouble was experienced with clogging of the grinder by scales and bones. Yields of ground salted meat to whole fish were calculated by multiplying (yield of ground salted meat to fillets) by (yield of fillets to whole fish).

Table 4–Total investment required in pilot plant

Item	Cost in U.S. dollars
Inventories[a]	850
Building, including installed utilities	4,000
Machinery and other equipment	6,110
Installation and organization costs[b]	700
Total	11,660

[a]Inventories include 1 ton of raw material at an average price of $0.10 per kilo, and 15 days' inventory of cakes (2250 kilos) at an average price of $0.33 per kilo.
[b]Installation and organization costs include installation of press plus other start-up expenses.

Table 9 lists data on the pressing operation. In all runs 650g of ground salted material was added to the press in order to form a cake. Pressing times and number of applications of pressure were subjectively determined by noting when little or no further liquor was expressed from the cakes. Pressing rates were calculated by dividing weights of wet cakes by pressing times, while yields were calculated by dividing the same wet cake weights (in grams) by 650. All runs were performed by the same operator. Total pressing times, consisting of loading the press, pressing and removing the cakes may be seen to have been approximately equal for all species, of the order of 1.5 min. Table 9 shows that yields of wet cakes to ground salted meat were also approximately equal, so that production rates of wet cakes for the different species differed little from each other. One possible explanation for the approximate equality of pressing times and production rates for all species is that their proteins, having been coagulated by the high amounts of added salt, were all in a similar dehydrated condition and behaved similarly when pressed.

Table 5–List of machinery and other equipment required for pilot plant

Item	Cost in U.S. dollars
Meat grinder	480
Hydraulic press	5,000
Table	20
Utensils	40
Sun drier	250
Ice box, 1 ton capacity	320
Total	6,110

Table 6–Production costs of quick-salted fish cakes in U.S. dollars per kilo of dry cakes

Species	Whole fish	Salt[a]	Direct labor[b]	Indirect labor[c]	Amortization-depreciation[d]	Total cost[e]	Cost of fresh fish per kilo[f]
Chac-chic	0.15	0.01	0.04	0.06	0.03	0.29	0.05
Rubia	0.42	0.01	0.04	0.06	0.03	0.56	0.20
Mojarra	0.20	0.01	0.04	0.06	0.03	0.34	0.10
Small mero	0.19	0.02	0.04	0.06	0.03	0.34	0.06
Sardine	0.23	0.02	0.02	0.06	0.03	0.36	0.20
Rejects	0.18	0.02	0.04	0.06	0.03	0.33	0.06
Trimmings	0.02	0.01	0.02	0.06	0.03	0.14	0.02

[a]Cost of salt is $0.02 per kilo.
[b]Direct labor: pilot plant requires 2 persons per 8-hr shift, each paid $2.50 per 8-hr day; production of cakes is 160 kilos per 8-hr shift. Filleting cost is $0.024 per kilo of fillets.
[c]Indirect labor: One person for management of entire operation at $240 per month. Production of cakes per month = 160 kg/day × 24 days/month = 3840 kg/month.
[d]Amortization-depreciation of capital investment ($11,660) in 10 yr, equal to $1166 per year.
[e]Manufacturing costs (electricity) were negligible in all cases.
[f]Cost of fresh fish is reported in U.S. dollars per kilo of raw material, be it whole fish or fillets, as in the case of trimmings.

Table 7–Data on filleting

	Chac-chic	Rubia	Small mero	Mojarra	Sardine	Rejects	Trimmings
Production of fillets, kg/min-person	0.28	0.24	0.21	0.15	–	0.23	–
Yield, fillets/whole fish, %	28.6	41.9	32.6	46.0	100.0	30.3	100.0

Table 8–Data on salting and grinding

	Chac-chic	Rubia	Small mero	Mojarra	Sardine	Rejects	Trimmings
Salt dose, %	75	75	75	75	100	75	75
Production of ground salted meat, kg/min	3.65	5.18	4.39	4.03	0.50	5.83	2.07
Yield, ground salted meat/fillets, %	172	170	172	173	172	175	198
Yield, ground salted meat/whole fish, %	49.4	71.0	56.0	79.8	172	53.1	198

Table 9 also shows that roughly 30% of the weight of the ground salted meat was removed by pressing. Since the press liquor consisted mostly of a saturated salt solution (approximately 26.5% NaCl), it may be calculated that approximately 52% of the water originally present in the fillets was removed by this operation. Finally, it should be noted that high pressures (2100 psi) were applied in all cases in order to expel the maximum amount of water mechanically and form compact, coherent cakes which did not disintegrate. Yields of wet cakes to whole fish reported in Table 9 were calculated by multiplying (yield of wet cakes to ground salted meat) by (yield of ground salted meat to whole fish).

Table 10 reports data on sun drying of the cakes. Times reported in the table were those necessary to dry the cakes to constant weight. Ambient conditions during the duration of the runs were dry bulb temperature, 33–37°C and relative humidity, 65–75%. 10 cakes from each species were used in each run. Yields of dry cakes to wet cakes were obtained by dividing the wet weight by the dry weight of the 10 cakes. All runs were performed by the same operator. It may be seen that some cakes dried faster than others. These differences were possibly due to differences in water-holding capacity of the flesh proteins of the different species. Similar differences were found and discussed in a previous paper (del Valle and Gonzalez-Iñigo, 1968). In any case, average drying time for the cakes was of the order of 3–4 days. Table 10 also shows that average weight losses in drying were approximately 15%; evidently, these losses were due to evaporation of water from the cakes. If this figure is considered together with that of weight losses in pressing, it may be calculated that 75% of the water originally present in the fresh fish flesh was removed in converting the fillets to dry cakes. Quite interestingly, final yields of dry cakes to fillets were all approximately 1:1. This shows that salt simply replaced water in the conversion of fillets to dry cakes.

Table 9—Data on pressing[a]

	Chac-chic	Rubia	Small mero	Mojarra	Sardine	Rejects	Trimmings
Pressing time, minutes	1.25	1.22	1.52	1.25	1.66	1.48	1.50
No. applications of pressure	2	2	4	2	2	4	4
Pressure applied, psi	2100	2100	2100	2100	2100	2100	2100
Avg weight of wet cake,g	478	470	459	471	419	452	431
Production of wet cakes, kg/min	0.389	0.385	0.303	0.376	0.253	0.305	0.286
Yield, wet cakes/ground salted meat, %	73.8	72.4	70.8	72.3	64.7	69.4	66.1
Yield, wet cakes/whole fish,%	36.4	51.9	39.8	57.8	111.3	37.0	129.5

[a]In all of these runs 650g of ground salted meat were pressed to form a cake.

Concerning calculated analyses of the wet and dry cakes, all except sardine were found to have very similar composition. This is reasonalbe in view of the fact that equal doses of salt were applied in all cases except sardine. Cakes from this last species, which received more salt, showed higher salt and lower protein contents than cakes from the other species. Analytical data on wet and dry cakes can be summarized as follows: Wet cakes, 20% protein, 50% salt, 30% water; dry cakes, 25% protein, 58% salt, 17% water. Figures for the dry cakes are consistent with the finding that yield of dry cakes to fillets was approximately 1:1; fresh fish flesh contains approximately 25% protein and 75% water, while the dry cakes contained the same 25% protein. Thus, water in fresh fish flesh was replaced by salt in the dry cakes.

Concerning results of taste tests among adults, Table 3 reports data on hedonic ratings. It may be seen that acceptance was excellent in all cases. The few persons who said they disliked the product were mostly people who had never before eaten fish and refused to taste the samples. Their scores reflected their prejudice against fish. Table 3 shows that overall order of preference for the cakes was: small mero > trimmings > rejects > trimmings + rejects. As might have been expected, cakes made from fresh fish were preferred to those made from rejects. It should be pointed out, however, that differences in preference for the different species were small. Concerning finally, color, flavor and texture, scores were assigned by interviewers based on opinions expressed by tasters for each of the attributes. Results obtained showed that nearly perfect scores were assigned by most tasters to all samples.

In a way, acceptance was better than measured by the taste tests. In all villages, many persons asked for more samples, and a few ate as many as 10 tacos. These observations point as much to product acceptability as to the great hunger which exists among Mayan Indians. One woman told the senior author that this was the first time in her life that she had been able to eat to her satisfaction.

Table 10—Data on sun drying

	Chac-chic	Rubia	Small mero	Mojarra	Sardine	Rejects	Trimmings
Sun-drying time, days	6	5	3	4	3	3	3
Yield, dry cakes/wet cakes %	89.6	91.2	80.4	89.4	78.1	89.6	83.7
Yield, dry cakes/whole fish %	32.6	47.4	32.0	51.1	86.9	33.1	107.4
Yield, dry cakes/fillets %	1.14	1.14	0.98	1.12	0.87	1.00	1.07

Table 11—Results of taste trials with pre-school children

Group no.	Type of meal	No. of tasters	Liked (%)	Disliked (%)	Repeated (%)
1	Breakfast	25	98	2	28
2	Breakfast	51	96	4	49
3	Lunch	62	90	10	11
4	Lunch	58	100	0	9
All breakfasts		76	96	4	42
All lunches		120	95	5	10
All children		196	95	5	22

Table 12—Cost per kilo of protein for quick-salted fish cakes and other protein foods available in Mexico

Food	Price per kilo of edible portion, U.S. dollars	Protein content per kilo of edible portion (%)	Cost per kilo of protein, U.S. dollars
Quick-salted fish cakes[a]			
Chac-chic	0.29	25	1.16
Rubia	0.56	25	2.24
Mojarra	0.34	25	1.36
Small mero	0.34	25	1.36
Sardine	0.36	17	2.12
Rejects	0.33	25	1.52
Trimmings	0.14	25	0.56
Other protein foods[b]			
Milk	0.20	3.5	5.68
Eggs	0.68	12.4	5.36
Fresh fish	1.60	22.3	7.20
Beef	1.60	17.7	9.04
Chicken	2.40	20.0	12.00
Cheese	2.40	18.0	13.36

[a]Values for protein contents of fish cakes taken from calculated analyses reported in this work.

[b]Data on protein contents of "Other protein foods" taken from FAO, Food Policy and Food Science Service, Nutrition Division, 1970. Amino-acid content of foods and biological data on proteins, FAO, Rome.

Table 11 reports results of tests among pre-school children. It may be seen that acceptance in these tests was as good as that obtained in tests with adults. Data on percentage of testers who ate more than one serving were also obtained. As may be seen from the table, these percentages were appreciable in all cases, although they were higher with breakfasts than with lunches. This is reasonable, since children are normally more hungry in the morning than at noon.

Concerning costs, Table 4 lists total investment required in the pilot plant, Table 5 lists costs of equipment for the plant, and Table 6 reports production costs per kilo of dry salted cakes. Raw material costs have been included in Table 6 for comparison. It may be seen that total investment required for the plant is quite low. Production costs of the dry cakes vary, depending mostly upon raw material cost. Based on data given in Table 6, the most suitable raw materials for the plant would be trimmings, while the least suitable would be rubia. All other materials, including sardine, have approximately equal costs which lie between those for rubia and trimmings. In terms of absolute cost, all cakes are seen to be cheaper than either fresh-iced or frozen fish, which retails in Mexico at $1.20–2.00 (U.S.) per kilo. In terms of cost per kilo of protein, prices of cakes are still quite low when compared with those of other protein foods available in Mexico, as shown in Table 12.

Concerning, finally, the low cost of a plant for making the cakes, the senior author believes that such plants could be nearly family operations, and visualizes the possibility of establishing a number of small plants in different locations along the coast of a developing country.

CONCLUSIONS

JUDGING from the results of this work, it is considered that it would probably be possible to produce quick-salted fish cakes in an industrial or semi-industrial scale employing low-cost raw materials. Considering calculated production costs, retail prices of the cakes would probably be quite low, and certainly within the economic reach of most low-income groups. Extrapolating finally results of the taste trials reported in this work, acceptance of the cakes would probably be assured, as much among adults as among children.

In summary, it may be said that the purpose of the quick-salting process has been satisfied, since it provides a quick and inexpensive method for preserving fish which is applicable to developing countries. In this way it makes available a high-protein low-cost food to the undernourished population of these countries.

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OXIDATIVE RANCIDITY IN DRY-CURED HAMS: EFFECT OF LOW PRO-OXIDANT AND ANTIOXIDANT SALT FORMULATIONS

INTRODUCTION

THE ROLE OF oxidative rancidity in the flavor of dry-cured country hams was reported by Kemp et al. (1961) and Turner et al. (1954). Their studies showed that hams with greater oxidative rancidity had lower palatability scores. Thus, the reduction of oxidative rancidity would have a desirable effect on the acceptance of these hams.

From work reported by Denisov and Emanuel (1960), heavy metal ions (principally iron, copper and chromium) are responsible for catalizing oxidative rancidity. Iron and copper particularly are usually present in flake salt (NaCl) used in meat curing.

Two possible alternatives for retarding oxidative rancidity are: (1) removal of the heavy metal ions from the salt and (2) the addition of antioxidants.

Butylated hydroxanisole (BHA), butylated hydroxytoluene (BHT), citric acid and propylene glycol were shown to be effective antioxidants in meat products by Bentz et al. (1952); Hanely et al. (1953); Lew and Tappel (1956); Tollenaar and Vox (1958); and Kraybill et al. (1949). That heme pigments may also catalyze oxidative rancidity as reported by Younathan and Watts (1959) and Lew and Tappel (1956) would indicate that the use of salt mixtures containing antioxidants might prove more effective than the removal of the heavy metal ions alone.

This research was conducted to determine the effects on oxidative rancidity and flavor using a regular flake salt, an antioxidant salt and a salt with a low concentration of heavy metal ions in the curing mixture when applied to dry-cured hams.

EXPERIMENTAL

Ham treatment

Three lots of nine hams each were selected at random from a meat packer's normal production. They were left unskinned, and the shank was removed at the hock joint. The hams were in the 6.37–7.26 kg weight range.

The hams were rubbed with the following curing mixture: 3.63 kg salt, 1.36 kg sugar and 85.2g sodium nitrate, applied at the rate of 80g per 1 kg of ham.

Salts

Only the salt differed in each curing mixture. Lot I contained flake salt (copper 1.5 ppm and iron 1.5 ppm) and was used as the control group. Lot II contained Morton Culinox 999, food-grade salt having a low content of heavy metals, with copper at 0.1 ppm and iron at 0.4 ppm. Lot III contained Morton Star Flake R.G. Dendritic 1.14 salt, containing the following antioxidants: BHA, 0.253%; BHT, 0.253%; citric acid, 0.253%; and propylene glycol, 0.03%. Copper and iron content was the same as for the Culinox 999.

During smoking and aging

The curing mixture was rubbed on the surface of the hams in three applications: 50% of the mixture initially, 25% after 3 days and the remaining 25% after 10 days. The hams were shelf-cured for 30 days at a temperature of 3°C.

After the curing period, the hams were placed in stockinettes and hung at a temperature of 3°C for the "salt equalization" period of 30 days. The hams were soaked for 6 hr at room temperature (22°C) to remove excess surface salt and heat-processed in a steam heated air-conditioned smokehouse at a temperature of 32°C and 65% RH for 72 hr. Dense smoke generated from mixed hardwood sawdust was applied for approximately 1 hr until the hams became dark mahogany-brown. After the smoking, the hams were aged at a temperature of 18°C and 45% RH for 90 days.

Chemical and sensory analysis

One ham in Lot I was removed during aging to determine the extent of "characteristic" flavor development. Two hams from each lot were selected at random after aging for taste-panel evaluation. The remaining hams were used for chemical determinations:

1. Salt and moisture content of an entire 1.9 cm thick center slice was determined as described by AOAC (1955).
2. TBA values were determined separately for fat and lean from one 1.9 cm-thick center slice. Another 1.9 cm-thick center slice was analyzed in its entirety. The method used for determining TBA values was that reported by Tarladgis (1960).

A 43-member taste panel selected at random, was used to score the center slices from the two hams for flavor, saltiness, tenderness and overall acceptability. For scoring, the taste panel used a 9-point hedonic scale ranging from 9 "like extremely" to 1 "dislike extremely." Each 1.9 cm-thick center ham slice was broiled in an electric oven broiler to an internal temperature of 50°C on one side, turned and then broiled to 70°C final internal temperature.

RESULTS

Weight loss

The combined average weight loss (from green weight) of hams in each lot was 17.23%. This loss is lower than is normally found under similar conditions (Kemp et al., 1968; Rogers et al., 1965). Weight loss occurring from the green weight to the end of the salt equalization period was only 4.81%. A greater average weight loss normally is expected during this period. The authors could find no apparent explanation for this difference.

Chemical tests

Center slices from six hams in Lot I and seven hams in Lot II and Lot III were analyzed chemically. At the time all hams were cut they were given visual appraisal for color difference, but none was observable. Table 1 shows the least-squares means and standard errors for chemical

Table 1—Least squares means and standard error for factors in chemical analysis for treatments

Chemical Factors	(Lot I) Regular flake salt		(Lot II) Low pro-oxidant salt		(Lot III) Salt with antioxidant		
	Means	S.E.	Means	S.E.	Means	S.E.	LSD[a]
% Water	48.11	1.42	46.60	1.31	48.02	1.31	
% Fat	21.26	2.20	23.09	2.04	22.61	2.04	
% Salt	4.35	0.16	4.87	0.15	4.58	0.15	
Muscle TBA[b] (mg/kg fresh wt)	0.65	0.04	0.52	0.04	0.55	0.04	
Muscle-fat TBA[b] (mg/kg fresh wt)	2.24	0.32	1.60	0.30	1.45	0.30	
Fat TBA[b] (mg/kg fresh wt)	3.02	0.56	3.73	0.51	1.18	0.51	0.99

[a] Least squares different—minimum value needed for significant difference ($P < 0.05$) between means.
[b] TBA values

Table 2—Mean squares of the analysis of variance for factors in chemical analysis for treatments

Source	d.f.	% Water	% Fat	% Salt	Muscle TBA	Muscle-fat TBA	Fat TBA
Treatment	2	4.83	5.71	0.45	0.03	1.09	12.01*
Residual	17	12.10	29.01	0.16	0.01	0.62	1.86

*P < 0.05

Table 5—Correlation coefficients of organoleptic scores of flavor, saltiness and tenderness with overall satisfaction

Organoleptic factors	Correlation coefficients
Flavor and overall satisfaction	0.43
Saltiness and overall satisfaction	0.70
Tenderness and overall satisfaction	0.93**

**P < 0.01

Table 3—Least squares means and standard errors for organoleptic flavor, tenderness, saltiness and over-all satisfaction for treatments

Organoleptic factors[a]	(Lot I) Regular flake salt		(Lot II) Low pro-oxidant salt		(Lot III) Antioxidant salt		
	L.S. Means	S.E.	L.S. Means	S.E.	L.S. Means	S.E.	LSD[b]
Flavor	4.55	0.17	5.32	0.17	5.56	0.17	0.54
Tenderness	4.70	0.81	5.04	0.81	4.87	0.81	
Saltiness	4.76	0.51	5.56	0.51	4.88	0.51	
Overall Satisfaction	4.57	0.27	5.42	0.27	5.12	0.27	

[a]Scale used: 9-like extremely; 8-like very much; 7-like moderately; 6-like slightly; 5-neither like nor dislike; 4-dislike slightly; 3-dislike moderately; 2-dislike very much; 1-dislike extremely.

[b]Least squares difference—minimum value needed for significant (P < 0.05) difference between means.

Table 4—Mean squares of the analysis of variance for organoleptic flavor, tenderness, saltiness and overall satisfaction for treatments

Source	d.f.	Flavor	Tenderness	Saltiness	Overall satisfaction
Treatment	2	0.56*	.06	0.38	0.37
Residual	3	0.06	1.33	0.53	0.14

*P < 0.05

analyses. Mean squares of the analysis of variance for percentage of fat, water, salt, TBA values for muscle, TBA values for muscle and fat and TBA values for fat are shown in Table 2. Only the TBA values for fat were significantly different between treatments (P < 0.05).

Table 1 shows that Lot III had significantly (P < 0.05) lower mean TBA values for fat than either Lot I or Lot II. Mean fat TBA values for Lot II were the highest of all three lots, indicating that rancidity occurs even with the reduction of heavy metal ions in the salt.

Taste panel

The least-squares means and the standard errors for organoleptic flavor, saltiness, tenderness and overall satisfaction are shown in Table 3. Mean squares of the analysis of variance are shown in Table 4. A significant difference (P < 0.05) for flavor was found between the treatments. Lot III was scored higher for flavor than either Lot I or Lot II (Table 3), and Lot I was scored the lowest.

The correlations of flavor, saltiness and tenderness with overall satisfaction are shown in Table 5. Flavor and saltiness are not significantly correlated with overall satisfaction. Tenderness is highly correlated with overall satisfaction (P < 0.01).

DISCUSSION

RANCIDITY was significantly reduced in the lot containing the antioxidants (Lot III) as shown by the TBA values for fat. The TBA values for muscle and for muscle and fat combined, however, showed no significant difference (Table 2). This lack of statistically significant differences in the TBA values for muscle or the muscle and fat combination may be due to the relatively high standard error for total fat content of these samples (Table 1) or to the fact that the antioxidants had their principal effect on surface fat.

The taste panel scores indicate a significantly greater flavor preference for the samples in Lot III. These results indicate that the salt containing antioxidants has a definite beneficial effect on flavor. The greater flavor acceptability might be due to the reduction of oxidative rancidity. This conclusion is questionable since Lot II, containing the low heavy metal ion salt, was scored higher for flavor than Lot I, but the TBA values for fat in Lot II showed a greater degree of rancidity than Lot I. Because samples used in the chemical analysis were from different hams than those used in the taste panel, no correlation between rancidity and flavor could be established.

Only the taste-panel flavor scores and rancidity, indicated by the TBA values for fat, were significantly different (P < 0.05) between the treatment lots. The different salts used in each lot did not affect the hams for the other factors measured.

Even though a significant difference was found for flavor between lots (Table 4) overall satisfaction was not significantly different. The overall satisfaction is apparently affected to a greater extent by factors other than flavor in dry-cured hams as shown by the correlations in Table 5.

Since oxidative rancidity supposedly only affects flavor, its role in overall satisfaction for dry-cured hams is relatively less important than other factors, particularly tenderness. The control of oxidative rancidity in this type of product may be unnecessary.

The fact that the salt containing the antioxidants does have a significant effect on flavor and does significantly retard oxidative rancidity warrants further study of the effects of antioxidants in products of relatively high fat content and those where flavor has a significant role in overall acceptability. This would be particularly true for products, such as cured ham and bacon, that may be subjected to prolonged freezer storage.

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EFFECTS OF FREEZING AND PACKAGING METHODS ON SHRINKAGE OF HAMS IN FROZEN STORAGE

INTRODUCTION

THE PRACTICE of holding hams in intermediary frozen storage aids in balancing pork supplies during periods of high and low production. Hams are especially suitable to frozen storage because they become more porous upon thawing and are more receptive to curing solutions (American Meat Institute Foundation, 1960).

A serious problem associated with freezing and storing hams is shrinkage resulting from moisture evaporation which may be caused by a number of factors. A major cause of shrinkage is the difference between vapor pressure of the water or ice at the surface of the hams and the air surrounding the hams which allows the moisture vapor to move out of the hams into the air and subsequently condense on the refrigeration coils (Hulland, 1970; Peters, 1970). High air velocity over cooling hams also will greatly increase moisture loss (Kastner et al., 1970). Ramsbottom (1947) and Peters (1970) showed that storage temperature is an important factor affecting shrinkage: the lower the storage temperature the less shrinkage occurs. This was attributed to the reduced capacity of low temperature air to hold water vapor. Dunker and Hankins (1953) reported that increasing the freezing rate of ground pork also would reduce shrinkage. Powell (1940) found that the shape of the object under refrigeration affects shrinkage. The more spherical an object, the greater the rate of moisture evaporation from its surface. Peters (1970) also reported that the geometric configuration of the product in frozen storage may have considerable effect on shrinkage.

Packaging is a well recognized preventative against moisture loss from meat under refrigeration (ASHRAE, 1968; Bouton and Howard, 1956; Doordan et al., 1969; Peters, 1970; Ramsbottom, 1947; Ziemba, 1946). For maximum protection, however, the packaging material should: (1) Be relatively impermeable to moisture; (2) Adhere to the product so as to reduce free air space within the package; and (3) Be tightly sealed.

The amount of shrinkage pork incurs during freezing and storage may vary considerably depending largely on handling procedures and environmental conditions in the storage rooms. A large American meat packer reported a 0.50–0.75% weight loss when freezing unwrapped pork picnic shoulders (TRRF, 1966). Cooper (1970) reported a freezing shrinkage of 0.75% for unwrapped and 0.50%

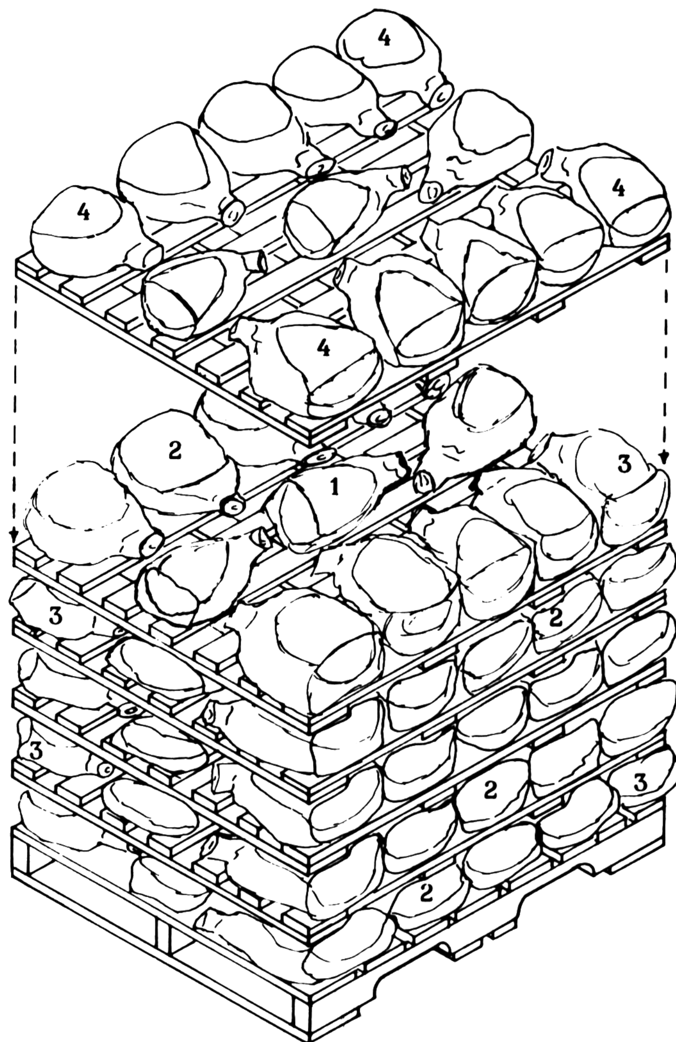

Fig. 1—Hams stacked on a pallet for freezing. Numbers denote amount of exposure to water spray and air around the exterior of the pallet: 1 = no sides exposed; 2 = 1 side exposed; 3 = 2 sides exposed; and 4 = 3 sides exposed.

Table 1—Changes in weight of hams as influenced by freezing, packaging and duration of storage[a]

9-pallet set	Wt change during initial freezing[b] %	Duration of storage months	Wt change during glazing and storage[c] %	Net wt change upon removal from storage %	Wt change after thawing[d] %	Total shrinkage after storage and thawing[d] %
1	−1.05	2	+0.33	−0.72	+0.25	0.47
2	−1.04	4	+0.60	−0.44	−0.39	0.83
3	−1.00	6	+0.53	−0.47	−0.63	1.10

[a]Each value represents the average of 144 hams.
[b]Each value is an average of all three freezing methods.
[c]Significantly different at ($P < 0.05$)
[d]Significantly different at ($P < 0.01$)

for wrapped bacon sides. After 2 months' storage at 0°F, the unwrapped bacon sides shrank 3.1% while the bacon sides wrapped in polyethylene lost no weight. Roussel and Sarrazin (1970) found that after 355 days in forced air storage at −4°F unwrapped hams shrank 8.2%. However, unwrapped hams stored in still air at −15°F shrank only 3.8%. Kunis and Placzek (1970) reported that East German industry norms for shrinkage of frozen pork carcasses in storage were about 0.4% at the end of 2 months, 0.8% after 4 months and 1.2% after 6 months.

There are considerable gaps in research reporting the amounts of shrinkages occurring to hams during commercial refrigerated warehousing. This research attempts to identify the areas in the warehousing chain where the greatest amount of shrinkage occurs, and the effects of different freezing and packaging methods on shrinkages of hams.

EXPERIMENTAL

27 PALLET LOTS of fresh chilled hams (78 hams per pallet) were used for a 3 × 3 × 3 factorial design experiment with an additional nested factor to study the effect of position of individual hams on the pallets. Shrinkage data were analyzed by analysis of variance.

The experiment was designed to study shrinkage of hams under actual commercial conditions in a refrigerated warehouse. Hams for the experiment were selected at random from a typical day's kill in a commercial slaughter house. All freezing, packaging and handling methods applied were typical of those used in the industry.

The hams came from hogs that were graded USDA 1, 2 and 3. The hogs were slaughtered 2 days before the test started. The carcasses were chilled overnight, broken into cuts and the hams were graded by weight. Hams in the weight grade 14–17 lb were used in the experiment. They were skinned leaving a 55% collar covering. Fat was trimmed from the edge of the collar to a depth of ¼ in. at the butt end. The hams were trucked in stainless steel tubs for a 60-mi distance to the public refrigerated warehouse used for the experiment.

Upon arrival at the warehouse, the hams were stacked on 40-× 48-in. wood pallets. Each pallet was loaded six layers high with 13 hams stacked on each layer. A wood divider rack was placed between each layer to stabilize the load and allow air circulation between the layers. Each pallet and wood divider previously had been washed and covered with polyethylene film to meet USDA meat inspection requirements.

As the hams were stacked on each pallet, 16 sample hams were randomly selected, weighed and tagged for identification. The sample hams were divided into four groups (four hams in each group) and stacked on the pallet so that each group would be subjected to one of the following four types of exposure to spray glazing and air around the outer surfaces of the pallet: (1) No sides exposed (located in the interior of the stack); (2) one side exposed (located in the middle side of the stack); (3) two sides exposed (located at any corner, except top layer); and (4) three sides exposed (located at any corner on the top layer) (Fig. 1).

For freezing, the 27 experimental pallet loads were divided into three sets of nine pallets. Each set was assigned to a different freezing method and frozen until the hams on the corners of the top layers reached a center temperature of 0°F. One set of nine pallets of hams was placed in a still air freezer storage room (−5 ± 1°F.) and held for 5 days.

Another set of nine pallets of hams was put in a freezer storage room (−7 ± 1°F.) refrigerated by fan forced air. Air velocity across the top of the pallets ranged from 150–200 fpm (feet per minute), and from 10–50 fpm at areas along the bottom edges of the pallets. Freezing time was 3 days.

The remaining set of nine pallets of hams was placed in a blast freezer and frozen within 2 days at −16 ± 1°F. Air velocity over these pallets ranged from 250–300 fpm across the top of the pallets and 100–150 fpm along the bottom edges of the pallets.

After freezing, each pallet load was broken down and the sample hams weighed. In turn, each pallet load was reconstructed with the sample hams returned to the assigned locations on the pallet.

The 27 pallet loads of hams were divided into three sets of nine for packaging treatments. In one set, each of the pallets was fully enclosed in a 2-mil polyethylene bag (polybagged). Air was evacuated and the bags were tied with a string. Another set of nine pallets was ice glazed by placing each pallet in a specially designed spray-rack for 5 min. The spray-rack was equipped with nozzles that uniformly sprayed a fine mist of water around each pallet. After spraying, each pallet in this set was polybagged. The remaining set of nine pallets also was subjected to 5 min of spray, but was not polybagged.

After the <2–5-day freezing and the glazing and/or polybagging, all 27 pallets of hams were placed in the still air freezer room (−5 ± 4°F.) for storage. The pallets were again divided into three sets of nine pallets according to storage period treatment of 2, 4 and 6 months.

At the end of 2 months, the first set of pallets was removed from storage. The sample hams were weighed, thawed for 20 hr at 60°F., then weighed for the final time and returned to the processing plant. The procedure for handling and collecting the test data on the sample hams at the end of the 4- and 6-month storage periods was identical to that followed at the end of the 2-month period.

RESULTS & DISCUSSION

WEIGHT LOSS during initial freezing and subsequent reabsorption of glaze and condensation moisture during storage and thawing were the most important factors influencing the total shrinkage of hams during refrigerated warehousing.

Effect of time in storage

After initial freezing, hams stored under all treatments showed weight gains which appeared to level off after 4 months' storage (Table 1, column 4). A

Table 2—Effect of freezing methods on shrinkage of hams[a]

Freezing method	Shrinkage during initial freezing[b] %	Total shrinkage after thawing[c] %
Still air room	1.22	0.92
Forced air room	1.09	0.91
Blast freezer	0.79	0.58

[a]Each value is an average for all storage periods.
[b]Significantly different at ($P < 0.01$)
[c]Significantly different at ($P < 0.05$)

Table 3—Effect of packaging method on total shrinkage of hams[a]

Packaging method	Total shrinkage after thawing[b] %
Glazed and polybagged	0.74
Polybagged only	0.87
Glazed only	0.80

[a]Each value is an average for all storage periods

[b]Not significantly different (P < 0.05)

Table 4—Effect of location on pallet and packaging on percent total shrinkage of hams[a]

Location on pallet	Packaging method			
	Glazed & polybagged	Polybagged only	Glazed only	Overall mean[b]
Interior	1.07yz	1.11yz	1.18z	1.12W
Middle side	0.98wxy	0.82vwx	0.94vwxy	0.91X
Corner	0.72u	0.80uvw	0.78uv	0.77Y
Corner (top layer)	0.18t	0.75uv	0.31t	0.41Z

[a]Values in the body of the table not followed by a common lower case letter or letters are significantly different (P < 0.05), (Duncan, 1955).

[b]Values in this column not followed by a common upper case letter are significantly different (P< 0.05), (Duncan, 1955).

large amount of this weight gain was attributed to glaze, and condensation moisture which accumulated on the hams while they were being weighed in an unrefrigerated room. Upon removal from storage and thawing the hams gained additional weight, but only up to the first 2 months in storage. They then began to lose weight again when storage was extended to 4–6 months (Table 1, column 6). The same type response was noted by Ramsbottom (1947). It appears, therefore, that net shrinkage of hams upon removal from storage actually decreases during the first 4 months' storage (Table 1, column 5), but total shrinkage after thawing, increases steadily with storage time (Table 1, column 7). Apparently the biochemical mechanisms that favor reabsorption of moisture by the muscle break down steadily as time in storage increases. Thus, total shrinkage was more a function of the decreasing reabsorption ability of the hams than it was of moisture loss during storage.

Effect of freezing method

During initial freezing hams frozen in the blast freezer incurred significantly (P < 0.01) less shrinkage (0.79%) compared to those frozen in the forced air (1.09%) and still air (1.22%) rooms (Table 2). Shrinkage in the blast freezer was nearly the same as that reported by Cooper (1970). The results agree with previous research which showed that the faster meat is frozen, the less shrinkage occurs (Alyamovsky, 1970; Cooper, 1970; Dunker and Hankins, 1953; Lorentzen and Rosvik, 1959). The relative effect of the freezing method on weight retention remained during packaging and storage so that the total shrinkage after thawing was still significantly lower (P < 0.05) in the blast frozen hams than those frozen in either the forced air or still air rooms (Table 2).

Effect of packaging

The data in Table 3 indicate a slightly improved weight retention for the glazed hams over the polybagged, and a further slight improvement for the hams that were both glazed and polybagged. These differences, however, were not statistically significant, so that it may be concluded that all three methods provided a similar degree of protection against moisture loss. That the amount of protection was substantial may be deduced by comparing these losses (range of 0.74–0.87%) to those reported by Cooper (1970) who found a shrinkage of 3.1% in unwrapped bacon sides after only 2 months' storage at 0 °F., and practically no weight loss in polyethylene wrapped bacon.

The failure of the polybagging technique to provide more effective protection against shrinkage in this instance may be attributed to the fact that all of the bags were punctured in some manner during handling through the warehouse. Moreover, the bagging technique allowed considereable free air space between the film and the meat, thereby permitting some water vapor to escape from the meat and condense as frost on the inside of the polyethylene film.

Interactions

No statistically significant first order interactions were found for total shrinkage between freezing and packaging, freezing and storage, or packaging and storage treatments. Significance of the second order interaction could not be tested since it was used as the error term. An examination of the detailed data, however, showed a tendency for the hams that were glazed only to shrink more when frozen in still air (1.09%) than in forced air (0.74%). The reverse was indicated for the hams that were polybagged only (0.97 vs. 1.03%, respectively).

Locations on pallet

Location on pallet, or exposure to glazing and air surrounding the pallet, was a nested factor in this experiment. There were significant differences (P < 0.05) in shrinkage of hams stacked at different locations on the pallet. Table 4 shows that hams stacked in the interior of the pallet where they froze slowly shrank more than those stacked on the outer surfaces of the pallet where faster freezing occurred. This again shows the merit of fast freezing in reducing shrinkage.

The spray glaze on the outer surfaces of the pallet load did not materially decrease the shrinkage of hams in any position except those on the top layer. Glazed hams on the top layer lost only ¼–½ as much weight as those that were not glazed (Table 4). This was expected because the hams on the top layer had considerably more glaze available for reabsorption than glazed hams stacked in other positions.

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EFFECTS OF FREEZING AND PACKAGING METHODS ON FREEZER BURN OF HAMS IN FROZEN STORAGE

INTRODUCTION

IN THE FOREGOING study (Ashby et al., 1973) we investigated the effects of various freezing methods, packaging methods and storage periods on the shrinkage of hams in commercial frozen storage. The shrinkage of hams was found to be substantial. Even more loss may be caused during the freezing and storage of hams if "freezer burn" develops.

Freezer burn develops on the surface of exposed frozen meat, in the form of patches of light-colored tissue, when ice crystals evaporate and leave air pockets between the fibers of the meat. A small amount of freezer burn at the termination of storage may be acceptable, because rehydration and curing will counteract it. However, if the freezer burn is excessive, the hams must be sold at reduced prices to compensate for trimming losses.

The lighter color of burned areas is explained, technically, as an optical effect that results from the scattering of light by the honeycomb-like structure formed by evapoated ice crystals (Moran, 1934). Histological studies by Kaess and Weidemann (1961) provided more information on the development of freezer burn than had been revealed in previous studies. Analysis of sections from samples of liver fixed in the frozen state showed that dehydration of the surface brings about the formation of a condensed layer of tissue, caused by the merger of fibers and cells, and that freezer burn develops from this layer of tissue. As dehydration continues, the condensed layer of tissue advances toward the interior of the meat, causing freezer burn of increasing depth.

In the past, the main remedies for protecting meat from freezer burn have been to prevent dehydration by maintaining high humidity and low temperature differentials over the coil during cold storage (Christensen and Cook, 1958; Kaess and Weidemann, 1963) and by application of packaging films (Moran, 1934; Ziemba, 1946). A traditional method of protecting pork bellies, hams and picnics is to give them a coating of ice glaze by dipping them in water after they are frozen (American Meat Institute, 1957). Kaess (1961) reported that early stages of freezer burn may disappear when the humidity of the storage is raised. Subsequent work by Kaess and Weidemann in 1969, however, indicated that maintaining a 97% relative humidity during storage had little influence on the amount of freezer burn occurring.

Kaess and Weidemann (1963) showed that freezer burn is reversible if moisture loss in the surface layer does not exceed about 0.5 oz per 100 sq in., but if the freezer burn is more severe it is irreversible because of damage to the muscle enzyme systems and concentration of tissue salts (Callow, 1952; Kaess and Weidemann, 1961).

Kaess and Weidemann (1969) showed that meat frozen slowly, with evaporative weight loss, developed less freezer burn than meat frozen rapidly, without weight loss. Slow freezing helped to form a condensed layer on the meat surface which restricted the development of freezer burn. When freezer burn cavities were present, they occurred below the condensed layer. With rapid freezing, the positions of the freezer burn cavities and the condensed layer that form during storage were reversed. Freezing and storing at low temperatures retarded the development of the condensed layer. Less evaporation weight loss was needed at lower temperatures than at higher temperatures to produce a definite intensity of burn. At the lower temperatures there was less tissue flow or displacement into spaces earlier occupied by ice crystals (Kaess and Weidemann, 1969).

A considerable amount of technical information is available concerning the development of freezer burn on meat tissue during freezing and storage under laboratory conditions. However, very little practical information is available to the meat industry on the approximate amount of freezer burn that can be expected to develop as the result of the handling methods used during refrigerated warehousing. Information is also needed for use in determining the amount of trim weight loss from meat as a result of freezer burn. The purpose of this paper is: (1) To develop information on the effects of various freezing and packaging methods on the amount of freezer burn that occurs on hams during frozen storage; and (2) To determine whether a relationship exists between the amount of freezer burn that occurs on the surface of hams and the amount of trim weight loss that results from the burn.

EXPERIMENTAL

THE AMOUNT of freezer burn on the surface of the cushion, butt end and butt face of hams frozen, packaged and stored as described in the preceding paper (Ashby et al., 1973) was measured at the end of the 4- and 6-month storage periods. Data were analyzed by analysis of variance and by linear regression and correlation.

Measurement of burn

A USDA meat grader measured the number of square inches of freezer burn with a clear acetate grid marked off in 1-inch squares. He measured the depth of the burn with a stainless steel ruler having graduations of 1/100 of an inch.

Trim loss

Hams that had been held in storage for 6 months were thawed, weighed and then trimmed to remove freezer burn. They were then weighed again to determine the amount of trim weight loss.

Table 1—Effect of freezing method and time in storage on development of freezer burn on hams[a]

Time in storage	Freezing method[b] Still air room Sq in./ham	Forced-air room Sq in./ham	Blast freezer Sq in./ham
4 months	5.69y	6.44y	5.61y
6 months	11.69yz	19.83z	10.72yz

[a]Each value is the average of 48 hams.
[b]Values not followed by a common letter are significantly different ($P < 0.05$) (Duncan, 1955).

RESULTS & DISCUSSION

Effect of time in storage

The amount of visible freezer burn on the hams at the end of the 2-month storage period was negligible, but increased significantly to an average of 5.91 sq in. per ham after 4 months' storage, and to 14.08 sq in. per ham after 6 months' storage. Even after 6 months' storage, some of the hams had no visible freezer burn, and none of the hams were burned severely or deeply enough to disqualify them from being traded on the Chicago Mercantile Exchange (CME, 1969).

Freezing method

The freezing method exerted a significant ($P < 0.01$) influence on the ultimate amount of freezer burn that occurs on the hams. The forced-air freezing method caused increased amounts of freezer burn to form on the hams between the 4- and 6-month storage periods. This fact was indicated by the significant ($P < 0.01$) interaction between freezing and time in storage. Table 1 shows the effects of this interaction. At the end of 4 months, the amount of freezer burn on hams frozen by all three methods was about the same. However, at the end of 6 months, the burn had doubled on hams frozen in both the still-air room and the blast freezer and tripled on hams frozen in the forced-air room.

The freezing method also significantly ($P < 0.01$) affected the freezer burn trim weight loss of the meat stored for 6 months. Hams frozen in the blast freezer had a 1.74% trim loss as compared with a 2.39% loss for those frozen in the forced-air room and a 2.54% loss for those frozen in the still-air room.

Packaging method

No significant differences were found in the amount of freezer burn sustained by the hams as a result of any of the three packaging methods tested. However, a significant ($P < 0.05$) interaction between packaging method and time in storage showed that between the 4- and 6-month storage periods the amount of freezer burn more than tripled on hams that were stored on open pallets with only ice glaze for protection; whereas the amount of freezer burn only doubled on hams protected with polybags (Table 2). This finding emphasizes the need to reglaze meat stored on open pallets when the storage period exceeds 4 months.

Location on pallet

Highly significant differences ($P < 0.01$) were found between the amount of freezer burn on hams stacked at different locations on the pallet. Table 3 shows that hams stacked on the corners of the top layers of the load, where they froze faster, sustained more than three times as much freezer burn as those stacked in the interior of the load, where they froze more slowly. Futhermore, research on shrinkage (weight loss) (Ashby et al., 1973), using the same hams, showed that the hams stacked on the corners of the top layers of the load sustained a total shrinkage of only 0.41%, as compared with 1.12% for those stacked in the interior of the load. Apparently, an inverse relationship exists between freezer burn and shrinkage with respect to location of the hams on the pallet. This relationship evidently is due to the effect that evaporative weight loss during freezing exerts on the formation of freezer burn. These results under actual warehousing conditions confirm the findings of Kaess and Weidemann (1969), which showed that reducing evaporative weight loss through rapid freezing increases the development of freezer burn; whereas allowing weight loss through slower freezing retards the development of burn.

Table 3 also shows that packaging treatments did not significantly ($P < 0.05$) affect the amount of freezer burn that occurs on hams in any of the locations except those on the corners of the top layer of the load. Hams stacked in this location with only a polybag for protection sustained more freezer burn than those that were glazed only or glazed and polybagged.

Depth of burn

Of the 144 sample hams checked for freezer burn at the end of each storage period, 96 had visible burn at the end of 4 months. Of these, 15 hams had burn of measurable depth. This burn was on the butt face and ranged from 0.05–0.10 in. in depth.

At the end of 6 months, 124 hams exhibited visible freezer burn. Although the average number of square inches of burn had more than doubled, still only 15 hams had freezer burn of measurable depth. This burn also was confined to the butt face, but was deeper–ranging in depth from 0.20–0.25 in. Since hams are cut across the fiber at the butt face, these results correspond with those of Kaess and Weidemann (1961), who found that development of freezer burn tends to be greatest along vessles in the tissue and along fibers.

Freezer burn and trim loss

An attempt was made to develop a method of predicting the amount of trim weight loss from a given amount of freezer burn by determining the regression of percentage of trim loss on square inches of freezer burn. A significant ($P < 0.05$) but low correlation coefficient ($r=0.30$) was obtained between trim loss and freezer burn. The data for this correlation are plotted in Figure 1, showing that a relatively large increase in freezer burn increases trim loss very little.

The coefficient of determination ($r^2 = 0.09$) indicates that 91% of the varia-

Table 2–Effect of packaging method and time in storage on development of freezer burn on hams[a]

Time in storage	Packaging method[b] Glazed and polybagged Sq in./ham	Polybagged only Sq in./ham	Glazed only Sq in./ham
4 months	5.56yz	8.16yz	4.01y
6 months	12.72yz	13.63yz	15.88z

[a]Each value is the average of 48 hams.
[b]Means not followed by a common letter are significantly different ($P < 0.05$) (Duncan, 1955).

Table 3–Effect of location on pallet and packaging on the square inches of freezer burn that occurs on hams during frozen storage[a]

Location on pallet[b]	Packaging method: Glazed and polybagged Sq in./ham	Polybagged only Sq in./ham	Glazed only Sq in./ham	Overall mean[c] Sq in./ham
Interior	5.20vw	4.41v	5.80vw	5.14
Middle side	10.78wx	10.37wx	8.96vwx	10.04
Corner	7.73vwx	9.74vwx	9.00vwx	8.82
Corner (top layer)	12.85xy	19.07z	16.03yz	15.98

[a]Values in body of table not followed by a common letter are significantly different ($P < 0.05$) (Duncan, 1955). Each value is an average of 24 hams.
[b]See Figure 1 in preceding article (Ashby et al., 1973) for illustration of ham positions on the pallets.
[c]Significantly different ($P < 0.01$); each value is an average of 72 hams.

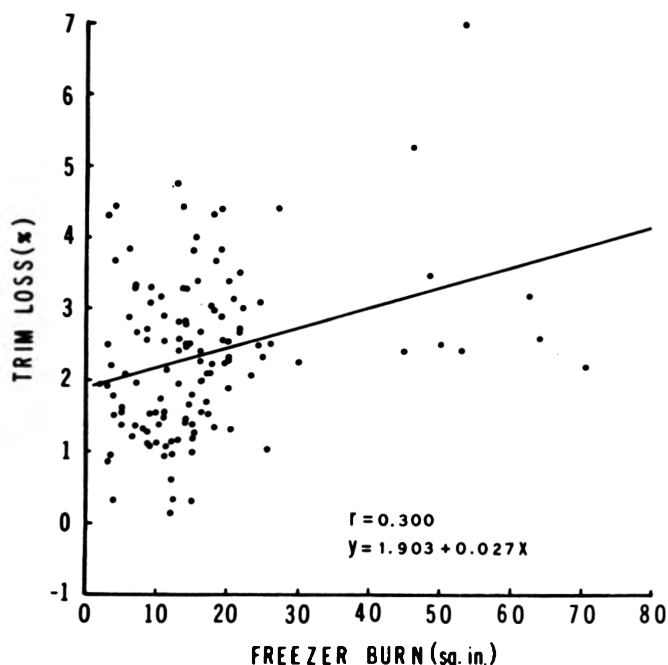

Fig. 1—Scatter diagram showing relationship between amount of freezer burn and percentage of freezer burn trim loss.

tion in trim loss is not necessarily associated with freezer burn, but with other factors or error. Much of this error, however, may be attributed to excessive trimming. After the trimming test was started, it became apparent that the ham trimmer in the processing plant was trimming more than just freezer burn from the hams. If a better trained person were used for the trimming test, a more accurate prediction of trim weight loss in terms of square inches of freezer burn might be obtained.

Freezer burn and total shrinkage

A correlation coefficient (r) of 0.05 indicates practically no relationship between the amount of freezer burn that occurs on hams and the amount of total shrinkage. These results agree with those of Kaess (1961), who found that freezer burn is essentially independent from rate of evaporation during storage.

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REDUCTION OF BACTERIA ON PORK CARCASSES

INTRODUCTION

THE PROBLEM of reducing the number of salmonellae organisms from meat animal carcasses has been approached in a variety of ways. Kampelmacher et al. (1965) found that animals fed decontaminated (salmonellae-free) feed were free of salmonellae before and after slaughter. Galton et al. (1954) were able to reduce salmonellae contamination of hog carcasses by a thorough cleaning of the dehairing machine. Dixon and Pooley (1961) reported a reduction of salmonellae in a poultry processing plant by using a 200 ppm chlorination program in the chilling water. Shotts et al. (1961) used a hot water wash (165°F) on the outside of hog carcasses before and after evisceration but their results were inconsistent. Thomson et al. (1967) studied the effects of chlorine, antibiotics, B-propiolactone, citric and succinic acids on breast meat inoculated with *S. typhimurium* and found that all these agents reduced the incidence of recovery of the test organism.

Mountney and O'Malley (1965) in studying the effects of ten organic acids as poultry meat preservatives, determined that acetic, adipic and succinic acids were the most effective and extended shelf life by 6 days more than controls. They considered acetic acid unacceptable because of its pungent odor and its effect on the skin and concluded that adipic and succinic acids gave best overall results both from their ability to inhibit microflora and still have an acceptable taste.

The present study was undertaken to develop methods which could be used to effectively reduce the total number of microorganisms and the incidence of salmonellae on pork carcasses.

MATERIALS & METHODS

THIS STUDY was conducted in two stages: (1) a pilot plant phase during which selected chemicals and steam were evaluated for their abilities to inhibit *S. enteritidis* on pork carcasses; and (2) in-plant studies where pork carcasses were treated with various pH levels of acetic acid and reduction in total bacterial numbers and incidence of salmonellae were determined. To attain levels of pH below pH 2.4 hydrochloric acid was added to 0.1N acetic acid solutions.

Culture

S. enteritidis culture (obtained from the National Center for Disease Control, Atlanta) was activated, prior to use, by transferring into tryptic soy broth (TSB-Difco) once every 24 hr for a period of not less than 72 hr.

Pilot plant studies

Surface skin of freshly slaughtered hogs was examined for microbial population and for the presence of salmonellae. Various designated areas (8–10 cm in diameter) were swabbed with a cotton tipped applicator after being moistened with sterile physiological saline. In order to test for control of salmonellae, some of these areas were inoculated with the test organism (10^6 organisms/ml) by swabbing with a cotton swab. Subsequent sampling of these areas showed that the total aerobic plate count averaged 10^6 organisms/cm^2.

Stannous chloride (5%), hydrogen peroxide (5%) and acetic acid (pH 1.5 and 2.0) were applied in a form of a continuous spray of fine particles. All applications were made at approximately 8 cm from the carcass for 10–12 sec. No attempt was made to determine the volume applied but treated areas were well covered. There was no overlap of treatments and runoff from one area could not recontaminate other treated or untreated areas. The carcasses were then swabbed to determine the total microbial population and incidence of salmonellae and stored in a chill room at 4°C. The following day the carcasses were examined as previously described. A 1 ml quantity from each swab tube was inoculated into Brilliant Green Tetrathionate Broth (BGTB) and incubated at 37°C for 24 hr for the enrichment of salmonellae as described by Galton et al. (1968). Following incubation (37°C for 24 hr) aliquots from each BGTB tube were streaked onto brilliant green (BG) agar and bismuth sulfite (BS) agar and again incubated (37°C for 24 hr). Suspected colonies were selected and inoculated into triple sugar iron (TSI) agar and incubated at 37°C for 24 hr. Slants of TSI agar showing typical growth as described by Galton et al. (1968) were then forwarded to the Southeastern Salmonella Serotyping Laboratory, Atlanta, for positive identification.

Application of steam onto the inoculated areas was made in the pilot plant for 30 sec at approximately 1 in. from the carcasses. Total plate counts and incidence of salmonellae as described earlier were recorded.

A second trial was conducted using acetic acid (pH 2.0) only varying the amount of time the acid was sprayed on pork carcasses, both inoculated with 10^6 *S. enteritidis* and uninoculated. One-half of all the carcasses were washed with water following treatment with the acid while the remaining carcasses received no washing. Time intervals of 30 and 60 sec were used for acetic acid application. Total bacterial numbers and incidence of salmonellae were determined.

Industrial studies

Of the treatments compared in pilot plant studies, acetic acid was selected to be used in a local slaughter house. After each pork carcass was inspected by meat inspection personnel, an area approximately 20 cm in diameter on the left ham was sprayed with acetic acid of varying concentrations of H^+ ions (pH) and the corresponding area on the right ham was used as control. The carcass was then placed in the chill room (7°C). After 100 carcasses had been sprayed each was swabbed in the area treated and in the identical area on the control side. All swab samples were kept in the chill room until transported to the laboratory in an insulated container with ice. After the samples arrived at the laboratory (about 2 hr later) they were immediately prepared for total plate counts and

Table 1—Effects of various treatments on pork carcasses inoculated with saline suspension of S. enteritidis in pilot plant

Treatment	No. of samples	Total aerobic plate count (Cm^2) After evisceration	Before treatment	After treatment	Incidence of Salmonellae after treatment
Acetic acid (pH 1.50)	6	2.3×10^3	2.7×10^6	3.3×10^2	1/6
Acetic acid (pH 2.00)	72	5.9×10^2	4.3×10^6	5.5×10^2	11/72
Stannous chloride (5%)	6	2.7×10^3	2.7×10^6	8.8×10^4	2/6
Hydrogen peroxide (5%)	6	2.7×10^3	2.7×10^6	1.3×10^4	2/6
Steam 10 sec (application 1 in. from carcass)	6	2.0×10^4	2.7×10^6	TFC[a]	0/6

[a]All areas sampled after 24 hr in cooler were TFC (too few to count).

incidence of salmonellae. 500 samples were collected over a period of 6 wk.

RESULTS & DISCUSSION

Pilot plant studies

All treatments used in the pilot plant studies showed significant reduction in total aerobic plate counts of microorganisms and incidence of salmonellae (Table 1). Both acetic acid treatments, pH 1.5 and pH 2.0, reduced the total aerobic plate count by 10^4 organisms; stannous chloride reduced total numbers by 10^2; hydrogen peroxide by 10^3 organisms; and steam effected greater than 10^5 reduction in total aerobic organisms counted. All areas tested were positive for salmonellae due to inoculation and the reduction in incidence ranged from two positive areas of the six treated with stannous chloride and hydrogen peroxide, one of six and 11 of 72 treated with acetic acid, pH 1.5 and pH 2.0, respectively, to zero incidence in those areas treated with steam. The reduction due to washing effect or runoff was not determined; however, these areas, when sampled 24 hr later, still showed the same results indicating that the effects were of a permanent nature. The test animals were slaughtered by students and consequently were subjected to unusually good care. This along with the sudden change in temperature to which the carcass was exposed when it passes into chill room, may account for some of the reduction in organisms after 24 hr. It is possible that if hog carcasses were subjected to better sanitation in the plant by a more thorough cleaning before passing into the chill room, salmonellae contamination and the overall bacterial population could be reduced to a minimal level.

While all treatments were relatively effective in controlling the salmonellae and the overall bacterial population, not all of the treatments were acceptable from the standpoint of appearance and effects on the carcass. Acetic acids, at various concentrations, left noticeable runoff marks on the skin of the carcass. However, this effect would be overcome by spraying the entire external surface of the carcass. Hydrogen peroxide (5%) on the other hand caused a marked discoloration of the skin. Steam, while it did not mar the carcass intially, caused a noticeable shrinkage of the skin, darkening of the blood vessels in the treated areas and a slight yellow discoloration 24 hr after treatment. Stannous chloride (5%) produced the least adverse effects on the appearance of the carcass and there was only a slight discoloration due to runoff from this chemical.

There seemed to be no advantage in spraying acetic acid on carcasses for 60 sec as compared to 30 sec (Table 2). Likewise washing carcasses subsequent to spraying did not seem to affect total plate counts; however, the incidence of *S. enteritidis* was higher on carcasses treated with acetic acid for 30 sec followed by water washing. There were no differences in those carcasses treated with acid for 60 sec. Treatment with acid whether followed by water wash or not resulted in lower plate counts. The incidence of *S. enteritidis* was lower in most cases upon sampling 24 hr after treatment. Although this trial was designed as a 2 × 2 × 3 factorial these results were not analyzed statistically because most values were too few to count (TFC).

Industrial studies

Table 3 lists the results obtained from treatments with acetic acid on 500 pork carcasses at a local meat processing plant. This acid, at relatively low pH, was effective in reducing the overall carcass contamination. At pH of 3.0 no reduction in total plate counts was noted whereas pH 2.5 gave an average of one log unit lower than the control and a pH of 2.0 showed a two-fold long reduction in contamination.

As may be seen from the data in Table 3, there was a decline in incidence of salmonellae in the pork carcasses used for controls from the first weeks of sampling. There was no apparent technical reason for this. All personnel and methodology in the plant remained essentially un-

Table 2–Total plate count[a] and incidence of salmonellae from areas on pork carcasses (naturally occurring microflora and inoculated with S. enteritidis) after treatment with acetic acid (pH 2.0) for 30 or 60 sec and left unwashed with water

		Naturally occuring microflora			Inoculated with *S. enteritidis*		
		Time of spraying with acetic acid (sec)			Time of spraying with acetic acid (sec)		
		0	30	60	0	30	60
Unwashed	A[b]	51.7×10^3 (0)[d]	TFC (0)	TFC (0)	38.5×10^3 (6/6)	TFC (1/6)	TFC (2/6)
	B[c]	48.6×10^2 (0)	TFC (0)	TFC (0)	25.2×10^2 (4/6)	TFC (0/6)	TFC (2/6)
Washed with water	A[b]	98.4×10^2 (1/6)	TFC (0/6)	TFC (0/6)	62.0×10^3 (6/6)	20.5×10^2 (6/6)	TFC (2/6)
	B[c]	22.5×10^2 (1/6)	TFC (0/6)	TFC (0/6)	79.1×10^2 (4/6)	TFC (2/6)	TFC (2/6)

[a]Each value in the table represents the average of six locations sampled on pork carcass.
[b]A–count taken 1 hr after treatment
[c]B–count taken 24 hr after treatment
[d]Number in parenthesis indicates incidence of *S. enteritidis* from each treatment (six areas per treatment).

Table 3–Total plate counts and incidence of salmonellae on 500 pork carcasses from a local processing plant after treatments of acetic acid of various concentrations.

Treatment		No. of samples	Avg. TPC Before treat.	Avg. TPC After treat.	Incidence of Salmonellae Before treat.	Incidence of Salmonellae After treat.	Reduction in TPC due to treatment	Reduction in Salmonellae due to treatment	Sampling day
1st wk	(pH 2.5)	40	1.29×10^6	1.63×10^5	22	15	0.79×10^1	7	Monday
2nd wk	(pH 2.5)	60	1.26×10^6	1.55×10^5	28	18	0.81×10^1	10	Monday
3rd wk	(pH 3.0)	100	6.44×10^5	9.14×10^5	6	3	0	3	Tuesday
4th wk	(pH 2.0)	100	1.58×10^6	1.48×10^4	0	0	1.06×10^2	0	Tuesday
5th wk	(pH 2.0)	100	2.62×10^5	1.51×10^3	0	0	1.73×10^2	0	Monday
6th wk	(pH 2.5)	100	4.60×10^5	3.97×10^4	7	3	1.15×10^1	4	Monday

Table 4—Salmonellae species isolated from 500 pork carcasses

Serotypes	Week of isolation	Frequency of isolation
S. heidelberg	1, 2, 3, 6	30/60
S. munster	1, 2, 6	10/60
S. indiana	1, 2, 3	8/60
S. derby	1, 2	4/60
S. anatum	1, 2	4/60
S. livingstone	1, 2	3/60
S. uzanamo	6	1/60

changed throughout the sampling period. One reason for this decline could be the particular season of the year or perhaps the day on which the samples were collected. The first 100 samples were collected on consecutive Mondays. The second 200 samples, collected in lots of 100 each, were obtained on consecutive Tuesdays. The final 200 samples were collected on Mondays again. There was no apparent relationship between the incidence of salmonellae and the day of sampling.

The season of the year seems to be critical for the isolation of salmonellae. There is apparently some factor(s), perhaps environmental, which occurs each year at this time causing a reduction of salmonellae in the herd before the animals arrive at the abattoir.

Industrial studies showed that applications of acetic acid applied to hog carcasses by spraying were effective in reducing salmonellae contamination and overall bacterial population. While a pH of 3.0 was found to be ineffective, a treatment of pH 2.5 showed an average drop of almost 1 log unit in overall contamination and a reduction in salmonellae to the extent of 33%. Although the effects of acetic acid pH 2.0 on salmonellae were not determined in the processing plant, the overall bacterial population on the carcass were reduced by 2 long units. Even though these treatments at the lower two concentrations caused discoloration of the skin and some darkening of the meat, it was felt that application of acetic acid (pH 2.0) subsequent to washing is both a practical and economical method of reducing microbial contamination of hog carcasses.

Table 4 lists the various species isolated from the 500 pork carcasses sampled from the processing plant. There was no relationship between week of isolation and the serotype isolated. Salmonellae were isolated in 1.2% of the samples taken, a lower incidence than has been reported by other authors (Galton et al., 1954; Shotts et al. 1961; Weissman and Carpenter, 1969).

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EFFECT OF CARCASS SUSPENSION METHOD ON SENSORY PANEL SCORES FOR SOME MAJOR BOVINE MUSCLES

INTRODUCTION

THE NORMAL METHOD of carcass suspension (from the Achilles tendon) allows major muscles of the carcass to shorten during the onset of rigor morits (Hostetler et al., 1972). Shortening of the muscle increases the possibility that the cooked meat will be less tender than if shortening had been prevented. Herring et al (1965) found that muscle shortening was reduced and the tenderness increased if the carcass side was laid on the table, bone side down, with the limbs perpendicular to the vertebrae. Using five methods of carcass suspension Hostetler et al. (1972) found that suspending the side from the obturator foramen was the most practical method of minimizing muscle shortening and thus significantly increasing post rigor sarcomere lengths and decreasing shear force values.

The improvement in tenderness manifested in decreased shear force values is of little value if persons eating the cooked meat cannot detect the improvement. The present investigation was designed to measure tenderness components of muscles from beef carcasses suspended by various methods as determined by a trained sensory panel.

EXPERIMENTAL

THE DESIGN of this experiment was described previously by Hostetler et al. (1972). Each side of 40 beef carcasses was suspended by one of five methods: conventional suspension of the pelvic limb (vertical); laying the side on a table, bone side down, with limbs perpendicular to the vertebrae (horizontal); suspending from cervical vertebrae with limbs tied together to bring the pelvic limb perpendicular to the vertebrae (neck-tied); suspending from the obturator foramen with limbs hanging free (hip-free); and, suspending from the obturator foramen with the limbs tied together to bring the thoracic limb perpendicular to the vertebral column (hip-tied).

Steaks, one from each of nine muscles from 80 sides, were placed in two groups. The first group included the longissimus (LG), semimembranosus (SM) and triceps brachii (TB). In the second group were the remaining six muscles: the semitendinosus (ST), adductor (AD), gluteus medius (GM), psoas major (PM), biceps femoris (BF) and rectus femoris (RF). The 240 randomized samples from group one were cooked, six in the morning and six in the afternoon over a period of 4 wk. The 480 randomized samples of group two were then cooked, either six or 12 per day over a period of 10 wk. Paired samples, i.e., muscle samples from the right and left sides of an animal but having had different treatments, were not compared at a single panel session. In this study, samples from paired muscles may have been tested from a few days to several months apart.

A 7.5 × 5.0 × 2.5 cm sample cut from each steak was heated uncovered for 46 min to approximately 70°C in a 175°C oven. After the cores for shear determinations had been taken from the ends, parallel to the fibers (Hostetler et al., 1972), six samples approximately 2 × 2 × 0.4 cm were cut from the remaining center portion and randomly assigned to six judges.

The six panel members (four male, two female) were trained over a period of 2 wk to recognize and score components of tenderness on a nine-point scale as described by Cover et al. (1962). Two members of the panel had at least 10 yr experience on this type of panel. The same six panel members were present for all sessions during the study. The members ranged in age from 20 to 57 yr.

The sensory analysis included: (1) Juiciness–a first impression; (2) Softness to tongue and cheek–determined by feeling the meat primarily with the tongue immediately after placing in the mouth; (3) Softness to tooth pressure–judged by the force needed to sink the teeth into the meat during the first several chews; (4) Ease of fragmentation of muscle fibers–scored on how easily the fibers fragmented as chewing proceeded; (5) Mealiness–a measure of the presence in the meat sample of tiny, dry meat fragments that cling to the tongue and gums; (6) Apparent adhesion between muscle fibers–judged by the ease with which muscle fibers separated; (7) Amount and hardness of connective tissues–scored after sufficient chewing had taken place to remove the muscle fibers from the sample being tested (Cover et al., 1962).

Arithmetic means of the panel scores for each steak for juiciness and the seven components of tenderness were used in the analysis of variance for each of the components. A standard, balanced incomplete block design (Cochran and Cox, 1957) requiring a minimum of four replications for tests of significance was used. Each replication utilized five blocks (carcasses). Use of eight replications, i.e., double the minimum design, increased the statistical precision. The design provided treatment comparisons that were independent of effects common to sides from the same carcass. Two sources of variation were included in the error: variation among the different muscles from the same side and thus the same treatment and variation between the same muscles from different sides given the same treatment. A posteriori tests of differences among treatments were made on a within-muscle basis using the Student-Newman-Keuls procedure and a significance level of 0.05 (Sokal and Rahlf, 1969). Shear force and sarcomere length determinations made on the same steaks were previously reported by Hostetler et al. (1972).

Table 1—Analysis of variance for sensory panel scores

Source of variation	df	Sensory Parameters							
		Juiciness	Tongue & cheek	Tooth pressure	Ease of fragmentation	Mealiness	Adhesion	Amount of connective tissue	Softness of connective tissue
Animal	39	.58*	.84*	2.75*	3.27*	1.89*	3.17*	1.99*	3.15*
Treatment	4	.24	.45	2.59*	4.65*	3.78*	4.94*	1.50*	2.25*
Muscle	8	14.25*	11.91*	22.45*	15.80*	12.14*	14.05*	143.09*	226.81*
Tr. × Mus. Int.	32	.55*	.36*	1.64*	2.38*	1.56*	2.71*	.46	.71
Error	636	.31	.24	.35	.37	.33	.40	.58	.92

*Significant at P< 0.05.

RESULTS & DISCUSSION

THE ANALYSES of variance (Table 1) indicated significant differences among animals for all sensory panel scores. Hostetler et al. (1972) also found significant animal variation in sarcomere lengths and shear force values. The correlated effect of animals on sarcomere length and sensory scores after adjustment for muscle-treatment differences was never greater than 0.38 for any of the components of tenderness. Thus knowledge of a mean sarcomere length for an animal would not give a good prediction of the tenderness of the animal as indicated by sensory scores. The low correlation indicates that at any given sarcomere length it is possible to have a range in tenderness. On the other hand, the correlated effect of animals on shear force versus sensory scores was 0.80 or greater for softness to tongue and cheek, softness to tooth pressure, ease of fragmentation mealiness and apparent adhesion. These values are similar to within muscle correlations for LG obtained between shear and these same sensory scores in earlier work (Cover et al., 1962).

The analyses also indicated differences among muscles (Table 1) for all sensory scores. This factor is overlooked by some investigators who make generalizations based on only one or two muscles. As suggested by Locker (1960) variation in contraction state may account for some of the differences between muscles but at the same time a large part of the differences between muscles is inherent in the individual muscle structure. There seem to be differences between muscles that are not dependent upon sarcomere length or connective tissue content and its distribution. Research workers, ignoring both the more obvious differences between muscles as well as this inherent difference, have been tempted to run simple correlations using data from muscles selected for their differences in tenderness. Thus, they cannot partition the relationship between sarcomere length and tenderness from the inherent effects of specific muscles on sarcomere length and tenderness.

Treatments produced differences (Table 1) detected by the sensory panel for all scores except for juiciness and softness to tongue and cheek. The correlated effects of treatments on sarcomere length and sensory scores after adjustments for animal-muscle differences were greater than 0.80, except for juiciness, mealiness and connective tissue scores for amount and softness. The similar correlation for sarcomere length and shear force was −0.64. The Warner-Bratzler shear cannot distinguish between connective tissue and muscle fibers while panel members should be able to do this. Thus, in muscles with large amounts of connective tissue the panel members could observe changes in tenderness of muscle fibers while these same changes in the tenderness of the muscle fibers as measured by the Warner-Bratzler shear would be obscured by the connective tissue.

The interaction between muscles and treatments was significant ($P < 0.05$) (Table 1) for all panel scores except the two connective tissue scores. As was stated in the previous paper, the interaction between treatments and muscles to change in sarcomere length was expected (Hostetler et al., 1972). If sarcomere length and tenderness are related, then the inteaction between muscles and treatments would be expected for these sensory measures of tenderness also.

It must be remembered that the interaction under discussion is not a measure of the interaction between change in tenderness and change in sarcomere length of muscles for the different measures of tenderness. This interaction was not measured. Some indication of this interaction is given in a later paragraph discussing differences in muscle response to the experimental methods of suspending carcasses.

The panel did not detect any effect of the experimental treatments on juiciness scores. Differences in juiciness of muscles remained constant for all treatments. The most juicy muscles were the TB, GM and BF and the least juicy, the AD, ST and SM muscles.

Scores for softness to tongue and cheek are dependent upon the water content of the meat (Ritchey and Hostetler, 1964). There was no relationship between juiciness and scores for softness to tongue and cheek across treatments since treatments had no effect on either score, but there was a close relationship between juiciness and tongue and cheek scores across both muscles (r=0.89) and animals (r=0.69).

The judgment of softness to tooth pressure is made soon after chewing is begun. Both the tenderness of the muscle fibers and the amount and softness of the connective tissue have an influence on this score. The panel generally gave lower scores for softness to tooth pressure to five muscles (SM, ST, BF, AD and TB) with the most connective tissue. At first, the connective tissue may have given an impression of firmness to the meat. However, after chewing, the panel found that muscle fibers in three of these muscles (SM, BF and AD) broke up easily and scores for ease of fragmentation, mealiness and apparent adhesion for these muscles were comparable to those for muscles with little connective tissue (higher scores).

The panel detected improvements in tenderness by the experimental methods of suspension for ease of fragmentation scores of the LG, SM, ST, AD and GM muscles (Fig. 1b) and in scores for softness to tooth pressure, mealiness and apparent adhesion (Fig. 1a and 1b) of the LG, SM, ST and GM muscles for this group of animals. When the experimental methods were compared with the vertical method the hip-free and hip-tied methods differed more frequently from the vertical method than the horizontal or neck-tied methods of suspension. The panel could not detect any difference in tenderness between the experimental methods and the conventional method of suspension for the ST and BF muscles in spite of the significant increase in sarcomere length produced by several methods for both muscles. A decrease in tenderness was detected by the panel for several of the experimental methods of suspension for the PM and TB muscles. It is clear however that the PM muscle remained highly acceptable and the hip-free method of suspension did not significantly decrease the tenderness of the TB muscle.

In this group of animals, certain muscles responded more to the experimental methods than did other muscles. In general the muscles with the least connective tissue (higher scores) (LG, RF, GM and PM) showed more response than did the muscles with the most connective tissue (lower scores) (ST, BF and AD) except for the SM and TB muscles. Thus while the panel generally may have been more sensitive to changes in the tenderness of muscle fibers produced by the experimental methods than the Warner-Bratzler shear, there were some rather notable exceptions, unless perhaps there was no improvement in the tenderness of muscle fibers of the ST, BF and AD muscles.

The ST muscle has been used in several sarcomere length-tenderness studies because of its shape and muscle fiber orientation. These data indicate that the ST muscle may not be suitable for muscle fiber studies since it did not respond well to increases in sarcomere length brought about by the experimental methods. With a sarcomere length of 3.3μ in the hip-tied method one would have expected higher tenderness scores, but it remained one of the least tender of the muscles studied. Apparently any improvement in tenderness brought about by increasing the sarcomere length of the ST muscle was masked by other factors, possibly connective tissue.

It is felt that randomizing the samples for the sensory panel provided a rigorous test of the change in tenderness produced by the experimental methods. In spite of the randomization the panel scored samples from muscles with the longest sarcomeres more tender compared with samples from the same muscles from the opposite side with shorter sarcomeres. A detectable difference in tenderness in a paired comparison test would justify the adoption of one of the experimental sus-

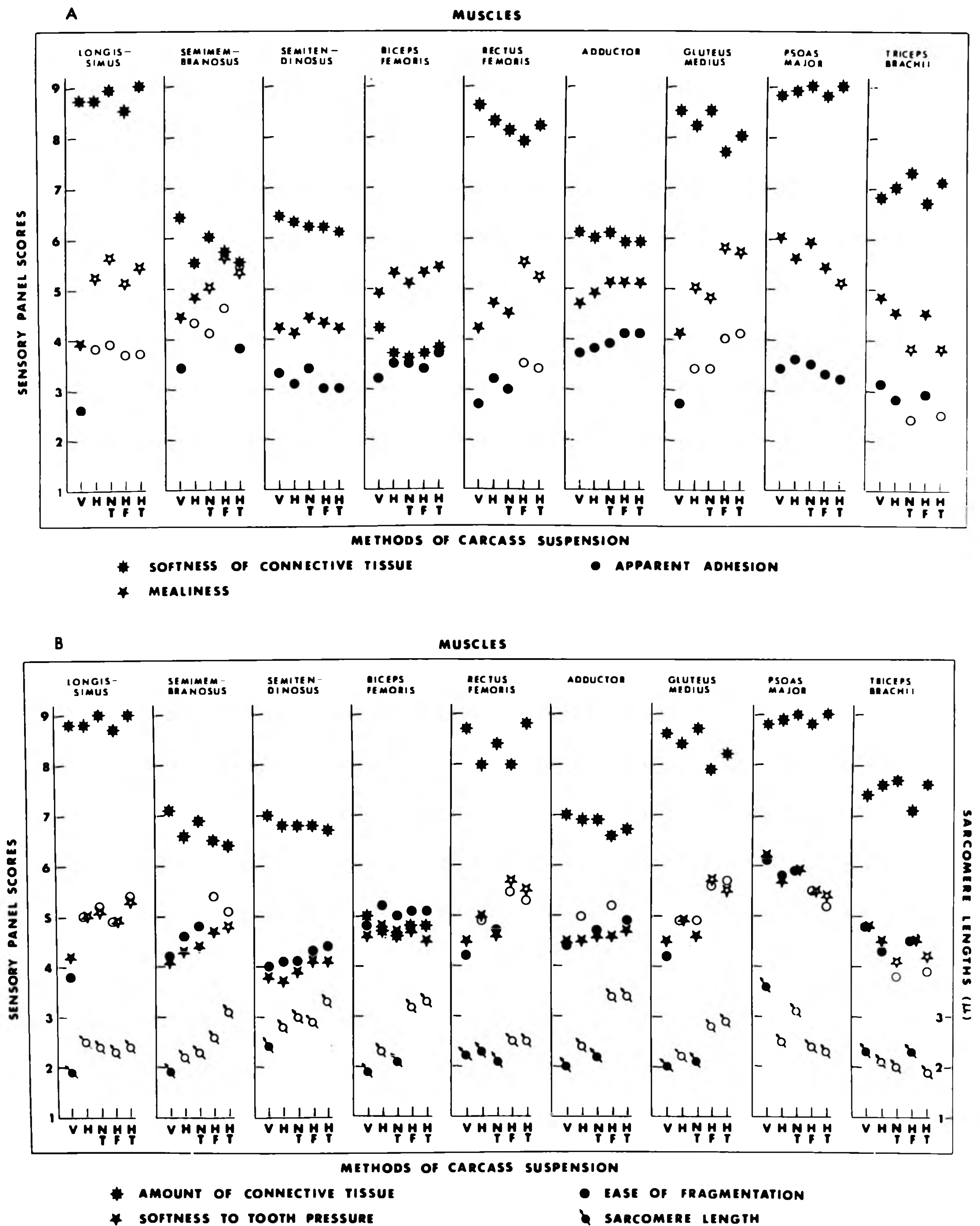

Fig. 1—Plot of sarcomere lengths and sensory panel scores (1=least desirable, 9=most desirable) of nine muscles for five methods of carcass suspension (V=vertical; H=horizontal; NT=neck-tied; HF=hip-free; HT=hip-tied). Open symbols indicate sensory panel values and sarcomere lengths of experimental methods of carcass suspension which are significantly different from the vertical method ($P < 0.05$).

pension methods. Since improvement in tenderness was obvious to the panel under conditions less favorable for detecting differences, it makes the use of a method such as suspension from the obturator foramen (hip-free), which is relatively easy to do, even more strongly justified.

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UTILIZATION OF BEEF AND PORK SKIN HYDROLYZATES AS A BINDER OR EXTENDER IN SAUSAGE EMULSIONS

INTRODUCTION

THE POSSIBILITY of utilizing collagen, found in great abundance in beef hide, has been discussed by Elias et al. (1970). They indicated that collagen fibers and granules could be isolated from beef hide and used as a possible binder/extender in meat products. Collagen fibers and granules may have a possible use in certain fabricated meat items, but could be detrimental when incorporated into emulsified meat products. An earlier study has shown that collagen, the major protein of skin, bone and connective tissue, was detrimental to the emulsifying capacity of poultry meat (Maurer and Baker, 1966). The inability of collagen to emulsify fat and its ability to convert to gelatin upon cooking make it an undesirable ingredient in sausage formulations.

Another property of collagen, its nutritional value, should be discussed when collagen is to be considered as a food additive. Collagen is known to be deficient in the essential amino acid tryptophan and limiting in other essential amino acids such as lysine, threonine and methionine. However, it has been shown (Ashley and Fisher, 1966) that chicks fed on a diet of 10% gelatin + 3% casein had the bodyweight gains equal to those fed on a diet of 13% soy protein + 0.2% methionine. Erbersdobler et al. (1970), using male rats as the experimental animals, showed when collagen or gelatin was incorporated into the diet at levels up to 5% of the total diet weight, there were slight improvements in daily gain and feed conversion. Therefore, collagen will not lower the nutritional quality, if used along with a balanced protein and maintained at a low level in the diet, such as would be the case when a collagen hydrolyzate is used as a binder or extender in meat emulsions.

Collagen in its native state is resistant to the proteolytic action of most enzymes, but when heated the resulting gelatin is easily enzyme degraded. Hydrolysis of a protein by means of an enzyme will also change the physical characteristics of the protein. Therefore, the present study was undertaken to investigate the possible use of enzyme hydrolyzed skin collagen as a binder or extender in emulsified meat products.

METHODS & MATERIALS

Dehairing beef skin

The beef skins used in this study were obtained from a local packing plant either in the fresh or the salted form. Prior to dehairing, the skins were extensively washed with water to remove any foreign material and salt. The washed skin (100g) was then limed overnight in 300 ml of distilled water containing 9.0g of $Ca(OH)_2$.

The procedure for enzymatic dehairing consisted of washing the limed skin to remove all excess $Ca(OH)_2$ and then placing the skin in another solution containing 300 ml water, 6g $(NH_4)_2SO_4$, 3g crude pancreatin. The skin was then incubated in this solution for 21 hr at 33°C, after which the hair was easily scraped off with a spatula.

After dehairing the skin was ground and then homogenized for 3 min in a Waring Blendor with an equal weight of distilled water. The slurry was then centrifuged at 7000G. The residue was resuspended in an equal weight of distilled water and the washing procedure was repeated two more times.

Defatting pork skin

The pork skins used were obtained frozen from a local packing plant. The thawed skin was cut into 0.5 cm strips and soaked in distilled water overnight. The skin was defatted using the following steps:

1. 100g of soaked skin was ground and then blended with 130 ml of methanol in a Waring Blendor for 5 min.
2. 65 ml chloroform was added and again blended for 5 min. This step was then repeated.
3. 65 ml of distilled water was added and the slurry was blended for 10 sec.
4. All solvents were removed from the pulp by suction filtration.
5. The pulp was repeatedly washed with distilled water until all organic solvents had been removed.

Enzymatic hydrolysis

The washed pulp from either beef or pork skin (50g pulp) was resuspended in 1 liter of distilled water and autoclaved at 15 lb pressure for 60 min. After cooling the autoclaved solution to 37°C, 1 liter of distilled water and a predetermined amount of enzyme were added. The pH of the suspension was adjusted to the desired value and maintained at that pH throughout a 12-hr incubation at 37°C. 500 mg of crude pancreatin (Viobin Corp., Monticello, Ill.) and a pH of 8.0; or 500 mg of crude papain (Type II, Sigma Chemical Company, St. Louis) and a pH of 7.5; or 30 mg of crystallized pure pepsin (Sigma Chemical Co.) and a pH of 2.0 was used for the enzymatic hydrolysis of the autoclaved solution.

The peptide content of the various hydrolyzates was observed qualitatively using thin layer chromatography on silica gel G with a solvent system of methanol-chloroform-ammonium hydroxide (4-4-1).

After enzymatic hydrolysis, the resulting solution was dried to a powder using a rotary evaporator and a 60°C water bath. The resulting powders will be referred to as the skin hydrolyzates.

Emulsion capacity

The emulsion capacities of the hydrolyzates, meat and nonfat dry milk (NFDM) were determined using the microemulsifier as described by Tsai et al. (1970) and a modification of the emulsion capacity procedure as described by Carpenter and Saffle (1964). The modifications introduced into the procedure were (1) 10 ml of oil was initially blended with the protein solution just prior to beginning the run, instead of the 25 ml as described in the original procedure; and (2) the blender speed was 5,500 rpm, instead of 13,140 rpm as described.

Emulsion stability

The emulsion stability test, as described by Townsend et al. (1968), was used in this study. The basic bologna sausage formulation used contained the following ingredients: 51g lean beef (20.1% protein), 40g pork (14.4% protein), 22 ml water, 2.3g nonfat dry milk (33.5% protein), 2.3g NaCl, 0.4g cure mix and 0.32g dextrose.

The shear strengths of the sausages were also determined using a L.E.E. Kramer shear press with the 114 kg compression ring and 10 blade knife.

Protein analyses

The protein or peptide analyses of the lean beef, NFDM and hydrolyzates were determined using the standard AOAC methods (1965). The factor used to determine the peptide content of the collagen hydrolyzates was 5.55, since collagen contains 18% nitrogen in its structure (Pikkarainen, 1968).

Statistical analysis

Statistical analysis for significant differences between sample means was determined at the 95% confidence interval using the Student t test as described by Steele and Torrie (1960).

Table 1—Peptide content of the various skin hydrolyzates

Hydrolyzate	Peptide content (%)
Beef skin:	
pancreatic digest	89.4
Pork skin:	
pancreatic digest	91.0
pepsin digest	88.0
papain digest	91.1

Table 2—Emulsion capacity of the various skin hydrolyzates, NFDM and beef muscle

Materials tested	Emulsion capacity[a] ml oil 100 mg proteinaceous material or protein	ml oil/g sample
Beef skin:		
pancreatin digest	9.2(.24)[c]	78.3(2.61)[c]
Pork skin:		
pancreatin digest	10.6(.26)[c]	96.7(2.32)[c]
pepsin digest	12.1(.37)[b]	101.5(2.94)[c]
papain digest	8.5(.19)[c]	77.0(2.82)[c]
NFDM (33.5% protein)	12.0(.49)[b]	40.2(1.71)[b]
Lean beef (20.1% protein)	23.5(1.47)	47.2(2.21)

[a]Results reported are the averages of triplicate analyses. The standard deviation is also shown in parentheses.

[b, c]Sample means having a superscript c are significantly different from the NFDM sample at the 95% confidence level.

Table 3—Emulsion stability and shear strength of sausages made by replacing NFDM with the various hydrolyzates

Skin hydrolyzate used to replace the NFDM	Emulsion stability[a] (ml released/10g emulsion) Fat	Water + Suspended solids	Shear strength[a] (kg force/g cooked sausage)
Beef skin:			
pancreatin digest	0.3(.12)[b]	2.0(.17)[b]	0.90(.10)[b]
Pork skin:			
pancreatin digest	0.5(.14)[c]	1.7(.30)[b]	0.84(.13)[b]
pepsin digest	0.4(.16)[c]	2.0(.29)[b]	0.76(.16)[b]
papain digest	ND[d]	ND[d]	ND[d]
Control:			
NFDM not replaced	0.6[e](.15)[c]	2.4[e](.12)[c]	1.17[e](.11)[c]

[a]Results reported are the averages of triplicate analyses, with each analysis composed of 3 sausages. Standard deviation also shown in parentheses.

[b, c]Sample means having the superscript b are significantly different from the control sausage at the 95% confidence level.

[d]ND—these values could not be determined.

[e]Results reported are the average of 12 analyses, with each analysis composed of 3 sausages. Standard deviation is given in parentheses.

RESULTS & DISCUSSION

KJELDAHL ANALYSIS of each of the skin hydrolyzates indicates that the nitrogen content is quite high in each hydrolyzate. The nitrogen content of each hydrolyzate is reported in Table 1 as peptide content, since each hydrolyzate is no longer protein but is a mixture of peptides as evidenced by thin layer chromatographic analysis. Therefore, for convenience, the term peptide content will be used as an index of the nitrogen content of the hydrolyzates. Table 1 indicates each hydrolyzate is high in peptidaceous material, ranging from 88–91%.

Other properties of the various skin hydrolyzates are: (1) tannish-white color; (2) bland flavor; (3) high solubility in water; (4) hygroscopic; and (5) when in solution, will not gel when cooled to 5°C. The emulsion capacities of the various hydrolyzates were compared to the capacities of lean beef and NFDM (Table 2). The emulsifying capacity of the hydrolyzates, when compared on a protein or peptide basis, is lower than the capacity of lean beef and equal to or only slightly lower than the emulsion capacity of NFDM. Since the hydrolyzates will be used to replace NFDM on a gram-for-gram basis, the emulsion capacities were also compared on a per g of material basis. Because of the higher peptide content of the hydrolyzates, when compared to protein content of NFDM, the emulsion capacity of 1 g of hydrolyzate was much greater than that of 1 g of NFDM.

Table 3 gives the data describing the emulsion stability and shear strength of sausages where the NFDM was replaced with the various hydrolyzates. The pancreatin and pepsin of pork skin and the pancreatin digest of beef skin, when used to replace the NFDM in a sausage formulation, yielded a sausage with a comparable or slightly better ability to retain fat and water + suspended solids, when compared to the control. The shear strength of these sausages was lower and this was possibly due to their greater fat and water content. The papain digest of pork skin, when used to replace the NFDM, produced a sausage with only a semisolid (mushy) texture. Because these sausages could not be handled without being damaged, data on the fat and water + suspended solids holding ability could not be determined. The mushy texture was possibly due to the presence of the undenatured papain in the skin hydrolyzate, which hydrolyzed the meat protein during the cooking of the sausage.

No alteration in color or evidence of off-flavors was detected by two of the authors in any of the sausages which contained the skin hydrolyzates.

CONCLUSIONS

IN CONCLUSION, hydrolyzates of beef or pork skin, when used to replace NFDM in a sausage formulation, will produce a sausage with a slightly better water- and fat-holding ability. Even though the emulsion capacity of the various skin hydrolyzates is slightly lower than the capacity of NFDM (on a per 100 mg protein basis), the greater protein content of the hydrolyzates gave the sausage emulsion improved stability during cooking, when compared to an emulsion containing NFDM.

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EFFECT OF COMPOSITION ON THE STABILITY OF SAUSAGE-TYPE EMULSIONS

INTRODUCTION

THE COMPOSITION of sausage emulsions, especially as relates to the relative amounts of continuous and disperse phases, is one of several factors affecting the stability of sausage emulsions. Saffle (1968) summarized the research literature on meat emulsions and stated that a straight-line relationship was found between protein concentration and emulsifying capacity until the system became overloaded. Morrison et al. (1971) reported that emulsion stability was highly dependent upon the level of added water. These workers also suggested that greater emulsion stability could be theoretically obtained by either removing some of the protein from the oil-water interface, or substituting a more efficient for a less efficient protein.

Several methods have been reported for evaluating sausage emulsions. The cook stability (Townsend et al., 1968), emulsifying capacity (Webb et al., 1970) and the subjective evaluation of physical properties (Morrison et al., 1971) are the basic tests which are presently considered to be relatively good methods for evaluating emulsions. Electrical conductivity and viscosity have been studied as possible methods for the evaluation of emulsions (Webb et al., 1970; Acton and Saffle, 1970; Morrison et al., 1971; Townsend et al., 1971). Emulsions with higher phase volume ratios show lower conductivities and higher viscosities (Becher, 1966; Acton and Saffle, 1970; Morrison et al., 1971).

Haq (1971) and Haq et al. (1972) reported that AC resistance was a better index of sausage emulsion stability than DC resistance or viscosity when cook stability and physical properties were used as indices of stability.

This investigation was undertaken to study the effect of selected compositions (Table 1) on emulsion stability as measured by cook stability, physical properties, DC resistance, AC resistivity and viscosity of sausage-type emulsions.

EXPERIMENTAL

Sample preparation

Sampling procedures and formula computations were carried out as described by Haq (1971) and Haq et al. (1972). The beef muscle tissue samples were taken from post-rigor beef top rounds (*semimembranosus*), trimmed free of surface fat and connective tissue, ground, mixed, divided in treatment lots and frozen until used. All experiments were conducted on a homogeneous sample prepared at the onset of the investigation. Moisture, protein and fat analyses were conducted on the beef samples as described by AOAC (1965) in order to establish accurate compositions for the experimental formulas.

Emulsion preparation

The prototype emulsion preparation system of Haq et al. (1972) was used to prepare 160-g batches of sausage-type emulsions of the compositions shown in Table 1. This system allowed for the control of chopping time and speed. Also, the sequence of ingredient addition was closely controlled as specified in the previously published procedure. This design and operation of this system resulted in the preparation of emulsions similar to commercial-type sausage emulsions. The lipid source was soybean oil for all emulsion preparations. The salt level was computed as 3% of the muscle tissue and added water.

Emulsion evaluation

All evaluations were made after the emulsion had been completed. The emulsion was evaluated for DC resistance, AC resistance and viscosity in the chopping bowl by first smoothing to remove voids and air pockets. The temperature sensing probe was attached to the inside of the wall of the Haq et al. (1972) prototype chopping bowl in order to determine and maintain control of the emulsion temperature. DC and AC resistances and viscosity measurements were made at two different temperatures within the range 18–21°C.

DC resistance. DC resistance of the emulsions was measured in kilohms on a triplet VOM meter (The Triplet Electrical Instrument Company, Bluffton, Ohio) by contact with plate electrodes as described by Haq et al. (1972).

AC resistance. AC resistance was measured in ohms at 1,000 Hz using a Y.S.I. (model 31) conductivity bridge (Yellow Springs Instrument Co., Yellow Springs, Ohio) connected to the plate electrodes.

Viscosity. All viscosity measurements were made on the emulsions with a Brookfield Synchro-lectric Viscometer (model RVF) using spindle #7 at 10 rpm. The viscosity reading was noted after 40 sec shearing time and recorded in centipoises.

The DC resistance, AC resistance and viscosity data were plotted against the temperature data and adjusted to a standard of 20°C. The AC resistivity in ohm-cm was then determined from the relationship

$$\text{Resistivity} = \frac{\text{measured resistance in ohm}}{\text{cell constant}} \text{ ohm-cm}$$

Cell constant for the plate electrodes, mounted in the chopping bowl (Haq et al., 1972), was determined using a standard 0.01 demal KCl solution.

Cook stability and physical properties. The cook stabilities and physical property ratings for the cooked emulsions were measured as described by Townsend et al. (1968) and Haq et al. (1972), respectively.

The data were statistically analyzed as described by Snedecor (1968).

RESULTS & DISCUSSIONS

MEAN VALUES for cook stabilities of the emulsions prepared with different lipid levels at two meat:water ratios (1.5:1.0 and 2.0:1.0) are presented in Figure 1.

Table 1—Composition formulae for sausage-type emulsions used to evaluate stability

	Meat:water ratio (1.5:1.0)		Meat:water ratio (2.0:1.0)	
Total lipids, %[a]	Water and/or ice, %	Beef muscle tissue, %[b]	Water and/or ice, %	Beef muscle tissue, %[b]
15.00	34.00	51.00	28.33	56.66
25.00	30.00	45.00	25.00	50.00
50.00	20.00	30.00	16.67	33.33
65.00	14.00	21.00	11.67	23.33
70.00	12.00	18.00	10.00	20.00
75.00	10.00	15.00	8.33	16.66

[a]Included beef tissue fat and soybean oil as calculated by Haq et al. (1972)

[b]Proximate composition: moisture = 76.01%; protein = 20.74%; fat = 2.1%

For both of the meat:water ratios, the cook stability of the emulsions was found to increase significantly ($P < 0.05$) as the lipid level was increased from 15% to 50%. That is, the 15, 25 and 50% lipid levels were each significantly different at the 0.05 level. The lipid level in commercial sausage emulsions is generally in the 25–30% range. At the 25% lipid level, the present study indicated that the emulsions yielded a slightly lower cook stability than at higher lipid levels. The emulsions were found to be most stable at the 50% lipid level, confirming the studies of Sutheim (1947). The cook stability of the emulsions with 70% lipids was higher than for those having 75%.

It was observed that more water was released at 15% lipid level than at the 25% lipid level but no oil was released at either lipid level (Table 2).

Extremely high levels of oil were released at the 75% lipid level, while no measurable water was lost. On the basis of previous work by Ivey et al. (1970) and Morrison et al. (1971), it was concluded that the continuous phase (protein-water) was more efficient up to the point that the emulsifying agent (protein) was extended to its limit of maintaining the emulsion system. It would appear that the system of this study exhausted the emulsifying capacity of the protein between 70-75% lipid levels.

The physical properties scores, as evaluated by the method of Haq et al. (1972), on the cooked emulsions with 15% lipid for both meat:water ratios were significantly ($P < 0.05$) lower than the scores for emulsions containing 25% lipid (Fig. 2). For the emulsions with lipids from 25% to 65%, the physical properties scores remained unchanged and were at a maximum. The 70% lipid level emulsions with a meat:water ratio of 2.0:1.0 yielded cooked samples which were slightly soft and the physical properties scores were, therefore, slightly lower than for emulsions prepared by use of a 1.5:1.0 meat:water ratio. At 75% lipid level, the cooked emulsions had extremely poor physical properties for both meat:water ratios which were significantly ($P < 0.01$) lower than the physical properties for the other emulsions.

The cook stability data (Fig. 1) and physical properties data by sensory evaluation (Fig. 2) were in close agreement and were used to evaluate DC resistance, AC resistivity and viscosity measurements.

Data for DC resistance terminal point measurements on emulsions with different lipid levels and two meat:water ratios are presented in Figure 3.

The DC resistance measurements on the emulsions with 15% lipids were highly variable (s.d. = ± 6.85) and did not differ significantly ($P < 0.05$) from the DC resistance measurements on the emulsions

Table 2–Water and oil pour-off measurements from cook stability on sausage-type emulsions[a]

	Meat:water ratio (1.5:1.0)		Meat:water ratio (2.0:1.0)	
Lipid level, %	Water pour-off, %	Oil pour-off, %	Water pour-off, %	Oil pour-off, %
15	6.90 ± 2.7	0.00 ± 0.0	5.20 ± 2.5	0.00 ± 0.0
25	3.13 ± 1.6	0.00 ± 0.0	1.43 ± 0.7	0.00 ± 0.0
75	0.00 ± 0.0	41.33 ± 4.1	0.00 ± 0.0	55.77 ± 8.5

[a]Percent water and oil pour-off was determined after each cook stability test using a graduated cylinder to measure the amount of water and oil pour-off which was converted to percent based on a 30-g sample.

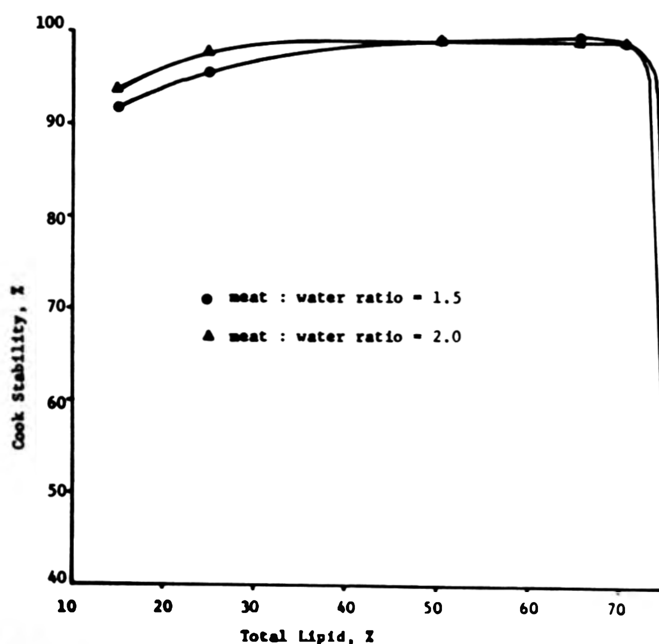

Fig. 1–Effect of percent total lipids on cook stability of sausage-type emulsions.

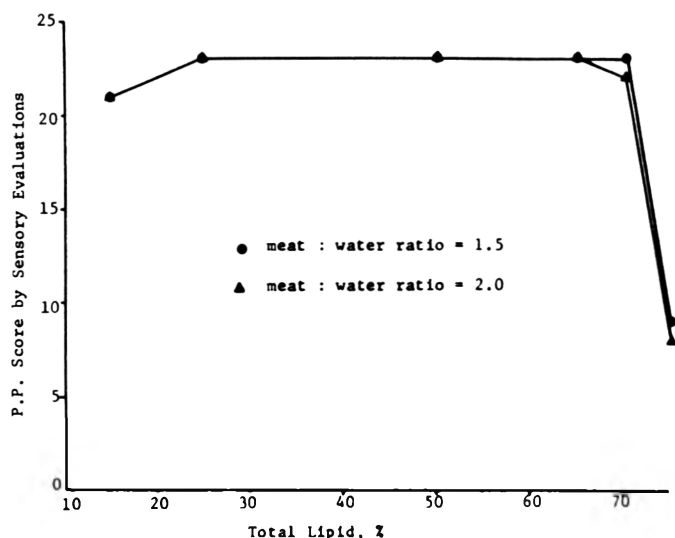

Fig. 2–Effect of percent total lipids on physical properties scored by sensory evaluation on cooked sausage-type emulsions.

with 25% lipids. At 25%, 50% and 65% lipid levels the DC resistance measurements did not differ significantly ($P < 0.05$) from each other. At 75% lipid level the emulsions were partially inverted and the oil-in-water system collapsed (Table 2 and Fig. 1) for both meat:water ratios. Since lipid is a nonconductor, the DC resistance increased sharply when the emulsions inverted and indicated the point of emulsion breakdown. However, on the basis of these results, the DC resistance measurements did not agree closely enough with the cook stability and physical properties data to be considered as a good indicator of emulsion stability in highly viscous emulsions. These results do not agree precisely with those reported for highly dilute emulsion systems as reported by Webb et al. (1970). This difference was attributed to the movement of free ions in dilute, agitated systems such as Webb et al. (1970) studied as opposed to more concentrated systems such as the sausage emulsions evaluated in this study.

The AC resistivity data for the two types of emulsions were plotted against the lipid level as illustrated in Figure 4. A visual comparison of the AC resistivity data with the cook stability values (Fig. 1) and the physical properties scores (Fig. 2) indicated that the three parameters are related and are in relatively close agreement as indicators of emulsion stability. The AC resistivity data indicated a more rapid decline and earlier indication of instability at the higher lipid levels than found by the cook stability and physical properties methods.

The AC resistivity of the emulsions with meat:water ratios 1.5:1.0 and 2.0:1.0 did not differ significantly ($P < 0.05$) from each other for the lipid levels 25%, 50%, 65% and 70%. At each of these lipid levels the amount of the continuous phase (muscle tissue and added water) was the same for each meat:water ratio. The resistivity of the emulsions was found to increase almost linearly ($r = 0.892$) with an increase in the lipid level in the range 25–65%. To increase the amount of lipids (nonconducting phase), the amount of the continuous phase (conducting phase) was reduced proportionally. This reduction in the amount of the continuous phase appeared to cause an increase in the resistivity. This was in agreement with the work of Becher (1966). Thus, the resistivity was believed to depend upon the amount of continuous phase.

The emulsion system was apparently more densely packed with dispersed particles at the 70% lipid level than at lower lipid levels. Apparently this was near the emulsifying capacity of the continuous phase and the dispersed particles had started to coalesce. This was reflected by a marked increase in the resistivity and a slightly unstable emulsion. The resistivity pattern could not be readily duplicated due to the instability of the emulsion system. When the lipid level was increased to 75%, the emulsion collapsed and the disperse phase no longer existed due to coalescence of the lipid particles. Lipid being a nonconductor, the resistivity of the inverted emulsion increased sharply. The results indicated that a relationship can be established between AC resistivity (or resistance) and emulsion stability.

The viscosity data are presented in Figure 5 for the various lipid levels and the two meat:water ratios. The expected trend in the figure was derived from data presented by Becher (1966). The measured viscosities at the 15% lipid level were very different from the expected trend reported by Becher (1966). The curves in the range of 25–65% lipid levels for both meat:water ratios indicated a sharp increase in the viscosity of the emulsions. The emulsions with 65% lipids showed the highest viscosity reading. Apparently the increase in dispersed particles (oil phase) of the emulsions resulted in an increase in viscosity. It was observed that the emulsions at the 70% lipid level had an oily feel. Although no oil was released when the emulsions were cooked (Table 2), it was not completely stabilized at the 70% lipid level. These emulsions showed slightly lower viscosity readings at the 70% lipid level. This would indicate that

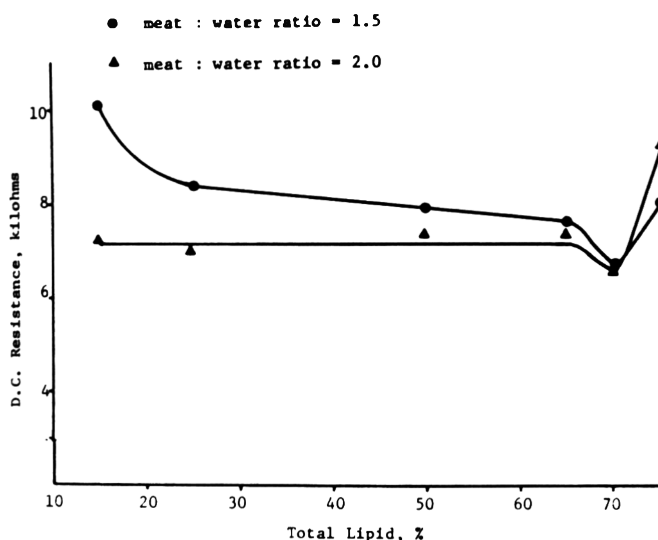

Fig. 3—Effect of total lipids on DC resistance of sausage-type emulsions.

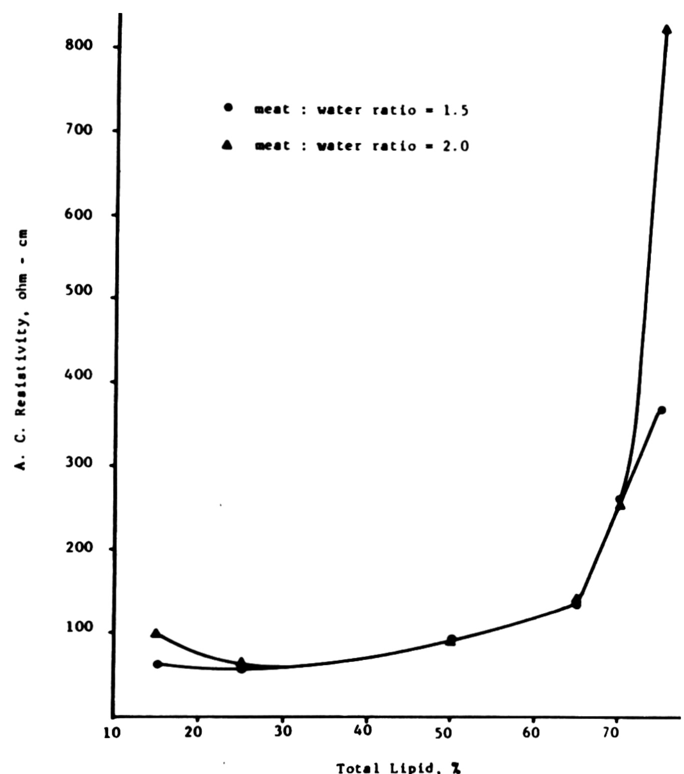

Fig. 4—Effect of percent total lipids on AC resistivity of sausage-type emulsions.

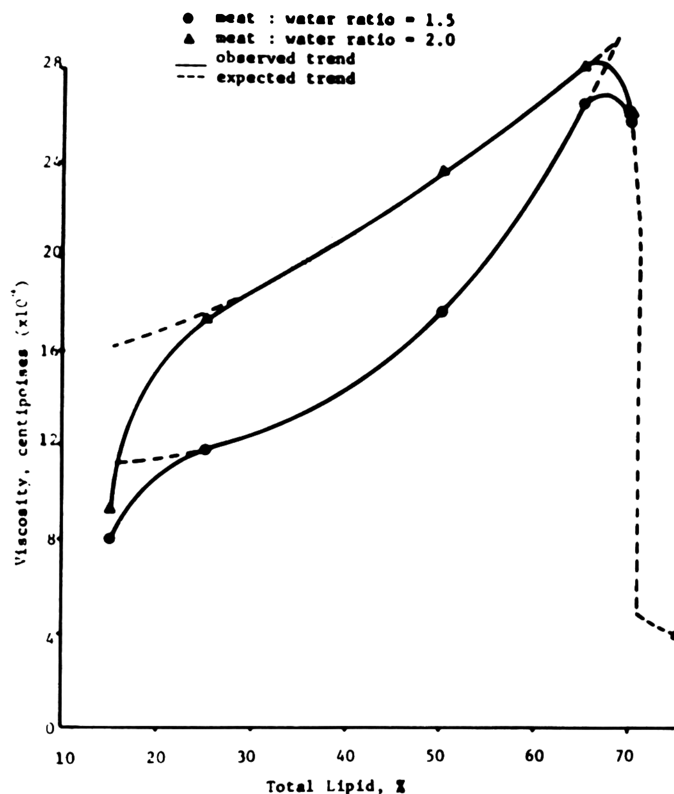

Fig. 5–Effect of percent total lipids on measured viscosity of sausage-type emulsions.

the dispersed particles had started to coalesce as postulated from the AC resistivity data (Fig. 4).

In the inverted emulsions (75% lipid level), the viscosity readings of the emulsions sharply dropped to a lower value than all previously studied lipid levels.

The emulsions with meat:water ratio 2.0:1.0 showed higher indicated viscosities than with meat:water ratio 1.5:1.0. This was believed to be due to the increased level of water in the system. Based on these data, viscosity was found to be a poor indicator of emulsion stability.

In conclusion, a significant result of these studies was the failure to obtain stable emulsions at the 15% lipid level. On the basis of the methods used to evaluate emulsion stability, it was theorized that the system at 15% lipid level might not be an emulsion but an entirely different system. It may be possible that at this lipid level the disperse phase droplets were so few in number in the system that an emulsion matrix was not established. However, as more lipids were added to the system, a critical point was reached which resulted in a stable continuous phase with the lipids being more uniformly dispersed, thus forming an emulsion. In the range of 25–65% lipid level, emulsions with the meat:water ratios studied were relatively stable. This would indicate that at lipid levels near 15% stable emulsions may not be formed, while at levels above 70% the protein-water (continuous) phase was insufficient to prevent coalescence of the disperse phase.

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QUALITY CHANGES IN PRERIGOR POULTRY AT –3°C

INTRODUCTION

ULTIMATE MUSCLE tenderness as influenced by chilling time prior to freezing has been studied in turkeys (Klose et al., 1959, 1961a, b; Stadelman et al., 1966; Scholtyssek and Klose, 1967; Marion and Goodman, 1967) and chickens (Pool et al., 1959). The chilling time and temperature necessary to achieve maximum tenderness in a given muscle of a given species is a function of carcass age, type of muscle, freezing rate, storage time and temperature, and thawing rate. In order to achieve frozen poultry of acceptable tenderness, the usual prefreezing procedure involves thorough chilling of carcasses in ice-slush for a period of 1–4 hr. Alternatively, carcasses can be tenderized by a slow freezing process or by holding them at a high subfreezing temperature. For example, Marion and Goodman (1967) produced turkeys (age 18–27 wk) of satisfactory tenderness by chilling them for 1 hr and freezing very slowly. The desired effect was attributed to continuation of the tenderization process during the 8 hr which elapsed while the large carcasses cooled from +4.5°C to –4°C. Similarly, poultry which is frozen with little or no chilling will undergo tenderization if held at –3.9 to –2.8°C. This tenderization has been reported for both chickens (Pool et al., 1959) and turkeys (Klose et al., 1959, 1961b). Klose et al. (1961b) reported that turkeys receiving a combination treatment of 1 hr in ice-slush and 14 days at –2.8°C were as tender as those treated 48 hr in ice-slush, and that 14 days at –2.8°C had no detrimental effect on flavor.

If a muscle is frozen prerigor, stored at a low subfreezing temperature and rapidly thawed, it will undergo a highly undesirable phenomenon called thaw rigor (DeFremery and Pool, 1960). During thaw rigor, biochemical changes such as depletion of ATP, breakdown of glycogen, accumulation of lactic acid, and decline of pH are greatly accelerated, and tenderness can be adversely affected. Moreover, muscle contraction and exudation of sarcoplasmic fluid (drip) are usually greater during thaw rigor than during normal rigor. Thus, the biochemical changes (glycolysis) accompanying rigor mortis must be essentially complete before poultry is thawed, or undesirable changes in quality will ensue.

In this study, preriogr poultry was frozen promptly after slaughter and glycolysis was allowed to proceed at a high subfreezing temperature. Although previous studies have established that tenderization will occur at high subfreezing temperatures, still unknown at these temperatures are the commercially important interrelationships among shortening, tenderness and drip, and the range of time-temperature conditions yielding satisfactory quality. This study provides information on all of these aspects and the results appear applicable commercially.

EXPERIMENTAL

LEGHORN HENS 18 months of age and raised together under identical conditions were sacrificed by breaking their necks and allowing blood to accumulate internally at the location of the break. Manual restraint was routinely applied during and immediately following killing to minimize the death struggle. The pectoralis major (breast) and biceps femoris (thigh) muscles were dissected from both sides of the carcass within 15 min postmortem. The lengths (parallel to general fiber direction) of all four muscles were promptly measured to the nearest quarter of a centimeter. The muscles were then labeled and wrapped in tightly-closed polyethylene bags. One muscle from each pair (the "test" muscle) was immediately transferred to an air-blast freezer at –23°C. The other muscle from each pair (the control) was chilled for 6 hr in a cold room at 2°C, frozen in an air-blast freezer at –23°C and stored in dry ice (solidified carbon dioxide). After 1 hr in a freezer at –23°C (center temperature of a typical muscle was –19°C), the "test muscles" were removed and given one of five treatments: (1) stored in dry ice, or (2) transferred to a –3°C ethanol-water bath for 1, 2, 3 or 4 hr, and then stored in dry ice. [Behnke et al. (1973) established that the rate of glycolysis in chicken breast muscle attained a subfreezing maximum at about –3°C.] 30 chickens were used in this study, providing six replicates for each of the five treatments described above.

While still frozen, all muscles were weighed to the nearest hundredth gram. The muscles were then completely thawed in a 60°C water bath for 20 min, unwrapped, re-wrapped in paper towels for 15 min (to absorb fluid), and their lengths and weights determined. Percent shortening and percent drip losses were calculated from these data.

The muscles were then labeled, wrapped in aluminum foil, and cooked to an internal (approximate geometric center) temperature of 82°C (periodically measured with a copper-constantan thermocouple and a millivolt potentiometer) using an oven at 163°C. Cooking required about 40–45 min for breast muscles and 25–30 min for thigh muscles. Breast muscles were then cut into standard-size pieces (6.5 cm length × 2.1 cm width × 1.0 cm thickness) using a stainless steel template. Thigh muscles were similarly cut into standard-size pieces (4.5 cm length × 1.3 cm width × 0.6 cm thickness). These standard-size muscles were weighed to the nearest hundredth gram, labeled and wrapped in polyethylene bags to prevent dehydration until tenderness values were determined.

A Lee-Kramer shear press, equipped with a 3,000-lb ring was used to assess tenderness of the above samples. Muscle samples were placed in a standard compression cell (6.7 cm × 6.7 cm × 6.3 cm height) and sheared perpendicular to the major surface of the sample and across the fibers at a rate of 18 cm/min. All measurements were performed with both the compression cell and muscle sample at room temperature. Tenderness results were expressed as pounds of shear per gram of muscle.

The tenderness of muscles treated while on the carcass was also investigated. These birds were slaughtered and dressed under commercial conditions at Brakebush Brothers Co., Westfield, Wisc. Birds from the flock described previously were used for this study. Chickens were electrically stunned, killed by slitting their throats, scalded (57°C for 2 min), and defeathered by a rubber-fingered revolving drum. Following dressing, birds were: (1) wrapped in plastic bags and frozen prerigor in still air at –18°C; (2) wrapped in plastic bags and frozen prerigor in crushed dry ice, or (3) chilled in unagitated ice-water for either 1 or 2½ hr, frozen in still air at –18°C, then stored in dry ice. Birds frozen prerigor in still air at –18°C were then packed in dry ice, transported to the University of Wisconsin and given one of the following treatments: (1) stored in dry ice, or (2) immersed in an ethanol-water bath at –3°C for ½, 1, 2 or 2½ hr, and again stored in dry ice. Birds frozen prerigor in crushed dry ice were transported to the University of Wisconsin, immersed in an ethanol-water bath at –3°C for 2 hr, and again stored in dry ice. 48 chickens, six replicates at each of the above eight treatments, were used in this study.

All birds were then thawed in warm (40°C) running water for 90 min. The chickens were roasted (breast side up) in an oven at 163°C until the internal temperatures of the thigh and breast muscles were at or slightly above 80 and 88°C, respectively. Roasting required about 1½

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hr. The pectoralis major (breast) and biceps femoris (thigh) muscles from both sides of the carcass were then dissected and cut into standard-sized pieces as described previously. These standard-sized muscles were weighed to the nearest hundredth gram and tenderness (lb/g) was evaluated with a Lee-Kramer shear press as described previously.

RESULTS & DISCUSSIONS

PLOTS of percent drip loss versus percent shortening of both breast and thigh muscles after various times at -3°C are shown in Figure 1. Figure 1 illustrates that: (1) shortening from about 6 to 20% had no appreciable effect on percent drip loss from either breast or thigh muscles; (2) increased shortening between 20 and 50% was accompanied by large increases in percent drip losses from both breast and thigh muscles; (3) muscles (breast and thigh) which were held at -3°C for 0, 1 or 2 hr (treatments A, B and C) exhibited greater shortening and larger drip losses than the controls; (4) muscles (breast and thigh) which were held at -3°C for 3 hr (treatment D) exhibited somewhat greater shortening, but no larger drip losses than the controls; and (5) muscles (breast and thigh) which were held at -3°C for 4 hr (treatment E) exhibited essentially the same shortening and drip losses as the controls.

Plots of tenderness (lb shear/g) versus percent shortening of both breast and thigh muscles after various times at -3°C are shown in Figure 2. Figure 2 illustrates that: (1) shortening from about 6 to 20% had no effect on tenderness of either breast or thigh muscles; (2) increased shortening between about 20 and 40% was accompanied by large decreases in tenderness of both breast and thigh muscles; (3) increased shortening between about 40 and 50% resulted in increased tenderness of both breast and thigh muscles; (4) muscles (breast and thigh) which were held at -3°C for 0, 1 or 2 hr (treatments A, B and C) exhibited greater shortening and less tenderness than the controls; (5) muscles (breast and thigh) which were held at -3°C for 3 hr (treatment D) exhibited greater shortening, but no less tenderness than the controls; and (6) muscles (breast and thigh) which were held at -3°C for 4 hr (treatment E) exhibited essentially the same shortening and tenderness as the controls.

Tenderness of both breast and thigh muscles increased with holding time at -3°C, with the greatest change occurring between 1 and 3 hr (Fig. 3). Thigh muscles held at -3°C for 3 hr (treatment E) or 4 hr (treatment F) were significantly ($p < 0.05$) more tender than corresponding muslces which were never held at -3°C (treatment B). In addition, there was no significant difference in tenderness between muscles (breast and thigh) which were frozen prerigor and held at

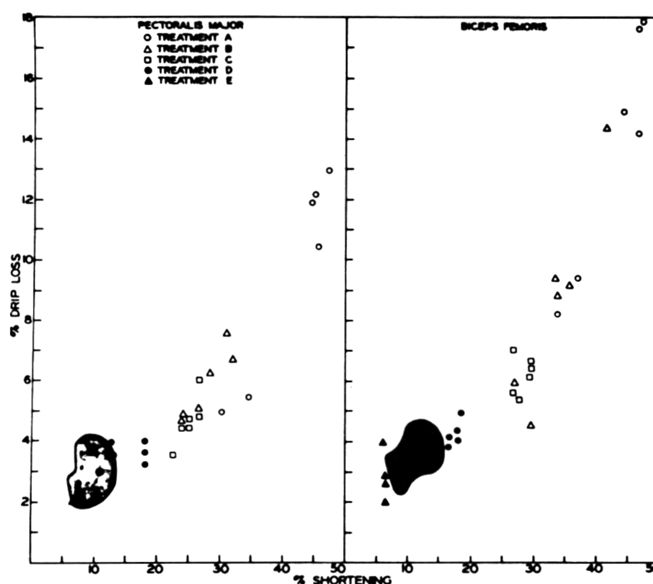

Fig. 1–Effect of percent shortening on percent drip loss of excised poultry muscles. Treatments: A = frozen prerigor in an air blast freezer at –23°C, then packed in dry ice (–78°C); B = frozen and packed prerigor (as in A), immersed in a refrigerated bath at –3°C for 1.0 hr, then stored in dry ice; C = frozen and packed prerigor (as in A), immersed (as in B) for 2.0 hr, then stored in dry ice; D = frozen and packed prerigor (as in A), immersed (as in B) for 3.0 hr, then stored in dry ice; E = frozen and packed prerigor (as in A), immersed (as in B) for 4.0 hr, then stored in dry ice. The irregular-shaped shaded area represents the range of 30 control values. Control muscles were excised, wrapped, chilled for 6.0 hr in circulating air at 1.7°C, frozen postrigor in an air-blast freezer at –23°C, then stored in dry ice. (Shortening–Initial lengths of excised pectoralis major muscles ranged between 13.0–17.5 cm, while those of excised biceps femoris muscles ranged between 7.5–10.0 cm. Drip Loss–Initial weights of excised pectoralis major muscles ranged between 38.98–67.17g, while those of excised biceps femoris muscles ranged between 8.65–17.91g.)

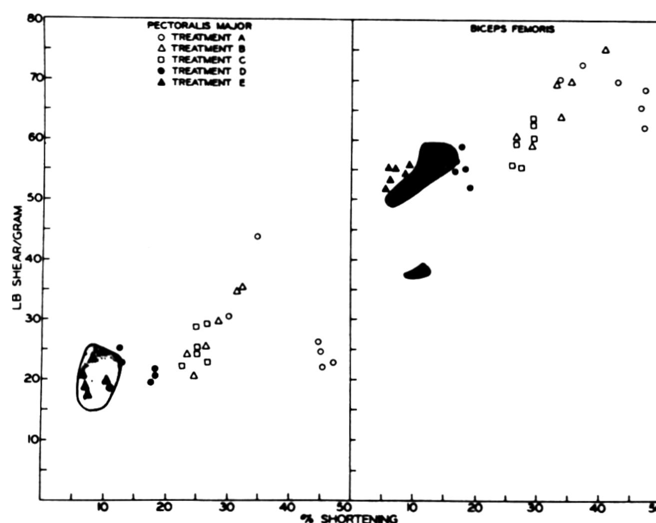

Fig. 2–Effect of percent shortening on tenderness of excised poultry muscles. (Treatments: See legend Fig. 1; Shortening–see legend Fig. 1; Tenderness (lb shear/g) was determined on standard-sized pieces of cooked pectoralis major which ranged in weight between 10.35–15.60g and standard-sized pieces of cooked biceps femoris which ranged in weight between 2.03–3.82g.)

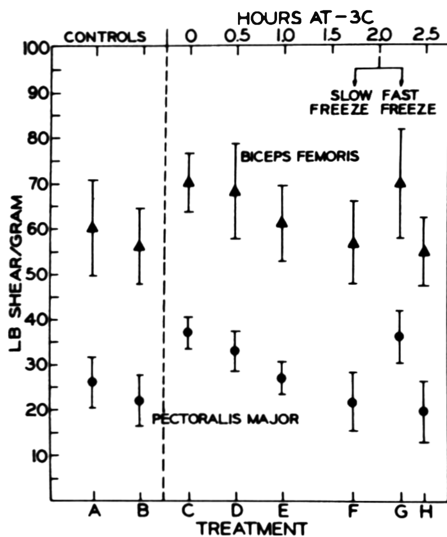

Fig. 3—Effect of time at −3°C on tenderness (mean ± 95% confidence interval) of excised poultry muscle. Treatments: A = chilled for 6.0 hr in circulating air at 1.7°C, frozen postrigor in an air-blast freezer at −23°C, then stored in dry ice (control); B = frozen prerigor in an air-blast freezer at −23°C, then packed in dry ice (−78°C); C = frozen and packed prerigor (as in B), immersed in a refrigerated bath at −3°C for 1.0 hr, then stored in dry ice; D = frozen and packed prerigor (as in B), immersed (as in C) for 2.0 hr, then stored in dry ice; E = frozen and packed prerigor (as in B), immersed (as in C) for 3.0 hr, then stored in dry ice; F = frozen and packed prerigor (as in B), immersed (as in C) for 4.0 hr, then stored in dry ice. (Tenderness—See legend Fig. 2).

−3°C for 3 or 4 hr (treatments E and F) and their respective control muscles (treatment A) which were chilled at 1.7°C for 6 hr before being frozen postrigor.

A study was undertaken to determine if the results obtained with excised poultry muscles were applicable to intact carcasses of birds slaughtered and dressed under commercial conditions. Tenderness values (means ± 95% confidence intervals) of both breast and thigh muscles (excised after roasting the entire carcass) following various times at −3°C are shown in Figure 4. In addition, Figure 4 depicts the comparative effects of slow freezing (placed in still air at −18°C for 24 hr, then packed in dry ice) and rapid freezing (layered in crushed dry ice) on the extent of tenderness achieved during a subsequent 2-hr period at −3°C. This comparison of freezing rates was done because previous results (Behnke et al., 1973) with excised chicken breast muscle established that the rate of postmortem glycolysis at −3°C was affected by the initial freezing treatment. Figure 4 illustrates that: (1) tenderness of both intact breast and thigh muscles increased as holding time at −3°C increased from 0.5 to 2.5 hr, with most of the increased tenderness occurring between 0.5 and 2.0 hr; (2) intact breast muscles held at −3°C for 1.0, 2.0 or 2.5 hr (treatments E, F and H) or intact thigh muscles held at −3°C for 2.5 hr (treatment H) were significantly ($p < 0.05$) more tender than corresponding muscles which were never held at −3°C (treatment C), (3) mean tenderness of intact control muscles (treatments A and B) increased as chilling time at 0°C (unagitated ice-water bath) increased from 1.0 to 2.5 hr, (4) there was very little difference in tenderness between intact muscles (breast and thigh) which were frozen prerigor and reacted at −3°C for 2.0 or 2.5 hr (treatments F and H) and corresponding control muscles which were chilled at 0°C (unagitated ice-water bath) for 2.5 hr (treatment B) before being frozen postrigor (similar to some commercial methods), and (5) intact breast muscles initially frozen at a rapid rate (treatment G) were significantly ($p < 0.05$) less tender after 2.0 hr at −3°C than corresponding muscles initially frozen at a slow rate (treatment F). Thus, investigations of both excised (Fig. 3) and intact (Fig. 4) poultry muscles have established that: (1) tenderness of muscles frozen prerigor increased with holding time at −3°C, and (2) tenderizing at −3°C (frozen prerigor) was at least as rapid as that at 1.7°C or 0°C (unfrozen).

The authors are unaware of previous studies of poultry muscle (cooked postrigor): (1) relating percent shortening to percent drip loss and tenderness, and (2) indicating that satisfactory ultimate tenderness of poultry can be achieved by holding promptly frozen poultry (no chilling) at −3°C for a time equal to or less than a conventional chilling period. The results illustrated in Figure 1 (percent shortening versus percent drip loss) and Figure 2 (percent shortening versus tenderness) are, however, in general agreement with those obtained by Marsh and Leet (1966) with beef muscles.

The results obtained with whole carcasses (Fig. 4) have possible practical application in the frozen poultry industry. Prior to freezing, birds are currently chilled in cold (0–5°C), agitated ice-water for various periods of time, depending upon: the type of poultry (e.g., broiler, mature hen, turkey), freezing rate, and frozen storage time and temperature. This chilling treatment is the most time consuming unit operation prior to freezing. On the assumption that all muscles of poultry behave as the two studied, the results in Figure 4 indicate that freshly slaughtered birds can be tenderized at −3°C (frozen) at least as rapidly as at 0°C (unfrozen). This suggests that it may be possible to eliminate the chilling procedure entirely, wrap the birds in plastic bags, freeze prerigor, and at some convenient point during frozen storage or marketing, hold the birds at −3°C for about 2 hr. Fortunately, −3°C approximates the temperature plateau encountered during freezing or thawing of poultry, thus the necessary treatment could be achieved by simply holding frozen poultry at an air temperature of 0°C or somewhat higher for 2 hr.

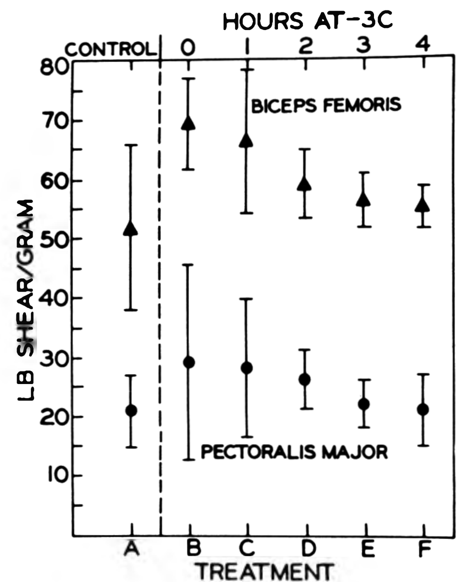

Fig. 4—Effect of freezing method and holding time at −3°C on tenderness (mean ± 95% confidence interval) of whole poultry. Treatments: A = chilled for 1.0 hr in ice-water at 0°C, frozen in still air at −18°C, then packed in dry ice; B = chilled for 2.5 hr in ice-water at 0°C, frozen in still air at −18°C, then packed in dry ice (control); C = frozen prerigor in still air at −18°C, then packed in dry ice (−78°C); D = frozen and packed prerigor (as in C), immersed in a refrigerated bath at −3°C for 0.5 hr, then stored in dry ice; E = frozen and packed prerigor (as in C), immersed (as in D) for 1.0 hr, then stored in dry ice; F = frozen and packed prerigor (as in C), immersed (as in D) for 2.0 hr, then stored in dry ice; G = frozen prerigor in crushed dry ice, immersed (as in D) for 2.0 hr, then stored in dry ice; H = frozen and packed prerigor (as in C), immersed (as in D) for 2.5 hr, then stored in dry ice. (Tenderness (lb shear/g) was determined on standard-sized pieces of pectoralis major muscles which ranged in weight between 8.89–14.45g, and standard sized pieces of biceps femoris muscles which ranged in weight between 2.09–4.17g.)

It is evident from Figure 4 that slow freezing (e.g., in air at −18°C) of prerigor poultry tends to minimize the required holding time at −3°C. Most of this difference probably can be accounted for by tenderization that occurred as the birds passed slowly through the zone of maximum ice formation (thus simply increasing the time spent at a high subfreezing

temperature). A supplementary cause may have been that glycolysis at $-3^{\circ}C$ tends to proceed more rapidly following slow freezing than following rapid freezing (Behnke et al., 1973). However, slow freezing will result in frozen poultry with an undesirable surface color. Thus, a more acceptable procedure would be to rapidly freeze the surface and complete freezing at a slower rate. This procedure is already used by some processors.

It should be noted that birds frozen prerigor and tenderized at $-3^{\circ}C$ do not absorb water during processing, and this of course would result in lower yields of product than now obtained. Compensatory advantages are that the process is more ethical from the consumer's view (no absorption of water), is potentially more sanitary, is more convenient, is perhaps less costly, and has no detrimental effect on product quality. The fact that chilling in ice-slush is not essential to good quality is well documented. Dodge and Stadelman (1960) demonstrated that packaged turkeys aged in air at $0^{\circ}C$ were at least as tender as unpackaged turkeys aged in water at $0^{\circ}C$. In addition, Baker (1957) reported that members of a taste panel preferred dry-packed broiler halves to ice-packed halves in regard to general appearance of the surface, aroma, flavor and juiciness of the flesh.

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IMPROVING THE QUALITY OF MECHANICALLY DEBONED FOWL MEAT BY CENTRIFUGATION

INTRODUCTION

THE ADVENT of mechanically deboned poultry meat has enhanced the utilization potential of poultry meat sources. Such parts as backs, necks, wings and hand-deboned frames may be mechanically deboned and the resulting meat incorporated into emulsified and other further processed products. Fowl carcasses may also be mechanically deboned and the resulting meat paste formulated into a variety of potential products. Thus, mechanical deboning has enabled production of an additional source of high quality protein. Nevertheless, the shortcomings of this meat, namely flavor instability and less desirable emulsifying properties, have limited its usage.

Modifications of mechanically deboned meat to improve its functional and flavor attributes would have merit. Froning and Janky (1971) altered mechanically deboned turkey frame meat by pH adjustment and salt preblending. Modification through pH adjustment to higher values and/or salt preblending was found to markedly improve emulsion capacity and emulsion stability.

Other work has pursued the combination of mechanically deboned meat with other hand-deboned poultry or red meat sources. Pauly (1967) reported that mechanically deboned meat is most suitable in combination with other meat forms. Froning et al. (1971) observed that red-meat frankfurters containing 15% fresh mechanically deboned turkey meat were comparable to all red meat frankfurters with respect to flavor and emulsion stability. This study indicated that utilization of mechanically deboned meat requires close adherance to a quality assurance program, since 90-day frozen stored mechanically deboned turkey meat produced unacceptable frankfurters at the 15% level in red-meat frankfurters (Froning et al., 1971).

If greater versatility is to be achieved with mechanically deboned meat, further modifications are necessary. This study was designed to investigate the effect of centrifugation of mechanically deboned fowl (regular and dwarf-sized fowl) meat on its subsequent functional and chemical properties with the hope of improving utilization of spent fowl meat.

Table 1—Experimental design for modification of mechanically deboned fowl meat

Group	Treatment
A	Mechanically deboned regular-size fowl
B	Centrifuged[a] mechanically deboned regular-size fowl
C	Mechanically deboned dwarf-size fowl
D	Centrifuged[a] mechanically deboned dwarf-size fowl
E	Hand-deboned regular-size fowl + mechanical deboning without bones
F	Hand-deboned dwarf-size fowl + mechanical deboning without bones

[a]Centrifuged at 20,000 rpm for 15 min in a refrigerated Sorval centrifuge (5°C)

EXPERIMENTAL

REGULAR and dwarf-size Single Comb White Leghorn fowl from the University of Nebraska's research farm were slaughtered at 18 months of age using a 59°C scald temperature. After evisceration, the carcasses were chilled overnight in ice-slush. Fowl (regular and dwarf) were divided into two groups: one was mechanically deboned immediately after chilling and the other group was deboned following two months of frozen storage (−29°C). The whole carcasses were vacuum packaged in polyvinylidene chloride bags for frozen storage. The whole carcass was preground through a 3/8 in. grinder plate and mechanically deboned with a Beehive BX-66 laboratory-size deboner. Mechanically deboned meat was vacuum packaged in polyvinylidene chloride bags and stored frozen at −29°C until ready for measurements. When ready for measurements, the meat was thawed overnight at 4°C. Treatments utilized in this study are outlined in the experimental design shown in Table 1. Two replicates for each of the fresh deboned and frozen deboned meat treatments were utilized involving a total of 150 regular fowl and 100 dwarf fowl.

Moisture content of mechanically deboned meat (MDM) from each of the treatments was determined as reported by Satterlee et al. (1971). Protein and fat determinations were measured as described in AOAC Methods (1970). The Kjeldahl method (24.010) for crude protein and ether extraction (24.005) for crude fat were used. Emulsion stability of fresh MDM was measured using the formulation reported by Froning (1970) and the method of Townsend et al. (1968). Emulsion capacity was determined using the method of Swift et al. (1961). TBA values were measured after 0, 4 and 8 wk frozen storage at −29°C as outlined by Tarladgis et al. (1960). Gardner L, a_L and

Table 2—Analyses of mechanically deboned meat[a]

Treatment group	Protein %	Moisture %	Fat %
A	13.9[a]	65.1[b]	18.3[a]
B	23.2[b]	70.5[a]	6.0[b]
C	14.4[a]	67.5[a]	16.1[a]
D	22.2[b]	69.7[a]	6.7[b]
E	14.3[a]	61.6[c]	22.1[a]
F	14.7[a]	66.7[a]	17.0[a]

[a]Values not having the same superscripts within each column are significantly different at the 5% level of probability using Duncan's multiple range test.

Table 3—Emulsification characteristics of mechanically deboned fowl meat[a]

Treatment group	Emulsifying capacity ml/2.5g meat	Emulsifying stability per 100g emulsion			Cooking loss %
		Gel-water ml	Solids g	Fat ml	
A	146[a]	15.8[a]	2.0[a]	4.1[a]	27.1[a]
B	160[b]	3.8[b]	0.4[b]	0.3[b]	9.1[bc]
C	148[a]	16.0[a]	2.2[a]	3.1[ac]	26.5[a]
D	150[a]	2.5[b]	0.2[b]	0.3[b]	7.5[b]
E	145[a]	13.9[a]	0.8[b]	4.9[a]	25.8[a]
F	147[a]	9.2[a]	0.5[b]	0.6[bc]	15.1[c]

[a]Values not having the same superscripts within each column are significantly different at the 5% level of probability using Duncan's multiple range test.

Table 4–Heme pigment content of mechanically deboned fowl meat[a]

Treatment group	Total pigments mg/g	Myoglobin mg/g
A	3.8[a]	0.8[a]
B	3.7[a]	0.7[a]
C	2.8[b]	1.0[a]
D	2.3[b]	0.9[a]
E	1.3[c]	0.6[a]
F	1.6[c]	0.8[a]

[a]Values not having the same superscripts within each column are significantly different at the 5% level of probability using Duncan's multiple range test.

Table 5–Gardner color values of raw mechanical deboned fowl meat[a]

Treatment group	L	a_L	b_L	a_L/b_L
A	52.1[a]	10.1[a]	9.8[a]	1.0[a]
B	48.4[b]	10.3[a]	9.0[a]	1.0[a]
C	55.8[a]	8.6[a]	9.2[a]	0.9[a]
D	45.0[b]	8.7[a]	8.1[a]	1.1[a]
E	56.5[a]	6.3[b]	10.5[b]	0.6[b]
F	56.1[a]	7.4[b]	9.4[a]	0.8[a]

[a]Values not having the same superscripts within each column are significantly different at the 5% level of probability using Duncan's multiple range test.

b_L values were ascertained with the Gardner model C-4 color difference meter. Total heme pigments and myoglobin concentration were analyzed by the method reported by Richansrud and Henrickson (1967).

The data were statistically evaluated by analysis of variance (Snedecor, 1957) and Duncan's (1955) multiple range test. All statements concerning significant differences are based on the 5% level of probability.

Table 6–TBA values of mechanically deboned fowl meat after 0, 4 and 8 wk of frozen storage

Treatment group[a]	TBA values mg malonaldehyde/1000g 0 wk storage	4 wk storage	8 wk storage	Overall average[b]
A	2.9	4.0	5.9	4.3[a]
B	3.3	2.5	4.1	3.3[b]
C	1.6	1.9	2.5	2.0[c]
D	1.2	1.3	1.6	1.4[c]
E	3.6	2.7	5.6	4.0[a]
F	3.0	4.0	4.7	3.9[b]

[a]Means not underscored by the same line within each treatment storage period are significantly different at the 5% level of probability using orthogonal comparison.

[b]Overall average values not having the same superscripts are significantly different at the 5% level of probability level using Duncan's multiple range test.

Table 7–TBA values of delayed (2 months storage as whole carcass before deboning) mechanically deboned fowl meat after 0, 4 and 8 wk of frozen storage

Treatment group[a]	TBA values mg malonaldehyde/1000g 0 wk storage	4 wk storage	8 wk storage	Overall average[b]
A	2.7	8.2	8.4	6.4[a]
B	2.7	4.5	4.2	3.8[c]
C	2.4	7.5	12.1	7.3[a]
D	2.8	3.6	3.9	3.4[c]
E	3.7	5.2	6.0	5.0[b]
F	3.0	4.9	5.0	4.3[b]

[a]Means not underscored by the same line within each treatment storage period are significantly different at the 5% level of probability using orthogonal comparisons.

[b]Overall average values not having the same superscripts are significantly different at the 5% level of probability using Duncan's multiple range test.

RESULTS & DISCUSSION

Analyses

Centrifugation significantly increased the protein content and significantly decreased the fat content of the end product (Table 2). Removal of bone prior to passing the meat through the deboner did not appreciably alter the composition of the MDM. The centrifugation process produced three fractions, which were the meat, an aqueous phase and a fat layer with yields of 36%, 30% and34% respectively. The aqueous and fat layers were discarded. Utilization of the fat in a commercial process would likely be feasible as a food grade fat source. The aqueous constituent appeared to contain a considerable quantity of heme pigments as indicated by a deep red color. The loss of these heme pigments in the aqueous phase may account for the increased oxidative stability of the remaining MDM.

Emulsifying characteristics

Emulsifying characteristics of meat from the various treatments are presented in Table 3. Emulsifying capacity of MDM from regular-size fowl was significantly improved by centrifugation. Emulsifying capacity of MDM from dwarf-size fowl was not significantly affected by centrifugation. With the similar composition of the two meat sources, one would have expected similar trends with respect to emulsifying capacity. The MDM from dwarf fowl did have a slightly lower protein content and higher fat composition after centrifugation which may have influenced the emulsion capacity. Groups E and F (hand-deboned meats) were comparable to the control (A and C) sources with respect to emulsifying capacity.

Emulsifying stability of MDM from both meat sources (regular-size and dwarf fowl) was significantly increased by the centrifugation treatment. Gel-water, solids, fat, and cooking losses were all significantly decreased by centrifugation. The improved emulsion stability was most likely related to the higher protein and lower fat content observed in the centrifuged samples. Emulsifying stability of group E was not significantly different than the control group A. Group F, however, did exhibit significantly lower fat release and cooking losses as compared to groups A and E.

Heme pigment content and color difference value

Heme pigment concentrations for all groups are shown in Table 4. Myoglobin content was not significantly influenced by any of the treatments. Total pigments on the other hand were significantly higher in MDM in groups containing bone prior to the deboning process. Since meats from groups E and F (hand-deboned prior to passing through the deboner) contained significantly less total heme pigments than meats from groups A, B, C and D, it would appear that conventional mechanical deboning contributes considerable pigment concentrations from the bone marrow.

Gardner color difference values are

presented in Table 5. Centrifugation significantly reduced Gardner L values over that observed for control groups A and C. Groups E and F exhibited a significant decrease in Gardner a_L values as compared to that observed in the other treatment groups. This lower trend for a_L values very likely reflects the differences in total pigment concentration. The Gardner b_L value and a_L/b_L ratio were also significantly affected in group E. These data indicate that Gardner values may possibly be utilized as an index to heme pigment concentration and perhaps pigment destruction during storage.

TBA values

Effect of centrifugation on TBA values of fowl meat deboned fresh and stored frozen for various periods is shown in Table 6. TBA values of mechanically deboned dwarf fowl meat were significantly lower than values for mechanically deboned regular-size fowl. MDM from dwarf fowl exhibited low TBA values thoughout all storage periods. Centrifugation significantly lowered TBA values of meat from regular-size fowl meat. TBA values of meat from all treatment groups significantly increased with the longer storage periods. Hand deboning prior to passage through a mechanical deboner did not significantly alter increases in TBA values in freshly deboned meat.

Delayed mechanical deboning of whole carcasses stored frozen for two months produced somewhat different trends than that observed in freshly deboned sources (Table 7). Centrifugation (groups B and D) significantly reduced changes in TBA values of the two fowl meat sources as storage time advanced. Groups A and C showed significant increases in TBA values during storage; whereas groups B and D had much more stable TBA values throughout the storage period. Although groups E and F showed significant increases in TBA values during storage, changes were not of the magnitude encountered with control groups (A and C). These results indicate that the heme pigments from the bone marrow apparently increased the instability of MDM. This was particularly true when delayed deboning was practiced. Previous work has shown that hemeproteins may act as biocatylists in promoting oxidative rancidity (Tappel, 1955; Maier and Tappel, 1959).

Utilization potential of fowl meat

This study has shown that the quality of MDM from spent fowl carcasses can be improved. The new dwarf fowl may be particularly adaptable to mechanical deboning. Their small size would limit the feasibility of a hand-deboning operation. Mechanical deboning of fowl (both regular and dwarf-sized fowl) offers a potential for upgrading the use of spent fowl meat sources into higher valued, further processed items. Cunningham et al. (1971) reported that mechanically deboned spent fowl meat could successfully be utilized in a variety of emulsified products.

Centrifugation greatly enhanced the functional and storage stability of mechanically deboned meats. Commercial scale continuous centrifugal separators are available and could be adapted to this purpose.

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TEXTURE STABILITY DURING STORAGE OF FREEZE-DRIED BEEF AT LOW AND INTERMEDIATE MOISTURE CONTENTS

INTRODUCTION

THE ACCEPTABILITY of low and intermediate moisture foods depends on stability to the many reactions which occur during product storage. Labuza (1968) has provided a review of the primary reactions which will occur in dry and intermediate moisture foods and their relationship to water activity. Although these reactions could influence product texture during storage, there appears to be only limited information available on the relationship between product texture and water activity during storage.

The texture properties of precooked freeze-dried beef were reported by Kapsalis et al. (1970) for samples stored for 5.5 months at 20°C and water activities of 0, 0.12, 0.23 and 0.66. In general, the results indicated that both maximum force for penetration and cohesiveness (as defined by texture profile analysis) increased with increasing water activity. There have been no reports on the texture changes which occur during the storage period at various water activities and temperatures.

The objective of this investigation was to determine the influence of water activity, temperature and freeze-drying plate temperature on the texture of precooked freeze-dried beef during storage.

EXPERIMENTAL

THE OBJECTIVES of the investigation were accomplished by conducting experiments designed to measure texture of freeze-dried beef at intervals during product storage.

Sample preparation

The freeze-dried beef samples were prepared as product cubes according to procedures described by Reidy and Heldman (1972). In general, portions of the longissimus dorsi muscle from beef were cooked, frozen and cut into cubes before freeze drying at plate temperatures of 105 and 145°F. The ½-in. cube samples were dried to less than 3% moisture content before dynamic equilibration to the desired water activity by previously described procedures, utilizing equipment illustrated schematically in Figure 1. The main component of the system was the air conditioning unit which provided air with known temperature and relative humidity for circulation through a layer of product cubes. The product was exposed to the conditioned air until weight change was negligible; usually less than 10 hr was required. After equilibration, the product cubes were placed in sealed containers for storage at the desired temperature and water activity.

Large quantities of the sample cubes were equilibrated at water activities of 0.25, 0.5 and 0.75 and temperatures of 39° and 100°F. The product samples were stored at each condition established as well as the dry condition (3% MC) at each temperature. The latter was considered to be very close to a water activity of zero.

Texture measurement

The texture parameters measured included hardness and chewiness from the texture profile analysis (Friedman et al., 1963; Bourne, 1968) and described by Reidy and Heldman (1972). An Instron Universal Testing Machine was utilized with a 3/16-in. diameter probe. The stroke length of the probe was set at 2.5 mm which corresponded to a total compressive strain of 20%. A crosshead speed of 5 cm/min was utilized during all experiments used to obtain results to be presented.

General procedures

The equilibrated precooked freeze-dried beef cubes were stored in sealed containers at the two storage temperatures (39° and 100°F). Each sealed container held 15 sample cubes to be used during a given texture evaluation. Samples from each condition (storage temperature, water activity and freeze-drying plate temperature) were evaluated at monthly intervals to determine the hardness and chewiness.

RESULTS & DISCUSSION

THE TEXTURE RESULTS are expressed as the mean value of 15 replicate measurements collected during each determination. Statistical analysis indicated that variation in parameters for replicates was usually less than variability from one condition to another. The influence of storage duration on precooked freeze-

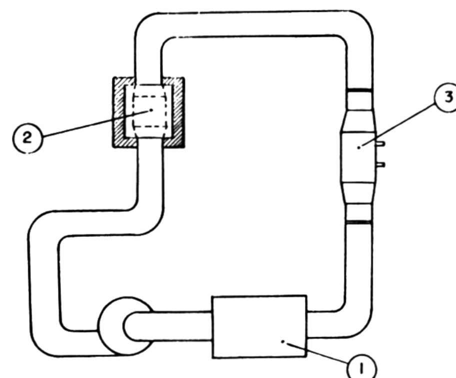

Fig. 1—Apparatus used for dynamic equilibration of precooked freeze-dried beef cubes. (1) air conditioning (Aminco) unit; (2) equilibration chamber for precooked freeze-dried beef cubes; (3) laminar air flow element for measurement of air flow.

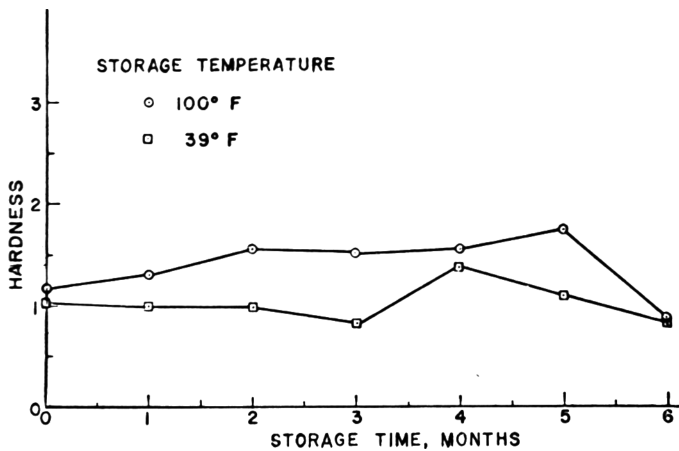

Fig. 2–Influence of storage temperature on hardness during storage of freeze-dried beef with water activity of 0.75.

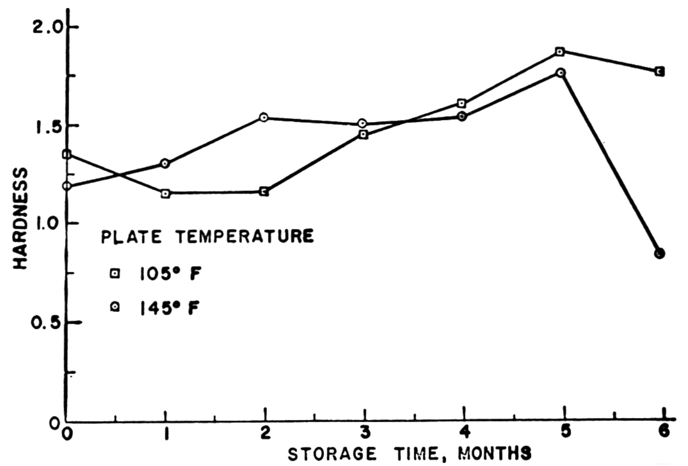

Fig. 4–Influence of storage on hardness of freeze-dried beef at 100°F with water activity of 0.75.

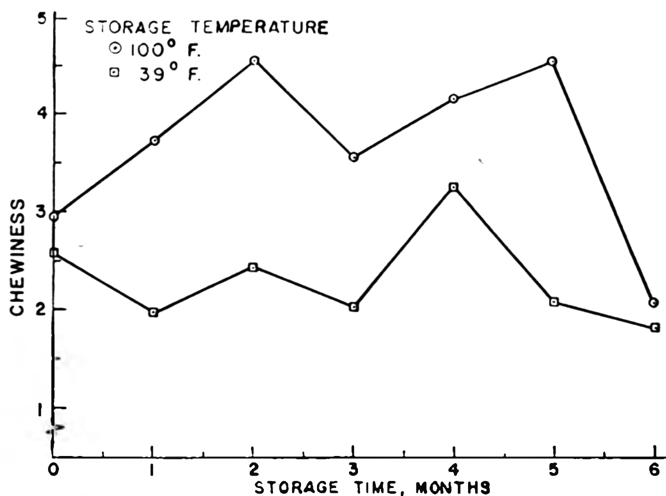

Fig. 3–Influence of storage temperature on chewiness during storage of freeze-dried beef with water activity of 0.75.

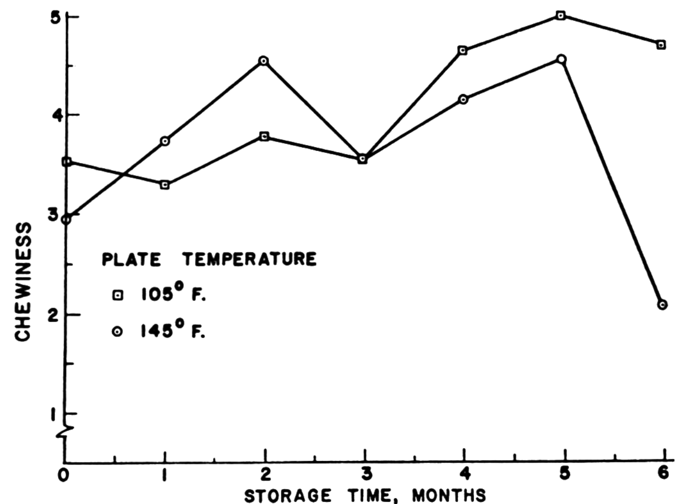

Fig. 5–Influence of storage on chewiness of freeze-dried beef at 100°F with water activity of 0.75.

dried beef texture was dependent on the processing and storage conditions. In all situations investigated, there was a tendency for hardness and chewiness to increase with duration of storage, but the influence of storage was much more evident under some conditions as compared to others. There was a consistent tendency for the influence to be maximum after 5 months of storage and both hardness and chewiness seemed to decrease after 6 months of storage. This feature of the results is illustrated in Figures 2 and 3 where the texture parameters reach the lowest values after 6 months of storage. The apparent change in texture between the fifth and sixth month of storage seems to be unrelated to the condition studied but the change was consistent in all samples involved in the texture evaluation during the sixth month.

Influence of storage temperature

The influence of storage temperature on precooked freeze-dried beef texture (hardness and chewiness) is illustrated in Figures 4 and 5. It is evident that the 100°F storage temperature resulted in higher hardness and chewiness values at each storage time interval measured. The results presented were obtained for samples freeze dried with plate temperature of 145°F and then equilibrated and stored at a water activity of 0.75. Results for the second plate temperature and the three additional water activities were similar to results presented, but differences due to storage temperature were less significant.

The hardness and chewiness values for product stored at 39°F are relatively constant with duration of storage with a slight increase during the fourth month of storage. The texture parameters for product stored at 100°F were higher at all intervals of storage. In addition, there was a tendency for each texture parameter to increase between the beginning of storage and the fifth month. The difference between the texture parameters measured for product stored at the two temperatures was most evident for the chewiness parameter. Based on this observation, it is suggested that the chewiness parameter may be more sensitive to texture changes or factors which influence product texture. The observed influence of temperature indicates that reactions occurring in the product to cause an increase in hardness or chewiness must be accelerated by higher temperatures as would be expected.

Influence of plate temperature

Precooked freeze-dried beef stored at 100°F and water activity of 0.75 is used

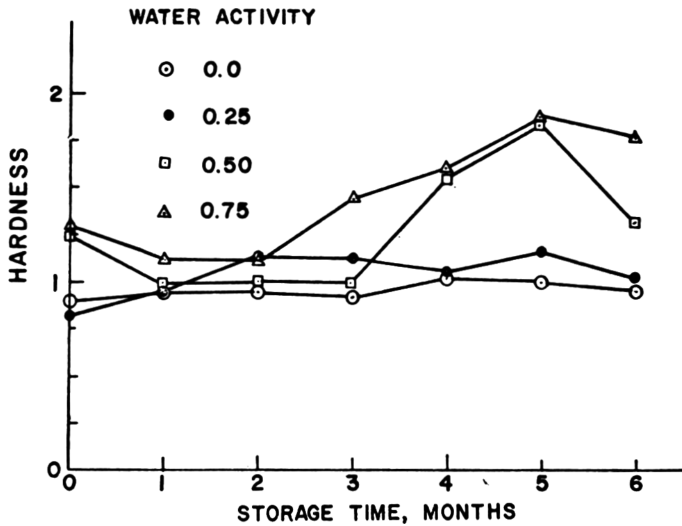

Fig. 6–Influence of water activity on hardness during storage of freeze-dried beef at 100°F (plate temperature = 105°F).

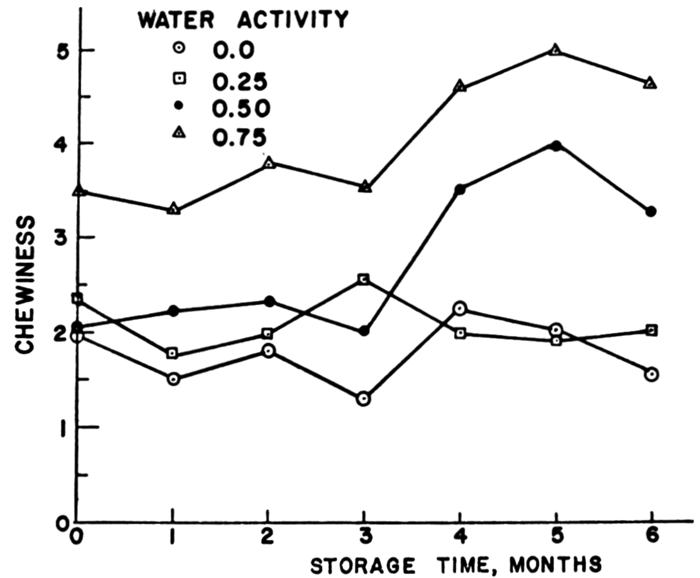

Fig. 7–Influence of water activity on chewiness during storage of freeze-dried beef at 100°F (plate temperature = 105°F).

to illustrate the influence of plate temperature on product texture during storage in Figures 4 and 5. During the first three months of storage, the hardness parameter was higher for the 145°F plate temperature product (Fig. 4). Beginning with the fourth month of storage, the precooked beef which was freeze dried at 105°F had the higher hardness values. The changes in the magnitude of the chewiness parameter during storage were very similar to those observed for hardness (Fig. 5). The texture results obtained at 39°F and all other water activities were similar to results presented in Figures 4 and 5.

Based on the results presented, additional experiments might be warranted. Assuming that changes in texture caused by reactions occurring during storage will become more evident with increased duration of storage, the relationship between the two plate temperature products existing during the latter stages of storage (4–6 months) should be more revealing. Based on this assumption, the product which was freeze dried with 105°F plate temperature would be expected to have higher values of hardness and chewiness anytime after 4 months of storage.

Influence of water activity

The texture parameters (hardness and chewiness) measured at various water activities during storage are illustrated in Figures 6 and 7. The results indicate that hardness and chewiness do not change significantly during the first 3 months of storage, although there appears to be a slight increase in hardness after 3 months. Beginning with the fourth month of storage, the hardness and chewiness of precooked freeze-dried beef stored at water activities of 0.5 and 0.75 increased significantly. The texture parameters of the same product stored at water activities of zero and 0.25 remained at nearly the same values throughout storage. The results obtained for product stored at 39°F did not reveal the types of changes observed for the 100°F storage.

The results presented in Figures 6 and 7 indicate that the reactions producing changes in product texture are both temperature and water activity dependent. Based on the results obtained, texture stability can be maintained by storage at 39°F and at 100°F as long as water activity is maintained at 0.25 or below. Additional investigations could better define the temperature at which texture stability is lost and the critical water activity between 0.25 and 0.5 at 100°F.

Statistical analysis of data

The data presented in Table 1 represent the results of an analysis of variance conducted on all hardness and chewiness data obtained in the investigation. The values in Table 1 indicate the level of significance which describes the assurance that a given variable causes a change in the hardness or chewiness parameter. In general, there is a tendency for the assurance of statistical significance to increase with duration of storage indicating that changes in product due to storage duration magnifies the influence of plate temperature, storage temperature and water activity. In addition, there appears to be some indication that assurance of significance level was established easier when analyzing chewiness values as compared to hardness.

The influence of plate temperature on hardness appears to become significant beginning after 1 month of storage and continues through 5 months. After 6 months of storage, the significance level was the same as variability among replicates. In general, the same relationships existed between plate temperature and the chewiness parameter. The overall significance levels indicate a very definite influence of plate temperature on product hardness and a somewhat less significant influence on the chewiness parameter.

The statistical analysis indicated that the storage temperature may not have a significant influence on product hardness until reaching a storage duration of 5 months. In the case of the chewiness parameter, the significance levels are sufficiently low at all times beginning with one month of storage supporting previous observations that the chewiness parameter may be more sensitive to variations in the product texture. An analysis of all data independent of storage time indicates that storage temperature has a definite influence on product hardness and chewiness.

The influence of water activity on product hardness seems somewhat questionable. The confidence levels are relatively close to corresponding levels for variations between replicate samples. This situation does not exist when analyzing the relationships between water activity and the chewiness parameter. Confidence levels obtained at storage times of 1

Table 1—Statistical significance of various processing and storage parameters on texture of freeze-dried beef

Variable	0	1	2	3	4	5	6	Overall
				Hardness				
Plate temp	0.720	0.021	0.001	0.002	0.061	0.019	0.065	<0.0005
Storage temp	0.224	0.018	0.011	0.636	0.857	<0.0005	<0.0005	<0.0005
Water activity	0.294	0.293	0.022	0.468	0.728	0.102	0.049	0.169
Replicates	0.252	0.976	0.165	0.503	0.898	0.408	0.064	–
				Chewiness				
Plate temp	0.072	0.003	0.297	0.295	0.767	0.365	0.162	0.003
Storage temp	0.223	<0.0005	0.183	0.098	0.033	0.002	<0.0005	<0.0005
Water activity	0.017	<0.0005	0.001	0.013	0.003	<0.0005	0.001	<0.0005
Replicates	0.060	0.758	0.749	0.880	0.966	0.170	0.384	–

month or more are very low indicating a very definite influence of water activity on chewiness of the product. Analysis of all product samples confirms these observations, although the lack of observable influence on hardness may be related to the lack of sensitivity in the measurement of that particular parameter.

Texture stability

The most obvious examples of the loss of texture stability in this investigation occur during the fourth, fifth and sixth month of storage at water activities of 0.5 and 0.75 and a temperature of 100°F. Under these conditions, the precooked freeze-dried beef experiences a hardening effect as indicated by a significant increase in both hardness and chewiness. A similar effect has been observed by Kapsalis et al. (1970) when storing precooked freeze-dried beef at 20°C (88°F) and water activities of 0.0, 0.12, 0.23 and 0.66. The hardening effect observed by Kapsalis et al. (1970) was most significant at the highest water activity and was attributed to the existence of less firmly bound water which was available to enhance physical and physico-chemical processes occurring in the product.

The results of the investigation being reported confirm the observations of Kapsalis et al. (1970). In addition, the present results indicate that the hardening effect is temperature dependent and can be reduced or eliminated by reducing the temperature from 100°F to 39°F. Based on these observations, the reactions or processes which cause the hardening effect are dependent on availability of sufficient moisture (water activity > 0.5) and an adequate temperature level. Although the temperature level is not well defined at this point, temperature may be the more important parameter since an increase in temperature decreases the moisture content at given water activity which should lead to less hardening effect. Apparently the accelerated reaction rates or processes at 100°F are sufficient to cause the hardening effect even with somewhat less available moisture. The importance of moisture content is confirmed by results obtained using product freeze dried at plate temperatures of 105°F and 145°F. The product obtained using the 145°F plate temperature had a lower equilibrium moisture content at all water activities (Heldman et al., 1968) and lower hardness and chewiness values after extended storage periods as reported in this investigation.

Texture parameter sensitivity

The availability of acceptable texture parameters to use in monitoring changes in product during investigations of the type being reported is of definite concern. Of the two parameters (hardness and chewiness) used in this investigation, all results obtained indicate that chewiness is significantly more sensitive to changes in texture. The raw data values are larger in magnitude, which contributes to the sensitivity. In addition, the statistical parameters presented in Table 1 indicate that it is easier to establish influence of a given parameter (plate temperature, storage temperature, water activity) on chewiness than on hardness. There is no indication that the two parameters are detecting different texture characteristics of the product.

CONCLUSIONS

1. An increase in storage temperature from 39°F to 100°F caused an increase in hardness and chewiness of precooked freeze-dried beef throughout the storage period of 6 months.
2. Precooked beef, freeze dried with a plate temperature of 105°F had higher hardness and chewiness values after three months of storage than product freeze-dried with 145°F plate temperature.
3. A definite increase in hardness and chewiness occurs in precooked freeze-dried beef stored at 100°F and water activities of 0.5 or 0.75 for 4 months or more.
4. The hardening effect which occurs during storage of precooked freeze-dried beef is dependent on temperature and the availability of sufficient moisture in the product.
5. The chewiness parameter appears to be more sensitive to changes in product texture than hardness.

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SYSTEMATIC VARIATION IN TOUGHNESS WITHIN THE BEEF LONGISSIMUS DORSI AND SOME OF ITS IMPLICATIONS

INTRODUCTION

THE HETEROGENEOUS structure of natural foods has given rise to the development of a number of empirical mechanical devices for evaluation of texture. The lack of homogeneity in biological materials would lead one to expect some difficulty in getting reproducible texture measurements. For example, the structured nature of muscle would tend to prevent one from obtaining the consistency among replicates on a given sample that would be expected in measuring a more homogeneous food such as jelly, processed cheese, margarine, etc.

The customary approach in mechanically testing the tenderness of a meat sample is to make a number of replicate determinations and average them. The expectation is that true differences between different samples will be demonstrated.

The fact that different muscles in a carcass tend to follow a rather consistent pattern with some being tougher and others more tender is well known. But, beyond this, end-to-end variation in tenderness within a given muscle has also been demonstrated (Ginger and Wier, 1958; Paul and Bratzler, 1955; Ramsbottom et al. 1945) as well as variation of tenderness at different locations upon a given cross section of the muscle (Alsmeyer et al. 1965; Hedrick et al. 1968; Tuma et al. 1962). These variations have been indicated using tests like the Warner-Bratzler shear device.

The Warner-Bratzler shear device has been used extensively to evaluate tenderness. The details of the test procedure such as cooking method, temperature of samples when tested, core diameter, positions where cores are taken from the sample, number of shear determinations made and averaged etc., varies somewhat between investigators. The shear test results however are usually analyzed by a statistical technique which assumes that variations in shear determinations on a given sample are normally distributed. This assumption implies that the material sheared does not contain systematic variation in texture.

As pointed out above, however, muscle is not homogeneous in texture, but contains a structured pattern of variation in tenderness. Practical attempts employed to get around this variability are to take core samples from prescribed locations and to avoid obvious visible connective tissue. Statistical analyses of data are generally done using classical methods with the assumption that the statistical method will be sufficiently robust to handle failure of measurement errors to conform to strict normality. It appears, however, that these assumptions may at times contribute to some confusing ambiguities.

The purpose of the experiment reported here was to study the variation of shear determinations within the longissimus dorsi of the beef short loin and to get an indication of how this variation affects precision of results when different numbers of shears are used per sample.

EXPERIMENTAL

A U.S. PRIME short loin with a longissimus dorsi of a large cross sectional area was obtained. Six 2 in. thick slices were cut from this loin and roasted in a 350°F oven to 150°F internal. After chilling to 40°F, the maximum possible number of good 1 in. diameter cores were taken from within the longissimus dorsi muscle. This number varied from seven to nine. Three shears were made per core. Consequently, from 21 to 27 shear determinations were obtained per slice. A total of 150 shear determinations were obtained from the entire short loin.

RESULTS & DISCUSSION

THE RESULTS of the shear measurements are summarized in Figure 1. The bars of the histograms indicate the number of shear determinations which occurred at the different pounds of force required. The values were grouped into groups at 1-lb intervals. Since shear force was read to the nearest ¼ lb, shear values of 9.5–10.25, for example, were grouped in the 10-lb bracket.

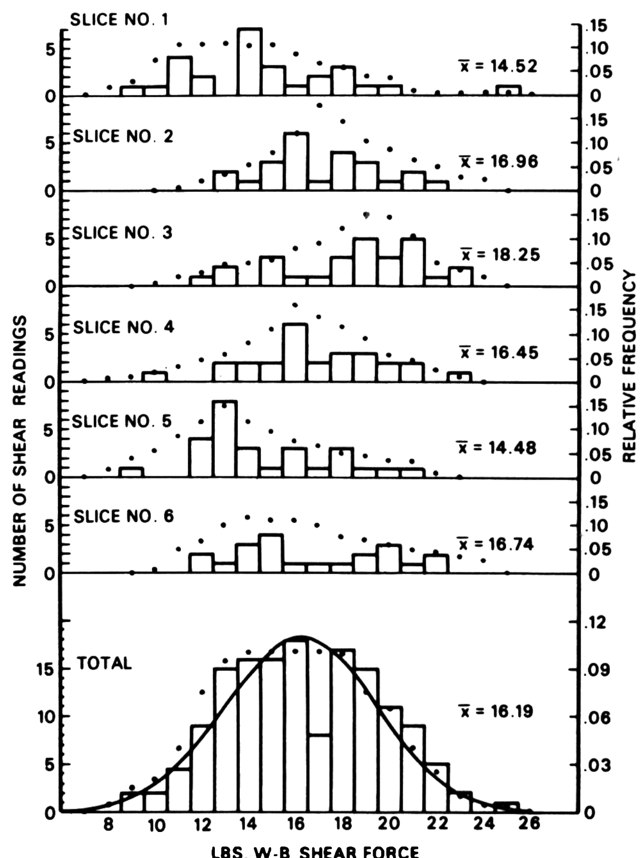

Fig. 1—Distribution of shear determinations in six slices from one short loin.

A technique for smoothing out sparse data to yield a frequency distribution (Schlaifer, 1959) was applied to try and smooth out the actual sample values. The results of these computations are indicated by the dots in Figure 1.

A normal curve was also fitted using the mean and standard deviation of the total data in the equation for the normal curve (Dixon and Massey, 1957). This fitted curve is drawn as the smooth bell shape curve superimposed on the histogram at the bottom of Figure 1.

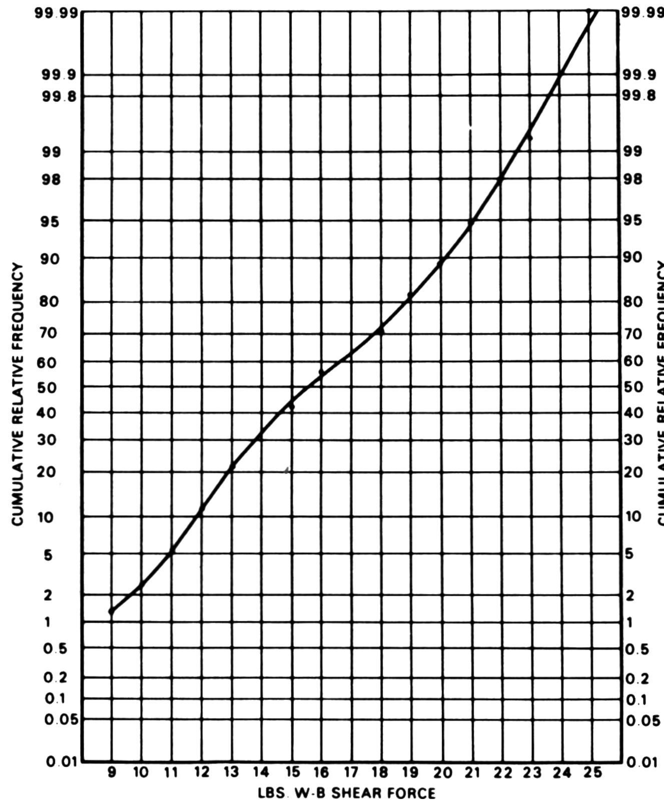

Fig. 2—Plot of individual shear determinations from one short loin.

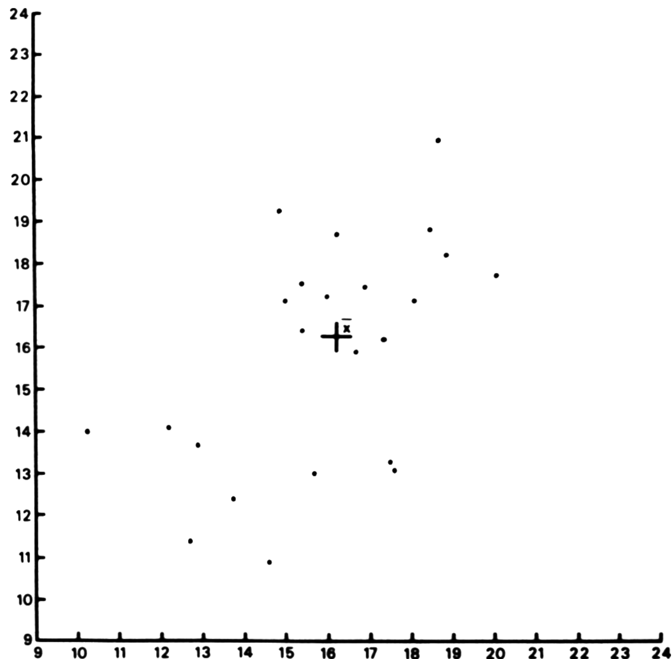

Fig. 3—Mean shear values from adjacent pairs of cores from within one short loin.

Some initial observations can be made viewing the results in this form: (1) Shear values within a given slice tended to be spread out over a rather broad range. (2) The small mode peak that was present in each slice was not necessarily centrally located. (3) The overall picture was a frequency distribution for the entire short loin with a somewhat flattened top. In other words, the frequency of observed shear readings at or near the mean was less, and the frequency of shear readings deviating moderately from the mean was greater than what would be expected with the assumption of a strictly normal distributed population.

Some tests of normality were made. In Figure 2 the cumulative relative frequency of shear values are plotted on normal probability paper. If the distribution were strictly normal, the line would be straight. In Figure 3 mean shear values of adjacent pairs of cores within slices were plotted. If the distribution were normal, there would be a more dense clustering of points near the mean but getting gradually more diffuse away from the mean. While the points are closer together near the mean, there appears to be a systematic linear pattern departing from the mean.

Since the above two tests for normality involve subjective judgments, some quantitative tests were tried. The results of tests for skewness and kurtosis are summarized in Table 1. The g_1 values bear out what was visually observed in Figure 1, i.e., the pattern of skewed asymmetry was not consistent between slices. The calculated t values do not indicate that skewness is a significant factor in explaining departure from normality.

The g_2 values were more consistent. All but one of these were negative indicating an excess of measurements deviating moderately from the mean. The calculated t values for kurtosis were not large enough to demonstrate significance however.

Table 1—Tests for symmetry and normality

Slice	Skewness		Kurtosis	
	g_1	t	g_2	t
1	.9249	2.100*	.7085	.8255
2	.1046	.2296	−.9472	1.068
3	−.5332	1.210	−.6408	.7466
4	−.2568		−.2526	.2801
5	.4358	1.2456	−.4911	.5722
6	.0455	.2202	−1.2557	1.8685
Overall	.1599	.8100	−.5918	1.5085

*$P < 0.05$

Table 2–Average shear force from two cores randomly chosen from within a slice[a]

Slice						Range
1	12.12	17.21	11.54	16.00	13.08	5.67
2	16.29	16.75	16.54	16.79	17.75	1.46
3	16.91	19.71	15.87	20.29	18.58	4.42
4	16.04	14.71	17.58	17.75	17.29	3.04
5	14.04	14.67	14.24	14.29	17.25	3.21
6	14.29	15.91	18.75	19.29	15.54	5.00

[a]Three shears were made per core so each value is an average of six shear determinations.

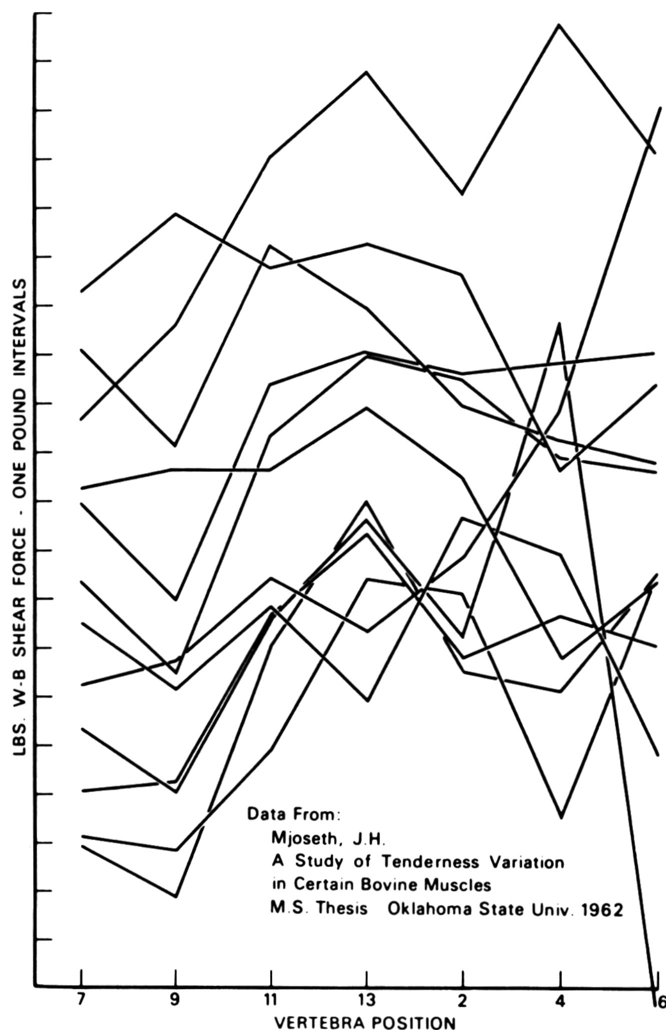

Fig. 4–Variation in shear force over length of longissimus dorsi.

As a whole, the above analyses of these data indicate that the experimental error in shear determinations deviates somewhat from normality. The structural variation within muscle apparently gives rise to an excess of moderate deviations from the mean. The curve for error variation therefore tends to be more flat near the mean.

An effort was made to see what the effect of this error distribution was on the consistency of average shear value when a relatively small number of shear determinations were made per sample. Since as few as six shear determinations per sample is not uncommon in the literature, this number was chosen. Two cores with three shears per core gave the six shear determinations per sample. Using a random numbers table, five pairs of cores were randomly selected from within each slice whose distributions are shown in Table 2. It can be seen that some of the differences are rather substantial in magnitude. The range of differences within slices varied, but the average range was 3.8 lb. The most extreme shear values within the whole loin were 11.54 and 20.29 or a range of 8.75 lb.

Since only one short loin was involved in this test, further confirmation would be desirable. Some corroboration may be found in a thesis by Mjoseth (1962). He reported shear values of cooked steaks from the longissimus dorsi through the rib and loin areas. He used three 1-in. cores and three shear per core, i.e., nine shear determinations per steak. The data in one of his tables are plotted in the graph in Figure 4. The erratic jumps in the curves suggest that a significant amount of experimental error is obscuring the true condition.

In conclusion, the work reported here supports the statement of Kinsman (1960). "Observing the shear data one is impressed with the variation in shear values that occurs within a given muscle." Kinsman recommends that " . . . . the number of cores per steak or muscle should be as many as feasible to not only randomize location but also to help average out extreme values." This should also be given serious consideration.

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CONVERSION OF BOVINE MYOGLOBIN INTO MULTIPLE, CHARGE-HETEROGENEOUS SUBFRACTIONS

INTRODUCTION

IT HAS BEEN NOTED (Hapner et al., 1968; Van den Oord et al., 1969) that myoglobin isolated from a large number of animal species consists of electrophoretically-distinct, multiple components. Beef muscle myoglobin preparations have been shown (Quinn et al., 1964) to consist of at least three components. The nature of the heterogeneity, and whether it occurs in vivo or is formed during the isolation and/or detection procedures, has not yet been made clear. Akeson and Theorell (1960) reported horse myoglobins to differ in their tryptic peptide patterns. Edmunson and Hirs (1961) suggested differences in amide content among sperm whale myoglobins.

A number of other proteins occur in charge-heterogeneous form. Among these are cytochrome c (Flatmark, 1964), prolactin and growth hormone (Lewis and Cheever, 1965), lactoperoxidase (Carlstrom, 1966), carbonic anhydrase (Funakoshi and Deutsch, 1969), L-amino acid oxidase (Hayes and Wellner, 1969) and aspartate aminotransferase (Bertland and Kaplan, 1970). For several of these proteins, the mechanism of charge-heterogeneity formation has been related to deamidation. The presence of free ammonia in solutions of prolactin undergoing conversion to more negatively-charged forms was noted by Lewis and Cheever (1965). Later (Lewis et al., 1970), a correlation between the rate of ammonia liberation and the rate of conversion of prolactin and of bovine and human growth hormone at alkaline pH was demonstrated. The kinetics of the nonenzymic conversion of cytochrome c into more negatively-charged forms was followed by Flatmark (1966) and found to indicate the hydrolysis of one amide group in each conversion step. The deamidation of asparaginyl-containing synthetic pentapeptides was found by Robinson et al., (1970) and McKerrow and Robinson (1971) to occur under the relatively mild conditions commonly encountered in protein experimentation.

It is now generally assumed (Vesterburg, 1967) that the multiple forms of myoglobin are also the result of nonenzymic deamidation reactions. While it was not the primary purpose of the following study to test this assumption, the characteristics of myoglobin heterogeneity were compared to those of the deamidated protein systems mentioned. For practical purposes, it was considered more important to obtain information on the instability of myoglobin to heterogeneity conversion reactions under experimental and storage conditions used in myoglobin research.

EXPERIMENTAL

Preparation of myoglobin samples

One cow round was trimmed of visible fat, cut into small cubes, and homogenized with two parts distilled water within 3 hr of slaughter. The slurry was adjusted from pH 6.1 to pH 6.7 with NaOH and filtered through Whatman no. 4 paper under vacuum.

Since $(NH_4)_2SO_4$ has been found to affect the heterogeneity patterns of lactoperoxidase (Carlstrom, 1966) and of cytochrome c (Flatmark, 1966), myoglobin samples for this study were prepared by two different methods; one utilizing and the other avoiding the use of this salt. The former method, involving $(NH_4)_2SO_4$ fractional precipitation and Sephadex G 75 chromatography was essentially that used previously (Quinn et al., 1964), except that the heating step was eliminated. The latter method was that of Hardman et al., (1966) and employed ethanol and lead subacetate for removal of protein impurities and zinc acetate for myoglobin precipitation.

The purified myoglobin samples were concentrated by ultrafiltration under argon pressure. All operations were performed at 6–7°C and samples were stored at 4°C.

Gel electrofocusing

The resolving power of isoelectric focusing was dramatically demonstrated by Vesterberg and Svensson (1966) who completely separated horse myoglobins differing in isoelectric point by only 0.06 pH units. Their density gradient column technique, however, was considered unsuitable for this study since the time involved in preparing, focusing and eluting the column contents would have permitted relatively few assay determinations. The principle of electrofocusing was successfully adapted by several researchers (Wrigley, 1968) for use with polyacrylamide gels as pH gradient-stabilizing media.

Initially, the gel focusing procedure of Catsimpoolas (1969) was employed in this study. However, inconsistencies in the photopolymerized gel structure were frequently observed and Pastewka et al. (1970) also found fluorescent lighting to affect the electrophoretic pattern of hemoglobin. The procedure finally adopted for routine use was essentially that of Wrigley (1968). Stock solutions of acrylamide (25% in acrylamide monomer and 1.0% in N,N'methylene bisacrylamide), N,N,N',N'-tetramethylethylenediamine (TMED, 1.0%) and ammonium persulfate (0.7%) were prepared. To make 40 ml of gelling solution, 8 ml of the acrylamide solution, 2 ml of TMED solution, 2 ml of ampholine concentrate (40%, pH 6–8, LKB-Produkter AB, Sweden) and 26 ml water were mixed, the mixture degased under vacuum and 2 ml of the persulfate solution then added. The mixture was pipetted immediately into the glass electrophoresis tubes and overlaid with a small amount of water. Polymerization occurred at room temperature within 20 min.

The acrylamide and methylene bisacrylamide reagents were purified before use by the method of Loening (1967). All reagent solutions were kept at 4°C. The persulfate solution was prepared fresh weekly. The glass tubes, 10 cm in length by 0.5 cm internal diameter, were of optical quartz (O.H. Johns Scientific, Toronto). They were set upright and stoppered at the bottom as described by Davis (1964) to receive the gelling solution.

After polymerization the tubes were inserted in a compartmented, plexiglas apparatus made essentially as described by Watkin and Miller (1970). Unpolymerized liquid was removed from both ends of the gel cylinder. The upper, or anode chamber, was filled with 0.1N HCl and the bottom with 0.4% ethanolamine. Approximately 20 µl of protecting ampholine solution (5% sucrose and 8% ampholine) was layered over the surface of the gel under the HCl electrolyte. The sample, diluted with loading ampholine solution (12.5% sucrose and 10% ampholine) was carefully injected between the gel surface and the protecting ampholine solution. The gels were maintained at 3–4°C by circulating cold water while electrofocusing was performed at an initial voltage of 25v per tube and, after 30 min, at a voltage of 37.5v per tube. Electrofocusing was completed in about 3 hr.

Isoelectric point determination

For the routine identification of the resolved myoglobins by their pI values the gels were removed from their tubes by rimming with a hypodermic needle while injecting water between the glass and the gel and the gel segments containing the visible myoglobin bands were excised and soaked in 0.6 ml distilled water. After at least 1 hr the pH of the solutions were determined by a Radiometer pH meter using the expanded scale and a micro combination electrode. Readings were taken at room temperature and no attempt was made to exclude atmospheric CO_2. For the more precise determination of pI values gels were made with 3% ampholine concentrations.

Nomenclature of the multiple myoglobins

The myoglobins were labelled by Roman numerals in order of their increasing acidities or negative charge. The myoglobin fraction of least mobility on gel electrophoresis at alkaline

pH values was, thus, labelled Mb I. This myoglobin was the fraction closest to the cathode end of gel electrofocusing patterns, ie., had the highest isoelectric point. Mb I was also the fraction present in greatest amounts in the original myoglobin sample preparations.

Spectrophotometric scanning and quantitation

The tubes were removed from the electrophoretic apparatus and the gels, still in their tubes, were immediately mounted and scanned at a rate of 2 cm per minute using a Gilford model 2400 spectrophotometer with gel scanning attachment. A slit width of 0.05 mm was used. The wavelength of the visible spectrum was selected depending upon the myoglobin heme derivative under measurement. Absorbancy was recorded at a rate of 2 in. per min utilizing, as much as possible, the full 10-in. width of chart paper.

The absorbancy areas on the recording paper were cut out, desiccated overnight, and weighed on a semi-micro balance. The weights of the various absorbancy areas recorded from a single gel were then used to calculate the relative proportions of the corresponding myoglobin fractions resolved in that gel.

To demonstrate the reproducibility of the quantitation procedure over a wide concentration range, a freshly-prepared myoglobin sample containing a large percentage of Mb I and a small percentage of Mb III was electrofocused at 16 different concentrations. The concentration range was reflected in Mb I chart peak height absorbancies ranging from 0.415–2.340. Peak areas were then used as described to calculate the relative proportions of the two fractions resolved in each of the 16 gels.

Mb I conversion rate assay conditions

Myoglobin samples were incubated at 1% concentrations in buffers of various pH values and at three different temperatures (see RESULTS & DISCUSSION). The effect of urea on the conversion rate was also studied. Buffers of pH 8.0 and less were made with phosphate, those of pH 9 and 10 with carbonate. All incubation solutions were made 1×10^{-3}M in KCN and 1×10^{-4}M in $K_3Fe(CN)_6$ to ensure uniformity among fractions as to their heme derivatives and to inhibit microorganism growth.

At designated times during the 7 wk incubation period aliquots of 0.1 ml were removed and mixed with 0.2 ml of the sucrose-ampholine loading solution. Within an hour, 10 μl of the mixture was loaded onto each of 2 gels and electrofocusing was begun. The percentage of Mb I relative to the total myoglobin concentration was determined for each of the duplicate focused patterns and an average value calculated. The use of percentage values made it unnecessary to begin with samples containing only Mb I. The initial percentage of Mb I in most of the incubation solutions was 84.2%.

The gels and the ethanolamine electrolyte solution used in the conversion assays were also made 1×10^{-3}M in KCN and 1×10^{-4}M in $K_3Fe(CN)_6$.

RESULTS & DISCUSSION

Preparation procedures

Hardman et al., (1966) found the zinc-ethanol procedure to yield preparations of sperm whale myoglobin almost entirely in the oxygenated state and with unusual stability to autoxidation. Similar results were found for the bovine myoglobin preparation. 12 days after muscle homogenization the zinc-ethanol preparation was calculated by the method of Broumand et al., (1958) to be 92% in the oxygenated state. In contrast, the myoglobin of an unfractionated muscle extract, stored for the same duration, had decreased in its oxymyoglobin content to 45%. The $(NH_4)_2SO_4$ fractionation procedure yielded myoglobin almost completely oxidized to the ferric state.

Since the electrofocusing assay procedure itself excludes contaminating proteins the preparative procedures were not designed to rigorously purify the myoglobin. It was noted, however, that the $(NH_4)_2SO_4$-Sephadex preparation contained significantly more hemoglobin than the zinc-ethanol preparation.

Contrary to observations reported on cytochrome c (Flatmark, 1966) and on lactoperoxidase (Carlstrom, 1966), the presence of $(NH_4)_2SO_4$ exhibited no apparent effect on the heterogeneity patterns of myoglobin. After six weeks at 4°C the proportion of Mb I relative to the total myoglobin concentration was 83% for the $(NH_4)_2SO_4$ preparation, 80% for the zinc-ethanol preparation and 85% for an unfractionated muscle extract.

The processes of freezing and of freeze-dehydration did not affect the myoglobin heterogeneity patterns. Samples were frozen and thawed and also freeze-dehydrated and reconstituted without apparent conversion of Mb I to charge-heterogeneous forms.

Gel electrophoresis

The assay of the conversion reactions of myoglobin required a method of quickly resolving the various fractions for their individual quantitation. The well-established technique of polyacrylamide gel electrophoresis was tested first because of its high resolving capability (Davis, 1964), and because the gels are transparent to visible radiation, permitting direct, in-gel spectrophotometric measurement.

Both the disc method of Davis (1964) and the gel slab method of Raymond and Nakamichi (1962) proved capable of readily resolving myoglobin samples into several component bands. Neither meth-

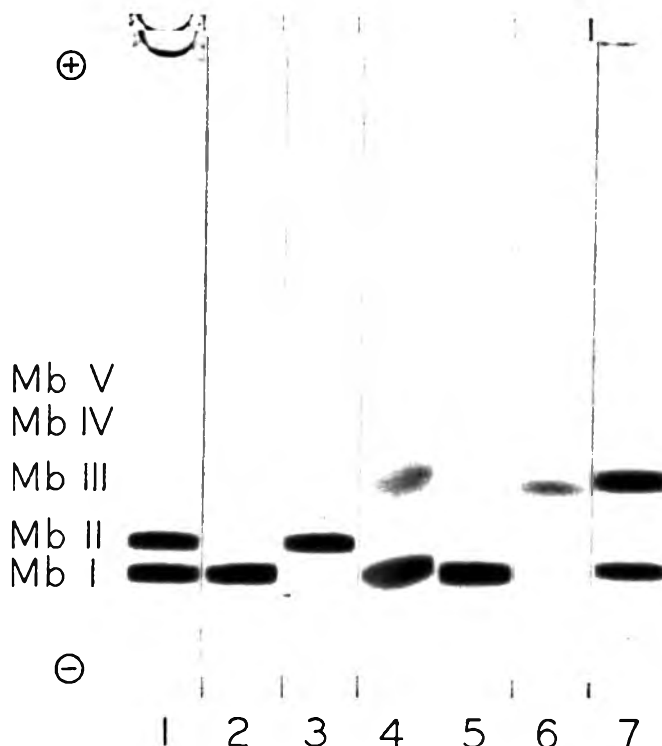

Fig. 1—Photograph of electrofocused gels in their glass tubes. The bands have the bright red color of cyanometmyoglobin. Gel patterns 2, 3, 5 and 6 were developed from gel segments, excised from previously focused gels, placed on top of new gels and re-focused. Gels 1 to 6 contain samples assayed in this study. Gel 7 demonstrates the pattern of an aged sample held in 4°C storage for over 3 yr as freeze-dehydrated material. For purposes of illustration, the samples were focused at much higher concentrations than normally used.

od, however, sufficiently separated the components for satisfactory quantitation. Many modifications of both methods were tested but offered no improvement. More importantly, the resolved bands, when subjected to re-electrophoresis under exactly the same conditions, always exhibited further heterogeneity, ie., the presence of bands migrating faster than the main component.

Although the resolved bands were heterogeneous, there was a correspondence between the electrophoretic pattern and the pattern resulting from electrofocusing. That is, the major component of the slowest migrating band was Mb I as identified by electrofocusing. The correspondence was proven to hold to at least the fourth band, or Mb IV. Thus, while electrophoresis proved inadequate for purposes of quantitation, it was suitable for use in the procedure of Hedrick and Smith (1968) to distinguish between size and charge isomerism. This procedure demonstrated that the relative mobilities of Mb I, Mb II, Mb III and Mb IV were unaffected by gel concentration (5, 8, 11 and 14% acrylamide) and, hence, these myoglobins were of equal molecular size.

Gel electrofocusing

The electrofocusing procedure consistently produced myoglobin resolution patterns suitable for quantitation. As seen in Figures 1 and 2, the fractions were separated into narrow band widths with sufficient distance between them to permit delineation of their individual spectrophotometric curves.

Figure 1 also demonstrates the homogeneity of the resolved fractions. Gel 1 displays the pattern of a pH 8, 37°C, 6-wk incubate. Gels 2 and 3 show the re-focused patterns of the Mb I and Mb II bands, respectively, isolated from a duplicate of gel 1. Gel 4 contains the pattern of a pH 10, 37°C, 6-wk incubate and gels 5 and 6 the re-focused patterns of Mb I and Mb III bands, respectively, isolated from a duplicate of gel 4. It is evident that, unlike gel electrophoresis, the electrofocusing procedure, itself, did not produce heterogeneity.

Under the conditions of the electrofocusing procedure, the myoglobins attained maximum resolution at about 3 hr. The focusing could be continued an additional several hours without affecting the pattern. Eventually, however, the cathodic migration of the pH gradient transports the myoglobin bands into contact with the alkaline electrolyte, causing denaturation.

Besides being easy-to-use and yielding excellent resolutions the electrofocusing technique offers the additional advantage of protein identification by simple pH measurement of isoelectric point.

Isoelectric points

Table 1 presents the pI values of the myoglobin fractions separated by electrofocusing. A sample of bovine hemoglobin (Hb, 2x crystallized, Sigma Chemical Co.) was electrofocused under the same conditions as used in the incubation studies and shown to have a pI value sufficiently distant from the CN metmyoglobins so as not to interfere in their identification or quantitation.

Van den Oord et al., (1969) reported the main component (Mb I) of bovine metmyoglobin to have a pI of 7.01 at 20°C. The value 7.05 reported herein is the average value of eight analyses which are seen to deviate from the mean to a greater extent than measurements on other fractions. There is an increased possibility of atmospheric CO_2 interference (Vesterberg, 1967) as the alkalinity of the test solution increases.

Van den Oord et al., (1969) also reported pI differences of 0.27 and 0.18 pH units between their first and second and between their second and third fractions, respectively. Although these myoglobins were focused as metmyoglobins and those studied here as cyanometmyoglobins, the pI differences between the respective fractions of the two systems should be similar. It is quite probable that the second fraction of Van den Oord et al. (1969) corresponds to the Mb III fraction of this study. As will be discussed later, Mb II was detected only under certain incubation conditions.

As shown in Table 1, the oxidation of ferrous myoglobin to metmyoglobin causes an increase in pI value of 0.21 pH units. Vesterberg (1967) reported the oxidation of horse myoglobin to be accompanied by a pI change of 0.50 pH units. Whether the pI change is due solely to the change in the valancy of the iron, however, is not established. Protein conformational changes as a result of the heme transformation may well contribute to the net charge difference. In agreement with Vesterberg (1967), the oxygenated form of ferro myoglobin was found to have the same pI as the deoxygenated form (Quinn, 1972).

Rate of Mb I conversion to charge-heterogeneous subfractions

The experiment designed to test the reproducibility of the assay procedure yielded 16 estimates of the Mb I percentage having a standard deviation of 1.3%.

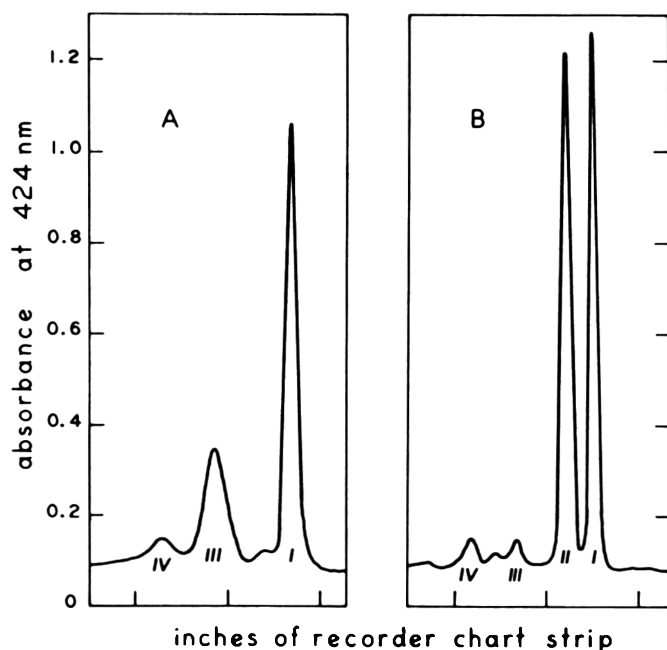

Fig. 2–Spectrophotometric scans of gel electrofocused myoglobin patterns. Part A is the chart recording of a pH 10, 37°C, 6 wk incubate and B is the recording obtained from a pH 8, 37°C, 6 wk incubate.

Table 1–Isoelectric points of myoglobin fractions

Myoglobin		pI[a,b]	Std dev
CN met	Mb I	6.81	0.012
	Mb II	6.70	0.014
	Mb III	6.59	0.022
	Mb IV	6.43	0.014
	Mb V	6.37	0.011
ferro	Mb I	6.84	0.014
met	Mb I	7.05	0.045
CN met	Hb	7.08	–

[a]pH measurements taken at 24–26° C
[b]Average of single determinations on eight gels

The content of Mb I as a percentage of the total myoglobin was constant throughout the wide concentration range used, indicating that possible ampholine-protein interactions (Kaplan and Foster, 1971) were not involved. The sample used in the reproducibility-testing experiment contained only two myoglobin fractions. During the later stages of the incubation period, as other fractions appeared, it became more difficult to extrapolate the absorbancy base line through the pattern recordings. The duplicate values of Mb I percentage were, hence, less precise as the heterogeneity reaction proceeded.

Table 2 presents the conditions of incubation and the half-life of Mb I under those conditions. The half-life is presented, rather than the rate constant, since it was found that the conversion reaction did not follow the expected first-order kinetics under all conditions. Under conditions where 50% or more of the initial percentage of Mb I was converted before the end of the incubation period (7 wk) the half-life (T½) was estimated directly from a plot of percent change versus time. Where less than 50% of the Mb I was converted, the T½ was calculated from the first-order rate constant. These slower conversion rates, encountered under the milder incubation conditions, were found to follow first-order kinetics, at least until the end of the measurement period.

The kinetic data presented in Table 2 represent single kinetic runs. Trends should, therefore, be noted rather than absolute values. In agreement with the studies on cytochrome c (Flatmark, 1966) and on prolactin and growth hormone (Lewis et al. 1970) the conversion of Mb I, within a given buffer system, increased with increasing pH and temperature.

Table 2–Half-life of Mb I under various conditions

Incubation	conditions[a]	T½ days
pH	6, 7°C	729
	6, 23°	252
	6, 37°	78
pH	7, 7°C	635
	7, 23°	175
	7, 37°	35
pH	8, 7°C	368
	8, 23°	164
	8, 37°	28
pH	9, 7°C	143
	9, 23°	128
	9, 37°	29[b]
pH	10, 7°C	239
	10, 23°	106
	10, 37°	80
	10, 37°, 0.6μ	36
pH	7, 7°C, 4M urea	155
	7, 7°, 8M urea	64
pH	10, 7°C, 4M urea	87
	10, 7°, 8M urea	29

[a] All incubations done at 0.1μ unless otherwise noted

[b] Protein precipitation noted

The buffer ion effect was marked. At 37°C, the conversion was faster in the phosphate buffers than in the pH 10 carbonate buffer. Phosphate buffer has been found (McKerrow and Robinson, 1971) to promote the deamidation of synthetic asparaginyl peptides.

The fact that shorter T½ periods were found at pH 9 (7° and 37°C) than at pH 10 is difficult to explain except by postulating denaturation or contamination. Visible precipitation was noted at pH 9, 37°C.

That individual proteins vary markedly in their susceptibilities to the charge-conversion reaction is evident in the literature. At 37°C and pH 10, prolactin had a rate constant (k) of 11.4×10^{-6} per second (T½ of 16.9 days) and bovine growth hormone a k varying from $0.9-2.1 \times 10^{-6}$ per sec., depending on the preparation (Lewis et al., 1970). At 37°C and pH 9.9, cytochrome c had a k of 1.87×10^{-6} per sec. (Flatmark, 1966).

That individual proteins vary with respect to the effect of various incubation variables on their conversion rates is, also, not unexpected. For example, at 37°C in phosphate buffer, the T½ values of myoglobin and prolactin were similar (35 and 32 days, respectively), but at 37°C in carbonate buffer the T½ values were 80 days and less than 1 day, respectively. Evidently, myoglobin is much less sensitive to alkalinity than prolactin.

From a practical viewpoint, it is seen that myoglobin is quite stable to charge-heterogeneity conversion under most conditions and may safely be kept in solution at low temperature for lengthy periods of time. The results also indicate that myoglobin would have reacted only to a slight extent during its extraction from muscle and purification at 6–7°C. Thus, the approximately 15% of converted forms found in the initial samples probably occurred in vivo. It should be noted that all samples used in the kinetic study were from the same animal. Possibly, the initial extent and, also, the rate of conversion would vary among animals.

Mechanism of the conversion reaction

As was stated, the Mb I conversion reaction did not always follow first-order kinetics. The deamidation reactions of asparaginyl and glutaminyl residues, however, are first-order (Flatmark, 1966; Robinson et al., 1970). While there is no direct evidence that the myoglobin charge-conversion reaction is due to nonenzymic deamidation, there appears no alternative explanation. The conversion is directed only towards increasing numbers and amounts of more negatively-charged fractions; that is, reversibility is never observed. The fractions do not vary in molecular weight. Similar reaction rate dependencies have been noted for other proteins, in which NH_3 evolution has been related to the reaction rate, and for synthetic peptide deamidations. The deviation from first-order kinetics, reflected in increasing rates during the latter stages of conversion under extreme incubation conditions, may well be caused by time-dependent protein structural changes with resultant increased exposure of the reactive amide group.

That protein conformation is important to the reactivity of incorporated amide groups is demonstrated by the fact that relatively few charge-hetereogeneous forms of the protein exist in relation to the total number of glutaminyl and asparaginyl residues present. The effect of urea on the rate of conversion of Mb I presents evidence of the importance of tertiary and secondary structure to amide reactivity.

As well as polypeptidyl configuration, the primary structure (sequence) is also rate-determining. Robinson et al., (1970) found the deamidation T½ values of 5 synthetic asparaginyl-containing pentapeptides, differing only in the amino acids next to the asparaginyl residue, to vary from 11 days to 3 months.

Figures 1 and 2 depict the two different heterogeneity patterns encountered in this study. The pH 10, 37°C pattern (little, if any, Mb II) was the type found under most of the incubation conditions. Mb II was never detected, in other than trace amounts, in myoglobin samples stored under refrigeration either in solution or as freeze-dehydrated material.

Mb II attained significant proportions during the incubations at pH 6 to 9, but only at the elevated temperatures employed. At 37°C, Mb II eventually attained higher concentrations than Mb I while, at 23°C, it occurred to a relatively smaller degree. Mb II did not occur in solutions incubated at 7°C, irrespective of pH, or in solutions at pH 10, irrespective of temperature. Mb III is formed from Mb II. This was established by incubating an electrofocused gel segment containing only Mb II and noting its gradual conversion to Mb III.

While it is possible that Mb II is a conformational variant of Mb I and Mb III is, then, the first deamidated protein, it seems more likely, based on similar net charge differences between Mb I and Mb II and between Mb II and Mb III, that Mb II has undergone one amide hydrolysis and Mb III two. Possibly, the first and second reactive amides have different responses to temperature and pH change, due to different responses of their respective polypeptidyl microenvironments.

Thus, at low temperature, the reactive Mb II amide would be hydrolyzed at a rate such that Mb II is not accumulated. But, at high temperature, because of the relatively greater temperature stability of its microenvironment, the Mb II amide would be hydrolyzed relatively less readily than the Mb I amide. It could also be postulated that highly alkaline conditions eliminate these temperature-induced conformational differences.

Obviously, a great deal more work is required to establish the mechanisms of myoglobin charge-heterogeneity reactions. No further work, however, is planned for this laboratory.

An interesting biological role for protein deamidation has been postulated by Robinson et al., (1970). Showing a correlation between the percentage of asparaginyl and glutaminyl residues in a protein and the biological half-life of that protein, they implicate deamidation reactions as providing a mechanism by which molecular and organismic development and aging are controlled. The biological half-lives of rabbit hemoglobin and human hemoglobin were reported to be 25–35 days and 60 days, respectively, and their amide amino acid percentages to be 6.3 and 4.9, respectively (Robinson et al., 1970). Coincidentally, bovine myoglobin has an amide amino acid percentage of 5.2 (Han et al., 1970) and, as reported in this study, a half-life at pH 7.0, 37°C of 35 days.

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PRE-RIGOR PRESSURIZATION OF MUSCLE: EFFECTS ON pH, SHEAR VALUE AND TASTE PANEL ASSESSMENT

INTRODUCTION

TENDERNESS is an important attribute of meat affecting its acceptability as a food. It is greatly influenced by the state of the connective tissue and the contraction state of the fibrillar proteins (Bouton and Harris, 1972a, b; Marsh and Leet, 1966). Hydrostatic pressure has been reported to influence the development of tension in muscle (Johnson et al., 1954; Brown, 1957; Johnson and Eyring, 1970), the tension being increased or decreased according to the temperature at which the muscle is pressurized. The tension-temperature relationship appears to be influenced by the animal species from which the muscle is obtained. Also, the chemical processes that occur in muscle have been reported to be influenced by pressure (Deuticke and Ebbecke, 1937).

From the studies referred to above it appeared that pressure applied to muscle before the onset of rigor could influence subsequent biochemical reactions and the contraction state of muscle, and consequently might affect the eating quality of meat. This paper reports the results of studies in this area which have been carried out on sheep and ox muscles.

EXPERIMENTAL

SHEEP were slaughtered at the laboratory and cattle at a nearby abattoir. After skinning, evisceration and washing operations, the muscles required were removed from the carcasses, cut to a size which fitted the pressure vessel, vacuum sealed in a polyethylene bag and placed in a water-bath at the same temperature as the pressure vessel. A relatively large bag was used for packing to avoid constricting the muscle during contraction or relaxation. The time required for temperature equilibration was usually 20–30 min. For each temperature this was checked by placing a thermocouple inside muscle samples of approximately the same dimensions as those to be treated. Sometimes muscles for pressurization were removed from only one side of the carcass so that they could be compared with the corresponding muscles, hereafter referred to as "standard" muscles, removed from the other side of the carcass post-rigor. Carcasses were hung from the Achilles tendon; sheep carcasses were held at 2°C; ox carcasses were processed and chilled in the normal manner at the abattoir.

For pressurizing samples, three pressure vessels were used. Two had chambers 2.54 cm in diameter and 7.62 cm long. The chamber of the other was 7.62 cm in diameter and 23.76 cm long. Pressure vessels were placed in individual water baths with temperature regulated to within ± 0.5°C. The working fluid in the small vessels was water and in the large vessel, water and water-soluble oil in the ratio 64:1. Unless stated otherwise, subsequent to pressurization samples were stored at 2°C.

For the measurement of pH, muscle samples were blended in 0.005N iodoacetic acid neutralized with sodium hydroxide. When it was desired to measure pH immediately after pressurization a time delay of 2–3 min between releasing pressure and blending the samples in the sodium iodoacetate solution was unavoidable. The ultimate pH was measured 2 days post-slaughter unless stated otherwise.

Taste panel assessments were carried out using expert laboratory panels of eight and of six members for the tests on ox and sheep muscles respectively. For these assessments, meat samples of nearly uniform dimensions (10–15 cm × 5 cm × 2 cm) were cooked for 20 min in an oven at 232°C with forced air circulation. Internal temperature after cooking was 75°C approximately.

Measurements of Warner-Bratzler (WB) shear values and cooking losses were carried out in a manner similar to that described by Bouton et al., (1971). Samples were cooked for 1 hr at 90°C. Moisture content of cooked samples was

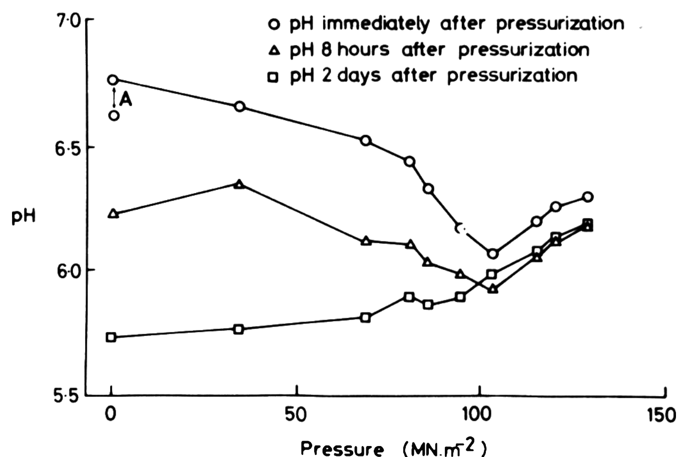

Fig. 1—pH changes in sheep semimembranosus muscle following pressurization at 30°C for 4 min. (A) Change in pH of controls over the period samples were being pressurized (approximately 1 hr).

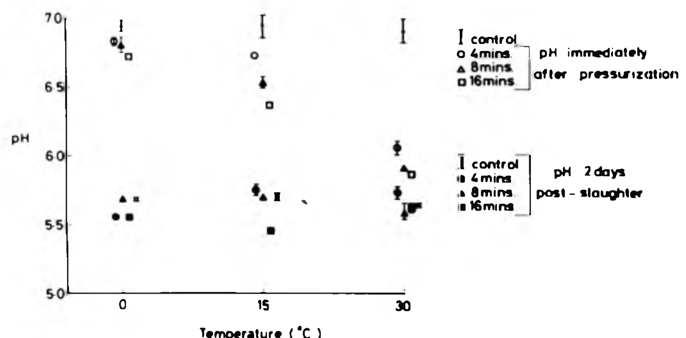

Fig. 2—Effect of temperature during pressurization and duration of pressurization at 103.5 MNm^{-2} on the pH of sheep semimembranosus muscle.

measured as the weight lost when samples were dried at 105°C for 18 hr. Fluid loss, or weep, from raw samples during storage was estimated from the weights of samples before and after storage. It is expressed as a percentage of the initial weight. Muscle shortening was estimated from the change in the distance between two pins inserted in the muscle near the opposite ends of fibers. Kymograph studies were carried out using two instruments of similar design to that described by Bate-Smith and Bendall (1949), under similar conditions to those described by Cassens and Newbold (1967).

RESULTS

Effect on pH

The changes in muscle pH following pressurizing were found to be dependent on pressure, temperature during pressurization and duration of pressurization. These effects are illustrated in Figures 1 and 2 for sheep semimembranosus muscle and Figure 3 for ox longissimus dorsi muscle. The values in each figure were obtained with samples from the one muscle. The experiments have been repeated several times and except as noted below with respect to Figure 1, have all given similar results. In Figure 1 the maximum pressure-induced change in pH is shown at 103.5 meganewtons/m^2 (MNm^{-2}), with a concomitant increase in ultimate pH. However, this effect was not always observed in sheep muscles and has not been observed in ox muscles. In the latter the pH change resulting from pressurization continued to increase with increase in pressure up to 138 MNm^{-2}, the highest pressure used. As indicated in Figures 2 and 3, the ultimate pH of the samples was similar to that of nonpressurized samples.

The pH drop in samples pressurized at 0°C was small and the magnitude of the effect increased as the temperature during pressurization increased, as can be seen from Figure 2. In these experiments, the duration of pressurization ranged from 4–16 min; at 30°C, most of the effect was achieved by 4 min. It can be seen from Figure 3 that pressurization of ox longissimus dorsi muscle to 103 MNm^{-2} and higher resulted in a large pH drop and during subsequent storage the pH of the muscle dropped rapidly to near its ultimate value. The effect of pressurizing on the pH of five muscles from each of six sheep can be compared from the data given in Table 1. Except for the semitendinosus muscle, a similar pressure-induced pH drop was found in all the muscles.

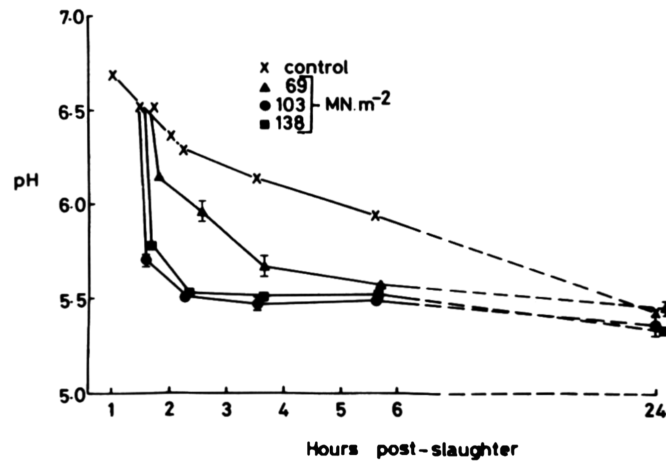

Fig. 3–The effect of pressurization at 35°C for 4 min on the pH of ox longissimus dorsi muscle subsequently stored at 25°C.

Table 1–Effects of pressurizing[a] on the pH[b] of various sheep muscles

Muscle	Mean[b] pH before pressurizing	Pressure induced pH drop	Ultimate pH pressurized	Ultimate pH standard
Semimembranosus	6.78 (0.05)	0.84 (0.07)	5.58 (0.07)	5.48 (0.02)
Longissimus dorsi	6.84 (0.04)	0.93 (0.07)	5.50 (0.10)	5.51 (0.03)
Biceps femoris	6.79 (0.04)	0.72 (0.08)	5.84 (0.05)	5.62 (0.03)
Semitendinosus	6.57 (0.06)	0.50 (0.11)	5.82 (0.08)	5.70 (0.10)
Adductor	6.74 (0.05)	0.83 (0.09)	5.62 (0.07)	5.48 (0.02)

[a]Conditions of pressurizing: 103 MNm^{-2} at 30–35°C for 2–4 min
[b]Values for each muscle are the means from 6 animals. Standard errors are given in brackets.

Kymograph investigations

The pH drop induced by pressurization of pre-rigor muscle at 30°C to 103 MNm^{-2} for 4 min indicated that post-mortem glycolysis had been greatly accelerated by pressurization. To investigate the effect of this treatment on the development of rigor, a sample of sheep semimembranosus muscle and a control sample from the same muscle, were attached to kymographs directly after completion of pressurization. Their extension-relaxation response to the periodic application and removal of a 25g load was followed at 21°C. The nonpressurized sample showed the expected shortening and loss of extensibility within a few hours. However, the pressurized sample showed an almost constant extensibility intermediate between that for pre- and in-rigor control muscle, over the two-day period that observations were made. Over this time there was an overall length increase. Similar results were obtained for two other experiments.

Warner-Bratzler (WB) shear values

Sheep. Warner-Bratzler shear measurements of the cooked meat indicated that under some treatment conditions the pressurized muscle was more tender than the corresponding standard muscle. Marked effects were obtained at pressures of 90 MNm^{-2} up to the highest investigated, 138 MNm^{-2}; temperatures above 25°C up to the highest investigated, 40°C; and times of pressurization of 1 min up to 8 min, the longest time used for these experiments. Comparable pressurization of post-rigor muscle was not found to affect tenderness. No effort has been made to establish the conditions required to yield the optimum effect, but the data presented in Table 2 for the ratio of Warner-Bratzler shear values of pressurized to those of standard samples illustrate the differences in the response to pressurization of various muscles. Thus values exceeding unity were usually obtained from semitendinosus muscles whereas all other muscles gave values less than unity. The pressure effect appeared to be greatest on the longissimus dorsi muscle although the value quoted is the mean from only three animals.

Ox. The WB shear values for a number of muscles from an ox carcass pressurized pre-rigor are presented in Table 3 along with those for standard muscles removed post-rigor. Also, the results from pre-rigor excised, but nonpressurized muscle (control sample) are included for comparative purposes.

The treated samples were pressurized at 103 MNm^{-2} for 2 min at 35°C 2½–3½ hr post-slaughter. On completion of pressurization, control and treated samples were held at 2°C for 2 days before cooking. The relatively high shear values of the control samples presumably reflects the

effect of cold shortening which is known to increase the toughness of meat (Marsh and Leet, 1966).

The range of values obtained for various measurements on pressurized, control and standard ox biceps femoris muscles obtained from four animals is given in Table 4. The WB shear results indicate pressurizing had a substantial tenderising effect. Shortening in the raw muscle was more marked in the pressurized than the control (cold shortened) samples. However, shortening as a result of cooking was least in tht pressurized samples. Although there was slightly more weep from the pressurized than from the other samples, the combined cooking and weep losses indicate there was greater moisture retention by the pressurized samples. This is confirmed by the estimate of the moisture content of the various samples following cooking.

Taste assessment

One set of longissimus dorsi and semimembranosus muscles was removed shortly after slaughter from each of 15 wethers, 4–5 yr old, and pressurized. Following pressurization, samples were stored at 5°C for 2 days together with the carcasses containing the standard muscles. The cooked samples were then assessed by a taste panel for tenderness, juiciness and acceptability. The results are reported in Table 5. Similarly, assessments were made on pressurized and standard samples from the longissimus dorsi muscle of five beef animals and the results are presented in Table 6. All pressurization treatments were found to improve the tenderness of the meat. Although there was an apparent decrease in juiciness, the pressurized samples were judged more acceptable than the standard samples. Even pressurization for 1 min gave a marked effect on the muscle.

Table 2–Mean ratios for Warner-Bratzler shear values of pressurized[a] and standard samples for various muscles from five sheep

Muscle	Mean value of WB press/WB std[b]	Standard error
Semimembranosus	0.57	0.13
Biceps femoris	0.42	0.12
Adductor	0.46	0.11
Longissimus dorsi	0.15	–[c]
Simitendinosus	1.31	0.31

[a]Conditions of pressurizing: 103–138 MNm^{-2} at 30–35°C for 2–4 min

[b]WB press/WB std: ratio of the Warner-Bratzler shear value of the pressurized sample to that of the standard

[c]Mean from muscles of three animals only; the individual values are 0.15, 0.09, 0.21.

Table 3–Warner-Bratzler shear values of standard, control and pressurized beef muscles

Muscle[a]	Treatment[b]	pH Before press.	pH After press.	pH Ultimate	Mean Warner-Bratzler values[c] (kg)
LD	C	6.48		5.31	17.14 (0.58)
	P		5.77	5.26	2.16 (0.03)
	S			5.34	15.1 (0.75)
BF	C	6.53		5.23	12.55 (1.35)
	P		5.73	5.25	2.47 (0.13)
	S			5.34	7.51 (0.43)
ST	C	6.57		5.44	14.87 (1.84)
	P		5.64	5.27	2.41 (0.08)
	S			5.54	5.56 (0.19)
A	C	6.52		5.41	17.09 (0.63)
	P		5.68	5.40	4.65 (0.45)
	S			5.37	8.06 (0.51)
SM	C	6.64		5.48	17.82 (0.87)
	P		5.73	5.30	3.0 (0.24)
	S			5.37	9.08 (0.42)
GM	C	6.25		5.25	16.46 (0.62)
	P		5.66	5.21	1.82 (0.12)
	S			5.26	8.83 (0.69)

[a]LD–longissimus dorsi; BF–biceps femoris; ST–semitendinosus; A–adductor; SM–semimembranosus; GM–gluteus medius

[b]C–control; P–pressurized at 103 MNm^{-2} and 35°C for 2 min, 2½–3½ hr post-slaughter; S–standard

[c]Data for control samples obtained from six measurements, for pressurized samples from 24 measurements, and for standard samples from 12 measurements. The standard error is given in brackets.

DISCUSSION

DURING THE course of these investigations it became apparent that many variables could affect the response of muscle to pressure; e.g., the actual pressure applied, the temperature during pressurization, the duration of pressurization, possibly the rate of pressure increase or decrease, restriction on the freedom of the muscle to contract, the time delay between slaughter and pressurization, the muscle pressurized and the animal species from which the muscle was obtained. The purpose of the present investigation was not to explore in detail the effect of, and the interaction between, these variables. Rather it was to show that conditions exist under which pressure applied to prerigor muscle can lead to a change in structure that may improve tenderness of meat.

The studies of pH changes indicate that under suitable conditions, e.g., muscle at 25–35°C pressurized to 103 MNm^{-2} for 2–4 min, glycolysis was virtually complete shortly after the finish of pressurization. On removal of muscle subjected to such treatments from the pressure chamber, it was seen to be contracted but no marked length changes occurred during subsequent storage (Table 4). The kymograph studies also indicated that such muscle either was effectively in rigor on removal from the pressure vessel or that myofilaments were so severely disrupted they were unable to exert a restoring force through the muscle length. A study of the pressurized muscle using an electron microscope, to be reported in a future publication, revealed pressurization resulted in extensive breakdown of myofilaments. This would account for the greater extensibility of the pressurized muscle compared to the 'in-rigor' control.

Of the sheep muscles investigated the data in Table 2 indicate that beneficial effects on tenderness usually were not apparent with the semitendinosus muscle. From Table 1 it is seen the pressure-induced pH drop of this muscle was least, and its pH at the commencement of pressurizing was usually lower than that of the other muscles investigated. These effects may be connected with a more rapid rate of rigor development in this muscle

Table 4—Range of values for various measurements on pressurized, control and standard ox biceps femoris muscles from four animals

Measurement	Treatment		
	Control	Standard	Pressurized[a]
Ultimate pH	5.61 ± 0.08	5.47 ± 0.04	5.49 ± 0.12
pH cooked meat	5.90 ± 0.10	5.69 ± 0.05	5.71 ± 0.23
Pressure-induced shortening, %			35.0 ± 3.2
Total % shortening before cooking	24.5 ± 8.5		37.4 ± 4.9
Shortening due to cooking[b], %	14.9 ± 2.5	16.4 ± 1.0	7.5 ± 2.1
Weep, %	1.3 ± 1.0[d]	1.0 ± 0.3[c]	2.0 ± 0.8[d]
Cooking loss, %	35.3 ± 2.3	38.4 ± 3.0	31.1 ± 1.8
Moisture content cooked meat, %	59.0 ± 2.2	57.3 ± 2.5	62.2 ± 1.5
Warner-Bratzler shear value cooked meat (kg)	13.57 ± 1.32	7.95 ± 1.82	4.11 ± 0.84

[a]Conditions of pressurizing: 103 MNm^{-2} at 35°C for 2 min
[b]Measured on three samples only
[c]After 1 day storage; muscles removed from carcass 1 day post-slaughter
[d]After 2 days' storage

Table 5—Taste panel scores for tenderness, juiciness and acceptability of pressurized and non-pressurized (standard) longissimus dorsi and semimembranosus muscles of sheep

Property	Mean taste-panel score[d]			
	Semimembranosus		Longissimus dorsi	
	Standard	Pressurized[e]	Standard	Pressurized[e]
Tenderness[a]	3.2	5.2	4.5	7.4
Juiciness[b]	6.8	6.2	6.6	5.8
Acceptability[c]	5.0	6.6	6.1	8.3

[a]9 point scale: 1—very tough; 9—very tender
[b]9 point scale: 1—very dry; 9—very juicy
[c]9 point scale: 1—nauseating; 9—acceptable in preference to others
[d]Mean of results from 15 animals; standard errors for the various assessments were: tenderness 0.26, juiciness 0.091, acceptability 0.22. The differences between standard and pressurized samples were all highly significant.
[e]Conditions of pressurizing: 83–138 MNm^{-2}, 30–35°C, 4 min

Table 6—Taste-panel scores for tenderness, juiciness and acceptability of pressurized and non-pressurized (standard) beef longissimus dorsi muscles

Property	Mean taste-panel score[a]			
		Samples pressurized[b] for		
	Standard	1 Min	2 Min	4 Min
Tenderness[c]	4.1 (0.22)	6.9 (0.27)	6.7 (0.15)	7.4 (0.22)
Juiciness[d]	6.0 (0.16)	5.6 (0.20)	4.5 (0.14)	5.2 (0.16)
Acceptability[e]	5.7 (0.21)	7.5 (0.24)	7.1 (0.12)	7.5 (0.21)

[a]Mean of results from five animals, standard error in brackets. The differences between standard and pressurized samples were all highly significant.
[b]Conditions of pressurizing: 103 MNm^{-2}, 35°C, 1–4 min
[c]9 point scale: 1—very tough; 9—very tender
[d]9 point scale: 1—very dry; 9—very juicy
[e]9 point scale: 1—nauseating; 9—acceptable in preference to others

than in the other muscles, as observed by van Eerd (1972) in investigations of semitendinosus and semimembranosus muscles. However, in the single experiment reported on ox semitendinosus muscle, pressurization had a tenderizing effect.

The data in Table 4 indicate that even though pressurized muscle shortened by near to 40% of its rest length, its shear value was less than one-third that of muscle contracted by about 25%. The studies of Marsh and Leet (1966) indicate that muscle shortened by about 40% of its rest length should show maximum toughness. It is apparent that by pressurization this source of toughness can be overcome.

That pressure would exert an effect on the contraction state of muscle is to be expected from the volume changes observed to occur when muscle contracts (Aradi, 1970; Baskin and Paolini, 1967; Baskin, 1967; Pasechnik, 1968; Sato, 1954). Both initial volume increases and initial volume decreases have been reported to occur when muscles are stimulated to contract and consequently the response to pressure would be expected to alter accordingly. Baskin (1967) observed that the initial volume decrease when a frog sartorius muscle was stimulated was maximal at "slack" or "end-free" lengths and decreased as the muscle was held near reference length. Thus the effect observed in the present experiments, with unrestrained muscle, might be modified if muscle contraction during pressurization were restricted.

One possible explanation for the tenderizing effect is that the myofibrillar structure is broken down by myosin filaments of the severely contracted muscle being forced into Z discs. Another possibility is that the actin filaments are weakened by a pressure-induced F-G transformation, as has been reported to occur by Ikkai and Ooi (1966).

Although the taste panels found reduced juiciness in the cooked pressurized ox longissimus dorsi muscle, measurements on ox biceps femoris muscle indicated that compared to standard or control samples, moisture retention was greatest in the pressurized samples. This may suggest that the disruption caused by pressurization results in the exposure of hydrophilic groups in the structural proteins. It is also noted that the pressurized muscle immediately following pressurization was at a relatively low pH and high temperature. This combination of conditions has been associated with pale soft exudative (PSE) muscle (Briskey and Kauffman, 1971). However the uncooked, pressurized muscle, when compared with the corresponding post-rigor standard muscle was noted (1) not to be noticeably paler in color; (2) to have a firmer feel when pressed by the fingers; and (3) not to suffer large weep losses (Table 4). Therefore there were no pronounced indications for the existence of the PSE condition in the pressurized muscle.

A pressurizing process such as that described here appears to offer a method for the rapid processing and tenderizing of meat. Following adequate pressurization of the warm pre-rigor muscle, increase in toughness as a consequence of muscle shortening apparently can be avoided.

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THE EFFECT OF CURING AGENTS, pH AND TEMPERATURE ON THE ACTIVITY OF PORCINE MUSCLE CATHEPSINS

INTRODUCTION

MANY COMPOUNDS have been identified which contribute to the basic meaty flavor and odor in all types of meats. Free amino acids have been recognized as precursors to many of the odor and flavor compounds. The concentration of free amino acids in country cured hams increases as the hams age and has been related to the flavor of the hams (McCain et al., 1968; Lillard and Ayres, 1969).

Only a small fraction of this increase in free amino acids is due to the proteolytic activity of microorganism in cured hams because of their limited growth in the decreased water content of dry cured hams (Ayres et al., 1967). The increase of amino acids in hams, therefore, may be due to the proteolytic enzymes, cathepsins, which are in the muscle tissue.

Cathepsins in various organ tissues have been studied extensively and many of their properties are known (Tallen et al., 1952; Press et al., 1960). Although cathepsins from muscle tissue have not been studied as extensively as those from organ tissues, recent work has indicated that many of the proteolytic enzymes from both types of tissue have similar properties (Tallen et al., 1952; Press et al., 1960; Parrish and Bailey, 1966; Iodice et al., 1966; Caldwell, 1970; Caldwell and Grosjean, 1971). Parrish and Bailey partially purified the major cathepsin in porcine muscle and found it very similar to cathepsin D in bovine spleen.

This investigation was undertaken to study the activity of porcine muscle cathepsins under conditions that exist in the curing and aging of country cured hams.

MATERIALS & METHODS

Preparation of enzymes

Porcine muscle tissue (loin of pork, obtained from a local retail market) was freed from all separable fats and cut into small sections. The tissue was mixed with 0.1M phosphate buffer (pH 6.0) in the ratio of two to one (v/v) and blended for 5 min at 4°C in a Waring Blendor. The meat-buffer mixture was centrifuged at 20,000 × G for 30 min and the supernatant filtered through cheesecloth to remove any remaining fat tissue. The filtrate was dialyzed for 24 hr at 4°C against deionized water. The dialyzed homogenate was again centrifuged for 30 min at 20,000 × G to remove any undissolved material and the supernatant was used as the crude enzyme preparation. The crude enzymes were fractionated with ammonium sulfate at three concentrations, 0–35%, 35–70% and 70–90% (Green and Hughes, 1960). Proteins precipitated with each salt concentration were removed by centrifugation, dissolved in small amounts of cold deionized water, dialyzed against deionized water for 24 hr at 4°C and retained for analysis. The enzymes precipitated with 35–70% ammonium sulfate were used in all experiments unless otherwise specified. The dialyzed solution of a 35–70% ammonium sulfate fraction was further purified with a DEAE-cellulose column (1.8 × 19 cm). The DEAE-cellulose column was first equilibrated with 0.005M phosphate buffer (pH 8.0). The enzymes were then eluted with phosphate buffer, pH 8.0, at concentrations of 0.005M, 0.01M, 0.05M, 0.1M, 0.15M and 0.2M. 5-ml fractions were collected at a flow rate of 45 ml per hr and the protein content of each fraction was determined by measuring the absorbance at 280 nm.

Assay of enzyme activity

The proteolytic activity of the extract was determined by using 3% salt soluble protein or 3M urea-denatured 2% hemoglobin as a substrate according to the procedure described by Sliwinski et al. (1959). The reaction mixture contained 2.0 ml of buffer solution of the desired pH (0.1M), 0.5 ml of the enzyme solution and 0.5 ml of 2% urea-denatured hemoglobin substrate. When 3% salt soluble protein was used as the substrate, 1.5 ml of buffer solution of the desired pH, 0.5 ml of the enzyme solution and 1.0 ml of 3% salt soluble protein substrate composed the reaction mixture. The substrate in buffer solution was preincubated at 37 °C for 10 min to obtain a constant temperature prior to the addition of enzyme solution. After incubating the mixture at 37°C for 90 min in a shaking incubator, the reaction was stopped by adding 3.5 ml of 10% trichloroacetic acid (TCA). The reaction mixture was centrifuged at 20,000 × G for 40 min at 4°C. Activity was expressed as the absorbance at 280 nm of the reaction mixture incubated at 37°C for 90 min and compared against a control at zero time of incubation. One enzyme unit is defined as that amount which caused a change in absorbance of 0.001 units per 90 min. Specific activity is expressed as the enzyme units per mg of protein as determined by a biuret procedure (Gornall et al., 1949).

The activity of the extract on the synthetic substrates benzoyl-L-argininamide and glycyl-L-phenylalanine were determined using the procedure described by Lutalo–Bosa and Macrae (1969). The enzyme assays in all experiments were done in duplicate.

pH optima of porcine muscle cathepsins

The urea-denatured hemoglobin and salt soluble protein were buffered to pH 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0 and 10.0 before the enzymes were added. The samples were assayed for cathepsin activity as given previously.

Effect of temperature and time on the activity of porcine muscle cathepsins

The urea-denatured hemoglobin in the buffer solution was preincubated at different temperatures in a water bath for 10 min before the enzyme was added. The reaction mixture was incubated at 20°C, 30°C, 40°C, 45°C, 47.5°C, 50°C and 55°C and the reaction was stopped by adding 3.5 ml of TCA after 10, 30, 50, 70, 90, 110, 130, 150 and 170 min incubation. The activity was expressed as the difference in absorbance at 280 nm of a reaction mixture and that of its control at zero time of incubation.

Heat stability was determined by measuring the activity of the enzymes after standing at various temperatures for different periods of time. The reaction mixture was incubated at 37°C for 90 min.

Effect of curing agents on the proteolytic activity of porcine muscle

Sodium acetate buffer (0.1M, pH 4.0) was prepared to contain the appropriate amounts of sucrose, potassium chloride and potassium nitrate. Glycine-sodium hydroxide buffer (0.1M, pH 9.0) was prepared to contain the appropriate amounts of sodium chloride. The reaction mixture contained 2.0 ml of sodium acetate buffer containing the desired amount of sucrose, potassium chloride and potassium nitrate, 0.5 ml of the enzyme solution and 0.5 ml of the 2% hemoglobin substrate or 1.5 ml of glycine-sodium hydroxide buffer containing the desired amount of sodium chloride, 0.5 ml of the enzyme solution and 1.0 ml of dialyzed salt soluble protein.

Salt stability was determined by measuring the activity of the enzymes after standing at various salt concentrations for different periods

Table 1—Proteolytic activity of enzyme solutions[a] isolated from six different samples of porcine muscle

Enzyme preparation	Absorbance[b]	Protein content mg/ml	Specific activity
1	0.101 ± 0.01	14	14.4
2	0.142 ± 0.05	18	15.8
3	0.115 ± 0.01	16	14.3
4	0.175 ± 0.05	19	18.3
5	0.116 ± 0.01	17	14.1
6	0.156 ± 0.03	19	16.2

[a]Precipitated with 35–70% ammonium sulfate
[b]Absorbance at 280 nm. Data in all tables and figures are the average of two experiments.

of time followed by dialysis to remove the salt. The reaction mixture (0.5 ml, dialyzed enzymes; 2.0 ml, pH 4.0, sodium acetate buffer; 0.5 ml, 2% hemoglobin substrate) was incubated at 37°C for 90 min.

RESULTS & DISCUSSION

THE FRACTIONATION of buffer extracts of porcine muscle was attempted with the use of ammonium sulfate at saturation levels of 35, 70 and 90%. After dialysis to remove the salt, the proteolytic activity was found mainly in the fractions precipitated with 35–70% ammonium sulfate. The enzymes in this fraction catalyzed the hydrolysis of urea-denatured hemoglobin but demonstrated very little activity on synthetic substrates for cathepsins B and C (benzoyl-L-argininamide and glycyl-L-phenylalanine, respectively). These findings agree with the results obtained by Parrish and Bailey (1966) who reported that the major cathepsin in porcine muscle was similar to cathepsin D. Six different enzyme preparations were used in this study. Only small differences in the protein concentration and specific activity were found in the six enzyme solutions (Table 1). This indicates that the proteolytic activity of the porcine muscle was similar even though the history of the tissue sample was not known.

When the 35–70% ammonium sulfate fraction was absorbed on a DEAE–cellulose column, the protein with the most enzyme activity was eluted with 0.1M, pH 8.0, phosphate buffer. This fraction from the column had a 27-fold increase in activity. The 0.1M, pH 8, phosphate buffer fraction hydrolyzed hemoglobin but showed no cathepsin B and C activity.

pH optima with denatured hemoglobin or 3% salt soluble protein as a substrate

The pH optima for cathepsin D activity against denatured hemoglobin were found at pH 4.0 and 9.0 (Fig. 1). The maximal activity occurred at pH 4.0. Therefore, the analysis used to measure the digestion of hemoglobin were run at pH 4.0. These pH optima are nearly the same as those of cathepsin enzymes isolated from various species of muscle and organ tissues. Parrish and Bailey (1966) indicated that porcine muscle cathepsin had maximal activity at pH 4.0 and two lower pH optima at pH 8.0 and 10.0. Press et al. (1960) reported that cathepsin D of bovine spleen had a pH optimum at 4.2 with albumin as substrate. Caldwell and Grosjean (1971) found a pH optimum of 4.0 for cathepsin D of chicken skeletal muscle. When 3% salt soluble protein was used as a substrate for the cathepsin, the greatest activity occurred at pH 9.0 (Fig. 1). This observation was consistent with the pH optimum found for the autolytic reaction occurring in crude and dialyzed rat muscle homogenates using a substrate of endogenous proteins (Koszalka and Miller, 1960), but does not agree with the pH optima at 3.8 and 4.8 found for the activity of catheptic enzymes in bovine skeletal muscle using endogenous protein as a substrate (Lutalo-Bosa and Macrae, 1969).

Effect of temperature and time on the activity of porcine muscle cathepsins

The activities of the enzymes incubated at different temperatures for dif-

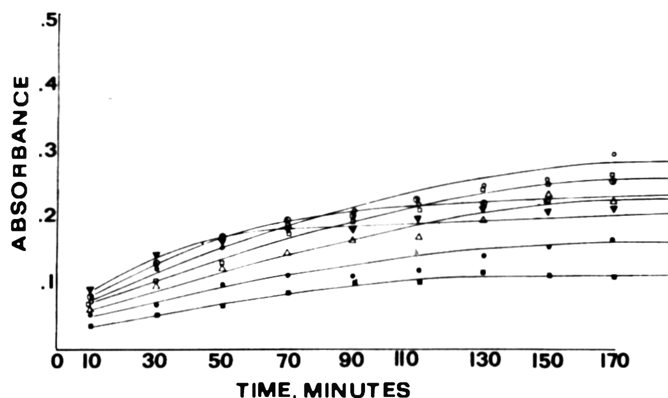

Fig. 2–Relationship between temperature and length of cathepsin reaction. ▪ = 20°C, • = 30°C, △ = 40°C, □ = 45°C, ○ = 47.5°C, ◗ = 50°C, and ▾ = 55°C. Absorbance was determined at 280 nm.

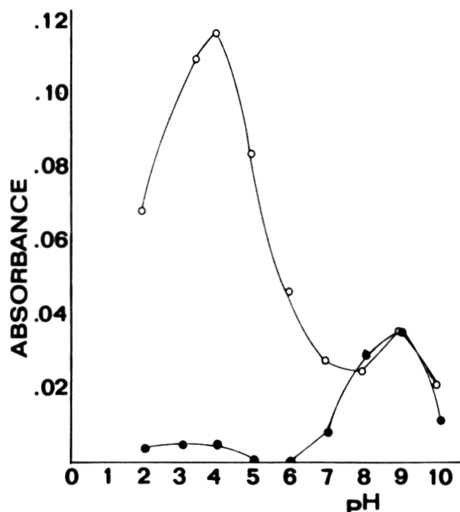

Fig. 1–Optima pH of porcine muscle cathepsins with 3M urea-denatured 2% hemoglobin (○) and 3% salt soluble protein (•) as substrates. Absorbance was determined at 280 nm.

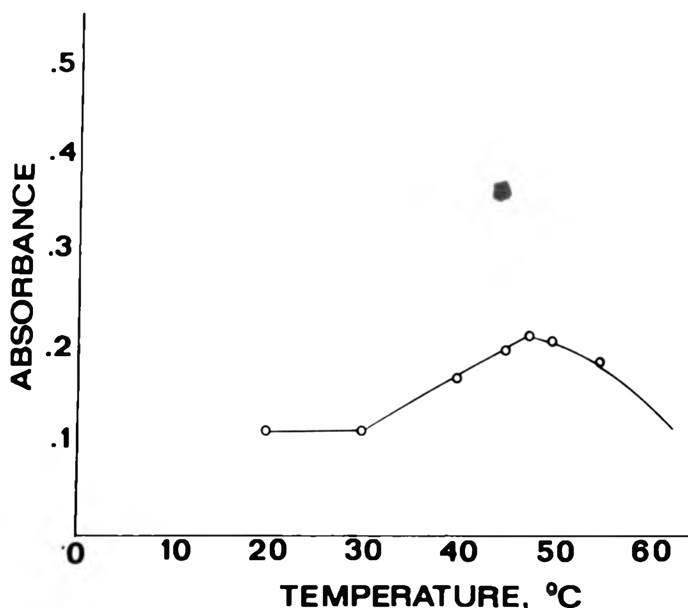

Fig. 3–Optimum reaction temperature of porcine muscle cathepsins. Absorbance was determined at 280 nm after 90 min reaction.

ferent time intervals are shown in Figure 2. For short reaction times the maximum activity was obtained at 50–55°C. The cathepsin D activity began to decrease as the length of the reaction increased past 70 min. At all other temperatures, enzyme activity continued to increase past 70 min. Since the optimal temperature is determined by the balance between the effect of temperature on the rate of the enzyme reaction and on the rate of destruction of the enzyme, the optimum temperature of cathepsins is 47.5°C (Fig. 3) when the reaction mixture was incubated for 90 min.

The cathepsin D activity of the 35–70% $(NH_4)_2SO_4$ fraction after standing at 37°C, 45°C and 50°C, for different periods of time is shown in Figure 4. The porcine muscle cathepsin D activity did not change when exposed to 37°C for 2 hr. When the enzymes were held at 45 and 50°C, the activity of the enzymes decreased linearly as the length of heat treatment increased. This indicated that the cathepsin D in porcine muscle is not denatured at 37°C. Temperatures which are used in the aging of country cured hams range from 20–35°C (Christian, 1963; Skelley et al., 1964) and, therefore, cannot adversely affect the activity of porcine muscle cathepsin D.

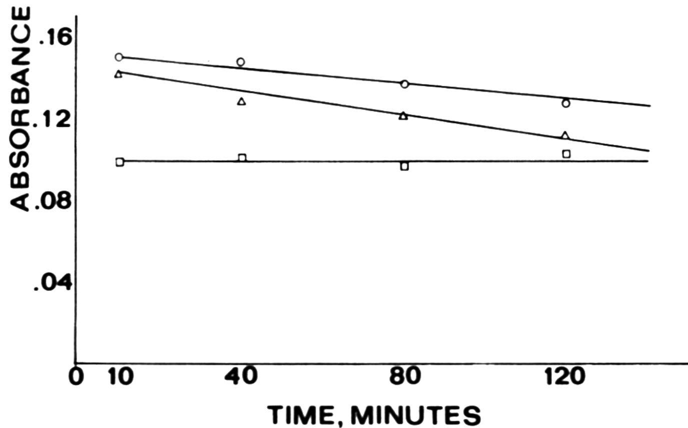

Fig. 4–The activity of porcine muscle cathepsins after holding at 37°C (□), 45°C (○) and 50°C (△). Absorbance was determined at 280 nm.

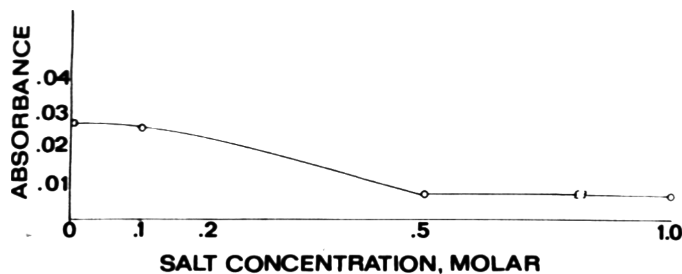

Fig. 5–Effect of sodium chloride on the activity of porcine muscle cathepsins. Absorbance was determined at 280 nm.

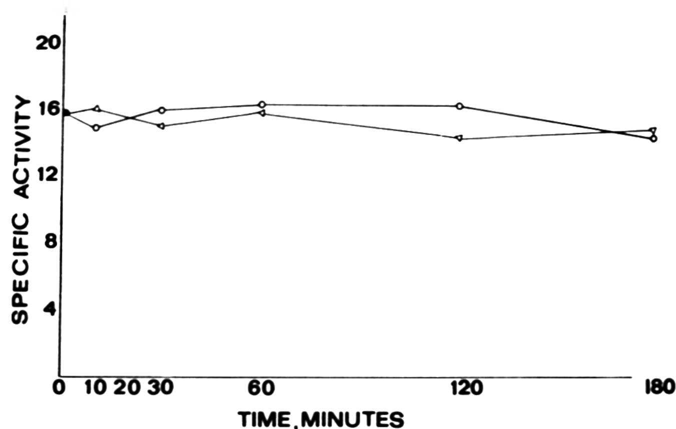

Fig. 6–The activity of porcine muscle cathepsins after holding in 0.1M (○) and 1.0M (△) sodium chloride solutions. Absorbance was determined at 280 nm.

Effect of curing agents on the proteolytic activity of porcine muscle

Urea-denatured hemoglobin could not be used as the enzyme substrate in studying the effect of sodium chloride on enzyme activity because the hemoglobin precipitated when salt concentrations above 0.5M were used. A substrate of 3% salt soluble protein extracted directly from fresh pork was used as a substrate in these experiments. The optimum concentration of 3% salt soluble protein was 18.30 mg/ml. The proteolytic activity of porcine muscle was not affected at sodium chloride concentrations below 0.1M but 0.5M sodium chloride reduced the activity of the enzymes. (Fig. 5).

The stability of the enzymes was determined by measuring the activity of the enzymes after standing at various sodium chloride concentrations for different periods of time followed by dialysis. Dialysis of the treated enzymes and controls resulted in slight variations in protein concentration. Therefore, the specific activity rather than activity was used to show the results (Fig. 6). The results indicated that the activity of the enzymes was not changed after standing in 0.01M and 1M sodium chloride concentrations

Table 2–Effect of sucrose on the activity of porcine muscle cathepsins

Treatment	Activity[a] Enzyme grade sucrose
0 (control)	0.101
1×10^{-3} M	0.104
1×10^{-2} M	0.091
5×10^{-2} M	0.095
1×10^{-1} M	0.088
1.5×10^{-1} M	0.089
2.0×10^{-1} M	0.082
2.5×10^{-1} M	0.084
3.0×10^{-1} M	0.062
4.0×10^{-1} M	0.062
5.0×10^{-1} M	0.047
1M	0.027

[a] Absorbance at 280 nm

Table 3—The effect of potassium nitrate and potassium chloride on the activity of porcine muscle cathepsins

Treatment	Concentration	Activity[a]
KNO_3	1×10^{-3}M	0.115
	1×10^{-2}M	0.099
	1×10^{-1}M	0.070
	1.0M	0.000
KCl	1×10^{-3}M	0.114
	1×10^{-2}M	0.118
	1×10^{-1}M	0.084
	1.0M	0.010
Control	0.0M	0.115

[a]Absorbance at 280 nm

for various periods of time. Therefore, it was concluded that the porcine muscle cathepsin D is very salt stable and will recover its activity when separated from the salt.

The enzyme activity was not affected with enzyme grade sucrose at low concentrations but decreased as sucrose concentration increased with the greatest decrease occurring at concentrations above 0.3M (Table 2).

The activity of porcine muscle cathepsin was not affected at concentrations lower than 0.001M, but decreased at concentrations higher than 0.01M of potassium nitrate and at concentrations higher than 0.1M potassium chloride (Table 3). In 1966, Parrish and Bailey studied the influence of metal ions on proteolytic activity of partially purified porcine muscle cathepsins. They indicated that 0.001M calcium chloride as well as 0.001M to 0.05M ferrous ammonium sulfate stimulated activity. However, cathepsin activity was diminished slightly by 0.01M concentrations of calcium chloride, magnesium sulfate, and zinc acetate. Koszalka and Miller (1960) also found that the divalent cations, barium, calcium, magnesium, manganese and zinc, at 0.01M concentrations, inhibited proteolytic activity of rat muscle cathepsins. Results of this study show that, as with the divalent ions, higher concentrations of the monovalent potassium has an inhibitory effect on cathepsin activity; whereas, lower concentrations, 0.001M or less, has no effect on cathepsin activity.

The results of this study indicate that the amount of curing agents and temperatures used in the curing and aging of country ham (Christian, 1963) do not inhibit the activity of the cathepsins in porcine muscle. Although the high salt content of country cured hams may reduce the activity of cathepsins in the final stages of aging, it is unlikely that the interior of the hams attain high enough sodium chloride levels in the beginning of the aging period to reduce cathepsin activity. Future work needs to be done to determine the cathepsin activity in country cured hams during aging.

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PROPERTIES OF GRAM NEGATIVE AEROBES ISOLATED FROM MEATS

INTRODUCTION

MANY STUDIES have shown that gram negative, aerobic bacteria predominate in the associations developing on the surface of refrigerated meat (Mossel, 1968). The literature lists many species of *Pseudomonas* which have been recovered from these associations (Ayres, 1960) but one can surmise that definitions of limited taxonomic significance were used for their identification. Consequently, there has been a tendency in recent years to use the scheme of Shewan et al. (1960) for grouping pseudomonads isolated from proteinaceous foods. The older literature on meat microbiology contains frequent reference to the occurrence of *Achromobacter* (Ingram and Shewan, 1960). In many cases this reflects the failure of workers to accord priority to the position of the flagella on an organism. Thus the study of Brown and Weidemann (1958) indicated that a large number of the achromobacters isolated by Empey and Scott (1939) were polarly flagellate. The study of Thornley (1967) casts further doubt on the identification of isolates from meat with *Achromobacter*. In the present study, organisms from a wide range of meat products were examined with the object of establishing a simple scheme for the identification of *Pseudomonas* and organisms which formerly would have been assigned to *Achromobacter*.

EXPERIMENTAL

Organisms

They were recovered on Plate Count Agar (Oxoid)–incubation 20 or 22°C–containing dilutions of homogenized beef, lamb, pork or British fresh sausage. Isolates were streaked on PCA or Heart Infusion Agar (Difco) until pure and then stored on slopes of HIA or PCA at 4°C.

Oxidation/fermentation test

Cultures were stab-inoculated in the medium of Hugh and Leifson (1953) containing filter-sterilized glucose (final concentration, 1.0% w/v).

Morphology

The Jensen (at Colworth) or Hucker (at Bath) modification of Gram's stain was used. Flagella were stained by the methods of Hodgkiss (personal communication) or Rhodes (1958).

Reducing compounds from gluconate

The utilization of this substrate was tested by the methods of Haynes (1951) and Paton (1959); the demonstration of reducing compounds by Benedict's reagent in 10d cultures was taken as a positive result.

Pigment production

This was tested with media A and B of King et al. (1954).

Oxidase

The method of Kovacs (1956) was used.

Catalase

Hydrogen peroxide was added to colonies on HIA or PCA and the evolution of gas was taken as evidence of the action of catalase.

Extracellular hydrolases

The methods of Frazier (1926) and Cowan and Steel (1965) were used for testing for gelatin digestion and starch hydrolysis respectively.

Carbon and energy sources

The substrates (123 in all) were sterilized by filtration through sintered glass and added (final concentration, 0.1% or, in the case of sugars, 0.2% w/v) to the mineral base of Stanier et al. (1966). Nutrient Agar plates were spot inoculated at their surface with 20 isolates and, after overnight incubation at 22°C, the organisms were harvested on sterile velvet and pressed gently on the surface of nine plates of mineral base each containing a different substrate, and finally on a plate of Nutrient Agar. Prior to inoculation, the plates were dried at 37°C for 24 hr. The inoculated plates were incubated (20°C) for 3 days and the presence or absence of growth recorded. A selection of the isolates were retested so that reproducibility of the results could be assessed.

Growth temperatures

Inoculated broths were held in water baths at 4 or 41°C, and observed for turbidity.

Cleavage of diphenols

The method of McLeod (1966) was used.

Poly-β-OH-butyrate as cellular reserve

The method of Stanier et al. (1966) was followed.

Hydrogen sulphide production, reduction of litmus milk and arginine test

The methods of Thornley (1967) were used.

RESULTS & DISCUSSION

231 POLARLY FLAGELLATE, gram negative rods were studied. The properties common to 100 meat isolates are given in Table 1. These organisms had the principal characters used in the definition of *Pseudomonas* (Stanier et al., 1966). Within this group, 19 formed fluorescent pigments on Medium B of King et al. (1954) and the eight which hydrolysed gelatin were identified with *Ps. fluorescens*. The nonproteolytic fluorescent isolates were identified with *Ps. putida*. 62 of the 81 nonpigmented isolates were

Table 1—Properties common to all the pseudomonads isolated from beef, pork and mutton[a]

Character	Result
Morphology	Rods, single and pairs
Gram reaction	–
Motility in hanging drop	+
Flagella (and numbers)	Polar (1 or > 1)
Oxidation/fermentation test	Oxidative
Catalase	+
Oxidase	+
Reducing compounds from gluconate	+
Starch hydrolysis	–
Growth at 4°C	+
Growth at 41°C	–
Cleavage of diphenols	Ortho
Poly-β-OH butyrate as cellular reserve	–
Methionine requirement	–

[a]Number of isolates, 100.

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proteolytic. Data derived from an analysis (Table 2) of the utilization of carbon and energy sources by these organisms did not permit identification with the nonfluorescent species listed by Stanier et al. (1966). It is noteworthy that, although the strains studied by Stanier and his coworkers were isolated from a broad spectrum of environments, organisms belonging to the group II of Shewan et al. (1960)–the dominant organisms on spoiled proteinaceous foods–were not included (Shewan, 1971).

A similar picture emerged from a study of 131 organisms isolated from British fresh sausages. Of the 16 fluorescent strains eight (proteolytic) were identified with *Ps. fluorescens* and eight (nonproteolytic) with *Ps. putida*. The remaining 115 were nonpigmented and nonproteolytic but they had nutritional properties similar to those of the isolates taken from whole meat (Table 2).

Table 2–Organic compounds used by meat pseudomonads as sole carbon and energy source

Carbohydrates and sugar derivatives	Hydroxyacids	Aliphatic amino acids
D-glucose (100) 100[a]	D-malate (100) 99	L-aspartate (100) 98
gluconate (100) 100	DL-lactate (100) 94	L-glutamate (100) 99
2-ketogluconate (100) 100	D-tartrate (16) 22	γ-aminobutyrate (100) 99
D-ribose (90) 90	meso-tartrate (16) 22	β-alanine (97) 99
D-xylose (88) 90	DL-β-hydroxybutyrate[b]	DL-arginine (87) 91
D-fructose (83) 98	DL-glycerate[b]	D-α-alanine (84) 97
D-mannose (81) 79		L-ornithine (84) 91
L-arabinose (79) 72	Miscellaneous organic acids	DL-serine (80) 75
mucate (78) 77	citrate (100) 95	L-valine (50) 52
D-galactose (66) 59	α-ketoglutarate (100) 100	DL-leucine[b]
D-arabinose (58) 72	pyruvate (100) 100	iso-leucine[b]
trehalose (54) 50	itaconate (44) 30	glycine[b]
sucrose (25) 8	mesaconate (44) 30	L-lysine[b]
inulin[b]	aconitate[b]	DL-citrulline[b]
		Amino acids and related compounds containing a ring structure
Fatty acids	Polyalcohols and glycols	L-histidine (100) 98
caprate (98) 96	glycerol (100) 97	L-proline (100) 100
caprylate (98) 96	mannitol (87) 72	L-tyrosine (100) 99
acetate (90) 99	meso-inositol (63) 46	L-phenylamine (98) 96
pelargonate (63) 63	2,3 butyleneglycol (29) 29	Amines
valerate (32) 63	propyleneglycol (11) 30	putrescine (100) 91
propionate (10) 15	sorbitol (4) 8	spermine (100) 96
butyrate (9) 15	Alcohols	ethanolamine (74) 93
isovalerate[b]	none	Miscellaneous nitrogenous compounds
caproate[b]		
Dicarboxylic acids	Nonnitrogenous aromatic and other cyclic compounds	creatine (95) 90
succinate (100) 97	p-hydroxybenzoate (87) 87	hippurate (88) 89
fumarate (100) 96	benzoate (83) 85	betaine (72) 67
glutarate (100) 95	quinate (80) 81	sarcosine (25) 40

[a]Figures, percentage of isolates utilizing compound. Figures in parenthesis, isolates (100) obtained from pork, beef or mutton and figures out of parenthesis, isolates (131) derived from sausages.
[b]Growth on these substrates was scanty and difficult to score.

These observations suggest that the majority of commonly occurring pseudomonads on meat and meat products do not conform with the species proposed by Stanier et al. (1966). Our results suggest, however, that they have affinity with the *fluorescens-putida* group of these authors. It is questionable at this time whether, in the absence of detailed physiological data, the exact identification of an organism is likely to contribute to a better understanding of its role in bringing about spoilage. In this context, however, the information on substrate utilization by meat pseudomonads (Table 2) should help those who seek to correlate the development of microbial associations on and chemical changes in meat held under refrigeration.

The properties of 110 nonmotile gram negative aerobes are given in Table 3. The majority of isolates (Group A) were similar to the organisms included in Phenon 4i of Thornley (1967) and the remainder (Groups B & C) to her Phenon 4ii or definition of *Moraxella lwoffi*. Using the nomenclature of Baumann et al. (1968a, b), our oxidase positive isolates (Group A) would be referred to as *Moraxella* and those of Groups B and C (oxidase negative) as *Acinetobacter*.

This study has indicated that from a routine control standpoint, the gram negative aerobic bacteria developing on chilled meat can be assigned to major groups on the basis of the following tests: gram stain, shape, motility, position of flagella (if any), oxygen requirements, oxidase test and action on gluconate.

Table 3–Properties of nonmotile rod shaped aerobic bacteria isolated from beef, pork and mutton

Property	Group A	Group B	Group C
Morphology	Coccobacillus	Coccobacillus	Coccobacillus
Gram reaction	a	a	a
Motility	–	–	–
Oxidation/fermentation test	Inert towards glucose	Inert towards glucose	Inert towards glucose
Growth at 0°C	+	+	–
Growth at 37°C	–	–	+
Reducing compounds from Gluconate	–	–	–
Reduction of litmus milk	+	– (19)[b]	+
H_2S production	–	+	– (21)[b]
Oxidase	+	–	–
No. isolates	61	26	23

[a]Tendency to retain crystal violet complex
[b]Number of strains negative

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UTILIZATION OF HIGH PROTEIN TISSUE POWDERS AS A BINDER/EXTENDER IN MEAT EMULSIONS

INTRODUCTION

MUCH ATTENTION has been given to the development of a high protein powder (75–90% protein) from fish tissue (FPC), which can be used to fortify plant-based foods. In recent years investigators have applied the techniques used in making FPC towards the better utilization of protein from the animals slaughtered in the meat packing plant. Levin (1970a) described an azeotropic extraction/distillation process for removing the lipid and water from by-products of the beef and pork processing operations. When dried at a relatively low temperature this system yields a high protein powder for possible use as a fortifier of human food. Levin (1970b) described the nutritive value of the protein concentrate powder obtained from both pork and beef. The PER value for powder from either the beef or pork powder was about 70% of the PER obtained for casein. When either protein powder was fed in combination with cereal protein, the PER value increased so that it was from 80–85% of the casein PER. The addition of 1% methionine to either protein powder increased the PER to 95% of the value for casein. Olson (1970) determined the essential amino acid composition of the various beef and pork by-product tissues that were used to make the protein powder described by Levin (1970a). Olson (1970) found that almost all of the by-product tissues, except blood, were low in the essential amino acid tryptophan, when compared to the standard, whole egg protein. Although the author (Olson, 1970) does not discuss the methionine content of the various by-product tissues, from his data it would seem that all tissues were low in methionine, when compared to whole egg protein.

Other investigators have looked into the food uses of certain animal processing by-products. Pals (1970) described how blood could be removed from immobilized hogs and cattle at time of slaughter by severing the neck, clamping off the esophagus and then collecting the blood in precleaned containers containing an anticoagulant (citric acid). The blood was then centrifuged to remove cellular material and the resultant supernatant (serum) spray dried. Pals (1970) estimated that for each beef animal slaughtered, 1 lb of blood serum powder could be obtained.

If a new protein source is to be incorporated into a sausage emulsion, it must be known if the protein has emulsifying capacity and stability, as well as if it will impart an off color or flavor to the finished sausage product. Inklaar and Fortuin (1969) and Pearson et al. (1965) determined the emulsifying capacity and stability of three presently used sausage binder/extenders: soy protein isolate, casein and nonfat dry milk (NFDM). Froning (1970) and Froning and Janky (1971) described the emulsifying characteristics of mechanically deboned meat, a by-product of the poultry processing industry. Thus far no information is available which describes the emulsifying

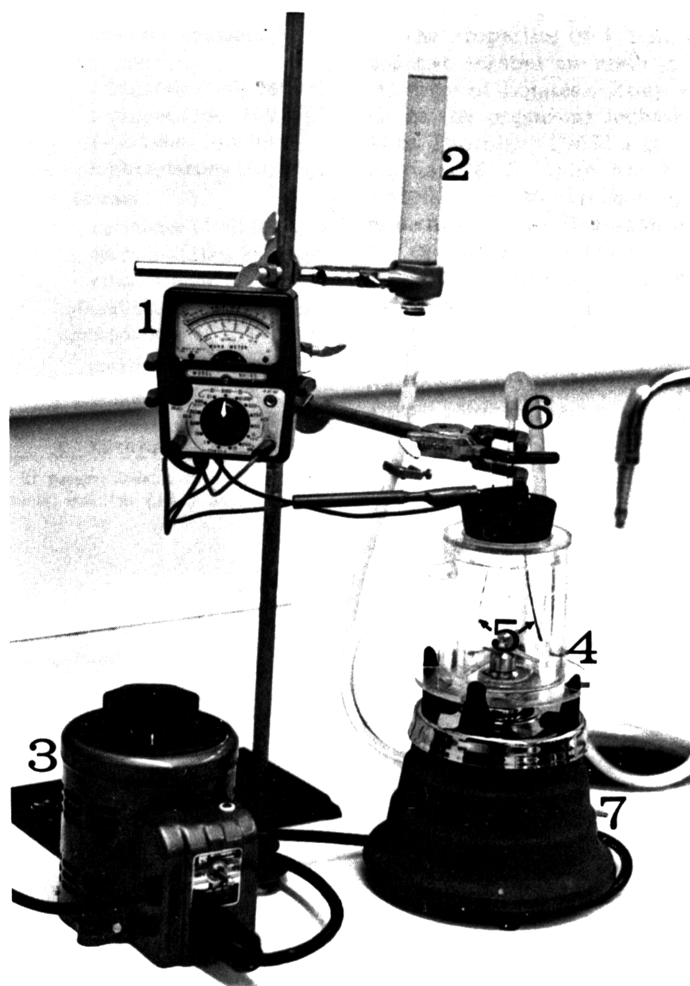

Fig. 1—Microemulsifier and allied equipment: (1) Ohm meter; (2) Graduated oil reservoir; (3) Variable transformer; (4) Microemulsifier chamber with cooling jacket; (5) Electrodes; (6) Oil delivery tube; and (7) Waring Blendor base.

characteristics of the high protein powders that can be obtained from beef and pork by-products.

This study was undertaken in order that information concerning the type of protein present, the emulsion capacity, emulsion stability and the organoleptic properties of emulsions made from the various by-product tissues could be obtained and used to evaluate the future of these powders in emulsified meat products.

EXPERIMENTAL

Tissue powders studied

The various tissue powders studied were obtained from inspected organs that were defatted and desiccated according to the procedure described by Levin (1970a). The individual powders studied were: pork (brain, duodenum, heart, liver, lung, kidney, spleen and stomach); beef (whole blood). All powders were stored at 5°C throughout the duration of the study.

For comparison purposes, post-rigor beef semitendinosus muscle was analyzed along with the tissue powders.

Total and % soluble protein

Total protein analysis for each sample was performed by the Kjeldahl method (AOAC, 1965).

The % soluble protein in each sample was determined by the technique developed by Saffle and Galbreath (1964).

Emulsion capacity

The emulsion capacity of each tissue powder sample as well as the lean beef was determined using a water-cooled microemulsifier developed for this study. The microemulsifier and its allied equipment are shown in Figure 1. The apparatus was cooled by tap water so that the sample temperature during the emulsification never rose above 25°C. Corn oil was the lipid source. The sample to be emulsified was suspended in 33 ml of 1M NaCl containing 0.01 phosphate buffer pH 7.0, to give a final protein concentration of 3 mg/ml. Emulsification of the protein and the corn oil was performed at a speed of 5,500 rpm. During the emulsification the DC resistance of the emulsion was monitored with a Cole-Parmer model NH-65 ohm meter. During formation of the emulsion the DC resistance ranged 1.0–1.5 Kohm, whereas when the emulsion broke, the DC resistance rapidly rose to 10–12 Kohm.The end point or capacity of the emulsion was determined by measuring the amount of corn oil needed to break the emulsion.

All emulsion capacities were performed in triplicate.

Histological analyses

The emulsions that were made by blending each tissue powder as well as beef with the corn oil were also observed histologically. After the emulsion capacity was determined for each sample, one more emulsion at 80% capacity was made. The emulsion was then carefully diluted 1:1 (w:w) with 1M NaCl containing 0.01M phosphate buffer pH 7.0 and carefully reblended. The resulting diluted emulsion was stained according to the procedure described by Hanson (1960). Photographs were taken of each stained emulsion. Using the stained emulsions, the average globule size was determined by randomly selecting and measuring 25 globules on each of three slides made from each emulsion.

Photographs were obtained using a microscope magnification of 100 × and enlarged 3.4 ×. The globule size determinations were made using a total magnification of 64 ×. All measurements are reported in microns (μ).

Emulsion stability

The emulsion stability test was performed using the technique described by Townsend et al. (1968) and a sausage formulation described by Satterlee et al. (1973). The stability test was performed by using each tissue powder to replace the NFDM in the basic formulation on per gram basis.

Subjective evaluations of the sausages

A panel consisting of seven individuals evaluated sausages which had each tissue powder incorporated into the formulation as a replacement for the NFDM. The sausages were evaluated on a five point hedonic scale (5–excellent down to 1–poor) for the following characteristics: flavor, texture, appearance, juiciness and oiliness.

Included with each sensory evaluation were one or more control sausages where the unsubstituted formulation was used in making the sausage.

RESULTS & DISCUSSION

KJELDAHL PROTEIN analysis of each tissue powder indicated each was quite high in protein. Table 1 gives the % protein composition of each powder as well as the lean beef studied. The amount of soluble protein differed greatly between the individual powders. Soluble protein, as % of the total protein, is also given in Table 1 for each powder. The amount of soluble protein in a sausage ingredient is a good initial indicator of the ingredient's ability to emulsify fat (Hanson, 1960). The next paragraph describes the emulsion capacity of the various tissue powders, and during that discussion it will be shown how well percent soluble protein correlates with the emulsion capacity of each tissue powder.

The emulsion capacities of each tissue powder, both on a ml oil emulsified per 100 mg total protein and on a ml oil emulsified per 100 mg soluble protein basis, are given in Table 2. When the emulsion capacities (per 100 mg total protein) are compared with the percent soluble protein (Table 1), it can readily be seen that as the amount of soluble protein decreases, the emulsion capacity of the tissue powder also decreases. Therefore, it should be emphasized that it is the soluble protein in these tissue powders that is solely responsible for their ability to emulsify fat. When emulsion capacities are compared on a per 100 mg of soluble protein basis, the quality of the soluble protein can then be determined. The emulsion capacities per 100 mg soluble protein, as shown in Table 2, indicate that those powders which have the greatest amount of soluble protein, also have the protein with the greatest ability to emulsify fat. Those tissue powders containing small amounts of soluble protein

Table 1—The protein composition of the tissue powders and lean beef

Tissue powder	Total protein (%)	Soluble protein[a] (% of total protein)
Blood	83.95	69.71
Brain	70.54	18.91
Lung	80.00	16.23
Stomach	74.81	16.12
Duodenum	85.64	12.40
Kidney	76.53	8.75
Liver	79.73	8.17
Spleen	84.28	3.25
Heart	85.32	2.88
Beef muscle	21.90	22.68

[a]Soluble in 3% NaCl

Table 2—Emulsion capacity of the whole tissue powders and their respective soluble protein

	Emulsion capacity ml oil/100 mg	
Tissue powder	total protein	soluble protein
Blood	43.82	49.57
Brain	19.17	35.50
Lung	12.50	36.67
Stomach	15.82	35.16
Duodenum	17.31	31.27
Kidney	13.25	23.76
Liver	12.83	28.70
Spleen	9.46	28.00
Heart	9.57	25.67
Beef muscle	15.96	35.33

Table 3—Histological measurements of the 80% completed tissue powder emulsions

Tissue powder	Avg globule size[a] (μ)	Globule size range (μ)
Blood	80.5 ± 42.0	35.0–122.5
Brain	89.2 ± 22.7	15.8–112.0
Lung	47.2 ± 19.2	17.5–70.0
Stomach	98.0 ± 26.2	35.0–175.0
Duodenum	145.2 ± 47.2	52.5–297.5
Kidney	255.5 ± 89.2	110.0–402.5
Liver	152.2 ± 49.0	70.0–262.5
Spleen[b]	NM	NM
Heart	189.0 ± 110.2	70.0–402.5
Beef muscle	87.5 ± 33.2	52.5–122.5

[a]The standard deviation is also given along with the mean particle size.

[c]The spleen emulsion was too unstable to obtain accurate measurements.

Table 4–Emulsion stability of sausages prepared using individual tissue powders to replace the NFDM in the formulation

Tissue powder	ml Fat released / 10g emulsion	ml H_2O + suspended solids released / 10g emulsion
Blood	.03	1.43
Brain	.08	1.26
Lung	.02	.74
Stomach	.04	1.01
Duodenum	.12	2.30
Kidney	.17	2.03
Liver	.01	1.00
Spleen	.11	1.72
Heart	.12	1.49
Control	.02	1.14

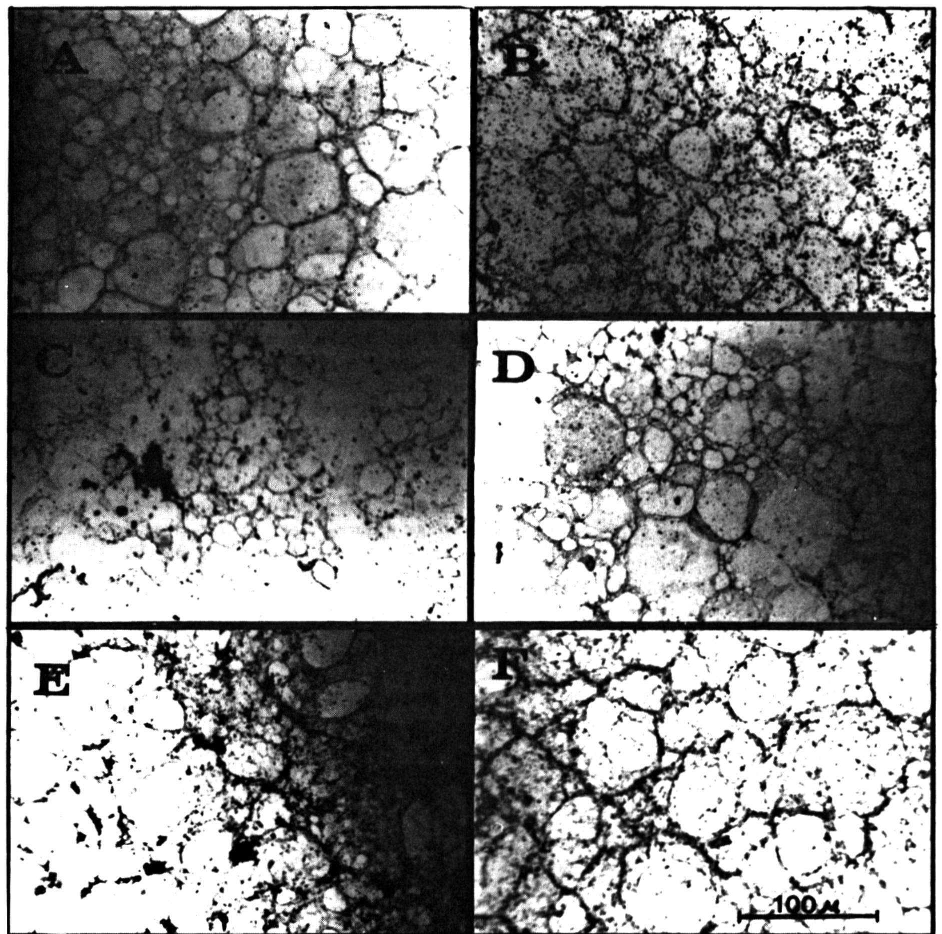

Fig. 2–Photographs of the 80% completed emulsions of: (A) blood; (B) brain; (C) lung; (D) stomach; (E) duodenum; and (F) beef semitendinosus muscle. Total magnification (220 ×).

also contain soluble protein with a lesser ability to emulsify fat. The soluble protein of blood excels that from any other tissue in its ability to emulsify fat. The soluble protein from kidney has the least ability to emulsify fat.

Histological examination of the 80% completed emulsions that were produced in the microemulsifier yielded data concerning the size of the protein-lipid globule formed by each tissue powder. Table 3 gives the data concerning the average globule size and the range in size noted for the globules produced by each tissue powder. Examination of the data in Table 3 indicates that the tissue powders whose soluble proteins possess the greatest emulsion capacity also produce the emulsions with the smallest globule size. For example, blood whose soluble protein can emulsify 49.57 ml oil per 100 mg has an average globule size of 80.50µ, whereas kidney, with a much lower emulsion capacity (23.76 ml oil/100 mg soluble protein), produces a large lipid-protein globule (255.50µ). The emulsions produced by all tissue powders as well as by lean beef consisted of globules of varying sizes. The range of globule sizes for each emulsion is also shown in Table 3.

Figures 2 and 3 show photographs of all emulsions (80% completed) formed in the microemulsifier. The photographs show:

(1) the relative sizes of the protein-lipid globules in the emulsion;
(2) the integrity of the protein film surrounding the lipid globule; and
(3) the thickness of the protein film which surrounds the globule.

Figure 2 shows photographs of six tissue powder emulsions which possess from moderate to high emulsion capacity. The most noticeable features seen in these photographs is the small compact globule possessing a uniformly thick protein coat. Figure 3 shows photographs of four tissue powder emulsions which have a low emulsion capacity. The main noticeable feature seen in these photographs is the large globule size. In the case of the heart, spleen and the lung emulsions, the globules are partially disrupted.

The emulsion stability test indicated that certain tissue powders which had a large emulsion capacity (blood, lung and stomach) could be incorporated into a sausage formulation, replacing the NFDM, and yield a cooked sausage with stability values very similar to the control sausages (unsubstituted formulation). Tissue powders from brain and duodenum, which had from high to moderate emulsion capacity, produced relatively unstable sausages, an unstable sausage being one with a greater fat release, when compared to the control sausage. Tissue powders which had a low emulsion capacity (spleen, heart and kidney) also produced sausages with a low emulsion stability.

The emulsion capacity test tells how well a protein can emulsify fat, whereas the emulsion stability test tells how well the protein can maintain the emulsion during a heating process. The mere fact that a protein is able to emulsify fat well does not always determine how well that protein will maintain that emulsion during the formation of an actual sausage product. Tissues such as brain and duodenum perform well in establishing an emulsion, but the emulsion they do form is not as heat stable, when compared to other tissue powders such as blood, lung and stomach, which have high emulsion capacity and stability.

The subjective evaluation of various sausages, by a seven member panel, showed that the sausages containing the tissue powders were comparable to control sausages in oiliness, juiciness and texture. The appearance of all sausages, except the sausage containing blood powder, was comparable to the control. The sausage containing blood powder possessed an undesirable dark reddish-brown color. Subjective evaluation of flavor indicated that sausages made from kidney, spleen and liver powders were equal to or better than the flavor of the control sausage. All other sausages had a slightly lower flavor score, but no one sausage had a definite objectionable flavor.

In conclusion, the desiccated, defatted powders obtained from certain byproduct tissues of beef and pork are high

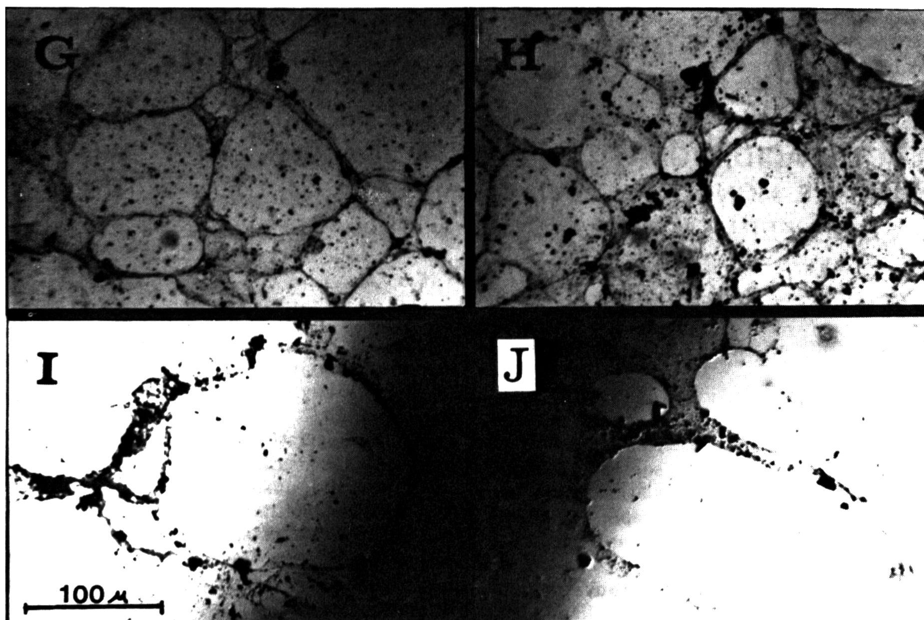

Fig. 3–Photographs of the 80% completed emulsions of: (G) kidney; (H) liver; (I) spleen; and (J) heart. Total magnification (180 ×).

in protein and do possess emulsifying properties. Powders from tissues such as blood, lung, brain and stomach possessed an emulsion capacity comparable to that of lean beef. Histological analysis showed that the high capacity emulsions possessed small and closely packed globules, whereas the low capacity emulsions possessed globules which were large and oftentimes partially disrupted. Some, but not all, of the tissue powders which had a high emulsion capacity also possessed a high emulsion stability.

This study indicates that certain tissue powders excel others in their ability to form stable emulsions and in their ability to form sausages which have acceptable organoleptic properties. The powders from blood, lung and stomach tissue had high emulsion capacity and stability and should be definitely considered in the blending of tissue powders to produce a tissue protein concentrate for future use as a binder or extender in meat emulsions. Tissue powders from heart, spleen and kidney have poor emulsifying properties and should be omitted from the tissue protein concentrate blend.

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FORMATION OF LACTIC ACID, VOLATILE FATTY ACIDS AND NEUTRAL, VOLATILE MONOCARBONYL COMPOUNDS IN SWEDISH FERMENTED SAUSAGE

INTRODUCTION

IN SWEDEN there exists a type of fermented sausage, "isterband," which can be classified as a genuine Swedish meat product. Production of the product, by a variety of different processing techniques, is widespread. The main difference between processes is the final treatment of the sausage, done either by drying, smoking or a combination of these processes.

With a future control of the fermentation process in mind, we decided to study the effect of appropriate process variables on the formation of some flavor-contributing substance classes. This article treats the formation of lactic acid, volatile fatty acids and neutral, volatile monocarbonyl compounds in two batches of sausages during ripening. In one batch the fermentation was initiated by a smoking procedure while in the other this process was carried out by drying.

MATERIALS & METHODS

Production and storage conditions

The sausage batches used in this investigation were produced on a pilot plant scale from the following components:

Component	Quantity (kg)
Beef meat	4.0
Pork meat	6.6
Potatoes, boiled	2.6
Barley grain, boiled	9.4
Salt-nitrite mixture (0.6% $NaNO_2$)	0.4
Sugar	0.2
Spices	0.2

The barley grains were boiled in the grain-water ratio 1:1.7 for use in the drying sausages and in the grain-water ratio 1:3 for use in the smoked and stored sausages.

The meat used was of high hygienic quality. After a gentle pre-cutting of the meat the ingredients were mixed into it in a bowl chopper. The processed meat was stuffed in oxygen impermeable casing of about 3 cm diam. The batch used for drying was stored at 11–13°C with 55–70% relative humidity for 3 wk while the other was smoked at 20–25° for 2 days and stored at 4–5°C with about 85% relative humidity for 3 wk.

Analytical methods

Lactic acid determination was done spectrophotometrically according to Harper and Randolph (1960).

Isolation and evaluation of the volatile fatty acid (VFA) composition was carried out according to the principles given in Figure 1. The VFA fraction was isolated as sodium salts after steam distillation. After ethylation the VFA composition was gas chromatographically evaluated. Details of this procedure are published elsewhere (Halvarson, 1972).

The neutral, volatile monocarbonyl fraction was isolated and characterized according to the scheme given in Figure 2.

Isolation of the carbonyls from the meat product was accomplished by steam distillation and selective trapping in a receiver containing 2,4-dinitrophenylhydrazine dissolved in a two-phase system.

After regeneration of the carbonyls in a reactor described earlier (Halvarson, 1971), the qualitative and quantitative composition was determined. Quantitative analysis of individual components was accomplished by gas chromatography with use of an internal standard and identification was carried out by combined gas chromatography-mass spectrometry. The isolation and evaluation steps were described earlier (Halvarson, 1972).

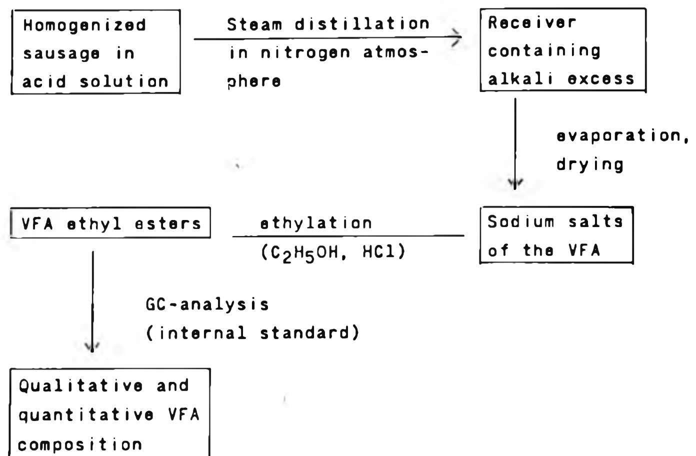

Fig. 1—Scheme showing the principles used in the isolation and evaluation of VFA.

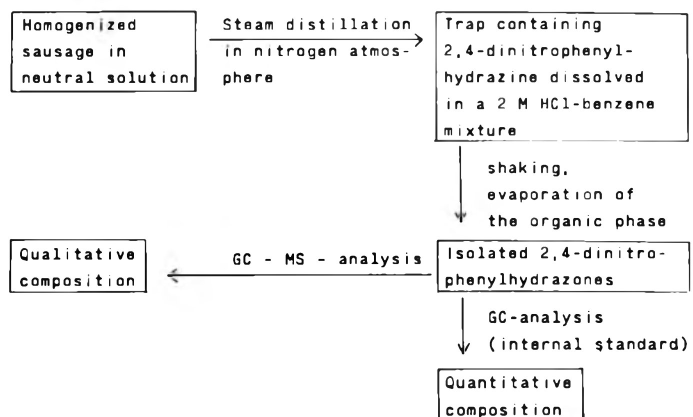

Fig. 2—Scheme illustrating the applied technique in order to characterize the neutral, volatile carbonyl fraction.

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Table 1—Lactic acid formation during the experiment

Storage time	Drying sausage	Smoke and stored sausage
0	3.3	2.7
2	3.3	4.3
3	3.4	4.6
6	4.2	5.1
11	5.6	5.6
17	6.5	6.2
21	6.7	5.9

Table 2—VFA formation during the experiment

Storage time (days)	Levels of VFA components[a] (mg/g sausage)[b] Drying sausage				Smoked and stored sausage			
	C^1	C^2	C^3	n-C^4	C^1	C^2	C^3	n-C^4
0	0.14	0.23	0.002	0.006	0.09	0.20	–	–
2	0.12	0.28	0.004	0.002	0.40	0.94	0.02	0.005
3	0.14	0.23	0.002	0.003	0.26	0.86	0.03	0.007
6	0.18	0.40	–	–	0.28	1.1	0.03	0.004
11	0.22	0.49	–	–	0.31	1.2	0.03	0.005
17	0.31	0.58	0.005	0.004	0.40	1.1	0.03	0.003
21	0.25	0.70	0.004	0.004	0.42	1.2	0.03	0.007

[a]The levels of C_5 and C_6 fatty acids were < 0.001 mg/g sausage in both types of sausages throughout the experiment.
[b]Detection limit ≈ 0.001 mg/g sausage

RESULTS & DISCUSSION

THE RATE of lactic acid formation as shown in Table 1 was rather similar in the two batches of sausages investigated. However, the higher smoking temperature apparently favored growth of lactobacilli bacteria during smoking compared with the initial growth rate in the drying sausages. During the further fermentation the lactic acid levels in the two batches gradually reached about the same levels. Thus the final lactic acid concentrations in the dried and in the smoked and stored sausages were 6.7 mg/g and 5.9 mg/g respectively which are normal values for this product.

Table 2 illustrates the VFA production in the sausage batches during fermentation. In this case also the smoking process stimulated VFA production compared with drying during the same phase. This difference can also probably be attributed to the higher smoking temperature. In this case, however, the differences remained throughout the experiment. Thus, in the smoked and stored sausages the acetic acid level rose during smoking from 0.2 mg/g to 0.9 mg/g and remained after 6 days at 1.1–1.2 mg/g while in the drying sausages the acetic acid level rose smoothly from 0.2 mg/g to 0.7 mg/g.

The formic acid concentrations rose to final concentration of 0.3 mg/g and 0.4 mg/g in the dried and in the smoked and stored sausages respectively.

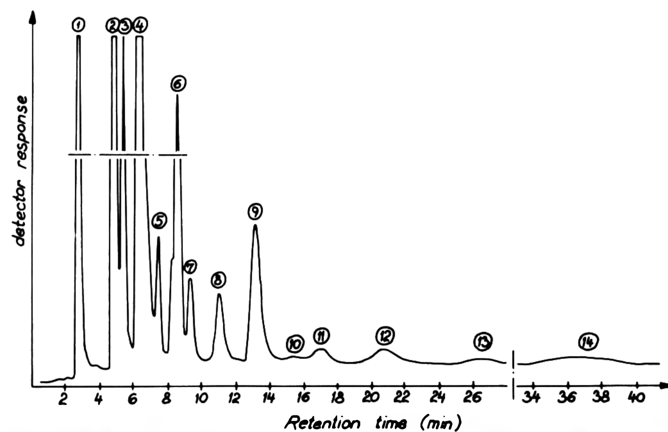
The concentrations of the other VFA were rather low and constant, but it is interesting to note that the propionic acid concentration in the smoked and stored sausages was nearly 10-fold higher compared with the level in the drying sausages.

The differences related above concerning lactic acid formation and VFA development during the two fermentation processes were of the same magnitude in a preliminary study under the same process and storage conditions and seem therefore to be conclusive.

The differences observed can be explained by assuming existence of different dominating bacteria strains in the two types of fermented sausages. Besides homofermentative lactobacilli there exist e.g., heterofermentative lactobacilli strains which produce the VFA analyzed by carbohydrate fermentation.

The compositions of the neutral, volatile monocarbonyl fractions isolated from the sausages are summarized in Table 3. All the straight-chain alkanals up to octanal, with the exception of butanal, were detected as well as several methyl ketones, 2-alkenals and 2,4-alkadienals.

Figure 3 shows a gas chromatogram of the volatile monocarbonyl content of the dried sausages.



1 = CH_3CHO
2 = C_2H_5CHO
3 = $(CH_3)_2CO$
4 = $(CH_3)_2CHCHO$
5 = $CH_3COC_2H_5$
6 = $(CH_3)_2CHCH_2CHO + C_2H_5CH(CH_3)CHO$
7 = n-C_4H_9CHO
8 = C_4H_7CHO(2-enal, branched)
9 = n-$C_5H_{11}CHO$
10 = C_5H_9CHO(2-enal, branched)
11 = C_5H_9CHO(2-enal, straignt chained)
12 = n-$C_6H_{13}CHO$
13 = n-$C_6H_{11}CHO$(2-enal, straight chained)
14 = n-$C_7H_{15}CHO$

Fig. 3—Gas chromatogram showing the volatile monocarbonyl pattern in sausages dried for 21 days after addition of the internal standard (For details see Halvarson, 1972).

The quantitatively dominating substances in the raw sausages were ethanal, propanal, propanone and in the drying sausages 3-methyl butanal in concentrations of 1.1–3.6 mg/kg. Their effect on the aroma quality, however, is probably rather limited. A comparison of the content of carbonyls with stronger potential influence on the aroma quality in the raw and in the ripened sausages reveals that the concentrations of n-hexanal, n-octanal and some of the 2-alkenals have increased significantly in the dried sausages.

Table 3–Monocarbonyl content in the beginning and at the end of the experiment

	Quantitative composition after storage[a] (mg/kg sausage)			
	Drying sausage		Smoked and stored sausage	
Qualitative composition	0 days	21 days	0 days	21 days
HCHO	trace	trace	trace	trace
CH_3CHO	3.6	2.3	1.7	2.2
C_2H_5CHO	1.8	2.4	1.3	1.6
$(CH_3)_2CHCHO$	0.1	0.4	0.1	0.2
n-C_4H_9CHO	0.2	0.3	0.1	0.2
$(CH_3)_2CHCH_2CHO$	1.1	1.0	0.4	0.4
$C_2H_5CH(CH_3)CHO$	0.6	0.5	–	0.2
n-$C_5H_{11}CHO$	0.2	0.5	0.1	0.3
n-$C_6H_{13}CHO$	0.1	0.1	–	0.1
n-$C_7H_{15}CHO$	–	0.1	–	–
$(CH_3)_2CO$	1.5	0.6	2.4	1.3
$CH_3COC_2H_5$	–	0.1	trace	0.2
CH_3COn-C_3H_7	0.1	–	0.02	0.03
CH_3COn-C_5H_{11}	–	–	0.02	–
$(CH_3CO)_2$	+	–	+	+
$(C_2H_5)_2CO$	–	–	–	0.2
C_4H_7CHO (2-enal, branched)[b]	0.8	1.9	0.5	0.5
n-C_5H_9CHO (2-enal)	–	0.6	0.04	–
C_5H_9CHO (2-enal, branched)[b]	–	0.05	–	–
n-$C_6H_{11}CHO$ (2-enal)	trace	0.3	trace	–
C_5H_7CHO (2, 4-dienal)	+	–	–	+
C_6H_9CHO (2, 4-dienal)	+	–	trace	–

[a] – sign indicates that the substance could not be traced; + sign indicates that the substance was detected but not quantified. Trace limit ≃ 0.01 mg/kg sausage.
[b] Tentatively identified

These substances can have different origins. Hornstein and Crowe (1960, 1963) found e.g., ethanal, propanal, n-hexanal, n-octanal, 2,4-heptadienal and propanone from beef fat, pig fat and lamb fat, and Gaddis et al, (1961) and Ellis et al. (1966, 1968) found alkanals, 2-alkenals and 2,4-alkadienals in model experiments with unsaturated fatty acid units and authentic fats. Several of these aldehydes are formed as secondary degradation products from unsaturated fatty acids such as oleic acid, linoleic acid and linolenic acid via intermediate hydroperoxides. This mechanism explains the relative high proportion of carbonyls formed as autoxidation products in the dried sausages in the end of the experiment. It is a well-known fact that certain organic substances such as phenols which are transferred to the sausages during smoking, act as fat antioxidants because they are oxidized according to the same mechanism, i.e., radial mechanism, as unsaturated fatty acids.

Another pathway to the carbonyls investigated is via Strecker degradation of α-amino acids as in the examples below.

Precursor	Carbonyl compound
$CH_2(NH_2)COOH$	HCHO
$(CH_3)_2CHCH(NH_2)COOH$	$(CH_3)_2CHCHO$
$C_2H_5CH(CH_3)CH(NH_2)COOH$	$C_2H_5CH(CH_3)CHO$

The lowest carbonyls such as ethanal and propanone may also be produced by microbiological activity.

The significance of the analyzed substances on the flavor quality of Swedish fermented sausage has thus been evaluated to a certain degree.

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POSTMORTEM CHANGES IN DARK TURKEY MUSCLE

INTRODUCTION

THE DARK MUSCLES of the avian leg and thigh possess a greater proportion of red muscle fibers than the pectoralis major, which is composed almost entirely of white fibers (Ashmore and Doerr, 1971). Red muscles, compared to white, exhibit a more well developed aerobic metabolism and display slower postmortem pH decline rates. Red muscle may exhibit greater sensitivity, in terms of shortening, to postmortem environmental temperature (Bendall, 1966) than white muscle (Henderson et al., 1970). These biochemical and physiological differences between muscles should be considered, but frequently are not, in the processing of intact avian carcasses.

It is well established that chilling or freezing of excised prerigor muscle results in severe muscle toughening. de Fremery (1963) reported that excised chicken breast muscle, frozen prerigor, experienced severe "thaw rigor" rendering it tough. Muscles which have experienced thaw rigor, as determined by Luyet (1966), exhibit short sarcomeres, loss of acid fluid, and formation of masses of irregular bands. Marsh and Leet (1966) reported that subjecting excised prerigor beef muscle to cold resulted in severe shortening and toughening.

Sparse information is available on the possible effects of cold shortening and/or thaw rigor on intact muscle. Welbourn et al. (1968) demonstrated that 0°C chill treatment increased shear force significantly in the biceps femoris muscle. Correlations between sarcomere length and shear were not significant, however.

The objectives of this study were (1) to identify specific patterns of postmortem changes in dark turkey muscle; (2) to investigate possible relationship of these changes to electrical stimulation; and (3) to determine effects of some commercial processes on intact prerigor muscle.

EXPERIMENTAL

Experiment 1

Toms (6 months of age), restrained in metal cones when killed, were dispatched and determination of time course of rigor, response to electrical stimulation, and sarcomere length were made on biceps femoris and peroneus longus as described by Ma et al. (1971). Measurements of sarcomere length were conducted hourly until lengthening occurred. Excised muscle was vacuum packaged and cooked in a water bath to an internal temperature of 80°C. Warner-Bratzler shear press was used to evaluate muscle tenderness. Electrical stunning, scalding, picking and evisceration were not performed in this experiment.

Experiment 2

Ten rigor and ten prerigor birds, as determined by tactile evaluation, were selected from a commercial processing line. Five birds from each group were chilled at 2°C for 3 hr prior to freezing. The other five birds from each group were frozen immediately with no chilling period. Carcasses were thawed at 2–4°C and samples for precooked sarcomere length determinations (Hegarty and Allen, 1972) on biceps femoris, peroneus longus and gastrocnemius were secured. Opposite sides were wrapped in aluminum foil and cooked in a 180°C oven to an internal temperature of 80°C. Shear was determined as in Experiment 1.

Data were subjected to analysis of variance (significance of means determined by "t" test) and correlation analysis (Steel and Torrie, 1960).

RESULTS & DISCUSSION

A HIGH DEGREE of variation was noted for threshold and duration of response to stimulation and time course of rigor for both muscles (Table 1). The 4 and 5 hr rigor time courses for biceps and peroneus muscles may be a finding of practical importance. Thin, superficial leg and thigh muscles could conceivably experience prerigor freezing during the rapid freezing process used by some plants. Also possible is a cold shortening effect if prerigor muscle is quickly chilled postmortem. The importance of both phenomena to meat tenderness depends upon whether or not the sarcomeres can shorten significantly in the intact state.

Rigor time course was correlated to excitability threshold (Table 1) in b. femoris ($r = -0.41$, $P < 0.05$) and p. longus ($r = -0.56$, $P < 0.05$) and to contractility duration in the p. longus ($r = 0.91$, $P < 0.01$). These results agree with those of Ma et al. (1971) on the pectoralis muscle. They suggest a possible mechanism for objective, rapid determination of rigor state in avian muscle which might be related to tenderness, based on electrical properties (Zachariah et al., 1971). Both muscles exhibited apparent postmortem sarcomere lengthening (Fig. 1) patterns similar to but generally slower than pectoralis major muscle, as determined by Ma et al. (1971). These data are difficult to interpret due to the probability that contraction occurred during homogenization of prerigor myofibrils. A very complete discussion of the problems involved in this procedure has been published (Ma et al., 1971). Thus, sarcomere length, as determined on prerigor muscle (1 or 2 hr postmortem) by this method is not equivalent to a 24 hr postmortem measurement. These findings could explain the lack of correlation between sarcomere length and shear in some previous studies (Welbourn et al., 1968).

Shear value of b. femoris was significantly correlated to sarcomere length at 24 hr postmortem ($r = -0.45$, $P < 0.05$). The finding of high shear in muscle with short sarcomeres has been reported by several investigators (Herring et al., 1965). The same relationship was highly significant ($r = -0.68$, $P < 0.01$) in the peroneus. It should be emphasized that both muscles remained intact until 24 hr postmortem at which time sarcomere length and shear samples were removed.

Experiment 2 was designed to determine whether commercially practiced methods of chilling and/or freezing of prerigor, intact muscle could result in toughening. Minimal effects were noted

Table 1—Means for electrical stimulation response, time course of rigor mortis and shear of biceps femoris and peroneus longus

	Biceps femoris (n = 17)		Peroneus longus (n = 13)	
	Mean	Std dev	Mean	Std dev
Rigor completion (min)[a]	163	79	241	85
Excitability threshold (volts)[b]	20	30	6	4
Contractility duration (sec)[c]	204	104	405	151
Shear value (kg)	5.2	1.7	2.2	0.0

[a]Time to complete the decrease in extensibility
[b]Minimum voltage that stimulated contraction
[c]Time that muscle maintained ability to contract upon stimulation

for chilling, freezing and heating on sarcomere length of biceps femoris muscle (Table 2). However, shear value was significantly ($P < 0.05$) higher in muscle, frozen without chilling, which was in rigor at time of freezing as compared to prerigor muscle. This was an unexpected result. It is possible that the postmortem behavior of the biceps femoris muscle may be similar to the anaerobic pectoralis major in which case rapid completion of rigor and anaerobic glycolysis may be associated with toughening. There is need for histochemical determination of fiber type distribution of the muscles of the turkey leg and thigh. This information would be useful in explaining patterns of postmortem changes in muscles with different fiber distributions.

The peroneus longus experienced a significant ($P < 0.05$) increase in sarcomere length due to cooking in both prerigor groups. As determined by Hegarty and Allen (1972), the effect of cooking on sarcomere length depends upon the length of the uncooked sarcomeres. Thus, this result may have occurred because muscles had undergone thaw rigor (during homogenization) and, therefore, possessed very short sarcomeres in the uncooked state. Also noted are shorter sarcomeres in prerigor uncooked samples compared to rigor uncooked samples. There was a similar increase in sarcomere length due to cooking on the unchilled-prerigor group for the gastroncnemius. Again, shorter ($P < 0.05$) sarcomeres were noted in prerigor groups as compared to rigor groups. In peroneus longus and gastrocnemius, there is a definite trend that prerigor muscles have high shear values, with those in the gastrocnemius being significantly ($P < 0.05$) higher. It should be emphasized that all sarcomere length determinations in Experiment 2 were made on postrigor muscle. Thus, the short sarcomeres noted for some treatments were not the result of homogenization of prerigor myofibrils. These results suggest that in peroneus and gastrocnemius, chilling and/or freezing of prerigor muscle causes shortened sarcomeres and toughness in thawed muscle. Therefore, cold-induced or freeze-induced contracture does occur in muscle treated prerigor, even when muscles are intact. Such sarcomere shortening may induce toughening although considerable variation occurs between muscles. Subsequent

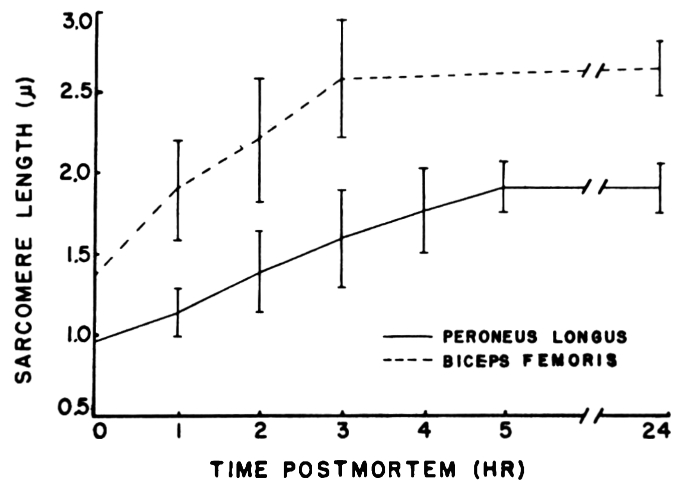

Fig. 1—Graph of sarcomere length as a function of time postmortem for biceps femoris and peroneus longus muscles.

Table 2—Effects of chilling and freezing prerigor and rigor intact muscles on cooked and uncooked sarcomere length and cooked shear value

	Prerigor No chill (n = 5)	Rigor No chill (n = 5)	Significance between treatments[a]	Prerigor chill (n = 5)	Rigor chill (n = 4)	Significance between treatments[b]
Biceps femoris						
Sarcomere length (μ)						
uncooked	1.73 ± 0.17[d]	1.87 ± 0.15	NS	2.11 ± 0.25	1.80 ± 0.33	NS
cooked	1.80 ± 0.35	1.67 ± 0.30	NS	1.90 ± 0.11	1.90 ± 0.24	NS
Significance due to cooking[c]	NS[e]	NS		NS	NS	
Shear value (kg)	2.01 ± 0.25	2.61 ± 0.63	P < 0.05	2.17 ± 0.87	2.10 ± 0.27	NS
Peroneus longus						
Sarcomere length (μ)						
uncooked	1.14 ± 0.16	1.26 ± 0.47	NS	1.14 ± 0.13	1.57 ± 0.36	P < 0.05
cooked	1.52 ± 0.14	1.46 ± 0.24	NS	1.47 ± 0.12	1.34 ± 0.13	NS
Significance due to cooking[c]	P < 0.05	NS		P < 0.05	NS	
Shear value (kg)	4.01 ± 0.33	3.41 ± 0.73	NS	3.88 ± 1.04	3.32 ± 0.75	NS
Gastrocnemius						
Sarcomere length (μ)						
uncooked	0.99 ± 0.09	1.59 ± 0.32	P < 0.01	1.29 ± 0.13	1.56 ± 0.46	NS
cooked	1.21 ± 0.05	1.53 ± 0.21	P < 0.01	1.24 ± 0.12	1.39 ± 0.13	NS
Significance due to cooking[c]	P < 0.01	NS		NS	NS	
Shear value (kg)	4.53 ± 0.80	3.56 ± 0.82	P < 0.05	4.83 ± 0.92	2.99 ± 1.11	P < 0.05

[a]Comparison of freezing prerigor and rigor muscle (unchilled); sarcomere length measurements made on postrigor muscle
[b]Comparison of chilling prerigor and rigor muscle; sarcomere length measurements made on postrigor muscle
[c]Comparison of sarcomere length of cooked and uncooked muscle
[d]Mean ± standard deviation
[e]NS = not significant (P < 0.05)

heating of muscle with short sarcomeres results in sarcomere lengthening. Nevertheless, the difference in shear between short- and long-sarcomere muscle is not overcome by cooking. Further studies are needed to determine the possible relationships among the postmortem changes in muscle, the mechanism of toughening/tenderization postmortem, and the proportion of red and white or fast and slow fibers.

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SORPTION HYSTERESIS AND CHEMICAL REACTIVITY: LIPID OXIDATION

INTRODUCTION

THE PHENOMENON of hysteresis has received considerable attention since the early part of this century. Rao (1941) has shown the hysteresis loop to be remarkably permanent and reproducible. A review of the literature shows numerous examples of hysteresis (Rao, 1941; Taylor et al., 1961; Bettleheim and Ehrlich, 1963; Berlin et al., 1969; MacKenzie and Luyet, 1971; Wolf et al., 1972). Labuza (1968) also made a review of the theoretical and practical aspects of water sorption and hysteresis in foods. However, the relationship between sorption hysteresis and chemical reactivity has not been explored until recent years.

In studying intermediate moisture foods, Labuza (1971) and Labuza et al. (1972) found that at the same water activity, reaction rates for deterioration can be very different depending on the direction of reaching the final water activity, either adsorption by going up the isotherm or desorption by going down the isotherm from the natural moisture content. They reported that the lipids in the system on the adsorption branch of the hysteresis loop oxidized about four to five times slower than the desorption systems at the same water activity.

Labuza et al. (1969) have reviewed the kinetics of lipid oxidation as a function of water activity. It was shown that water was protective for dehydrated foods and as water activity increased the oxidation rate decreased. However, it was also shown that in the capillary region of the isotherm (intermediate moisture foods range) the oxidation rate increased. Labuza (1971a) discussed the chemical stability of foods as a function of both moisture content and water activity. The protective effect of water at low water activity and low moisture content was attributed to hydration of metal catalysts decreasing their effectivenss and hydrogen bonding of peroxide thus slowing the chain reaction. The accelerative effect of water at higher water activity and moisture content was postulated to be due to the soluble solids content, viscosity of the liquid phase and swelling of the polyceric matrix. Normally, at higher water activities the water present presumably mobilizes the catalysts and swells the solid matrix exposing new catalyst sites so that the rate of oxidation increases over that of foods at lower water activity. Such a hypothesis might explain the mechanism of the faster oxidation rate for the desorption foods which are more swollen and contain more water than adsorption foods.

The purpose of this study is, first, to confirm the results found in actual food systems and, second, to elucidate the exact mechanisms and kinetics which control the rate of oxidation as affected by sorption hysteresis. The model system approach was used in order to provide conditions more amenable to control.

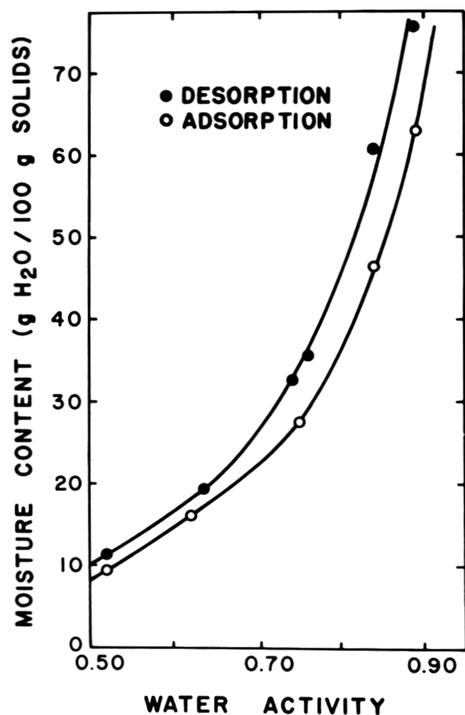

Fig. 1—Microcrystalline cellulose model system sorption isotherm.

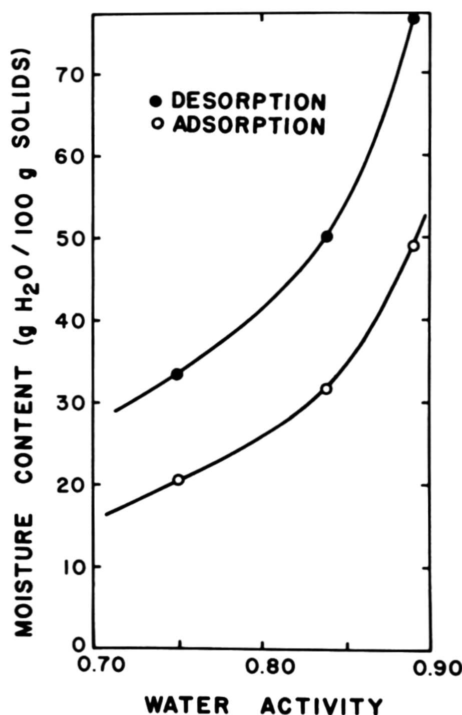

Fig. 2—Amylopectin model system sorption isotherm.

MATERIALS & METHODS

Isotherms

Methods similar to that reported by Labuza (1971) and Labuza et al. (1972) were used. The system compositions used are shown in Table 1. Methods used include:

System preparation. Glycerol and methyl linoleate are first mixed in a beaker using a glass stirring rod. To this the solid support (either microcrystalline cellulose (Avicel FMC Corporation) or amylopectin) is added and mixed thoroughly. For the direct mix system (DM) the amount of water necessary to achieve the desired water activity (as previously determined) is added. This gives the desorption system. For the humidified systems (DH) after mixing the support with the lipid and glycerol, the system is transferred into vacuum desiccators containing saturated salt solutions and held for 48 hr at 37°C.

A_W measurement. The vapor pressure apparatus described by Karel and Nickerson (1964) was used. It has an error of only ± 0.5% RH as determined with saturated salt solutions.

Moisture content. Moisture content in con-

Table 1—System compositions model systems

Avicel systems	
Methyl linoleate[a]	10
Glycerol	40
Microcrystalline cellulose	50
Water	Per isotherm
Amylopectin systems	
Linoleate	10
Glycerol	40
(Starch) Amylopectin	50
Water	Per isotherm

structing the isotherms was measured by GLC technique as described by Tihio et al. (1969). The isotherms are shown in Figures 1 and 2. The hysteresis effect is obvious. The samples after humidification were also checked by weight gain as a double check for moisture content before putting into storage.

Trace metal level

Trace metal analysis was performed by atomic absorption spectroscopy using a Perkin Elmer 303 A.A. spectrophotometer at the University of Minnesota Soil Science Agricultural Experiment Station.

Viscosity measurements

Viscosity was measured with the Brookfield Synchro-Leteric viscometer, Models BHT and LVF.

Oxidation measurements

Samples (in triplicate or duplicate) were prepared for oxidation studies by the method described in the isotherm system preparation above. The system was weighed directly into the Warburg manometer flasks before humidification to facilitate ease of handling. The direct mixed system was also held simultaneously in the desiccator so that the extent of oxidation is the same. Vacuum desiccators are used to prevent or to slow oxidation during the humidification procedure. After equilibration the flasks were connected to the manometers and oxidation was measured. The method of Umbreit et al. (1964) was used to calculate oxygen uptake.

RESULTS & DISCUSSION

IN THE FIRST three runs a repeat of previous studies done by Labuza (1971), Heidelbaugh and Karel (1970) and Heidelbaugh et al. (1971) was performed in order to validate the test methods. In addition, studies were made at higher humidities than previously done.

Figure 3 shows the results obtained for oxygen absorption in Warburg manometers for duplicate flasks from Run 1. Oxidation follows the typical autocatalytic pattern with a slow rate period followed by an increasingly faster rate. As expected the oxidation rate increases with increased A_w. In addition, the direct mixed (DM) systems oxidized faster than the humidified systems (DH) at similar water activity as had been reported by Labuza et al. (1972). The pro-oxidant effect of water at high A_w was presumed to be due to the increased mobility of the metal catalysts in the aqueous phase since the water content increase lowers the liquid phase viscosity. In addition, possible exposing of new catalytic sites during swelling of the solid matrix as A_w increases could also occur. Of course, in the microcrystalline cellulose system swelling should not occur to any large degree since cellulose particles are completely crystalline (Bluestein and Labuza, 1972). Thus, the mobility effect should be predominant for oxidation in this type of system.

It can be seen explicitly in Figure 3 that the rate of oxidation is faster at A_w = 0.84 than 0.75 for both desorption and adsorption systems. The difference is more easily illustrated in Table 2 where the kinetic constants for oxidation are reported. These constants, θ_i (the time to reach 1% oxidation on a molar basis which is called the induction time) and K_M (the monomolecular rate constant) which is the rate during the initial stages of oxidation up to the time of possible rancidity are useful in assessing the effect of various additives and conditions on lipid oxidation. Labuza (1971b) has reviewed the methodology in obtaining these values.

It can be observed in Table 2 that in all cases the dry mix humidified systems all show slower rates of oxidation as compared to the direct mix systems by about 1.5 times on the average. Although the magnitude of difference is somewhat less than that reported by Labuza et al. (1972) for actual liquid food IMF systems this confirms that oxidation is slower on the lower branch of the hysteresis loop. In Table 3 are shown the ratio of the theoretical viscosities of the systems in comparison to the ratio of the rates of oxidation. It can be seen that the viscosity effect can account for most of the decrease in oxidation in the humidified system but not all. This confirms that catalyst mobility is probably the major factor in the nonswelling cellulose system for control of oxidation rate.

In Run 4 amylopectin was used as the

Table 2–Microcrystalline cellulose systems

		Oxidation constants			
		θ_i Induction time (hr)		K_M $(M/M)^{1/2} \times 10^3$	
Run #	A_w	DM[a]	DH[b]	DM[a]	DH[b]
1	0.75	109	137	1.17	0.82
	0.84	73	109	1.81	0.88
2	0.52	183	197	0.82	0.49
	0.75	93	109	1.33	0.82
	0.84	72	110	1.51	0.92
	0.89	76	110	1.58	1.15
3	0.52	158	179	0.88	0.70
	0.68	95	111	1.10	0.93
	0.84	67	110	1.47	1.04

[a]DM–direct mix
[b]DH–dry mix (humidified)

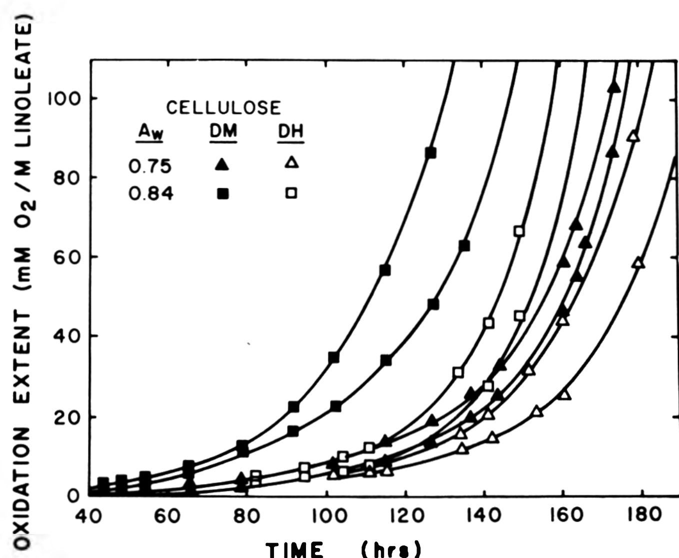

Fig. 3–Run 1–Extent of oxidation cellulose model system.

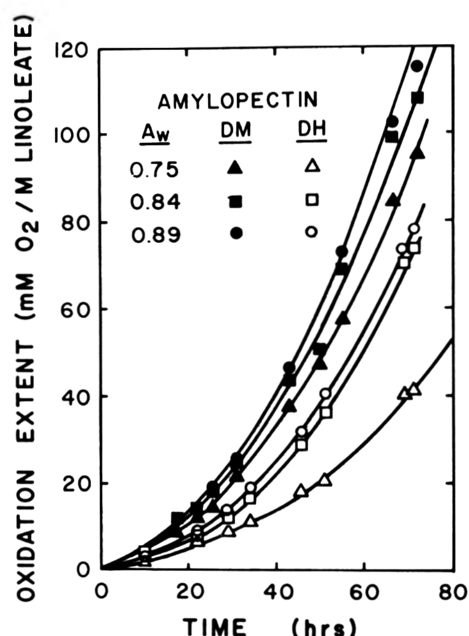

Fig. 4–Run 4–Extent of oxidation amylopectin model system.

Table 3–Run 1

	Theoretical viscosity[a] (centipoise)	K_M ($10^3 (M/M)^{1/2}/HR$)	Moisture content $gH_2O/100g$ solids
$A_w = 0.84$			
Direct mix	2.25	1.81	61
Humidified	3.00	0.88	47
Ratio	0.75	0.48	
$A_w = 0.75$			
Direct mix	4.16	1.17	32
Humidified	5.37	0.82	27
Ratio	0.78	0.70	

[a]Viscosities of aqueous phase due to glycerol

Table 4–Lipid IMF model systems oxidation constants

Run #	Support	A_w	Time to reach 1% oxidized–θ_i (hr) DM[a]	DH[b]	Monomolecular rate constant–K_M $(M/M)^{1/2}\ hr^{-1} \times 10^3$ DM[a]	DH[b]
4	Amylopectin	0.75	22	32	3.81	2.32
	Amylopectin	0.84	14	24	3.98	3.66
	Amylopectin	0.89	16	23	5.30	3.49
5	Microcrystalline cellulose	0.84	78	103	2.02	1.53
	Amylopectin	0.84	18	21	5.10	2.68

[a]DM–direct mix
[b]DH–dry mix (humidified)

Table 5–Time to reach 3% oxidation (hr), Run 6. (Total metal content–1000 ppm)

System	A_w	DM	DH	DH/DM
Microcrystalline cellulose[a]	0.75	11.0	9.7	0.882
Microcrystalline cellulose	0.84	18.5	12.5	0.676
Amylopectin	0.75	46.0	55.0	1.20
Amylopectin	0.84	38.0	43.5	1.14

[a]Soluble salts were added for each metal to give the same level as in amylopectin system.

Table 6–System viscosities[a]

% H_2O[b]	Spindle #[c]	rpm	Viscosity (cp x 10^3) Amylopectin system	Cellulose system
79.2	6	5	97	54
71.6	6	2.5	256	160
65.5	7	5	307	179

[a]at 20°C
[b]$gH_2O/100g$ solids
[c]Brookfield viscometer Model BHT

Table 7–Heavy metal contents (ppm)

Systems	Ca	Fe	Mg	Cu	Mn	Total
Amylopectin before dialysis[a]	285	90.5	570	5.86	4.13	955.5
Microcrystalline cellulose[b]	0.4	0.58	0.33	0.27	0.08	1.7
% Left after dialysis[c]						
Amylopectin	83.5	77.1	63.3	88.3	70.0	70.8
Microcrystalline cellulose	51.9	91.2	39.0	60.1	87.5	48.3

[a] Free metal salts were added to cellulose to those same levels
[b]Metal content before free metals added and before dialysis
[c]8-hr dialysis in dialyzer tubing (Fisher Scientific Co.) with distilled water

support. It has the same basic glucose unit as in cellulose but has a different linkage thus giving it swelling properties. It also shows a larger hysteresis loop (Fig. 1 and 2). Amylopectin also contains a very high concentration of trace metals, about 1000 ppm, as compared to the microcrystalline cellulose which has only about 3 ppm. As seen in Figure 4 oxygen absorption was very rapid with very little induction time. This can be attributed to the high trace metal concentration in the amylopectin (Table 7). In Run 5 both amylopectin and microcrystalline cellulose were compared simultaneously using the same batch of lipid and humidifying at $A_w = 0.84$. The oxidation constants of Runs 4 and 5 are reported in Table 4. The most significant finding of Runs 4 and 5 is that the amylopectin system follows the same pattern for lipid oxidation as microcrystalline cellulose with respect to water activity and method of preparation.

In order to establish whether swelling or catalyst mobility and concentration is the predominating mechanism in the increase in oxidation rate with A_w, the two support systems were studied in Run 6 in a simultaneous experiment. In this case in order to normalize the effects of catalysts, metal salts were added to the microcrystalline cellulose system in the amounts equal to that measured in the amylopectin system. Soluble salts were added for each metal to give a total concentration of about 1000 ppm with the same distribution of metals.

Oxygen uptake was measured by a Warburg manometric system. The rates were so fast that no measurement of K_M or θ_i was possible. In order to make comparisons the time to reach 3% oxidized is presented in Table 5. This time should be of value since an examination of most curves shows a very rapid rise in extent after 1% oxidation is reached.

In Table 5 it is noted that, as in previous runs, for the amylopectin system an increase in A_w decreases the time to reach a given extent of oxidation. In addition, the humidified samples oxidized slower than the direct mix system. However, for the microcrystalline cellulose exactly the opposite is found to that which was reported earlier. The rate decreased with an increase in A_w and the rate increased for the humidified system. Although at first this might be attributed to using the 3% oxidation level, an examination of all previous data (not reported here) at 3% oxidized always showed the same previous trend as reported at 1% oxidized.

These results can be used to explain the separate effects of swelling vs. catalyst mobility. In the cellulose system all the added trace metals are relatively free and are dissolved in the aqueous phase. At some point it should be expected that increasing the moisture content would

tend to dilute the concentration of metals in solution and increase their diffusion path. Simultaneously, the phase viscosity is reduced as more water is added which should increase the diffusion rate constant. These two mechanisms work in opposite direction. If swelling is not significant, i.e., no new catalyst sites exposed as the matrix absorbs more water and swells, then the two solution mechanisms presented above would predominate. The higher A_w system would have a lower catalyst concentration than the lower A_w system since it has more water present. At the high trace metal concentration the diffusion rate is no longer as strongly controlling as in the low trace metal systems previously studied so that the concentration effect predominates. Thus, an increase in A_w should reduce the rate because of a dilution of metals. As to the reason the humidified systems now oxidize faster, the same explanation holds true. The free metal concentration is higher than at the corresponding A_w system for the direct mix because less water is present. The increased viscosity although possibly slowing diffusion is masked by the concentration effect.

In the amylopectin system exactly the opposite happens. Physically, the gel structure of amylopectin system seems to have a higher viscosity than that of the cellulose system, so that any increase in moisture although diluting the metal level also reduces viscosity and increases the reaction rate. Although direct measurement of the system viscosity is very difficult (due to different solid support and system texture) in higher moisture content systems where the Brookfield BHT viscometer can be used the differences in viscosity between the two systems are obvious. Some of the measurements are shown in Table 6. In addition, the metals in amylopectin are more strongly bound to the solid matrix (see Table 7) and probably more hidden in unswollen pores so that swelling of the solid matrix should open up new catalytic sites and thus increase the effective catalyst concentration. The higher viscosity and more strongly bound, hidden metals in the amylopectin should explain the fact that at the same metal level, water activity and sorption isotherm branch, this system oxidized much slower than the cellulose system to which metal was added (Table 5).

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FATE OF WATER SOLUBLE SOY PROTEIN DURING THERMOPLASTIC EXTRUSION

INTRODUCTION

THE INCREASED ACCEPTANCE and use of texturized soybean products has been accompanied by studies of both a technical and basic nature. One area in which the literature seems to be deficient is in the linking up of basic and practical endeavors.

Glicksman (1971) and Wolf and Cowan (1971) provide excellent reviews on many facets of soybean technology. Water soluble soybean proteins are made up of four basic ultracentrifuge fractions present in differing amounts, i.e., 2S–22%; 7S–37%; 11S–31%; and 15S–11% (Wolf, 1970).

Much work has been done on the 11S fraction and many of its properties are well documented. It has been reported to undergo a 2S←7S⇌11S⇌15S transition (Catsimpoolas et al., 1969) and is thought to polymerize through disulfide linkage (Wolf and Tamura, 1969). The 11S protein reacts to heating by breaking into subunits at temperatures above 70°C, and forming insoluble aggregates (Catsimpoolas et al., 1970).

The 7S fraction consists of at least two immunochemically distinct moieties, β-conglycinin and γ-conglyanin (Catsimpoolas, 1969). Partially purified 11S protein contains a 15S fraction which reacts in the same manner as the 11S protein with regard to purification (Catsimpoolas et al., 1967). This is in agreement with earlier findings by Wolf et al. (1962).

Much work has been done on semipurified protein fractions in solution relative to heat stability and it seems that certain fractions are insolubilized by heating (Saio, 1968). With regard to molecular changes resulting from processing, little appears to have been published.

In this work we have attempted to ascertain the effect of thermoplastic extrusion on the water soluble soybean proteins by analyzing qualitatively and quantitatively changes in the soy protein from thermoplastic extrusion. Of prime importance was the correlation of theoretical findings for isolated protein with reactions occurring as a result of processing.

EXPERIMENTAL

EXPERIMENTAL MATERIAL was produced with a Brabender laboratory extruder equipped with a 7.5 in. tapered screw. All process parameters except extrusion temperature were held constant. The diameter of the die orifice was 0.125 in. and screw speed was 100 rpm. The formulation was kept as simple as possible to avoid the problems associated with determining cause and effect relationships in a complex system. Raw material for extrusion consisted of commercial defatted soybean meal with 30% added moisture. None of the suggested adjuncts such as sulfur compounds (Jenkins, 1970), reportedly required for good texturization, was employed. Nevertheless, heat and pressure provided a sufficiently texturized material to allow an investigation of texture parameters (Cumming et al. 1972).

The main tool employed in observing the effect of processing on water soluble proteins was polyacrylamide disc gel electrophoresis. A Model 1200 Canalco Disc Electrophoresis system with a Canalco Model 300 power source and Canalco Model 1800 quick gel destainer was employed.

Samples were prepared by extracting soy meal or pulverized extruded products with four volumes of distilled water for 1 hr. After extraction, the samples were centrifuged and the supernatant used for eletrophoresis. Samples of 5–15 μl generally gave proper loading. For characterization and determination of sample solubilities a standard 7% gel system was exployed (stacks at pH 8.9, runs at pH 9.5). A current of 5 ma/gel was used with the cathode in the upper bath. Migration was considered complete when the bromphenol blue tracking dye had

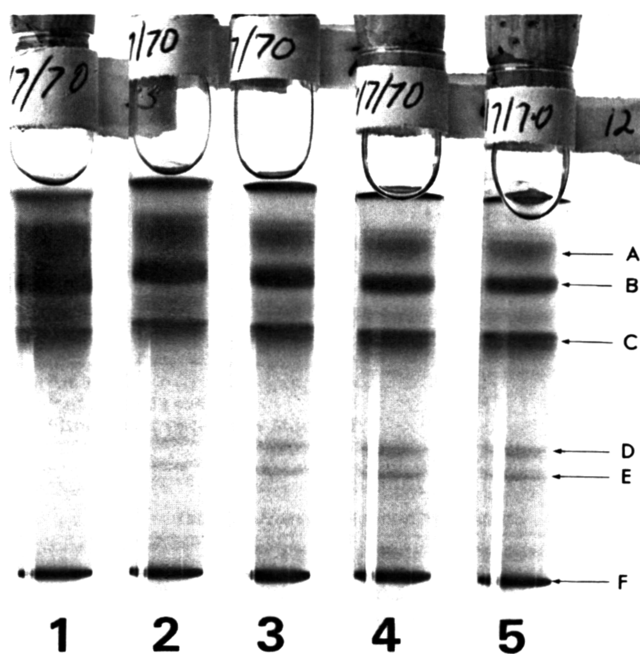

Fig.1—Effect of process temperature on electrophoresis pattern: (1) 93°C; (2) 121°C; (3) 149°C; (4) 177°C; (5) 204°C.

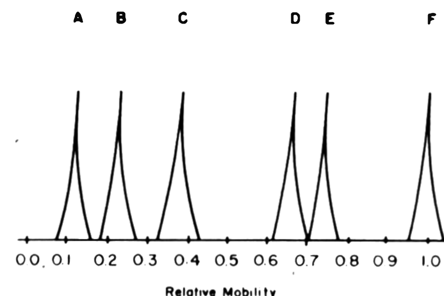

Fig. 2—Schematic diagram of the major water soluble soybean proteins found by polyacrylamide disc gel electrophoresis.

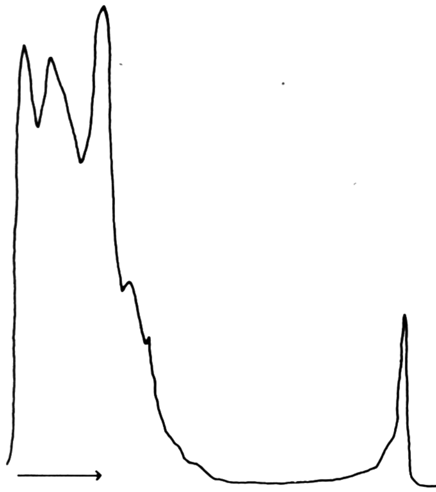

Fig. 3–Typical densitometer trace for unprocessed soybean meal.

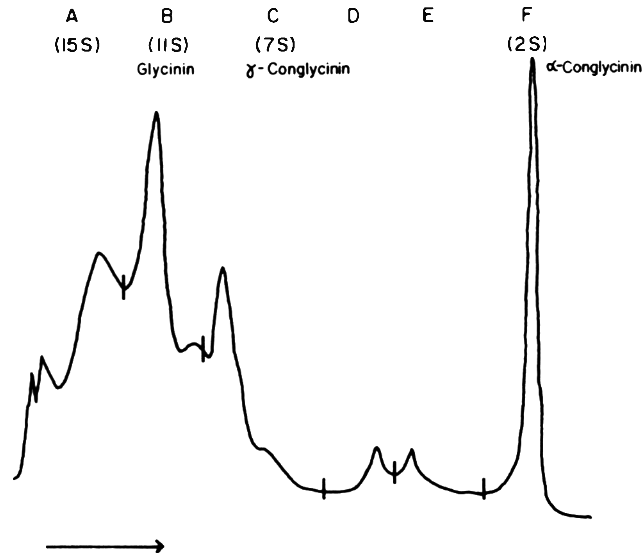

Fig. 4–Densitometer trace for product at 175°C.

travelled 3.8 cm through the running gel. The gels were stained for 1 hr with aniline blue-black, destained with the Canalco Model 1801 quick gel destainer and stored in 7% acetic acid.

A Joyce-Loebl Chromoscan densitometer was used to scan the gels at 620 nm. This instrument is equipped with an integrator and from the traces produced it was possible to obtain relative mobilities (Rm) and to estimate relative sample concentrations and individual peak intensities.

Molecular weights of various fractions were estimated by sodium dodecyl sulfate (SDS) electrophoresis. The method used combined the procedures and theories of Shapiro et al. (1967), Parish and Marchalonis (1970) and Zwaan (1967). A modified procedure for staining and destaining of gels was developed. After completion of electrophoresis, gels were fixed for 20 hr in 10% trichloroacetic acid, thoroughly washed with water and stained for 2 hr with aniline blue-black. Destaining was then accomplished with the quick gel destainer.

RESULTS & DISCUSSION

AS THE TEMPERATURE of processing rises, the concentration of many components changes drastically as is demonstrated by Figure 1. Those closer to the origin slowly disappear and ones with faster migration rates tend to increase. The six fractions of particular interest are indicated in Figure 1 and labelled A–F.

Twelve identical samples of defatted, unprocessed Altona soybean meal were examined electrophoretically to determine reproducibility of Rm values for replicate samples. The coefficients of variation for the four peaks chosen for examination averaged 0.84% and in terms of actual distance on the gels this amounted to 0.32 mm.

Figure 2 is a schematic diagram denoting the Rm range of the six fractions, A–F, which were found to comprise the major portion of the extracted water soluble proteins. These fractions are not found in all gels regardless of process temperature nor are they of equal intensity, i.e., fractions D and E appear and increase with increasing process temperature while A and B, particularly A, tend to decrease. Figure 3 is a typical densitometer trace for unprocessed meal and Figure 4 is a trace for meal processed at 175°C. Two naming systems have been applied to the fractions from literature reports and molecular weight studies. Where possible, fractions are designated according to the Svedberg constant and by the naming system suggested by Catsimpoolas (1969). It appears that the unprocessed meal has large quantities of 11S and 15S protein while at higher temperatures there is a reduction of 15S, 11S, and to a lesser extent 7S protein accompanied by a noticeable increase in breakdown products.

Samples produced at 230°C and at various stages of texturization were hand selected on the basis of appearance (Fig. 5). Processed samples were selected and labelled in ascending order of texturization as: (a) unprocessed meal; (b) 'ex-

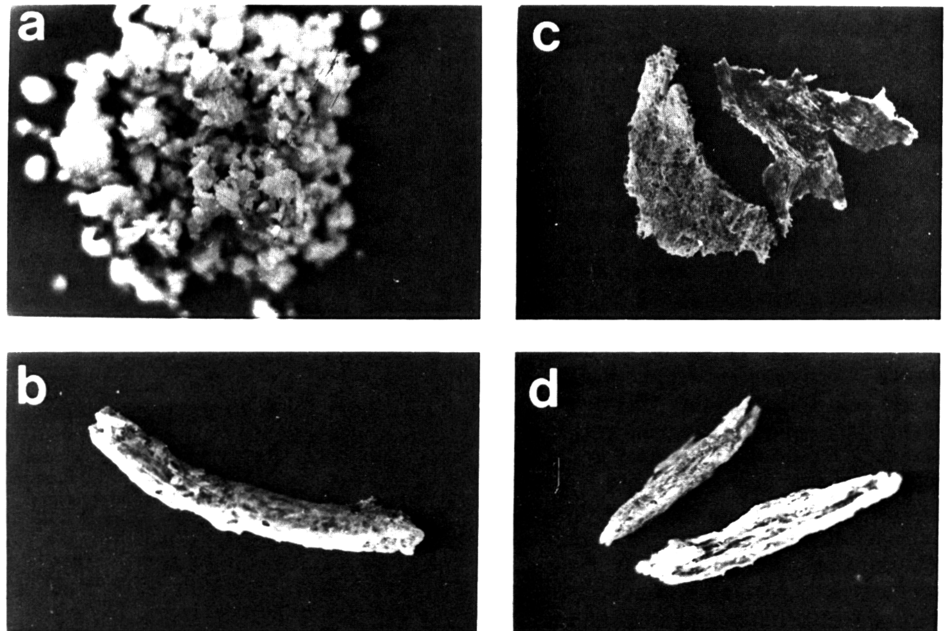

Fig. 5–Soy samples used for electrophoresis; (a) unprocessed; (b) extruded; (c) puffed; (d) texturized.

truded' (a continuous strand, porous in nature and showing some fiberization); (c) 'puffed' (irregular pieces of material exploded by escaping steam, showing some porosity and more fiberization); and (d) 'texturized' (discontinous strands 1–2 cm long but showing substantial fiber formation).

If experimental conditions are carefully controlled the integrator count from the densitometer can be used to approximate relative solubility of the samples. Such an experiment was carried out for products selected by physical appearance. To determine the feasibility of such work, 5, 10 and 15 μl replicates of the four samples were examined electrophoretically, If 1:2:3 ratios were obtained within samples then ratios between samples would also be meaningful within the limitations of the system. Table 1 demonstrates the effect of processing on total densitometer peak area and the ratios of the 5:10 and 5:15 μl samples. The experimental ratios approach the expected integer values of 2 and 3 respectively. Since the relationship of total peak area to sample concentration was close to the expected, the same total areas were compared between samples and in relation to unprocessed meal. Table 2 shows these results. The more highly texturized the sample the less soluble it becomes, to the extent that the texturized material shows only 27% as much soluble protein as the unprocessed meal.

By using the solubility ratios of Table 2 it was possible to express the protein content of the six major peaks (1–6) corresponding to the most prominent peaks of the six fractions (A–F) in g/100g based on the total soluble protein extracted from the unprocessed meal. Table 3 contains this information as well as percentage values for the particular peak area of a sample relative to the total peak area. There is an important distinction to be made between these two values, e.g., peak 4 of the puffed product was found to contain 15.8% of the extracted protein for that product but relating this back to the original protein content of the unprocessed material by applying the solubility ratio of 0.336 the extractable protein is found to be only 5.3 g/100g. As can be seen from the summed g/100g columns considerable protein becomes insoluble during the course of texturization. Table 3 also shows that as the degree of processing increased, peaks 1–3 (g/100g basis) decrease markedly, especially peaks 1 and 2. Peaks 4–6 show an overall rise under the same conditions. However, by the time full textuization is attained all fractions demonstrate a decrease in soluble protein from the maximum value whenever it may have occurred. Peak 6 actually remained relatively static at 7–8 g/100g.

Not all the water soluble protein is accounted for in Table 3. It may be noted for instance that in the case of unprocessed meal 23.8 g/100g of soluble protein is unaccounted for by the data presented. The reason for this is that only the area under the six major peaks was determined and recorded here. The missing 23.8 g/100g is accounted for by peaks and shoulders considered to be of lesser importance to the present investigation.

Another observation of interest is that the various peak intensities change at different rates, i.e., various proteins are affected unequally by processing, a fact which may prove to be of considerable practical importance.

Molecular weight determinations required the establishment of a calibration curve. A number of proteins of known molecular weight ranging from 13,000 (Cytochrome C) to 310,000 MW (Edestin) were used. When Rm was plotted against log MW a straight line was obtained with equation Rm = 4.300 – 0.770 log MW and a standard error of estimate of 0.065 Rm units.

In order to have a sample which contained all the fractions of interest, the product resulting from a process temperature of 175°C was used. The literature contains many molecular weights for the soy proteins. The major factors influencing these reported values are the environmental conditions of the sample and the method of determination. Okubo et al. (1969) claimed a basic subunit for 11S protein of 36,000 MW. However, Catsimpoolas et al. (1967) had previously claimed the subunit to be 26,000 MW. The results of our investigations suggest the subunit to be about 28,000 MW. Catsimpoolas et al. (1967) reported that 11S protein consisted of 12 subunits and has a molecular weight of 350,000. If the subunits were considered equal in size they would be about 29,000 MW which suggests that our value represents the subunit.

Table 4 shows the molecular weights of the material found in the fractions A–F. When examined electrophoretically some of the fractions yielded up to six distinct bands. Table 4 shows these bands as peaks within the separate fractions. Peaks 4, 3 and 1 could be polymers composed of 2, 3 and 6 of the 28,000 MW subunits. Peak 1 may be the 7S form of the 11S protein, since doubling it gives

Table 1—Effect of processing on total peak area

Sample	Densitometer count at known concentration			Count ratios	
	5 μl	10 μl	15 μl	5:10	5:15
Unprocessed	290	551	775	1.9	2.7
Extruded	125	238	326	1.9	2.6
Puffed	91	198	286	2.2	3.1
Texturized	78	147	246	1.9	3.2

Table 2—Effect of processing on relative solubility of protein as derived from electrophoretic analysis

Samples compared	Concentration series		Average
	5 μl	10 μl	
Extruded/unprocessed	0.431	0.432	0.432
Puffed/unprocessed	0.313	0.359	0.336
Texturized/unprocessed	0.269	0.268	0.269

Table 3—Effect of processing on protein distribution in selected samples

Peak	Unprocessed		Extruded		Puffed		Texturized	
	g/100g	%	g/100g	%	g/100g	%	g/100g	%
1	31.1	31.1	4.9	11.3	2.8	8.3	1.9	7.0
2	25.5	25.5	6.5	15.0	2.1	6.4	1.7	6.4
3	7.6	7.6	5.4	12.4	3.9	11.5	3.1	11.7
4	2.8	2.8	5.4	12.4	5.3	15.8	4.0	14.8
5	1.3	1.3	3.6	8.3	3.7	11.0	3.2	12.0
6	7.9	7.9	8.3	19.1	8.5	25.3	6.9	25.7
Sum	76.2	76.2	34.1	78.5	26.3	78.3	20.8	77.6

Table 4–Molecular weight distribution in the six major fractions of soy protein

Peak	Molecular weights (4 replicates) 1	2	3	4	5	6	7
Fraction							
A	170,000	93,000	80,000	55,000	49,000	–	28,000
B	–	93,000	78,000	53,000	47,000	–	27,500
C	–	–	–	52,500	–	–	27,700
D	–	–	–	–	–	29,300	27,000
E	–	–	–	–	–	–	28,000
F	–	–	–	–	–	–	28,300

Table 5–Molecular weight ratios (11S system)

Fraction	Peak 1/7	Peak 3/7	Peak 4/7	Peak 7/7
A	6.07	2.86	1.96	1.00
B	–	2.84	1.93	1.00
C	–	–	1.90	1.00
Closest interger	6	3	2	1

a protein with molecular weight of 340,000 which is very close to the literature value of 350,000 cited previously for 11S protein. Table 5 shows how the various peaks approximate the polymers suggested. From this information it is suggested that fractions A, B and C all contain 11S or glycinin subunits. Fraction A may be 15S protein. The moieties which appear as a result of SDS-electrophoresis indicate that fraction A consists of more than 11S protein and its subunits and polymers. Unfortunately, there is insufficient evidence to definitively indicate the origin of peak 2 or 5 but manipulation of either to only a slight extent allows them to be equated with beta or gamma-conglycinin.

Fraction B apparently consists of 11S protein combined with very small amounts of the two uncertain proteins. Fraction C and therefore peak 5 may be related to gamma-conglycinin; however, at this time our evidence is not strong enough to offer this as any more than a likely possibility. The remaining fractions consist in the main of the 28,000 MW subunit and D and E seem to stem from 11S protein as demonstrated by changes in distribution prompted by processing.

From molecular weight, relative solubility, and protein distribution data it seems that heating in a controlled extrusion process causes most of the water soluble soybean protein to break into subunits and/or become insoluble. Such a reaction was reported by Wolf and Tamura (1969) and Catsimpoolas et al. (1970) as a characteristic reaction of glycinin to heating in solution and certainly seems to hold true in the more complex and practical situation of extrusion cooking. For good fiberization to occur it is apparently necessary as a result of process heat and pressure for a considerable part of the water soluble protein to become redistributed and insolubilized as described here.

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FUNCTIONAL CHARACTERISTICS OF WHEY PROTEIN CONCENTRATES

INTRODUCTION

CONSIDERABLE EFFORT is being made to improve utilization of cheese whey by preparing undenatured whey protein concentrates (WPC) suitable for beverage and other formulated food applications. This work is being pressured by the need to reduce environmental pollution (Arbuckle and Blanton, 1967; Whey Utilization Conference, 1970), the high cost of whey disposal and the substantial economic and nutritional potential for this new protein source.

Commercial scale processes for preparing WPC are rapidly developing in these fractionation technique areas: electrodialysis (Stribley, 1963; Anonymous, 1970a, b); metaphosphate complex (Gordon, 1945; Wingerd, 1968; Hartman and Swanson, 1966); Sephadex gel filtration (Morr et al., 1967; Swanson and Ziemba, 1967; Ek, 1968; Horton, 1972); and ultrafiltration (Goldsmith et al., 1970; Horton et al., 1972; Fenton-May et al., 1971). Additional work is continuing in other less developed areas; carboxymethylcellulose complex (Hansen et al., 1971); iron complex (Jones et al., 1972); and ethanol precipitation (Morr and Lin, 1970).

The nutritional excellence of WPC has been reported (Anonymous, 1970a; Winston et al., 1970; Wingerd, 1971). Although the potential for WPC in carbonated beverages has been recently demonstrated (Holsinger et al., 1972), other claims for exceptional and unique functional properties in food formulations have not been as well documented. Hansen and Black (1972) reported on the whipping characteristics of spray-dried whey protein and carboxymethylcellulose complexes. This study was conducted to survey available WPC preparations for chemical composition and several important functional properties. Functional test procedures were arbitrarily selected and no attempts were made to optimize experimental conditions. Therefore, the findings are preliminary and final conclusions on the functional properties of WPC must await further and more detailed studies.

MATERIALS & METHODS

Source of whey protein concentrates

WPC samples were obtained from the following commercial suppliers. Electrodialysis WPC from Foremost Foods Company, San Francisco, California (acid, calcium neutralized and potassium neutralized Foretein) and Purity Products, Inc., Mayville, Wisconsin (LP 14 and LP 20). Metaphosphate complex WPC from Borden, Inc., Elgin, Illinois (acid, calcium neutralized and sodium neutralized Protolac). Sephadex WPC from Stauffer Chemical Co., Rochester, Minnesota (ENR-PRO 50). Ultrafiltration WPC from Abcor Inc., Cambridge, Mass. and Osmonics, Inc., Minneapolis, Minn. Samples of WPC were also obtained from the following noncommercial sources. Dialysis WPC was prepared in our laboratory by dialyzing pH 4.6 whey against an excess of distilled water for 72 hr and freeze drying. Sephadex WPC was also prepared in our laboratory by the procedure of Morr et al. (1967). CMC complex WPC was obtained from Dr. P.M.T. Hansen, Ohio State University, and iron complex WPC from Dr. E.B. Kalan, USDA Research Laboratory, Philadelphia, Pa.

Chemical and elemental analysis of whey protein concentrates

Ash content was determined by drying "dried" WPC in an atmospheric oven at 105°C and igniting in an electric muffle furnace at 600°C similar to Official Methods of Analysis (AOAC, 1970). Fat was determined by the Mojonnier extraction procedure (AOAC, 1970). Protein was obtained by micro Kjeldahl (AOAC, 1970) using a factor or 6.38 × total N. It was assumed that whey proteins in all WPC preparations are insoluble in 12% trichloroacetic acid (TCA) and therefore, nonprotein N was determined as soluble N in 12% TCA filtrate prepared from each WPC. Lactose was conducted by the phenolsulfuric acid method of Dubois et al. (1956). Phosphorus was determined by the molybdate colorimetric procedure of Sumner (1944). Elemental analysis was obtained by ashing about 100 mg WPC, dissolving the ash and making to 10.0 ml in 0.5% Li-1.5% HCl solution and examining in a 1.5m Jarrell-Ash Emission Spectrometer with

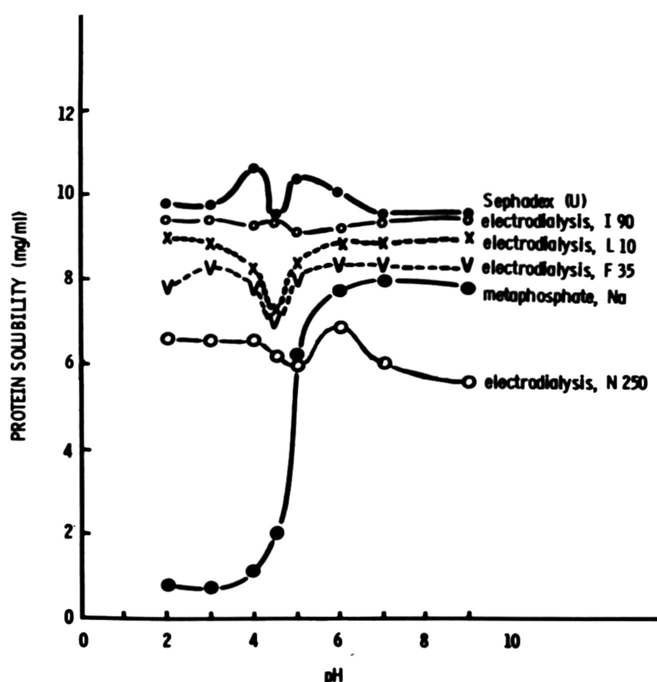

Fig. 1—Solubility of WPC dispersed in pH 6.98 phosphate buffer (1.0% protein concentration). Dispersion was stirred 2 hr, adjusted to pH from 2 to 9 with IN HCl or NaOH and centrifuged at 1,000 × G for 10 min. Supernatants were examined for total N by micro-Kjeldahl.

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direct readout, located in the Soils Dept., University of Minnesota. Available lysine was estimated by the trinitrobenzenesulfonic acid (TNBS) method of Wartheson et al. (1972).

Zonal electrophoresis

Whey proteins were examined by polyacrylamide gel electrophoresis (PAGE) in a vertical cell, Model EC470 (E-C Apparatus Corporation, Philadelphia, Pa.) using 8% gels in 0.02 ionic strength veronal buffer at pH 8.6 (Morr, 1967). Following electrophoresis for 4 hr at 250v, the protein bands were stained with Amido Black to permit visual comparison of protein zones.

Functional properties of whey protein concentrates

Protein solubility. The general procedure for determining protein solubility was to disperse dry WPC, equivalent to 0.5 or 1.0% protein, in distilled water or buffer at room temperature. The dispersions were stirred continuously for varying periods of time and then either filtered or centrifuged to remove insoluble proteins. The soluble protein fraction was adjusted to pH 4.6 and filtered or centrifuged to remove insoluble, denatured whey proteins. Soluble protein fractions were analyzed for total N by micro Kjeldahl. Variations in dispersion, pH adjustment and fractionation procedures are described with the solubility data.

Whipping properties. Whipping properties were determined by mixing 10g WPC with 5g egg white solids (Henningsen Foods, White Plains, N.Y.) in 100 ml distilled water at room temperature. The mixture was whipped at high speed in a Hamilton Beach mixer until a consistency of an egg white meringue was produced. The foam was immediately transferred to a tared 200 ml capacity Buchner funnel and weighed. Overrun was calculated from the

Table 1—Composition of whey protein concentrates

Preparation process	Number of samples	Protein (%)	Lactose (%)	Ash (%)	NPN[a] (%)	Fat (%)
Metaphosphate complex						
Range	3	54.1–58.0	12.7–13.2	10.6–15.5	1.1–1.3	3.3–7.2
Mean		55.7	13.0	13.7	1.2	5.3
Electrodialysis						
Range	5	27.3–37.0	40.5–60.0	1.4–19.7	5.4–7.7	2.4–4.3
Mean		32.9	51.8	9.0	6.7	3.3
Ultrafiltration						
Range	3	49.9–62.0	15.5–40.2	0.4–6.2	2.7–6.8	1.4–14.7
Mean		56.5	27.2	3.4	4.8	7.3
Sephadex						
Range	2	38.7–45.1	–	–	–	–
Mean		41.9	24.9	11.5	4.9	0.8
Dialysis	1	66.0	26.2	2.0	1.5	2.0
CMC complex	1	49.8	20.1	8.0	–	1.2
Iron complex						
Range	2	32.7–37.4	0.7–0.9	53.1–54.9	–	0.3–0.8
Mean		35.1	0.8	54.0	1.1	0.6

[a]Expressed as percentage of total WPC N

Table 2—Elemental analysis of whey protein concentrates

Preparation process	Number of samples	P	K	Ca	Na	Fe	Al	Zn	Cu
		(%)				(ppm)			
Metaphosphate complex	1	–[b]	1.05	–	0.30	–	34	14.1	47.9
Electrodialysis[a]	2	1.03	2.20	0.69	3.22	–	144	16.0	16.0
Ultrafiltration	1	0.59	3.29	0.67	0.98	–	–	59.1	80.7
Sephadex	2	0.94	2.89	0.75	1.91	–	202	41.8	167.2
Dialysis	2	–	2.54	0.39	0.63	–	59	23.5	41.6
CMC complex[a]	1	–	1.67	–	3.40	217	404	33.0	41.2
Iron complex[a]	1	0.87[b]	1.97	0.49	0.58	1079[c]	94	48.9	123.0

[a]Ash incompletely soluble in HCl/LiCl solution

[b]Phosphorus content determined by the Sumner Colorimetric Procedure was 4.0% (metaphosphate complex) and 12.5% (iron complex).

[c]Iron content by atomic absorption (Jones et al., 1972) was 9.0%.

weight of a given volume of WPC dispersion and foam by the expression:

$$\% \text{ overrun} = \frac{\text{wt mix} - \text{wt whipped mix}}{\text{wt whipped mix}} \times 100$$

Drip volumes were collected during the first hour after whipping.

Whipped topping mix. A whipped topping mix was prepared with the following composition according to Min (1969):

Ingredient	Percent
35% cream	15
Frodex 24[a]	10
Sucrose	7
NFDM or WPC (electrodialysis)	3
Water	64
Dariloid KB[b]	0.35
Carageenin[c]	0.05
Tween 65[d]	0.18
Tween 60[d]	0.10
PGM P-06[e]	0.20
Total	100

[a]American Maize Products Co., New York.
[b]Kelco Co., San Diego, Calif.
[c]Stein Hall & Co., Inc., Clifton, N.J.
[d]Atlas Chemical Ind., Inc., Wilmington, Del.
[e]Distillation Products Ind., Rochester, N.Y.

The whipped topping mix was prepared as follows: the dry ingredients were blended into the water plus cream and the mix was heated to 65°C. The melted emulsifiers were blended into the hot mix and it was pasteurized at 75–76°C for 5 min, homogenized in a double stage homogenizer at 1600 and 800 psi, cooled to 5°C and aged 24 hr. Whipping was conducted at room temperature in a Hamilton Beach mixer at "high" speed for 2 min. Relative viscosity of the mix was determined immediately after whipping with a Brookfield HBT viscometer using a 4.8 cm T-bar spindle at 50 rpm. Overrun was calculated from the weight of a given volume of mix and whipped topping by the expression above. Drainage through a course wire screen during a 24 hr period was used as the index of foam of stability.

Emulsion capacity. Emulsion capacity (EC) was determined by a procedure similar to that of Webb et al. (1970). Sufficient dry WPC was dispersed in 100 ml of 1.0M NaCl solution to provide a 0.1% protein concentration. Arcola corn oil was added from a buret at about 0.8 ml per second during continuous mixing at "blend" speed on an Osterizer. The electrical resistance of the dispersion was monitored with a voltmeter (Model R-1, Southwestern Industrial Electronics Co., Houston, Texas). The EC was taken as the weight of corn oil (g) required to produce infinite electrical resistance in the emulsion. A blank value of 37.2g corn oil for 1.0M NaCl solution was subtracted from the observed EC value for each WPC.

Buffer capacity and pH value. Buffer capacity and initial pH values were determined as follows. Sufficient dry WPC was dispersed in 100 ml distilled water to provide a 0.5% protein concentration. The initial pH value was determined with a Corning pH meter, Model 7, equipped with a combination electrode. The dispersions were then titrated to pH 3.0 with 0.1N HCl or to pH 8.0 with 0.1N NaOH using a Beckman Model 1062 Recording titrator. Buffer capacity was calculated for each 0.5 pH change (Δ pH) by the expression:

$$\frac{dB}{dpH} = \frac{\text{meq titrant}}{\text{wt of protein (g)} \times \Delta \text{ pH}}$$

RESULTS & DISCUSSION

TABLE 1 contains the compositional data for WPC from the various sources. Mean protein values ranged from 32.9% (electrodialysis) to 66% (dialysis). Mean lactose concentrations varied from 0.8% (iron complex) to 51.8% (electrodialysis). The apparent lactose content of CMC-complex WPC of 20.1% is probably contributed largely by complexed CMC since the phenol-sulfuric acid method is general

Table 3–Available lysine content of whey protein concentrates

Prepration process	Number of samples	Available lysine content (mg/100 mg WPC)	Available lysine content (mg/100 mg protein)
Metaphosphate complex	1	5.15	8.9
Electrodialysis			
Range	2	1.30–2.30	4.8–6.2
Mean		1.80	4.5
Ultrafiltration	1	4.45	7.9
Sephadex			
Range	2	2.85–3.60	7.4–8.0
Mean		3.22	7.7
Dialysis	1	4.50	6.8
CMC complex	1	4.80	9.6
Iron complex	1	4.30	11.5

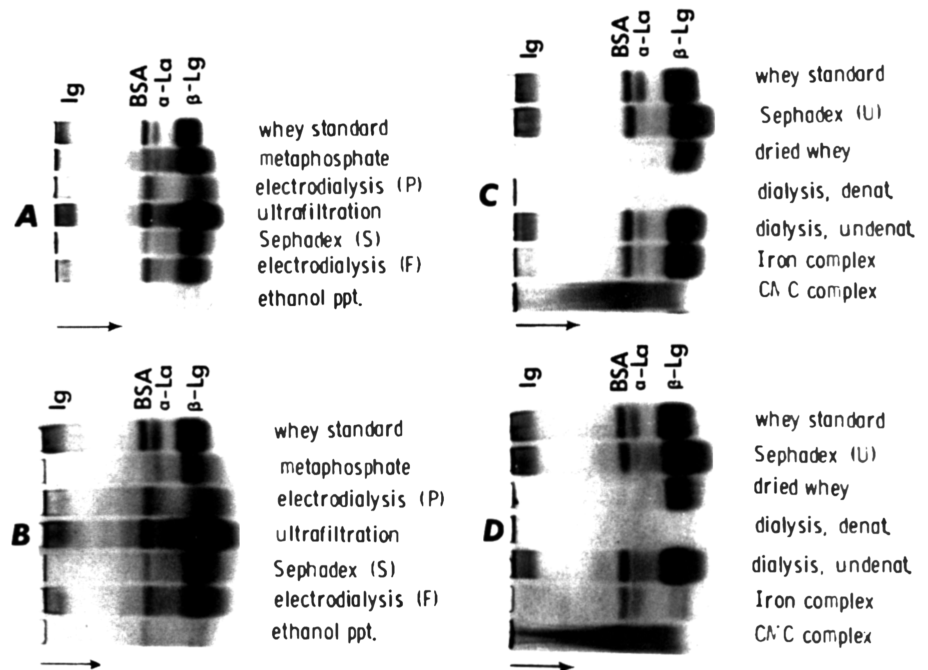

Fig. 2–PAGE patterns of WPC dispersed in pH 6.98 phosphate buffer (1.0% protein). Dispersions were stirred overnight at 0–5°C, dialyzed 48 hr against two changes of fresh buffer and centrifuged at 1,000 × G. 50 µl of each supernatant (soluble proteins) was examined by PAGE–patterns A and C. The above supernatants were adjusted to pH 4.6 and centrifuged at 1,000 × G and 50 µl of each final supernatant (undenatured proteins) was examined by PAGE–patterns B and D. Dialysis, denatured WPC, was heated at 90°C for 30 min to denature the proteins. Dried whey was a commercial source of spray-dried whey solids.

Table 4—Initial pH and solubility of whey protein concentrates

Preparation process	Number of samples	Initial pH[a]	Protein solubility[b] pH 2	pH 4	pH 6	pH 8
				(%)		
Metaphosphate complex						
Range	3	3.2–6.9	4.1–48.8	6.2–55.2	65.0–87.1	68.9–96.5
Mean		5.5	31.1	24.0	73.1	85.9
Electrodialysis						
Range	5	5.0–7.2	83.5–94.2	88.1–95.6	87.2–95.6	87.2–100.1
Mean		6.6	90.4	92.2	91.1	93.6
Ultrafiltration						
Range	3	4.6–8.3	72.8–92.6	66.6–91.7	70.3–88.9	61.9–94.9
Mean		5.8	80.0	77.9	77.3	76.7
Sephadex						
Range	2	6.6–7.1	81.2–94.9	85.8–93.1	86.2–94.2	87.6–94.9
Mean		7.0	88.0	89.5	90.2	91.2
Dialysis						
	1	6.1	79.4	91.2	–	81.2
CMC complex						
	1	6.5	21.2	57.7	80.9	86.2
Iron complex						
Range	2	5.0–5.1	–	0.7–1.6	21.0–32.9	55.6–76.6
Mean		5.0	0	0.7	26.9	66.1

[a]WPC dispersed in distilled water (0.5% protein concentration)

[b]WPC dispersed in 100 ml pH 2, 4, 6 or 8 buffer (1.0% protein concentration) at room temperature and stirred 30 min. The pH was readjusted and stirring continued 30 min. The solution was filtered through S & S 590 White Ribbon filter paper and examined for total N by micro-Kjeldahl.

for carbohydrates including polysaccharides. Mean ash content varied from 20% (dialysis) to 54% (iron complex). The NPN content (soluble N in 12% TCH) of electrodialysis, ultrafiltration and Sephadex WPC was between 4.8 and 6.7% of their total N content, whereas for all other WPC it was about 1–1.5% of their total N. Mean fat content ranged from 0.6–7.3% for the different WPC. The data show wide compositional differences among WPC prepared by a given process and even wider differences in composition of WPC from different processes. The high lactose and ash:protein ratios in the original whey makes it extremely difficult to prepare WPC with more than 50–60% protein content, even by processes that utilize extensive dialysis and protein complex precipitation techniques.

Elemental analysis data for selected WPC are in Table 2. Metaphosphate complex and electrodialysis WPC were of the acid types not neutralized with calcium, sodium or potassium. Phosphorus values obtained by emission spectrograph were abnormally low for metaphosphate complex and iron complex WPC. Phosphorus values obtained by the Sumner colorimetric procedure compared closely to values provided by the respective manufacturers. A similar discrepancy was noted in the iron content of iron complex WPC (see footnote, Table 2 for corrected values). Part of this discrepancy may be due to the incomplete dissolution noted for the iron complex WPC ash in the LiHCl solution for emission spectrometry (see Table 2). It is also possible that the presence of polyphosphate in the dissolved ash matrix adversely affected phosphorus and iron determination by the emission spectrometer. Both electrodialysis and CMC complex WPC contained high levels of sodium. Elemental analysis data were also obtained for magnesium, molybdenum, manganese and boron. The values of these element concentrations were ⩽ 0.22% (Mg), ⩽ 50 ppm (Mo), ⩽ 40 ppm (Mn) and ⩽ 40 ppm (Bo).

The available lysine content of WPC in Table 3 varied from 4.5 (electrodialysis) to 11.5 (iron complex) mg/100 mg protein. The total lysine content of total whey proteins is in the order of 10–11% (Anonymous, 1970a; and others). Heating and drying treatments during WPC preparation may result in Maillard type browning with concomittant reduction of available lysine. However, the available lysine procedure is known to be affected by the presence of glycoproteins (Holsinger et al., 1970) and carbohydrates (Wartheson et al., 1972). Although a special reagent blank was included to cor-

Table 5—Solubility and apparent denaturation of whey proteins in whey protein concentrate

Preparation process	Number of samples	Solubility at initial pH[a] (%)	Apparent denaturation[b] (%)
Metaphosphate complex[c]	1	57.3	90
Electrodialysis			
Range	5	83.1–98.1	12–40
Mean		92.4	22.4

[a]WPC dispersed in distilled water (1.0% protein concentration), stirred 30 min and allowed to stand overnight. Supernatant examined for total N by micro-Kjeldahl.

[b]Supernatant from a adjusted to pH 4.6 with 1N HCl and centrifuged at 1,000 × g for 20 min to sediment insoluble (denatured) proteins. Final supernatant was examined for total N by micro-Kjeldahl and the percent depletion (apparent denaturation) calculated.

[c]Sodium neutralized metaphosphate complex WPC

Table 6–Emulsification and whipping properties of whey protein concentrates

Preparation process	Number of samples	Emulsion capacity[a] (g)	Whipping properties Overrun (%)	Time (min)	Drainage (ml)
Metaphosphate complex					
Range	3	32–40	–	–	–
Mean		37	–	–	–
Electrodialysis					
Range	5	39–42	0–1020	2–11	9–35
Mean		41	632	4.5	17.5
Ultrafiltration					
Range	3	36–49	460–900	0.5–15	10–36
Mean		40	453	7.8	23
Sephadex					
Range	2	–	600–760	3.5–10	13–30
Mean		42	680	6.8	22
Dialysis	1	41	760	10	25
CMC complex	1	64	400[b]	2	0
Iron complex					
Range	2	26–41	520–600	–	12–16
Mean		34	560	3	14

[a]Grams corn oil required to produce infinite electrical resistance in the emulsion
[b]Due to extremely high viscosity, CMC complex WPC was dispersed in twice as much (200 ml) water as other WPC.

rect for browning during the acid hydrolysis step, available lysine values obtained here are apparently influenced by the level of lactose in the WPC. Electrodialysis WPC, which had the highest lactose concentration (see Table 1) had the lowest available lysine content. Similarly, the available lysine content of the other WPC also appear to be inversely related to their lactose contents. These results are in agreement with those of Holsinger et al. (1971), who reported that low lactose WPC had 10.9g lysine per 100g proteins and that 80% of the total was available by the TNBS procedure. Present available lysine results are questionable and additional work is required to devise a procedure to reliably differentiate available lysine in WPC from that reacted with lactose during WPC preparation.

As shown in Table 4, mean initial pH values of WPC dispersed in distilled water varied from 3.2 (acid metaphosphate complex) to 8.2 (ultrafiltration). Protein solubility for iron complex, CMC complex and metaphosphate complex WPC was highly dependent on pH, whereas protein solubility for the other WPC was essentially independent of pH. The low protein solubility for the former three WPC is due to the presence of residual precipitant ions, e.g., metaphosphate and CMC, which complex and precipitate the positively charged protein molecules at or below the isoelectric point. A second solubility experiment (see Table 5) showed that sodium neutralized metaphosphate complex and electrodialysis WPC, dispersed in distilled water (pH 6–7), had protein solubilities of about 57 and 92%, respectively. Apparent protein denaturation, determined by adjusting to pH 4.6 and centrifuging (Nielsen et al., 1972), was from about 22–90% for electrodialysis and metaphosphate complex WPC, respectively. It is obvious from these data and those in Table 4 that the pH 4.6 solubility procedure is not reliable for assessing protein denaturation in metaphosphate WPC. However, these data do confirm substantial apparent protein denaturation in electrodialysis WPC. A third solubility experiment was conducted in which the solubility of metaphosphate complex, Sephadex and electrodialysis WPC was determined at pH values ranging from 2 to 9 (see Fig. 1). All WPC, except metaphosphate complex WPC, exhibited a dip in their solubility curves between pH 4 and 5. As indicated above, metaphosphate complex WPC has a drastic reduction in solubility at pH values below pH 6, with its minimum solubility below about pH 4.

Figure 2 shows the PAGE patterns for WPC proteins soluble at pH 6.98 (A and C) and those soluble at pH 4.6 (B and D). The proteins in most of the WPC examined, including those in WPC prepared by dialysis and freeze drying, have undergone detectable alteration (apparent denaturation). Dialysis, denatured WPC, which was intentionally heated to denature the proteins, ethanol-precipitated WPC, and commercial spray-dried whey solids, all exhibited maximum protein alteration as revealed by PAGE. As indicated above, metaphosphate complex, CMC complex and iron complex WPC are essentially insoluble at pH 4.6 and therefore, PAGE patterns of pH 4.6 soluble proteins do not provide a reliable assessment of their protein denaturation. Inadequate resolution of the proteins in CMC complex WPC was obtained for both pH soluble fractions which was probably due to residual CMC and its affect on viscosity and protein charge. α-Lactalbumin was the protein component that was most susceptible to denaturation in nearly all WPC preparations examined. Thus, it appears that drying and storage of WPC results in apparent protein denaturation. Further work is needed to establish the mechanism and means for blocking the reaction.

Table 6 contains the emulsion capacity (EC) data for the different WPC. All WPC had similar EC values of 34–42, except CMC complex WPC which was about double that of the others. The whipping properties of the different WPC (Table 6) showed considerable variation under the experimental conditions used here. Metaphosphate complex WPC failed to form a stable foam. CMC complex WPC formed a thick paste when dispersed by the normal procedure and was therefore diluted with twice the normal volume of water.

Table 7–Whipping properties of whipped topping mix containing whey protein concentrate or nonfat dry milk solids for protein source[a]

Protein source	Number of samples	Whipping properties Overrun (%)	Relative viscosity (cp)	Drainage (%)
Nonfat dry milk solids	1	219	18	6.4
Electrodialysis WPC				
Range	3	150–175	8–13	11–51
Mean		158	10	37.8

[a]See Materials & Methods section for details on composition, preparation and whipping procedure used.

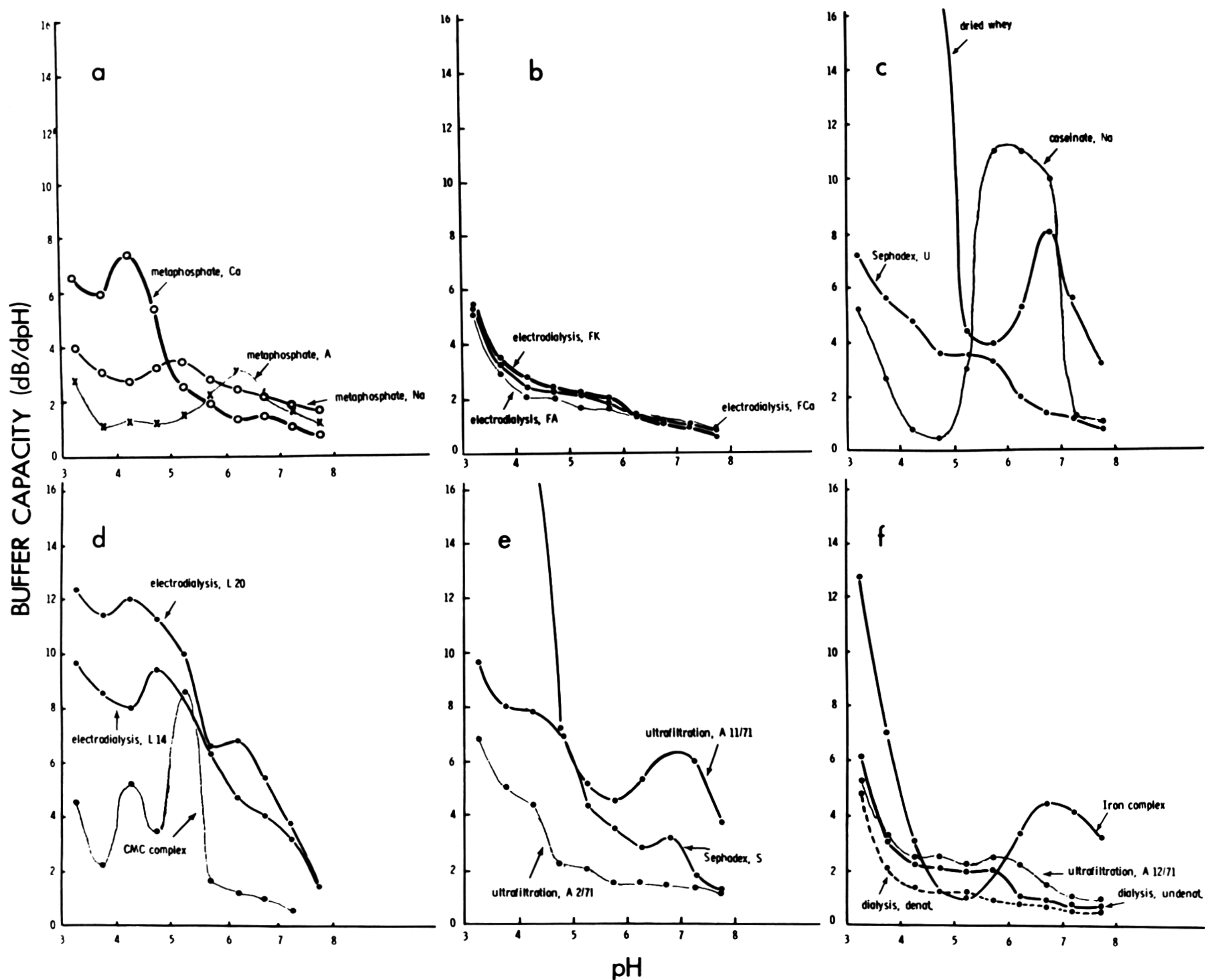

Fig. 3–Buffer capacities of whey protein concentrates dispersed in distilled water (0.5% protein) and titrated with 0.1N HCl or NaOH.

Whipped CMC complex WPC had a more stable foam than any of the others. Mean overrun values ranged from 0–760%, but none of the WPC produced as high an overrun as did sodium caseinate at 1120%. An overrun value of 900% was produced by the egg white solids under these experimental conditions.

The whipping properties of the whipped topping mixes containing commercial NFDM or electrodialysis WPC as their main protein source are presented in Table 7. These toppings contained about 5.25% milk fat and about 27% total solids. No attempt was made to equalize the final protein levels in the different mixes; however, all protein source materials contained similar protein concentrations of 30–35%. Whipped topping mixes containing WPC produced lower overrun, and foams with lower viscosity and less stability than did the mix containing NFDM as the protein source. Thus, under present experimental conditions, casein, which is the principal protein in NFDM, appears superior to whey proteins for formulating a whipped topping mix.

Buffer capacity (BC) versus pH graphs are presented in Figure 3. Electrodialysis, ultrafiltration, Sephadex and dialysis WPC all had generally similarly-shaped curves with gradually increasing BC toward the lower pH regions of the graph. WPC with lower degrees of demineralization, e.g., ultrafiltration A 11/71 WPC (Fig. 3e) and electrodialysis L 14 and L 20 (Fig. 3d) had high mineral content and also high BC. Metaphosphate Ca WPC (Fig. 3a) had a peak in its BC curve at about pH 4.5 and also had higher BC than either of the other two metaphosphate WPC. CMC complex WPC had a uniquely shaped BC curve with peaks at about pH 4.5 and 5.5 (Fig. 3d). Iron complex WPC (Fig. 3f) had a broad BC peak between pH 5.5 and 7.5, which was totally different than was observed for metaphosphate WPC. Sodium caseinate (Fig. 3c) had a broad and excessively high BC peak between pH 5 and 7 but very low BC between pH 4 and 5, the isoelectric region where it is virtually insoluble. Dried whey (a commercial source of spray dried whey solids) had its BC peak between pH 6 and 8 and had excessively high BC below pH 5.5. pH buffering in WPC dispersions is probably due to ionization equilibria involving mainly protein carboxyl groups (pKa 1.8–2.5), citrates (pKa 3.08, 4.74 and 5.40), and phosphates (pKa 2.12, 7.21 and 12.67), (Handbook of Chemistry and Physics, 1964) as well as metaphosphate and CMC poly ions in WPC utilizing these protein precipitants. Minor pH buffering would also be contributed by ionization equilibria involving residual organic acids and other protein ionizing groups having their pKa's in the pH 3–8 range.

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CHEMICAL NATURE OF STONE CELLS FROM PEAR FRUIT

INTRODUCTION

SCLEREIDS or stone cells are contained in the pulp of most pear varieties where they impart a gritty texture. Early studies of these "tartarecious grains" revealed they were similar in paracrystallinity to fiber cells (Crist and Batjer, 1931) being composed of successive thin layers. The intractable solubility properties of these particles led Crist and Batjer to suggest they were lignocellulosic in nature. Similarly, Smith (1935) observed that stone cells were insoluble after extraction with benzene, alcohol, alkali and water and concluded they were chemically analogous to wood lignin. There is, however, no definitive information on the chemical composition of stone cells.

We have observed an inverse relationship between free phenolic substances, mainly chlorogenic acid, and the quantity and size of stone cells in several pear varieties (Ranadive and Haard, 1971). This finding led to a suggestion that the cellular localization of peroxidase delimits the metabolism of phenolic substances into lignin (Ranadive and Haard, 1972). A practicable outgrowth of these studies is that calcium nutrition and uptake of the tree may relate the development of stone cells in pears. The present investigation was undertaken to discern the chemical nature of stone cells.

MATERIALS & METHODS

Isolation of stone cells

A 200-g sample of peeled and cored pear fruit (*Pyrus Serotina*, var. "Yuzuhada") was homogenized with 500 ml distilled water in a Waring Blendor for 5 min. The homogenate was diluted with 1,000 ml water and made 0.1M with NaCl. The suspension was incubated for 30 min at 20–22°C and the supernatant phase decanted. The sediment was incubated for 30 min with 500 ml of 0.5N NaOH and decanted. Finally, the sediment was suspended in 500 ml of 0.5N HCl for 30 min, decanted and washed with water. The above washing operations were repeated several times until the stone cells were free of extraneous cell debris.

Histochemical test for stone cells

Stone cells were observed to form a bright red conjugate when incubated with 1% phloroglucinol in 3N HCl. A similar chromophore develops when wood lignin is treated with this reagent. (Johansen, 1940).

Oxidation of stone cells

Nitrobenzene oxidation of stone cells was carried out according to the methods described for analysis of wood lignin (Stone and Blundell, 1951). The stone cells were pulverized to pass through 125 micron sieve. A 40-mg sample of stone cell powder, 0.06 ml of nitrobenzene and 2 ml of 2N NaOH were heated at 160°C for 5 hr in a sealed stainless steel bomb (25 ml capacity). The cooled mixture was filtered, brought to slightly acidic pH with HCl and analyzed by chromatography.

Oxidation was also carried out using cupric sulphate instead of nitrobenzene to determine the oxidation products of nitrobenzene itself.

Paper chromatography of oxidation products of stone cells

Unidirectional descending paper chromatography was used for separation of oxidation products. A 25 to 50 μl sample was spotted on Whatman No. 1 paper. Chromatograms were developed in each of the following systems: (1) butanol:acetic acid:water (4:1:5) (2) butanol:ethanol:water (5:1:4); and (3) water saturated with benzene (Fukumuzi, 1960). Papers were developed for 16–18 hr in the case of solvent systems (1) and (2) and 3.5–4 hr with the third solvent system. The papers were air dried and sprayed with chromogenic reagents. Chromogenic reagents used were: (1) 1:1 mixture of potasium ferricyanide and $FeCl_3$ (0.5%); (2) Diazosulfanilic acid; (3) 2,4-dichlorophenylhydrazine; and (4) 2,4-dichlorophenolindophenol (Fukumuzi, 1960).

Thin layer chromatography of oxidation products of stone cells

Unidirectional thin layer chromatography was carried out on 8 in. × 8 in. thin layer plates of Silica gel G. A 25 μl sample was applied to plates which were developed in each of three solvent systems: (1) benzene:ethanol (150:22); (2) benzene:acetone (3:2); and (3) methanol: chloroform (3:7) (Barton, 1967).

After drying, the plates were sprayed with a 1:1 mixture of $K_3Fe(CN)_6$ and $FeCl_3$ (0.5%) or diazosulfanilic acid.

Infrared spectroscopy of stone cells

15–20 mg of stone cell powder, passed through a 120 micron sieve, was mixed and pulverized with approximately 70 mg potassium bromide, dried with a hair dryer to remove traces of water and pressed to form a pellet. Samples were examined with a Perkin Elmer model 421 grating infrared spectrophotometer.

Table 1—Paper chromatography of nitrobenzene oxidation products

Solvent[a]	Rf	Color reactions: Diazosulfanilic acid	Color reactions: $K_3Fe(CN)_6$ $FeCl_3$	Color reactions: 2,6-dichloro-indolphenol	Relative conc
(1) Sample	0.91	Yellow	Blue	–	+++
	0.88	Pinkish-orange	Blue	–	+++
	0.86	Orange-pink	Blue	–	+++
	0.83	Brown	Blue	–	++
	0.82	Purple	Blue	–	+
	0.78	Orange	Blue	–	+
	0.71	Brown-tan	Blue	–	+
	0.01	Brown	–	–	+
p-hydroxybenzaldehyde	0.94	Yellowish-orange	Blue	–	
Vanillin	0.88	Orange	Blue	–	
Syringaldehyde	0.85	Pink	Blue	–	
(2) Sample	0.84	Pink	Blue	Purplish pink	
	0.67	Yellow-orange	Blue	–	+++
	0.63	Pink	Blue	–	+++
	0.32	Yellow	Blue	–	+++
p-hydroxybenzaldehyde	0.61	Yellowish	Blue	–	
Vanillin	0.67	Yellow-orange	Blue	–	
Syringaldehyde	0.63	Pink	Blue	–	
(3) Sample	0.89	Brown	Blue	–	++
	0.79	Orange	Blue	–	+++
	0.68	Brown	Blue	–	+++
	0.36	Yellowish-brown	–	–	+
	0.16	–	Blue	Blue	+

[a]Solvent (1)—Butanol:ethanol:water (5:1:4); Solvent (2)—Water saturated with benzene; Solvent (3)—Butanol:acetic acid:water (4:1:5).

Table 2–Thin layer chromatography data of oxidized stone cells

	Solvent 1 Benzene:ethanol (150:22)			Solvent 2 Methanol:chloroform (3:7)			Solvent 3 Benzene:acetone (3:2)		
Compound	Rf	Color[a]	Relative conc	Rf	Color	Relative conc	Rf	Color	Relative conc
Sample	0.96	Yellow	++				0.94	Yellow	+
	0.83	Orange-brown	+++	0.84	Orange-brown	++	0.86	Orange-brown	++
	0.80	Pinkish-brown	+++	0.82	Pinkish-brown	+++			
	0.76	Orange-brown	++	0.73	Brown	+			
	0.55	Brick red	++				0.59	Faint yellow	+
p-hydroxy-benzaldehyde	0.76	Orange-brown		0.87	Yellow-orange		0.88	Brown-orange	
Vanillin	0.82	Orange		0.84	Orange-brown		0.87	Orange-brown	
Syringaldehyde	0.80	Pinkish-orange		0.82	Pinkish-brown		0.81	Pinkish-orange	

[a]Color: Color formed when plates were sprayed with diazosulfanilic acid.

Enzymatic digestion of stone cells

Stone cells (1g) were suspended in 20 ml of citrate-phosphate buffer, pH 4.0 containing 5 mg each of cellulase and hemicellulase (Wallerstein, N.Y.) with continuous shaking at 40°C. The reaction was stopped after 4 hr by boiling the mixture for 5 min. The digest was filtered and the residue was washed with water and dried at 98°C for 3 hr and weighed. The dried cells were pulverized to pass through 120 micron sieve and again treated with enzymes as described above. The remaining residue was consecutively extracted with benzene, 95% ethanol, distilled water and sodium hydroxide (2N). The insoluble residue was filtered, washed with water, dried and weighed. The residue was then moistened with 1 ml N,N-dimethylaniline for 12 hr and then 20 ml 70% ice cold H_2SO_4 was added (Ryugo, 1969). The mixture was allowed to react at 4°C for 24 hr. At the end of this period, the reaction mixture was diluted with 200 ml of distilled water and boiled for 15 min. The mixture was then neutralized with saturated sodium hydroxide to a pH between 6 and 7 and filtered. The residue was again washed with distilled water, dried at 150°F for 5–6 hr and weighed.

Paper chromatography of sugars

Filtrates of the enzyme hydrolyzed stone cells were analyzed for sugars by paper chromatography. A 25–50 μl sample was spotted on Whatman No. 1 paper, dried and the paper developed with butanol:acetic acid:water (4:1:5) for 18 hr. At the end of this period, the solvent front was marked and the paper dried in the air. The paper was then sprayed with aniline hydrogen phthalate (Partridge, 1949). Colored spots were marked with Rf values measured. Authentic samples of sugars were run simultaneously for comparison of Rf values with sample.

RESULTS & DISCUSSION

THE WIESNER reaction (Johansen, 1940) is not specific for lignin, itself, but for aldehyde groups of unpolymerized coniferyl aldehyde. Stone cells of pear turned red on treatment with this reagent and accordingly, appear to be ligninous in nature. This histochemical test was found useful in the present work to ascertain the purity of the isolated stone cells.

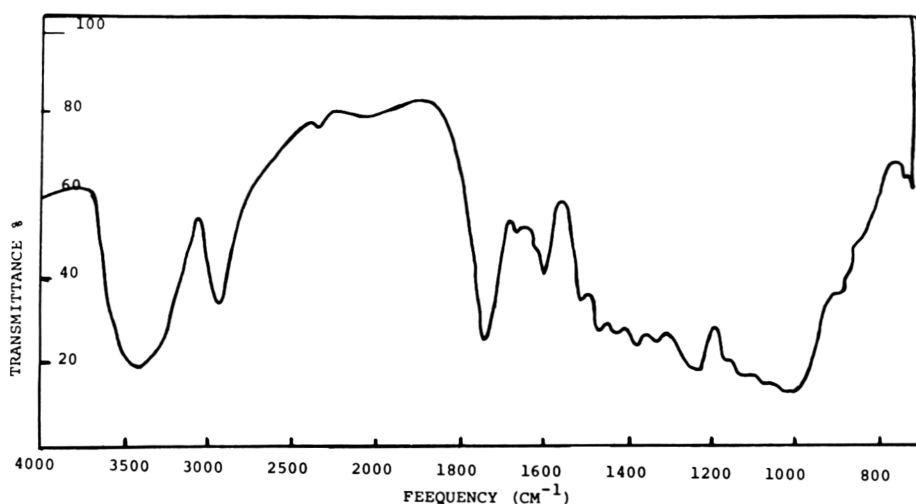

Fig. 1–Infrared absorption spectrum of stone cells.

Oxidation of stone cells

The major oxidation products of stone cells were vanillin and syringaldehyde. Their identification was based on color reaction and mobility during paper chromatography (Table 1) and thin layer chromatography (Table 2). Besides these two major components, chromatographic studies showed the presence of four to five other separable zones which were not identified. These minor spots turned blue when sprayed with $K_3Fe(CN)_6$ + $FeCl_3$ reagent, indicating their phenolic nature. One zone which turned yellow on spraying with diazosulfanilic acid (Rf 0.91 and 0.32 in solvent 1 and solvent 2, respectively) was later found to be an oxidation product of nitrobenzene itself.

Three different solvent systems were used to carry out thin layer chromatography. Benzene-ethanol gave maximum separation of the sample with five spots and a very faint spot at the origin. Methanol-chloroform and benzene-acetone solvents gave only three spots each and a fairly dark spot at the origin.

It is known that nitrobenzene oxidation of soft woods yields mainly vanillin; whereas, hardwoods are mainly degraded to syringaldehyde and some vanillin. The lignin of grasses degrade to p-hydroxybenzaldehyde, vanillin and syringaldehyde. The chromatographic data thus demonstrate the presence of lignin material analogous to that found in hardwoods.

Infrared spectroscopy of stone cells

The infrared spectra of stone cells (Fig. 1) showed extensive absorption at 1730 cm^{-1}. This is a region of absorption by carbonyl groups at the β-position. Bolkar and Terashima (1966) working on isolated wood lignin suggested that absorption in the region of 1700–1720 cm^{-1} is due to contribution from nonconjugated keto groups in the β positions of the phenylpropane side chain. The unconjugated β keto groups arise during iso-

Table 3—Carbohydrate material of pear stone cells

(A) Qualitative Tests of Enzyme Hydrolyzates

	Test	Observation	Inference
i.	Molisch reaction	Reddish violet ring produced at the interface	Carbohydrates present
ii.	Fehling solution test	Brick-red precipitate formed	Reducing sugars present
iii.	Seliwanoff reaction	Pinkish tinge	Ketose sugar absent
iv.	Phloroglucinol reaction	Chocolate-red color	Pentose, galactose or galactans present
v.	Bial Orcinol reaction	Green color formed in solution	Pentoses present

(B) Paper Chromatography of Enzyme Hydrolyzates

Solvent: Butanol:acetic acid:water (4:1:5)

Spot	Rf	Color with analine hydrogen phthalate	Inference
Sample	0.32	Pink	Xylose
	0.22	Brown	Glucose
	0.17	Pinkish-brown	Xylan
	0.13	Pinkish-brown	Xylan
Glucose	0.22	Brown	Glucose
Arabinose	0.28	Brown	Arabinose
Galactose	0.20	Brown	Galactose
Xylose	0.32	Pink	Xylose
	0.15	Pink	Xylan
	0.06	Pink	Xylan

lation of lignin from wood. Fukumuzi (1960) also observed greater absorption at wave number 1700 cm^{-1} for decayed wood lignin compared to that of Nord's lignin. He attributed this group because decayed lignin did not show purple red color with phloroglucinol-HCl reagent which is used to detect the conjugated coniferyl aldehyde group. The IR spectrum of stone cells was similar to Nord's decayed lignin (Fukumuzi, 1960) yet the stone cells reacted positively with phloroglucinol-HCl reagent.

The absorption bands (Fig. 1) at 1510 cm^{-1} and 1590 cm^{-1} indicate the phenyl ring skeletal vibrations with possible para substitution. The band at 1420 cm^{-1} may be due to the presence of aliphatic structure. The band at 2946 cm^{-1} is due to CH groups.

Cellulase, hemicellulase digestion of stone cells

Intact stone cells were first digested with cellulytic enzymes to remove adhering cell wall fragments not removed by washing. The observed loss in weight was 27.4%. The undigested material was considered to represent pure stone cells.

Treated stone cells were then pulverized and again treated with carbohydrases. An additional 15.3% of their weight was solubilized on this second treatment. The digested powder was extracted with benzene, ethanol and alkali with a further weight loss of 41.1%. Finally, treatment of the residue with 70% H_2SO_4 to remove additional cellulosic material resulted in 25.9% loss in weight on the basis of the original material. The residue remaining after these treatments was 17.7% of the original stone cell. This residue also gave a positive reaction for lignin when treated with phloroglucinol-HCl. The 82% weight loss resulting from these treatments presumably represented the cellulosic constituents, analogous to those found in wood lignin, as well as lesser quantities of lipid and protein substances.

Analysis of carbohydrates during enzyme digestion

Qualitative test showed the presence of reducing sugars, hexoses and pentoses in the enzyme hydrolyzate (Table 3A). The principle sugars in this fraction were identified as glucose and xylose and xylans of varied degrees of polymerization (Table 3B). These sugars are representative monomers of cellulose and hemicellulose material associated with woody plant tissues (Ling and Nanji, 1923).

CONCLUSIONS

RESULTS reported here demonstrate that stone cells are lignocellulosic, containing approximately 18% lignin and 82% of a material which was principally carbohydrate. The principal monomer units of lignin were vanillin and syringaldehyde and the carbohydrate hydrolyzate contained glucose and xylose residues.

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TEXTURE OF CUCUMBERS: CORRELATION OF INSTRUMENTAL AND SENSORY MEASUREMENTS

INTRODUCTION

CONSUMERS of cucumber pickles prefer a crisp, firm, hard-textured product. However, the lack of adequate instrumental methods for measuring texture of raw and processed cucumbers has long been recognized by research workers as well as picklers. Hand-operated fruit pressure testers have commonly been used for most of the objective textural evaluations of raw and processed cucumbers. The best known of these is the Magness-Taylor fruit pressure tester (Magness and Taylor, 1925). Haller (1941) reviewed the early history of fruit pressure testers and described their practical applications. Jones et al. (1954) applied the fruit pressure tester (FPT) to firmness measurement of salt stock pickles. Bell et al. (1955) devised firmness rating scales for salt stock pickles based on FPT values. Pflug et al. (1960) modified the FPT method to a mechanically operated recording pressure tester (MRPT) so as to eliminate part of the human element (i.e., variability among results obtained by different operators) from pressure testing and, at the same time, to record the result as a force-distance curve for a better understanding of product behavior during testing. Nicholas (1960), using pickles and other fruits as test samples, observed significant differences between FPT and MRPT results. Szczesniak and Bourne (1969), in reviewing objective firmness procedures, noted that while pressure or puncture methods had been assumed to be measuring firmness, there was little published evidence of attempts to correlate the objective data with sensory responses.

Szczesniak (1963) proposed multiple classification of textural characteristics based on Texture Profile Analysis (TPA) by means of the General Foods Texturometer (Friedman et al., 1963) and also on organoleptic texture profile methods (Brandt et al., 1963). Szczesniak et al. (1963) developed quantitative rating scales for correlating objective and sensory texture evaluations; objectively measured TPA parameteres correlated well with the same parameters measured subjectively by a trained panel. Szczesniak (1968) discussed statistical approaches by which better correlations might be made between instrumental and sensory methods through use of correlation coefficients along with suitable conditions and transformations of variables.

Bourne's (1968) procedures for deriving TPA parameters of pear parenchyma tissue from Instron UTM force-distance curves augmented the pioneering work done by Szczesniak's group. Henry et al. (1971) developed procedures for determining TPA parameters under tension of semisolid foods; they correlated the results statistically with sensory evaluations.

Breene et al. (1972) determined Instron TPA parameters of brittleness, hardness, cohesiveness, elasticity, gumminess, and chewiness to learn the nature and extent of textural differences in a wide range of cucumber genetic stocks. They found significantly different mean values among varieties for all parameters and observed that differences over the season appeared to be small. These workers suggested that textural quality might be adequately assessed by measuring one or more of (1) brittleness, (2) hardness, and (3) total work expended in sample compression.

Our work was undertaken to correlate sensory evaluation of cucumber texture with TPA values for brittleness, hardness, and total work of compression as well as with Magness-Taylor FPT firmness so that optimum texture testing procedures might ensue.

MATERIALS & METHODS

Cucumber samples

Six cucumber varieties selected for analysis on the basis of their textural characteristics (Breene et al., 1972) were grown in the field in St. Paul in 1971. Four of them (Explorer, Chipper, MSU 6902 G, and GY 3) had ranked relatively high in one or more of the TPA parameters; two (Green F and Mincu) had ranked low in all parameters.

During July and August, cucumbers approximately 1-in. diam were hand-harvested. Cucumbers for instrumental testing were handled essentially as described previously (Breene et al., 1972). They and samples for sensory tests were kept in sealed, moist chambers (100% RH) at room temperature until testing was completed which was, in all cases, on the day of harvest.

Instrumental measurements

Instron TPA. Three parameters–brittleness (BTL), hardness (HDN) and total work (TW) of compression–were determined as follows by procedures described in detail by Breene et al. (1972). Two cross-sectional slices 1-cm in length were cut from each of 30 cucumbers of each variety, one from the stem (S) side and the other from the blossom (B) side of the mid-length point. TPA values were obtained from curves plotted by the Instron TM-M during "one bite" compression of each slice to 0.25

Table 1–Analysis of variance for TPA and FPT data: Instron TPA brittleness (BTL), hardness (HDN) and total work (TW); FPT firmness (FRM)

Source	Degrees of freedom	Mean squares						
		TPA SKIN			TPA NO SKIN			FPT
		BTL	HDN	TW	BTL	HDN	TW	FRM
Stem and blossom	1	139.3*	179.6	3.38	47.3	47.2	0.89	134.3**
Varieties	5	1455.4**	1829.8**	39.5**	598.0**	219.3**	11.1**	62.2*
Residual	5	20.8	78.5	1.1	11.1	10.0	0.2	7.8
Sampling error	168	27.5	34.5	0.5	9.8	4.7	0.2	1.5

*Significant at 5% level
**Significant at 1% level

cm. Crosshead speed was 0.5 cm/min; chart speed was 5 cm/min. Full-scale setting was 100 kg.

To determine (for each variety) the contribution of cucumber skin to HDN, BTL and TW as well as to test the possibility of eliminating time-consuming slice area measurements, 30 slices in one series were compressed with skin on (SKIN) and 30 slices in a second series were compressed after removing the skin (NO SKIN) and a small portion of the outer parenchyma with a sharp 2-cm diameter cork borer. All test samples were compressed immediately after slicing and coring. The two slices from each cucumber were alternately assigned to the respective series to block out effects due to S and B. In the SKIN series, the two parallel faces of each slice were traced in outline on paper. Subsequently, these two areas were determined by planimeter; means of the two were recorded as slice area so that each replicate parameter value could be adjusted to a constant sample area of 1 sq inch (Breene et al., 1972). In the NO SKIN series, slice area was fixed; data were not adjusted.

Magness-Taylor firmness. The Magness-Taylor FPT (Ballauf Mfg. Co., Washington, D.C.) fitted with 0.79 cm (5/16 in.) diam tip was employed. Samples consisted of the stem and blossom end portions remaining after removal of TPA sample; each was punctured once in its geometric center. All measurements were made by the same operator. Caution was taken in original fruit selection to avoid those with lower than normal length/diameter ratio. For each variety, the same 30 cucumbers used in TPA were tested for FPT firmness; a total of 60 punctures were made.

Sensory evaluation. A design was used for sensory evaluation in which the six varieties were evaluated by a six-member trained panel on 6 days. For each variety on each day, five cucumber samples were cut in half across the long axis. Then each panelist received five stem end halves (S) of one variety and five blossom end halves (B) of another variety. Panelists were instructed to analyze each half-cucumber as follows: "Cut a slice approximately 1-cm long from the cut end and evaluate it for relative crispness (1=extremely lacking in crispness, 9=extremely crisp). Analyze the rest of each half-cucumber for relative firmness (1= extremely soft, 9=extremely firm) by pressing it between the thumb and fingers until it ruptures." Cucumbers must fall into the category of fruits which respondents evaluated for firmness primarily by testing for deformability (Szczesniak and Bourne, 1969). On each of the six days, five replicate judgments of crispness and firmness, respectively, were made on each variety by two different panelists. In all, each panelist evaluated 10 replicates of each variety (5S + 5B) for crispness and firmness, respectively; he also evaluated the same paired sets of samples as each of his fellow panelists. The varietal mean for each response is therefore based on a total of 60 replicate observations.

RESULTS & DISCUSSION

Varietal and sample differences

As shown in Table 1, analysis of variance (ANOVA) indicated highly significant differences ($P < 0.01$) between firmness of stem (S) and blossom (B) portions measured by FPT. FPT differences among varieties were significant only at $P < 0.05$. S and B slices subjected to TPA were not significantly different except for SKIN brittleness ($P < 0.05$). ANOVA showed highly significant ($P < 0.01$) differences among varieties for all TPA parameters and sampling methods. The lack of difference between TPA S and B was expected on the basis of previous experience (Breene et al., 1972). FPT punctures, however, were made at a sufficient distance from the mid-length points to detect differences due to thickness, relative seed cavity size, etc.

Results of instrumental and sensory texture measurements are summarized in Table 2. Mean values for TPA and sensory evaluations are based on 30 and 60 replicates, respectively. Although 60 FPT measurements were actually made on each variety, the mean of two readings (S and B) for each individual cucumber sample was used as one observation. Thus, each FPT mean (Table 2) is based on 30 replicates.

Variability within parameters

Variability within parameters is expressed by coefficients of variability (C.V.). This statistic, a ratio independent of any units of measurement, permits comparison of relative variations in the different types of measurements. C.V. were generally larger in the TPA NO SKIN method than in the SKIN method. Larger C.V. for NO SKIN were attributed to greater variability among cored samples in the proportion of relatively firm parenchyma tissue to relatively soft placental tissue. For example, NO SKIN samples of MSU 6902 G, which has a small seed cavity, contained relatively uniform proportions of the two types of tissue; it exhibited the lowest C.V. In Mincu and Green F, the combined effects of variation in sample dimension and large seed cavity produced more variation in the proportion of the two tissue types, resulting in high C.V. The smaller C.V. for FPT firmness as compared with TPA values were probably due primarily to a lower degree of sensitivity in the FPT

Table 2–Instrumental and sensory textural data: Instron TPA brittleness (BTL), hardness (HDN) and total work (TW); FPT firmness (FRM); sensory crispness (CRP) and firmness (FRM)

		Instron TPA[a]						FPT[a]	Sensory[b]	
		SKIN			NO SKIN					
Cucumber variety		BTL (kg)	HDN	TW (in.²)	BTL (kg)	HDN	TW (in.²)	FRM (lb)	CRP	FRM
Explorer	Mean	52.1	51.9	8.49	18.6	13.4	2.72	15.8	7.4	7.6
	C.V.	12.1	14.4	10.8	16.9	17.9	17.5	8.0	5.2	4.5
Chipper	Mean	57.1	52.1	9.03	19.3	14.0	2.79	16.6	7.3	7.6
	C.V.	10.1	12.4	9.3	19.3	14.7	18.4	8.8	8.1	9.0
MSU 6902 G	Mean	56.3	49.8	8.71	22.4	15.1	3.17	15.9	7.7	7.5
	C.V.	10.3	11.1	8.2	14.7	13.2	11.9	6.5	8.1	11.5
GY 3	Mean	53.3	51.1	8.65	17.1	12.3	2.52	15.4	6.8	6.6
	C.V.	10.0	12.8	9.3	18.5	19.7	16.0	8.1	15.8	18.8
Green F	Mean	40.1	33.5	6.26	10.7	8.1	1.61	13.4	5.5	5.3
	C.V.	11.7	14.0	7.6	24.2	30.7	22.2	9.3	27.8	28.5
Mincu	Mean	43.7	39.9	6.85	12.2	9.7	1.80	13.2	5.8	5.9
	C.V.	7.5	12.9	7.5	24.0	23.1	25.8	9.1	28.2	23.6
HSD[c]	($P < 0.05$)	3.07	4.27	0.46	2.33	1.67	0.32	0.77	0.57	0.57

[a]Mean values are based on 30 replicates.
[b]Mean values are based on 60 replicates.
[c]Tukey's ω-procedure or honestly significant difference.

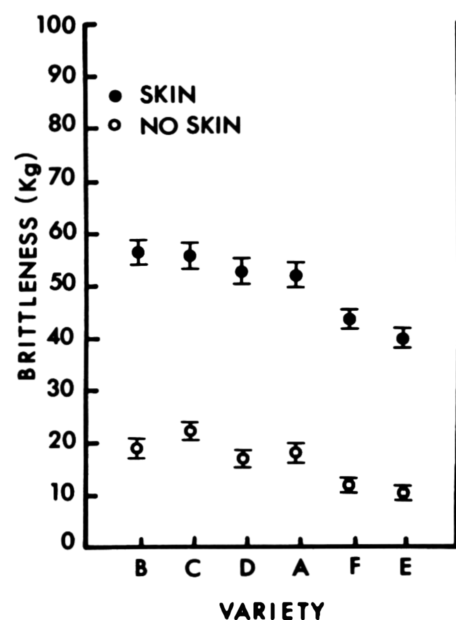

Fig. 1–TPA mean brittleness for SKIN and NO SKIN methods. A = Explorer, B = Chipper, C = MSU 6902 G, D = GY 3, E = Green F and F = Mincu.

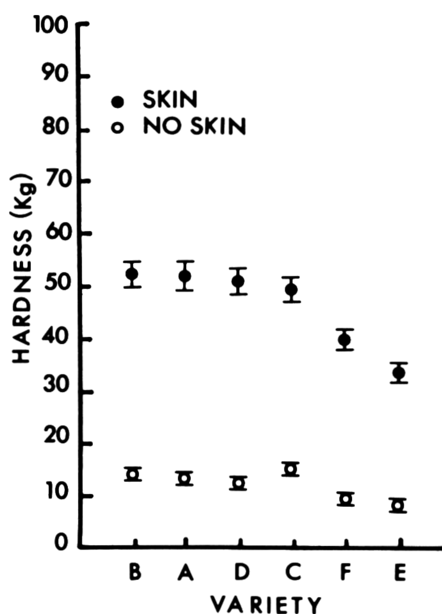

Fig. 2–TPA mean hardness for SKIN and NO SKIN methods. A = Explorer, B = Chipper, C = MSU 6902 G, D = GY 3, E = Green F and F = Mincu.

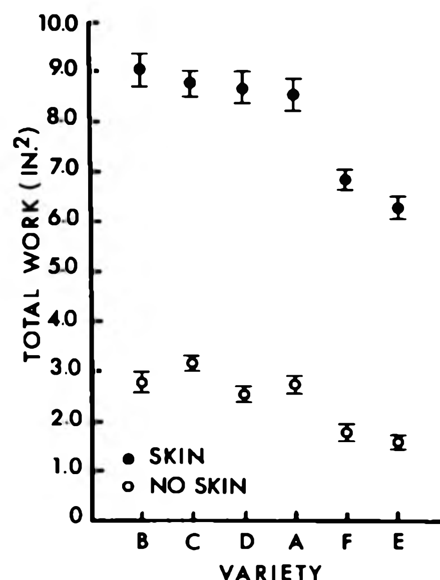

Fig. 3–TPA mean total work for SKIN and NO SKIN methods. A = Explorer, B = Chipper, C = MSU 6902 G, D = GY 3, E = Green F and F = Mincu.

method. This is in agreement with Nicholas (1960) who found MRPT variance to be significantly larger than FPT variance. Notwithstanding the narrow range of differences in mean FPT firmness, ANOVA of FPT results indicated no significant differences among replications but highly significant varietal differences. In addition, Tukey's ω-procedure indicated that the FPT is capable of detecting real differences among varieties.

Variability of data for sensory crispness (CRP) and firmness (FRM) was quite high in the "soft" varieties, Green F and Mincu, because a few panelists gave them relatively high scores which also increased their mean CRP and FRM to a certain degree. ANOVA, however, showed that differences among panelists were not significant for CRP and were significant only at the 5% level for FRM, but differences among varieties were highly significant (Table 3).

Influence of sample preparation method (TPA)

In Figures 1–3, varietal mean TPA SKIN values for BTL, HDN and TW are plotted in order of decreasing magnitude; vertical bars denote 95% confidence internals about the means. NO SKIN values are also plotted; though smaller, due in large part to smaller sample area, they show similar trends with notable exception of higher values for MSU 6902 G. These higher values might be explained as being due to differences among varieties in resistance to force offered by cucumber skin. That is, the other three "firm" varieties have tougher skin than MSU 6902 G and therefore their values were reduced to a greater degree by removal of skin. It is more likely, however, that this phenomenon resulted from the inherently large proportion of parenchyma tissue in cored MSU 6902 G samples.

Relationships among methods

Correlation coefficients (r) were computed to relate taste panel results to TPA values as well as to FPT firmness. Correlations were also made between FPT and TPA values. Mean values were used in all correlations; the results are shown in Table 4. All correlation coefficients except one (NO SKIN BTL vs. FPT) were significant at the 1% level. The highest r (0.988) was obtained between NO SKIN TW and sensory crispness. NO SKIN parameters showed slightly higher correlations with sensory tests than did SKIN parameters. FPT firmness showed good correlations with sensory scores as well as

Table 3–Analysis of variance for sensory crispness (CRP) and firmness (FRM)

Source	Degrees of freedom	Mean squares CRP	Mean squares FRM
Panel	5	12.40	21.54*
Varieties	5	48.61**	59.89**
Residual	25	7.87	7.31
Sampling error	324	1.17	1.20

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

Table 4–Correlation of instrumental and sensory measurements: Instron TPA brittleness (BTL), hardness (HDN) and total work (TW); FPT firmness (FRM); sensory crispness (CRP) and firmness (FRM)

		Correlation coefficients (r)[a]						
		Instron TPA						FPT
		SKIN			NO SKIN			
		BTL	HDN	TW	BTL	HDN	TW	FRM
FPT	FRM	.959	.932	.964	.908	.919	.920	
Sensory	CRP	.937		.929	.983		.988	.933
Sensory	FRM		.931	.931		.971	.950	.935

[a]Based on mean values of 30 observations from each variety for Instron and FPT; 60 observations for sensory evaluations.

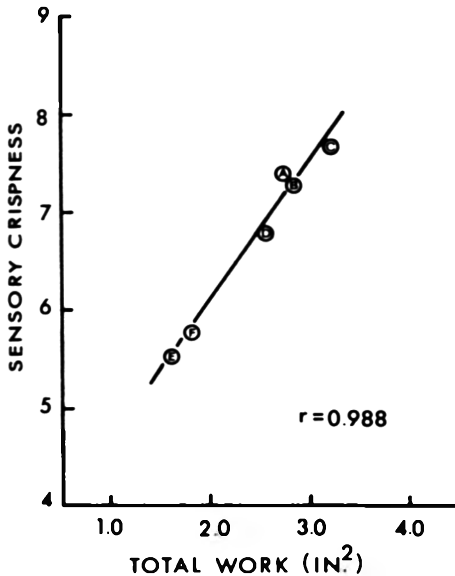

Fig. 4—Correlation of TPA mean total work of NO SKIN and sensory mean crispness.

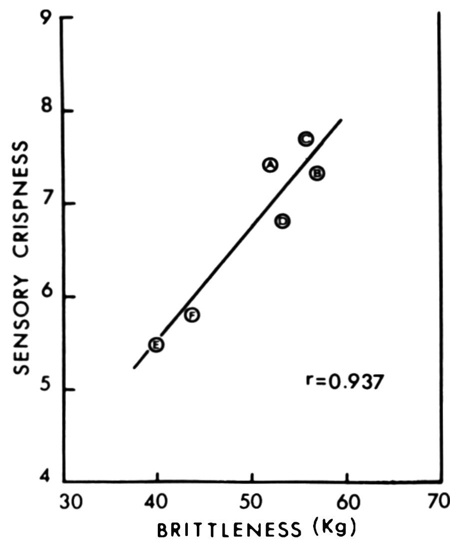

Fig. 5—Correlation of TPA mean brittleness of SKIN and sensory mean crispness.

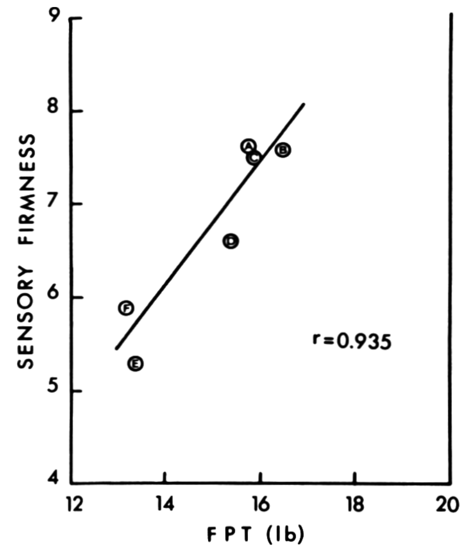

Fig. 6—Correlation of FPT mean firmness and sensory mean firmness.

with TPA parameters. The best correlations between each instrumental method (SKIN, NO SKIN, and FPT) and a sensory response are illustrated in Figures 4–6.

We concluded from these highly significant positive correlations that the sensory panel and any of the instrumental methods were measuring similar properties of cucumber texture. Certain advantages and limitations obtain for each of the instrumental methods. The TPA NO SKIN method has the advantage of being relatively rapid. In addition, force-distance curves from different samples can be compared directly. However, the method requires very uniformly-sized cucumber samples to avoid variability arising from differences in the proportions of parenchyma to placental tissue. Uniformity of shape is also important. Some varieties, e.g., Chipper, exhibit pronounced intercarpellary indentations which contribute to nonuniformity of composition in cored samples. The number of replications can be increased to reduce sampling variability but this increases testing time.

The point can be made that the SKIN method is a better measure of overall cucumber texture than is the NO SKIN method because texture derives from three principal edible tissues: skin, flesh or parenchyma, and seed cavity. The SKIN method suffers from being relatively slow and laborious; it requires tracing and measuring of slices and parameter adjustment.

Since FPT showed positive correlations with sensory and Instron procedures, its continued use for field purposes is recommended. However, in view of its low sensitivity and significant operator variability (Nicholas, 1960), its use in research for detecting small textural differences should be de-emphasized.

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MODIFIED METHOD FOR ANALYSIS OF SWEET POTATO α-AMYLASE

INTRODUCTION

TWO ACCEPTED METHODS of making sweet potato flakes (Deobald and McLemore, 1962; Hoover, 1966a) are no longer being used commercially but have been replaced by an "enzyme activation" technique developed by Hoover (1966b) utilizing the inherent enzymes in the sweet potatoes. Ikemiya and Deobald (1966) showed the presence of an α-amylase in sweet potatoes active at high temperatures. Deobald et al. (1968) reported on the control of this α-amylase to produce optimum quality sweet potato flakes and recommended determining the α-amylase present in the roots for predicting processing characteristics. A later study (Deobald et al., 1971) confirmed the necessity of determining α-amylase because of unpredictable contents due to handling and/or storage conditions.

The previously reported method for determination of α-amylase content is time-consuming, requiring specialized laboratory equipment not ordinarily found in sweet potato quality control facilities. A study was therefore undertaken to develop a method for use in the plant laboratory by modifying one of the known procedures, cutting down the time and technical equipment required, and adapting it for either pre-processing analysis or production control during processing.

EXPERIMENTAL

THE METHOD of analysis for α-amylase as reported in AOAC (1960) and modified for use with sweet potatoes by Ikemiya and Deobald (1966) is highly efficient for quantitative determinations made in a research laboratory, and this modified method was chosen for subsequent adaptation. Other methods for analysis of α-amylase in sweet potato juice require quite sophisticated techniques and are equally as time-consuming (Perten, 1966; Tipples, 1969).

Each step in the procedure of the Ikemiya method was studied for possible simplification. The incubation temperature of 70°C was not changed from the method of Ikemiya so that the results would be in comparable units (SDU). First, screening of substrates was undertaken by investigating their stability and reaction to the enzymes in an effort to select the one easiest to prepare or most readily available to processors. Raw Lintner starch, gelatinized Lintner starch and β-limit dextrin (made from both Lintner and sweet potato starches) were incubated with crude sweet potato juices which contained an excess of β-amylase. Also an "enzyme concentrate" as prepared by Ikemiya and Deobald (1966), in which most of the β-amylase was destroyed, was used in other experiments. As a result of this screening, use of the raw starch as a substrate was found to be too time-consuming and was therefore eliminated for determining amylase activity. A 2% solution of freshly made gelatinized starch from either source (Lintner or sweet potato) was quite satisfactory for use with crude juices, but such substrates were unstable, had to be made fresh daily and were impractical when assaying an "enzyme concentrate." Beta-limit dextrin prepared by the AOAC (1960) method using 2% Lintner starch gave good results with either crude or concentrated enzymes and had the advantage of good stability in solution. Since a processor of sweet potatoes would be determining activity only of a crude juice, he could choose either freshly made gelatinized starch or β-limit dextrin made weekly and refrigerated as the substrate.

In the second step, a difficulty was encountered during preparation of the sweet potatoes for extraction of the crude juice. In the usual method the sweet potatoes were shredded, then pressed under 600 psi on the ram using a hydraulic press. Since it is unlikely that a processing plant would have a hydraulic press, changes in the grinding method were sought. By macerating the sweet potato with a kitchen-type hand grinder, the resulting pulp could be weighed into a bag or cloth and the juice collected in a beaker by simple hand pressing.

The next step in the regular procedure was centrifuging or filtering for removal of the raw starch and other solids. With the macerating and pressing procedures, centrifugation, except in a high speed centrifuge, did not clear the juice sufficiently. Most processing plants would not have a centrifuge powerful enough and this method of clarifying was very time-consuming. Filtering was also much too slow. After addition of $CaCl_2$ for stabilization of the enzyme, a heat coagulation step was incorporated which greatly aided in this separation or clarification. It involved use of a hot plate, a larger beaker (400 ml) for a boiling water bath and a slightly smaller beaker (250 ml) which fit into the larger one without having to be supported. Juice was poured into the smaller beaker and with constant stirring the temperature of the juice was brought up to 71°C, just below the point of heat deactivation for the sweet potato α-amylase (Ikemiya and Deobald, 1966). The juice was then poured quickly down the inside of a graduated cylinder, with care being taken to eliminate air entrapment. Coagulation occurred within minutes and most of the solids precipitated yielding a supernatant juice that was suitable for α-amylase determinations. Since the juice has a pH close to 6.0, no buffering of juice or substrate was necessary in this modified method.

In the determination of amylase in the clarified juice a constant-temperature water bath is used in the laboratory method. Since the optimum temperature of the α-amylase varies with in about a 5° range (Ikemiya and Deobald, 1966), a beaker of water kept at about 70°C could be used for incubation of the enzyme-substrate reaction if a water bath is not available. Also a comparator equipped with an α-amylase color disk is used in precise quantitative determinations, but a solution of dextrin-iodine made to certain specifications and pipetted into a spotting dish can be substituted for the comparator. The dextrin solution is made by dissolving 0.12g of dextrin (Bacteriological, Eastman Org. Chem.) in 20 ml of boiling distilled water. 0.5 ml of this dextrin solution is added to 5 ml of diluted iodine solution (AOAC, 1960). 1 ml of the dextrin-iodine mixture is pipetted into the spotting dish for comparative color.

The method as it exists after various modifications, is summarized as follows:

For pre-processing use in a plant laboratory it becomes:

1. Wash and hand peel about 10 roots.
2. Macerate, using a kitchen type hand grinder.
3. Tare a 400 ml beaker with a double thickness of thin muslin or some cloth that would hold back solids but allow liquid to go through. Weigh into it about 400g of ground pulp and squeeze out 1/3 of the weight in juice.
4. Measure out a 100-ml portion of crude juice. Pour this into a 250 ml beaker, add 6 drops of 30% $CaCl_2$ for stabilization of α-amylase, immerse into boiling water in a 400 ml beaker. Stir juice constantly while checking the temperature. Remove the smaller beaker when the temperature of the crude juice reaches 71°C.
5. Pour the heated juice back into the 100 ml graduated cylinder. Allow about 5 min for starch and other solids to settle to bottom. (If a small laboratory centrifuge is available, it can be used to separate the coagulated solids more quickly than the "settling step" will).
6. Pour off about a 10 ml portion of supernatant from the clarified juice. Again, using a beaker as a 70°C water bath, heat 10 ml of the 2% solution of β-limit dextrin or gelatinized starch in a test tube to 70°C. While retaining that approximate temperature, pipette 2 ml (or any measured amount) of the juice into the substrate starting a stop watch at the same time. Measure the time it takes to match the color of 0.1 cc of this substrate in 1 cc of diluted iodine to the dextrin color in the spotting dish. Calculate the activity (12/time × ml) = SDU.

This modified technique was set up to allow a plant laboratory to operate as quickly as possible and with a minimum amount of equip-

[1] Deceased January 22, 1972.

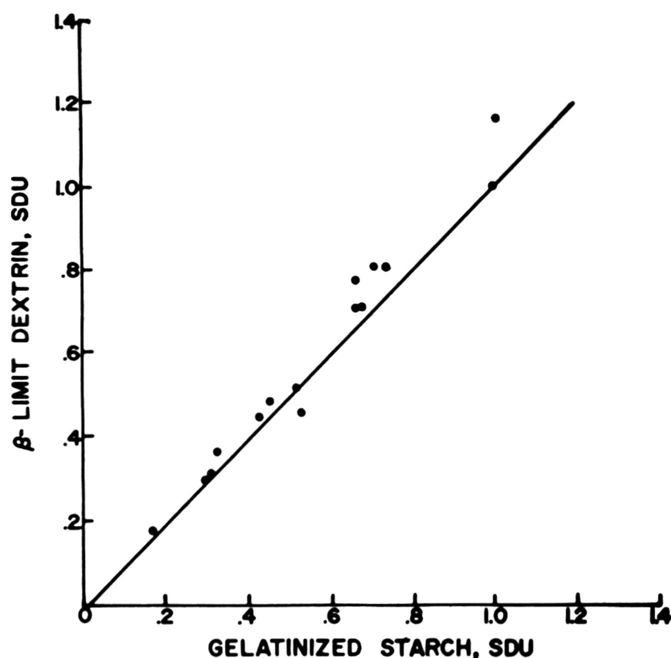

Fig. 1—Comparison of α-amylase values using different substrates.

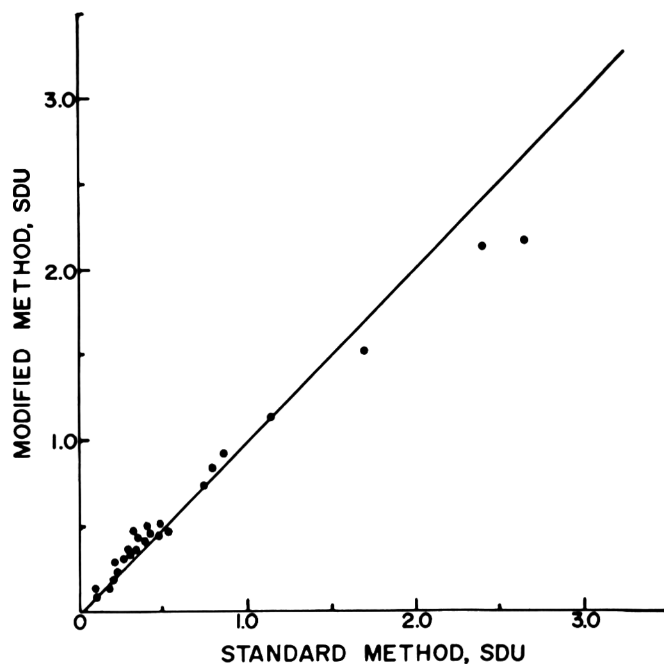

Fig. 2—Comparison of α-amylase values using different methods.

ment. It then became necessary to see if it could be used for in-line analysis during processing or if further adaptations would be required.

In order to make an in-plant test, a sweet potato processing plant was visited where the sample was taken from the processing line. The process used deep lye peel and hammer mill grinding. The puree showed the presence of soluble starch and β-limit dextrin in a thickened press juice. This made it difficult to obtain more than a small amount of juice by hand squeezing and produced an interfering color in the iodine tests. As soon as a few ml of juice were collected from the cloth bag, two drops of $CaCl_2$ were added, the juice was heated to 71°C, held at that temperature for 5 min or until interfering color was eliminated when checked in diluted iodine, then centrifuged or filtered and used for analysis.

To insure that enzyme activity was not affected in the process of heating-coagulation-centrifuging, β-limit dextrin was added to the juice after this clarifying procedure. The juice was again heated-coagulated-centrifuged and then reanalyzed, giving the same α-amylase value as obtained in the previous analysis.

RESULTS & DISCUSSION

CONSIDERATION of the following data will support the choices of substrates and procedures employed in the modified method.

Figure 1 is a comparison of α-amylase values obtained using gelatinized Lintner as the substrate in one instance and β-limit dextrin in the other. Duplicate samples of juices were used in each experiment. The line shows exact equivalence, thus deviations from the line may be viewed as possible errors or biases. The β-limit dextrin gives slightly higher values, but the differences in sweet potato dextrinizing units (SDU), were considered negligible. Thus, a processor could use either β-limit dextrin (made weekly and refrigerated) or gelatinized starch (made daily) as substrate.

Figure 2 shows the relationship of β-amylase contents determined by the method of Ikemiya and Deobald (1966) compared to the results derived by the modified method. The line shows exact equivalence, and it is seen that when the α-amylase content is very low (below 1 SDU) the modified method gives a value about 0.1 SDU higher than the standard method. Conversely, as the α-amylase content increases to 1.5 SDU and higher, the difference is reversed; the modified method then yields lower values than the regular method. It should be emphasized at this time that such differences in values derived by the two methods would be expected. The sweet potatoes were ground to a different degree. Because of this and the cloth thicknesses, the amount of starch in the juice varied. However, the heating step in the modified method should have affected only the β-amylase and not the α-amylase since sweet potato α-amylase is highly heat resistant, especially in the presence of $CaCl_2$ (Ikemiya and Deobald, 1966).

These differences, however, would be negligible to a processor. As stated by Deobald et al. (1968), good flakes can be made from sweet potatoes having as low as 0.2 SDU to as high as 19 SDU by varying processing conditions: the conditions being determined by the range in which the α-amylase values fall.

For quality control in sweet potato flake production it is essential that α-amylase content be determined as close to processing time as possible.

The simplicity of this modified method for laboratory use and its adaptability for production control during processing cannot be stressed enough.

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METHOD FOR DETERMINING NARINGIN CONTENT IN GRAPEFRUIT JUICE

INTRODUCTION

NARINGIN contributes to the bitterness of grapefruit juice, Horowitz (1964). Several tests have been devised to determine its quantity in grapefruit juice. A general test, developed by Davis (1947), for flavanone glycosides usually is used, but is not specific for naringin. Hagen et al. (1965) developed a chromatographic-fluorometric method for naringin which is very time consuming and complex. Fisher et al. (1966) developed a chromatographic-colorimetric method for naringin and its isomer, the 7-β-rutinoside of naringenin. This paper presents a simplified method for naringin based on the work by Fisher et al. (1966).

EXPERIMENTAL

ALL SAMPLES examined in this study were commercially canned single-strength grapefruit juice.

Baker-flex Polyamide 6 thin-layer sheets (20 × 20 cm, J. T. Baker Chemical Co., Phillipsburg, N.J.) were used for separation of naringin from its tasteless isomer. Solvent systems used in this work were: (1) 5:2 nitromethane-methyl alcohol, Hagen et al. (1965); (2) 5:1.5 nitromethane-methyl alcohol; and (3) 10:7:3 benzene-acetone-methyl alcohol. Rf values for naringin in solvents 1, 2 and 3 were 0.68, 0.40 and 0.45 and for naringenin 7-β-rutinoside were 0.73, 0.45 and 0.50. A 50 μl Hamilton syringe was used to spot 35 μl of whole juice across the plate which was then dried with a stream of nitrogen. Juice was applied twice to give a total of 70 μl. The chromatoplate was developed twice in solvents 1, 2 or 3. The TLC tank was paper-lined and well equilibrated before the plate was introduced. Each development required 30–45 min at 25°C. Plates were dried in a fume hood for 5–10 min between developments. After development and drying, the plates were sprayed with a 1% solution of $AlCl_3$ in ethanol. Naringin and naringenin-7-β-rutinoside, appeared as yellow fluorescent bands when viewed with UV light. The bands were marked and the plate or area to be collected was sprayed with water to prevent development of an electrostatic charge, which could cause loss in scraping and transferring the material to a centrifuge tube. The bands were scraped from the plates with a spatula and transferred to a centrifuge tube with the aid of a soft brush and a funnel. The test reagent used by Fisher et al. (1966) was modified slightly, and consisted of 125 ml methyl alcohol, 112 ml diethylene glycol and 20 ml of 4N aqueous sodium hydroxide. Two ml of test reagent were added to the scraped bands and the mixture stirred with a glass rod. The tubes were then capped and centrifuged for 3 min at 1800 rpm. The color began to develop as soon as the test reagent was added and reached a maximum after 15 min with no further change in absorbance after one hr. After centrifugation the supernatant was transferred with a disposable pipet to a 10 × 75 mm test tube. The intensity of the color (blue filter at 420 nm) was read using a Lumetron Colorimeter which has bushings in the sample holder to accommodate the 10 × 75 mm matched test tubes. This gave a direct reading of percent transmission or absorbance. On a clean plate a blank was prepared by spraying a 1 cm band with the $AlCl_3$ solution and treating as a sample.

A standard solution of naringin, containing 1 μg/1 μl, was prepared. Naringin, m.p. 167–171°C, reported 171°C by Asahina et al. (1929) contains two molecules of water of crystallization and is 94.15% naringin by weight. A standard curve was prepared by spotting known amounts of naringin on a plate and carrying these areas through the complete procedure. A plot of absorbance vs. micrograms of naringen gave a straight line; the slope was 0.01. At a load rate of 70 μl of whole juice this covers a range of naringin values from 0 to 571 ppm. If the load rate is halved effective concentration range doubles.

A standard curve for Davis (1947) values was prepared by adding known quantities (5 thru 40 μl) of the standard solution to test tubes, adding 2 ml of test reagent and reading the absorbance after 15 min. The blank was 2 ml of test reagent. These two standard curves were very similar but both are required if the Davis values are desired. The Davis test measures any compound in grapefruit juice that will give a yellow color with base and is generally considered a measure of total flavanone glycosides.

RESULTS & DISCUSSION

TABLES 1 AND 2 indicate the precision and accuracy of this method for naringin and its isomer. This simple analysis for naringin requires no sample preparation as the whole juice is spotted directly on a commercially available plate. Duplicate determinations on a single juice require about 2.5 hr. If only naringin values are required, and not the isomer, duplicate values for six juices can be carried out in 5 hr or less.

In a previous attempt to determine the precision of this method eight replicate determinations, each containing 70 μl of juice, were used and a mean value of 18.6 μg for naringin was found; the range was 2.0 μg with a standard deviation of 0.7. A mean value of 5.5 μg was found for naringenin-7-β-rutinoside with a range of 1.5 μg and the standard deviation was 0.7. Some slight variations in technique were found to improve reliability of the method. On some commercial plates the coating thickness is not completely uniform, i.e., the coating is thinner toward the sides. When spotted and developed across these different thicknesses the developed bands are not straight and not completely separated. With these plates the juice should be spotted along one thin side so the plate can be developed from thin side to thin side and separation will be achieved. If the plates are coated uniformly, any of the three solvent systems (see Experimental) will separate naringin and its isomer. Occasionally, the two compounds did not separate. This was usually because of: (1) failure to dry the plate thoroughly before placing in solvent tank; (2) plate placed in solvent tank before the tank was equilibrated; or (3) the plate was spotted from one thin side to the other thin side. The preferred solvent system was nitromethane-methyl alcohol (5:1.5) since separation was achieved each time it was used. Using these im-

Table 1—Replicate determinations of naringin and naringenin 7-β-rutinoside 70 μl aliquots of grapefruit juice

Sample no.	Naringin μg	Naringin ppm	Naringenin-7-β-rutinoside μg	Naringenin-7-β-rutinoside ppm
1	19.1	273	6.5	93
2	20.0	286	6.3	90
3	20.6	294	6.2	89
4	19.0	271	7.0	100
5	19.8	283	6.6	94
6	20.1	287	7.2	103
Mean	19.8	282	6.6	95
Range	1.6	23	1.0	13
s	.6		.4	

Table 2—Recovery of naringin added to a base sample of grapefruit juice

Sample no.	μg Naringin calculated value	μg Naringin found	% recovery
1	21.6	24.0	111.1
2	24.7	24.5	99.2
3	28.2	27.0	95.7
4	29.1	30.5	104.8
5	32.6	33.0	101.2
6	38.0	36.3	95.5

Table 3—Davis values, naringin (1–2) and naringenin-7-β-rutinoside (1–6) concentration in 10 commercial canned juices[a]

Sample no.	Davis ppm	1–2 ppm	1–6 ppm	1–2 / 1–6	Davis / 1–2
1	668	307	107	2.9	2.2
2	674	311	102	3.0	2.2
3	674	296	91	3.2	2.3
4	529	254	92	2.8	2.1
5	457	221	75	2.9	2.1
6	514	254	84	3.0	2.0
7	571	265	76	3.5	2.2
8	478	218	69	3.2	2.2
9	588	293	93	3.2	2.0
10	580	268	90	3.0	2.2

[a]Average of duplicate determinations

proved procedures the precision of replicates was that given in Table 1. This agreement of replicates, and the recovery of added naringen (Table 2) indicate that there was no problem with consistency in sampling. Recovery data also suggest this small sample is representative of the whole.

Up to 100 μl of juice has been spotted on a single plate and separation obtained, but this appears to be about the upper load limit of these plates. No interfering compounds were observed when naringin and its isomer were collected from plates run in the nitromethane solvent systems and rerun in solvent system 3.

To show the relationship between this method and the Davis test, Table 3 shows the Davis values, naringin and naringenin-7-β-rutinoside concentrations in ppm for 10 commercial grapefruit juices that were produced from November 1971 through February 1972. The glycoside concentration by the Davis value is approximately 2.2 times the concentration of the 1-2 naringin isomer.

This is a simplified and reliable method for naringin which can be performed easily on a routine basis, and might provide a basis for quality control tests in grapefruit juice.

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Mention of brand names is for identification only and does not imply recommendation by the U.S. Dept. of Agric.

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A NEW METHOD FOR THE DETERMINATION OF CAPSAICIN IN CAPSICUM FRUITS

INTRODUCTION

THE MEXICAN PEOPLE are large consumers of chili, fruit of the genus *Capsicum.* Chili peppers are used mostly as unripe fruits as a spice, especially in sauces. Sometimes the whole fruit is cooked to prepare some dishes or dried and ground to provide color and flavor, the pungent flavor being determined by the capsaicin content. Chemically, capsaicin is N-(3-methoxy-4-hydroxybenzyl)-8-methylnon-trans-6-enamide and the structure is shown in Figure 1. However, Benett and Kirby (1968), Kosuge and Furuta (1970) and Masada et al. (1971) have reported that capsaicin is actually a mixture of at least five closely related vanillyl amides.

A precise and accurate method for the quantitative determination of capsaicin has not been reported even though numerous scientific investigators have worked on the determination of this compound. Most colorimetric methods for capsaicin determination are based on the color reactions of capsaicin with vanadium oxytrichloride, a method first developed by von Fodor (1931) and modified by other investigators, Tice (1932), Prokhorova and Prozorovskaya (1939) and Hayden and Jordan (1941). Ting and Barrons (1942) and later van Blaricom and Martin (1947) developed colorimetric methods using synthetic color standards. North (1944) used a method based on the reaction of capsaicin with phosphotungstic-phosphomolybdic acid. Nogrady (1943) developed a procedure based on the titration of capsaicin with picric acid. Other investigators have combined chromatographic and colorimetric techniques to quantitate capsaicin, including Heusser (1964), Büchi and Hippenmeier (1948), Suzuki et al. (1957) and Spanyár and Blazovich (1969). Ríos and Duden (1971) have reported a method utilizing chromatography and fluorescence spectrophotometry to determine capsaicin content. Todd and Perun (1961), Morrison (1967), Kosuge and Furuta (1970), Hartman (1970) and Masada et al. (1971) have used gas-liquid chromatography (GLC) for the detection of capsaicin. Sensory methods have also been used, including methods developed and modified by Nelson (1919), Wirth and Gathercoal (1924), Munch (1930) and Newman (1953), but these methods have been shown to be even less accurate and reproducible than the chemical methods. The purpose of this study was to develop a simple, accurate and reproducible method for the quantitative measurement of capsaicin.

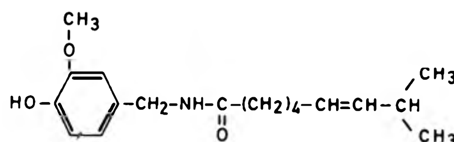

Fig. 1—Capsaicin.

EXPERIMENTAL

Materials and equipment

The fresh green chili peppers, *Capsicum annuum* L. variety Veracruz S-69 (Serrano), were obtained from the Horticulture Dept., INIA, México.

All chemicals used were of analytical reagent grade. Methanol, isopropanol and chloroform were of spectrophotometric grade (Uvasol), purchased from Merck-México, S.A. The capsaicin used as a standard was obtained from K & K Lab., New York. The absorbent charcoal No. 55159261 was obtained from Harleco Dare, México.

The samples were ground in a Sorvall Omnimixer Homogenizer. Evaporation was done in a Flash-evaporator from Buchler Instruments. Ultraviolet absorption spectra were determined on a Coleman-Hitachi Recording Spectrophotometer Model EPS-3T. The UV absorbance at 281 nm was determined by a Zeiss Spectrophotometer Model PMQ II.

Procedure

Capsaicin isolation and calibration of standard curve. 20g of finely ground chili peppers were placed in the extraction shell of a Soxhlet extractor and isopropanol was percolated through the sample until no more green color appeared in the percolated isopropanol. The extract was cooled and adjusted to a volume of 200 ml with isopropanol. 5g of charcoal was added to the colored extract. The slurry was heated to boiling for the duration of 3 min, filtered and the charcoal residue washed several times with isopropanol. The clear extract was then evaporated to dryness in the flash-evaporator. The oil residue was dissolved in petroleum ether and transferred to a separatory funnel and washed with distilled water. After discarding the aqueous layer, the ether layer was evaporated to dryness in the flash-evaporator. The purified oily residue remaining was dissolved in isopropanol, collected in a volumetric flask, and total volume brought up to 25 ml with isopropanol. The absorbance was read at 281 nm against an isopropanol blank. Capsaicin standard solutions containing 10, 20, 30, 40 and 50 μg/ml capsaicin were prepared in isopropanol and their absorbance values recorded at 281 nm. A standard curve plotting absorbance against μg capsaicin/ml was prepared and the quantity of capsaicin in the samples was determined from this standard curve.

Paper chromatography. The ascending technique of paper chromatography was used exclusively. 50 μl of each extract was spotted on Whatman #1 chromatographic paper 1.5 cm from the end of strips 2.5 cm wide × 17.5 cm long. Each strip was hung in a 30 × 200 mm corked test tube by means of hooked metallic pins inserted in the cork stopper. The developing solvent system was chloroform-methanol-acetic acid (95:1:5 v/v). Approximately 5 ml of the developing solvent was used and the paper was equilibrated with the solvent 15 min and when the solvent front reached a line 2.5 cm from the top of the paper, the strip was removed and air-dried. The chromatograms were examined under a 254 nm wavelength UV lamp. Capsaicin fluoresced and was located at the solvent front. The capsaicin was then eluted from the chromatograms with 2 ml spectrophotometric grade isopropanol. A spectrophotometric blank was prepared from an unspotted region of a blank chromatogram.

RESULTS & DISCUSSION

CHARCOAL was used to clean up the chili extracts. Adding less than 5g of charcoal per 20g of ground chili pepper in a volume of 200 ml isopropanol resulted in a yellow-green extract. Absorbance at 281 nm was too high when using less than 5g, indicating that impurities were absorbing at that wavelength as well as capsaicin. By using 5g of charcoal the extract appeared colorless and the differences between replications in absorbance at 281 nm were minimal. After drying the extract, the oily residue was slightly colored. It was found that water extracted these colored substances from capsaicin in petroleum ether.

From the solvent systems described in the literature, the following were tested for the capacity to separate capsaicin from other substances: butanol-isopropanol-acetic acid (10:75:25 v/v); chloroform-methanol-acetic acid (95:1:5 v/v); light

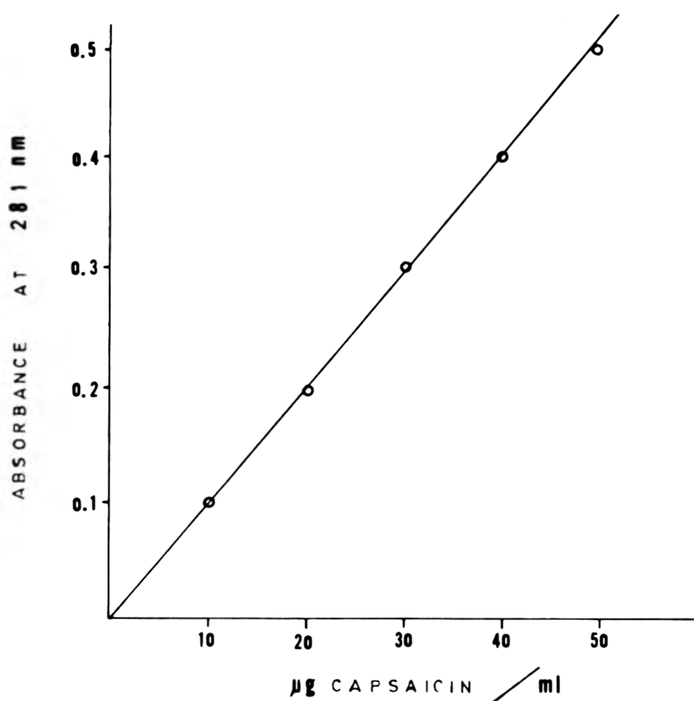

Fig. 2–Capsaicin standard curve at 281 nm.

Table 1–Capsaicin contents from the extracts of 10 chili pepper samples

Sample no.	Fresh wt (g)	Dry wt (g)	Capsaicin content[a]	
			amt in mg	% on dry wt basis
1	29.90	3.06	9.1	0.30
2	27.68	2.78	12.0	0.43
3	23.36	2.39	11.7	0.49
4	23.58	2.58	11.3	0.43
5	24.17	2.35	10.8	0.48
6	23.74	2.34	11.2	0.47
7	26.50	2.59	11.5	0.44
8	26.50	2.63	13.5	0.51
9	23.30	2.12	9.6	0.45
10	22.98	2.06	9.7	0.47
				0.45 ±0.06

[a]Relative standard deviation = 13.3%

petroleum (boiling range 60–100°C)-benzene-methanol-ethanol (10:2:1:1 v/v); and isopropanol. The best separation was achieved with the chloroform-methanol-acetic acid solvent system. Using this system, capsaicin moved with the solvent front leaving the interfering substances in the vicinity of the origin. The separation of capsaicin under these experimental conditions was relatively rapid, taking approximately 20 min.

The position of capsaicin on the chromatograms was initially established using the following sprays: (1) equal volumes, mixed immediately before use, of iron (III) chloride (1% in water) and potassium ferricyanide (1% in water). This resulted in a reaction yielding a blue color in the capsaicin region; (2) 0.5% solution of Fast Blue Salt B followed by 0.1N NaOH, yielding a red-brown color as the indicator of capsaicin. Neither method is specific for capsaicin, as other compounds containing phenolic groups may also give color reactions. Since these reactions are destructive, the detection of capsaicin for quantitative measurement was done using the 254 nm UV lamp. The spots of fluorescence at the solvent front and also at the origin were cut out and eluted in isopropanol.

The spectra of the isopropanol eluents were measured in the region of 200–300 nm. In comparing the spectra of pure capsaicin with capsaicin from the chili extracts, the same absorption maxima were found, one peak at 281 nm and another at 231 nm. Although the compounds at the origin of the chromatogram fluoresced under UV light, they did not exhibit absorption maxima at these two wavelengths. It was then considered that these other compounds would not interfere with capsaicin measurement if they were not separated from capsaicin by chromatography. Therefore, the chromatography of the extract was eliminated as part of the clean-up procedure. The wavelength of 281 nm was chosen for the capsaicin quantitation because a very distinct peak is obtained at that wavelength. The absorbance of different concentrations of pure capsaicin in isopropanol followed the Lambert-Beer law, as shown by the standard curve presented in Figure 2. At 281 nm a molar absorptivity of 3070 was obtained.

The capsaicin contents determined in the extracts of 10 chili pepper samples from the same lot are presented in Table 1. The average capsaicin content was 0.45% on a dry weight basis, with a standard deviation of 0.06.

To determine the percent recovery, 500 μg of pure capsaicin was added to ground chili samples. When the samples were carried through the extraction procedure and the charcoal clean-up, the added capsaicin was recovered to the extent of 86.5–99.0% with a standard deviation among 5 replicate analyses of 0.95 μg/ml or 5.2% (Table 2).

Table 2–Determination of percent recovery of capsaicin[a]

Capsaicin added	Capsaicin found	% Recovery
20 μg/ml	19.8 μg/ml	99.0
	18.0	90.0
	17.9	89.5
	17.3	86.5
	17.9	89.5
	18.2 ± 0.95	

[a]Relative standard deviation = 5.2%; Relative error = 9.0%; Total error = 19.4%

CONCLUSIONS

THE PROCEDURE for the capsaicin quantitation from green chili pepper extracts reported here has shown precision to a satisfactory degree, accuracy and simplicity. It was not necessary to utilize chromatographic techniques as has been reported by other investigators. The method is therefore well suited for the determination of capsaicin-containing extracts from fruits of various varieties of *Capsicum*. The capsaicin content determined in the Veracruz S-69 variety (Serrano) was, on the average, 0.45% on a dry weight basis. This value falls within the

range reported by Suzuki et al. (1957) with Mombassa chili peppers containing 0.80% capsaicin and the mild variety, Abyssinia, yielding 0.08% capsaicin, and within the range of values reported by Govindarajan and Ananthakrishna (1970) for Mysore variety yielding 0.12% capsaicin, and Guntur variety, yielding 0.07%.

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ISOLATION AND IDENTIFICATION OF VOLATILE COMPOUNDS FROM POTATO CHIPS

INTRODUCTION

THE CHEMICAL composition of the flavor of potato chips has been studied only recently. Dornseifer and Powers (1963) reported the presence of nine volatile carbonyl compounds in potato chips. Two years later, Mookherjee et al. (1965) identified 18 monocarbonyl compounds in fresh potato chips and 19 monocarbonyl compounds in a stale-but-not-rancid sample. The amount of alkanals, 2-enals and 2-alkanones, particularly of hexanal and 2-propanone, was found to increase significantly during storage. On the other hand, the amount of 2,4-decadienal, which had a characteristic deep fat fried flavor, was greatly reduced. Dornseifer and Powers (1965) also suspected the presence of alka-2,4-dienals in aged potato chips. Wishner and Keeney (1965) detected essentially the same alkanals, 2-alkenals, and 2,4-alkadienals in the volatiles produced by the deep fat frying of potatoes in fats and oils.

A number of sulfur compounds have been reported as components of cooked potato flavor. Casey et al. (1963; 1965) identified hydrogen sulfide, methane thiol, dimethyl sulfide and ethane thiol in cooked potatoes. The same compounds were identified by Swain and Self (1964) with the use of headspace analysis. Gumbmann and Burr (1964) detected four alkyl thiols, three sulfides, three disulfides and hydrogen sulfide in the volatile compounds isolated from freshly cooked potatoes. However, all these conclusions were based on gas chromatographic data only.

The volatile flavor compounds isolated from potato chips were found to contain 2.3% of nitrogen. The first nitrogen compound in the volatiles of potato chips was identified by Deck and Chang (1965). They reported that 2,5-dimethyl pyrazine had an earthy, raw potato flavor, at a concentration of approximately 10 ppm. Recently, Buttery et al. (1971) identified 18 pyrazine and pyridine compounds in the basic fraction of the steam volatile oil from potato chips. Guadagni et al. (1971) determined the minimum detectable amounts of some pyrazine compounds in dehydrated mashed potatoes. Pyrazine compounds were further identified by Sapers et al. (1971) in the volatile concentrates prepared from explosion puffed and conventionally dehydrated potatoes.

The present study is a systematic characterization of the volatile compounds in potato chips.

EXPERIMENTAL

Potato chips used

Eight samples of different brands of potato chips were evaluated organoleptically by a trained panel of six members. One sample was found to have a well-blended and pleasant, desirable flavor without any burned or harsh off-flavor. This sample of potato chips was manufactured from Kennebec potatoes by frying them in a mixture of 50% corn and 50% cottonseed oils at 185°C. An additional 200-lb batch of the Kennebec potato chips was then manufactured for use in this investigation. After the sample stood at room temperature for 1 day, it was sealed in 3-lb coffee cans under an atmosphere of nitrogen (1.70% oxygen). The cans were stored at −10°C until use. The color of the potato chips used was 4–5 on the proposed Potato Chip Institute International color chart.

Isolation of volatile compounds

The volatile compounds were isolated from 185 lb of potato chips, which were made into a slurry with 462 lb of water, with the use of a Waring Blendor. 6 liters of the water slurry were used as one batch. The water slurry was slowly added through a separatory funnel into a 12 liter round bottom flask evacuated to 12–25 mm Hg. The temperature of the material inside the flask was maintained at 15–30°C, with the use of a heating mantle. After 6 liters of the water slurry had been added at the rate of 100 ml/min, the distillation was continued for another 30 min. The volatile substances were collected in a series of traps consisting of a water cooled Friedrich condensor, four traps cooled with solid carbon dioxide and two traps cooled with liquid nitrogen. The apparatus used for this isolation step was made of all glass. The total condensate collected in the cooled traps was treated in a manner similar to that described by Herz and Chang (1966). It was saturated with sodium chloride and extracted with an equal volume of diethyl ether. The ethyl ether extract was separated into acidic and nonacidic fractions by extraction with a 10% aqueous solution of sodium carbonate. The diethyl ether solution of nonacidic compounds was dried with anhydrous sodium sulfate and then with anhydrous calcium sulfate. It was then concentrated to approximately 5 ml with the use of a 30-plate Oldershaw column. The ethyl ether solution of the acidic compounds was also concentrated to approximately 5 ml with the use of an Oldershaw column. The acidic compounds were then converted into their methyl esters by treatment with diazomethane, according to the method of Schlenk and Gellerman (1960).

Fractionation of isolated volatile compounds

The concentrated ethyl ether solution of the nonacidic compounds was separated into 19 broad fractions by preparative gas chromatography, using an Aerograph A-90-P-2, fitted with a 10 ft × 3/8 in. aluminum column, packed with 15% silicone gum SE-30 on 70/80 mesh Anakrom ABS (Analabs, Inc., Hamden, Conn.). The column temperature was programmed nonlinearly from 55–235°C, with a helium flow rate of 97 ml/min (Fig. 1).

Each of the broad fractions was collected with the use of a micro fraction collector (Deck et al., 1965), and chromatographed for the second time, with a Beckman GC-2A gas chromatograph connected to a Thermotrac temperature programmer. A 6 ft × ¼ in. aluminum column, packed with 20% Carbowax 20M on 60/80 mesh Anakrom ABS, was used for fractions No. 3-7, an 8 ft × ¼ in. aluminum column packed with 15% UCON 50 HB 280 X on 70/80 mesh Anakrom ABS was used for fractions No. 8-19 and an 8 ft × ¼ in. aluminum column packed with 20% Carbowax on 70/80 mesh Chromosorb W for fractions No. 1 and 2. The sub-fractions were collected in a specially designed micro fraction collector described by Smouse and Chang (1964). Each of the sub-fractions was then chromatographed for the third time, using an Aerograph 1520, fitted with an 8 ft × ¼ in. aluminum column packed with 20% silicone gum, SE-30 on 70/80 mesh Chromosorb W. The compounds collected after the third chromatography were generally pure compounds and were submitted for spectrometric identification.

The concentrated ethyl ether solution of the methyl esters of the acidic compounds was fractionated with a 6 ft × ¼ in. aluminum column, packed with 20% stabilized diethylene glycol succinate on 70/80 mesh Anakrom ABS. The column temperature was programmed nonlinearly from 50–150°C, with a helium flow rate of 120 ml/min. The gas chromatographic fractions were collected in a manner similar to the nonacidic volatile compounds, and chromatographed for a second time with a 6 ft × ¼ in. aluminum column packed with 20% silicone gum, SE-30. The fractions collected from the second chromatography were identified by spectrometric methods.

Identification of the gas chromatographic fractions

The procedure for the identification of the gas chromatographic fractions by the combination of infrared and mass spectrometry has

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been described previously (Kawada et al., 1966; 1967). The chemical structure postulated for a gas chromatographic fraction by the interpretation of its infrared and mass spectra was considered tentative. A compound was considered identified only when its retention time and infrared and mass spectra agreed with those of the authentic compound. When the authentic compound of a particular carbon number was not available, homologous plots of log retention time vs. carbon number were prepared with two different stationary phases by using available compounds of the same homologous series. Such compounds were also considered identified.

Synthesis of alkyl-substituted pyrazines

Two methods were used for the synthesis of alkyl-substituted pyrazines:

Bimolecular cycloamination of amino alcohols. By the method developed by Cenker et al. (1964), Langdon (1957) and Langdon et al. (1962; 1964), 2,5-dimethylpyrazine was prepared from 2-amino-1-propanol; 2,5-diethylpyrazine from 2-amino-1-butanol; a mixture of 2,5-dimethylpyrazine, 2,5-diethylpyrazine, and 2-ethyl-5-methyl pyrazine, from a mixture of 2-amino-1-propanol and 2-amino-1-butanol; a mixture of 2,5-dimethyl pyrazine and 2,6-dimethyl-pyrazine from a mixture of 2-amino-1-propanol and 1-amino-2-propanol; a mixture of trimethylpyrazine and tetramethylpyrazine from a mixture of 2-amino-1-propanol and 3-amino-2-butanol.

Alkylation of pyrazine derivatives. By this method, 2-ethyl-pyrazine was prepared fron pyrazine; 2-ethyl-3,6-dimethyl pyrazine from 2,5-dimethyl pyrazine; 2-ethyl-3,5-dimethyl pyrazine from 2,6-dimethyl pyrazine. This method, utilizing ethyl lithium, was originally described by Klein and Spoerri (1950; 1951).

0.01 Mole of the starting material was dissolved in 50 ml of ethyl ether in a 100 ml 3-necked round bottom flask. The flask was cooled to 0°C and the solution was stirred vigorously with a stream of nitrogen which provided an inert atmosphere. A slight excess of ethyl lithium in 0.01M benzene solution was added dropwise over a period of 40 min. A thick red precipitate was formed. The mixture was agitated for an additional 30 min. 50 ml of ice water was then cautiously added into the reaction mixture to yield the pyrazine compound.

RESULTS & DISCUSSION

A TOTAL OF 53 compounds was identified as the volatile components of a sample of potato chips, with a pleasant, desirable flavor. They included eight nitrogen compounds, two sulfur compounds, 14 hydrocarbons, 13 aldehydes, two ketones, one alcohol, one phenol, three esters, one ether and eight acids (Table 1). Many of the compounds identified have never been reported before as components of potato chip flavor. Furthermore, the aroma of many of them, particularly the alkyl-substituted pyrazines, the 2,4-dienals, phenyl acetaldehyde and furyl methyl ketone, indicated that they might play an important role in contributing to the desirable flavor of potato chips.

Nitrogen compounds

The most important group of compounds identified was definitely the seven alkyl-substituted pyrazines. The aroma of the fractions identified as 2,5-dimethyl pyrazine and 2-ethyl pyrazine was described by the organoleptic panel as either "strong potato" or "roasted peanut," depending probably upon the concentration. The fraction identified as 2-ethyl-5-methyl pyrazine was contaminated with a trace of 2,3,5-trimethyl pyrazine. The aroma of this fraction was described as "baked potato" or "peanut-like." The aroma of the fraction composed of 2-ethyl-3,6-dimethylpyrazine, with a trace of 2,5-diethyl pyrazine, was described as "earthy potato." The infrared spectra of the pyrazine compounds identified are shown in Figure 2.

The importance of pyrazine compounds to the flavor of foods was first reported by Deck and Chang (1965). They identified 2,5-dimethyl pyrazine in the volatile compounds of potato chips and reported that it had a characteristic potato flavor. Since then, a large number of pyrazine compounds have been identified in various foods. For example, Mason et al. (1966) reported the presence of five alkyl pyrazines in the volatiles isolated from roasted peanuts. The presence of pyrazine compounds in the flavor of roasted coffee was reported by a number of researchers (Viani et al., 1965; Reymond et al., 1965; Gianturco et al., 1966; Stoll et al., 1967; Gautschi et al., 1967; Goldman et al., 1967; Bondarovich et al., 1967). The presence of alkyl-substituted pyrazines in cocoa beans has also been intensively studied. Marion et al. (1967) discovered eight pyrazine compounds in cocoa beans and Rizzi (1967) six pyrazines in cocoa butter. In addition, Van Praag et al. (1968) described the identification of 11 alkyl-substituted pyrazines in the volatiles isolated from roasted cocoa beans. Van der Wal et al. (1968) also identified five pyrazine compounds in roasted cocoa. More recently, Buttery et al. (1971) identified 18 pyrazine and pyridine compounds in the basic fraction of the steam volatile oil from potato chips. They consider these compounds important to the characteristic flavor of potato chips. Pyrazine compounds have also been identified in popcorn (Walradt et al., 1970), hydrolyzed soy protein (Manley and Fagerson, 1970), deep fried soybeans (Wilkens and Lin, 1970), and in tobacco smoke condensate (Neurath and Duenger, 1969).

Another interesting nitrogen compound identified in the volatiles of potato chips is pyridine. It is probably produced

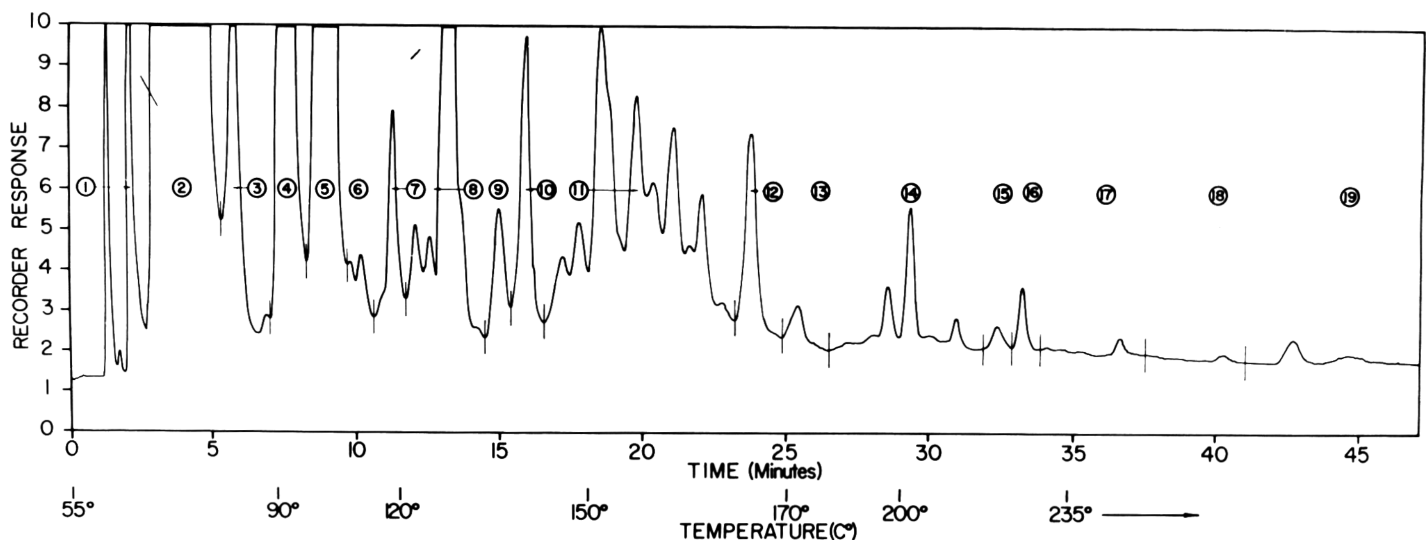

Fig. 1–Gas chromatogram of the preparative fractionation of the nonacidic volatile compounds isolated from potato chips.

by the decarboxylation of a component of potato, nicotinic acid, under the conditions of deep-fat frying. This compound has a strong, objectionable odor. Whether it could contribute to the total desirable flavor of potato chips at a low concentration, or should be eliminated to improve the flavor of potato chips, is difficult to ascertain.

Sulfur compounds

The low-boiling sulfur compound identified, dimethyl disulfide, was also identified by Gumbmann and Burr (1964) in cooked potatoes. This compound was probably produced by the decomposition of methionine. It had a cabbage-like aroma at higher concentrations and an onion-like aroma at lower concentrations. The compound, benzyl thiobenzoate, had a rather unpleasant, sulfur-like odor.

Hydrocarbons

The seven aliphatic hydrocarbons identified in the volatile compounds of potato chips are probably only of secondary importance to the potato chip flavor. Saturated hydrocarbons may be produced from potato lipids, as suggested by Buttery (1961). However, it is more probable that these hydrocarbons originate from the frying fat, as reported by Reddy et al. (1968). The acetylenic hydrocarbon, 1-decyne, is probably produced by the autoxidation of the frying oil in potato chips because it was identified in autoxidized soybean oil (Smouse et al., 1965), but not in the volatile decomposition products produced during deep fat frying (Krishnamurthy and Chang, 1967). The four identified aromatic hydrocarbons have rather unpleasant, kerosene-like, objectionable odors.

The identification of the three monoterpenes was rather unexpected. However, they might have originated from potatoes, as it was reported that various terpenes are present in roots, stems and leaves of many plants. They might also have been produced by a breakdown of higher terpenes under the conditions of deep fat frying (Gautschi et al., 1967). Only oxygenated monoterpenes have been reported so far (Buttery et al., 1970).

Aldehydes and ketones

Three of the aldehydes and the ketones identified may be of great importance to the flavor of potato chips. They are 2,4-decadienal, phenylacetaldehyde and furfuraldehyde. The 2,4-decadienal has been reported by Mookherjee et al., (1965), as having a characteristic deep-fat

Table 1—Volatile compounds identified in potato chips

Number of peaks[a]	Identified as	Size of peak[b]
	Nitrogen compounds	
10-4-1	2,5-Dimethyl pyrazine	XL
10-4-1	2,6-Dimethyl pyrazine	M
10-4-1	2-Ethyl pyrazine	S
11-7-1	2,3,5-Trimethyl pyrazine	S
11-7-1	2-Ethyl-5-methyl pyrazine	XL
11-8-6	2-Ethyl-3,6-dimethyl pyrazine	L
11-8-6	2,5-Diethyl pyrazine	S
8-10-2	Pyridine	M
	Sulfur compounds	
7-5-1	Dimethyl disulfide	XL
14-11-2	Benzyl thiobenzoate	S
	Hydrocarbons	
11-1-1	Heptane	S
11-2-1	Nonane	S
11-3-2	Decane	S
11-4-5	Undecane	S
12-4-1	Tetradecane	S
6-2-2	2-Methyl-1-butene	S
11-5-4	1-Decyne	S
11-5-6	Ethylbenzene	S
11-7-3	Butylbenzene	S
11-6-2	1,2,4-Trimethylbenzene	L
11-7-3	1-Ethyl-3,5-Dimethylbenzene	M
11-5-5	3-p-Menthene	S
11-5-5	D-Limonene	S
11-4-1	α-Terpinene	S
	Aldehydes	
1-1	Ethanal	XL
5-5-1	Butanal	XL
5-5-2	Pentanal	XL
8-9-1	Hexanal	S
8-10-4	Heptanal	S
8-10-5	2-Heptenal	S
11-6-1	2-Octenal	M
11-8-1	Benzaldehyde	L
7-5-1	3-cis-Hexenal	L
14-9-2	2,4-trans, trans-heptadienal	S
14-10-3	2,4-trans, trans-decadienal	XL
8-8-2	Furfuraldehyde	M
11-9-3	Phenylacetaldehyde	XL
	Ketones	
8-10-3	Cyclopentanone	M
10-5-1	Furyl methyl ketone	M
	Alcohol and phenol	
3-1-1	Ethanol	XL
16-5-1	2,6-di-tert-butyl-4-hydroxytoluene	S
	Esters	
4-4-1	Ethyl acetate	XL
6-3-1	Propyl acetate	M
8-4-1	Butyl acetate	M
	Ether	
14-12-4	Diphenyl ether	M
	Acids[c]	
A-1-4	Methylpropanoic acid[d]	S
A-1-7	3-Methyl butanoic acid	S
A-2-3	Butanoic acid	XL
A-3-1	Pentanoic acid	S
A-5-2	Hexanoic acid	S
A-6-3	Heptanoic acid	S
A-7-1	Octanoic acid	S
A-10-1	Decanoic acid	S

[a]The first, second and third numerals indicate the number of gas chromatographic peaks during the original chromatography and the first and second rechromatography respectively.

[b]Peak size: XL—indicates extra large, 100%, recorder response; L—indicates large, 76—100%, recorder response; M—indicates medium, 33—75%, recorder response; S—indicates small, 0—32%, recorder response.

[c]A indicates acidic fraction

[d]Tentatively identified

fried flavor. It is mostly produced from the oxidation of the oil under the conditions of deep-fat frying (Krishnamurthy and Chang, 1967). Phenylacetaldehyde was found to have a very strong, pleasant odor. It could be produced from phenylalanine in the potato by Strecker degradation, during deep-fat frying. Furfuraldehyde was found to have a sweet, baked food aroma, and furyl methyl ketone had a potato-like aroma, at low concentrations. These two compounds may be formed from reducing sugars in potatoes under the conditions of deep fat frying.

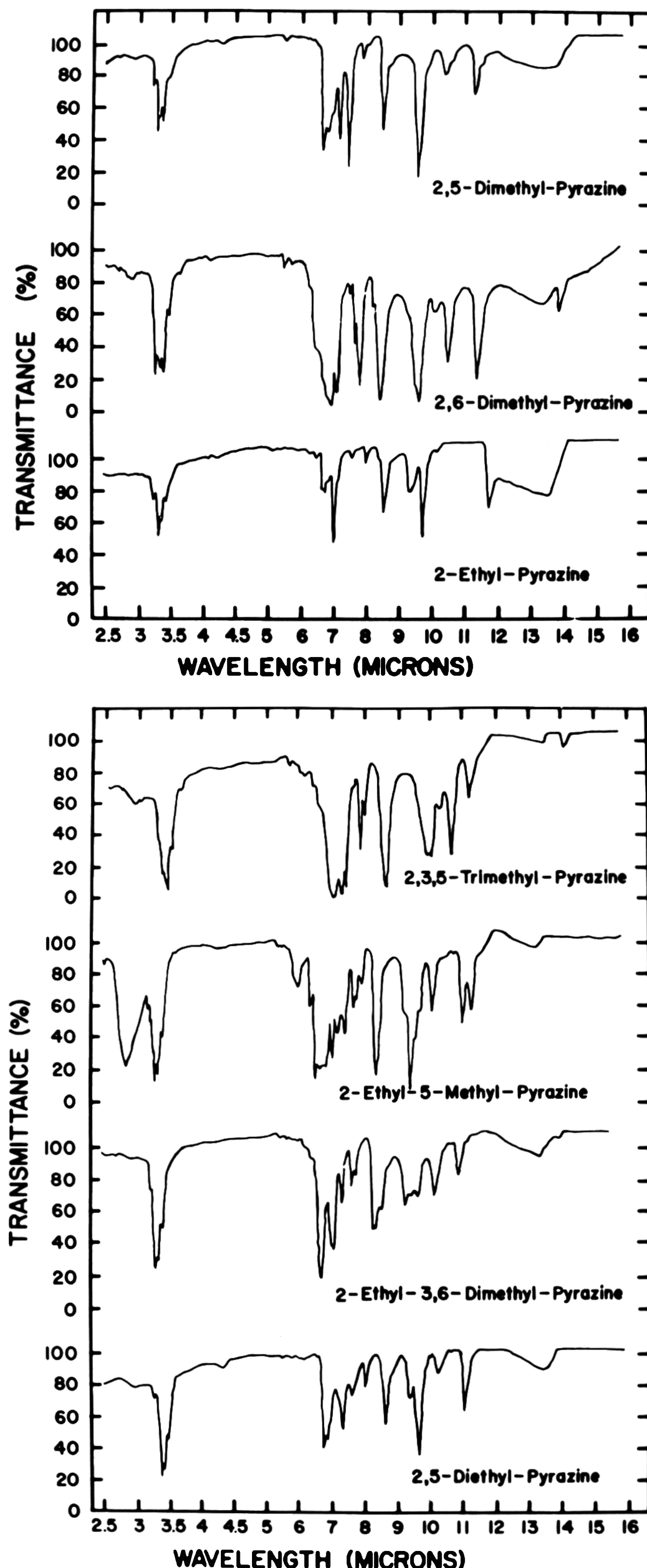

Fig. 2–Infrared spectra of the identified alkyl-substituted pyrazines.

Alcohol and phenol

No alcohol, except ethanol, was identified, even though other alcohols were expected to originate, both from the frying oil (Krishnamurthy and Chang, 1967), and from potatoes (Schormueller and Weder, 1966). A single phenolic compound, 2,6-di-tert-butyl-4-hydroxytoluene, was found. It was the common antioxidant, BHT, and was probably an additive in the frying oil.

Ether and esters

The only ether identified, dephenyl ether, had a camphorous odor. Among the three esters identified, the extra large peak of ethyl acetate was known to be an artifact from the ethyl ether used for the extraction of the volatile compounds.

Acidic compounds

Eight lower fatty acids were identified. They were all present as small gas chromatographic peaks, except that of butanoic acid, which was extra large in size. The straight chain fatty acids might be produced from the frying oil (Kawada et al., 1967; Yasuda et al., 1968). However, the presence of branch-chain fatty acids, and the unusually large amount of butanoic acid, indicated that amino acids are probably also a source of the free fatty acids identified.

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AN IMPROVED METHOD FOR THE DISCRIMINATION BETWEEN BIOGENIC AND SYNTHETIC ACETIC ACID WITH A LIQUID SCINTILLATION COUNTER

INTRODUCTION

DEVELOPMENTS in chemical and food technology have brought about extensive utilization of synthetic materials for the production of improved or reasonably priced foods. However, scientists at present are concerned not only with trying to help the normal development of industry, but also with checking on unsafe or unfair applications of scientific advancements.

In Japan it is not unlawful for a vinegar manufacturer or an industrial vinegar user to utilize some chemically prepared acetic acid as a substitute for acetic fermentation products. However, people generally appreciate 'natural' foods and dislike unfair representation. Rules for representation of vinegars have been enforced here (Fair Trade Commission, 1970), and we understand the situation is similar in many countries. The need for a scientific method to discriminate between a synthetic and biological material in a given condiment, is thus established.

It is well known that carbon-14 measurement of some biological materials has been useful for dating archeological samples. After a series of large scale nuclear experiments, the same method was applied for some geophysical studies (Broecker and Olson, 1960). For the latter, ethanol from wines (L'Orange and Zimen, 1968), from wines and spirits (Baxter and Walton, 1971), ethanol and essential oil (Kashida, 1970) have been used, because the radiocarbon content of the atmospheric CO_2 can be easily, though indirectly, estimated by determining radioactivities of these materials in a liquid scintillation counter.

Faltings (1952) was the first to demonstrate that natural acetic acid as well as ethanol has a measurable radioactivity, and suggested that this can be a useful criterion for the differentiation of biogenic from chemcial materials. Simon et al. (1968) determined carbon-14 in ethanol and acetate of the different origins in a liquid scintillation counter and reported the availability of the method for the checking of vinegars. Mecca and Vicario (1969) and Mecca (1969) briefly described similar work on some vinegar samples.

We also estimated the radiocarbon contents of acetate preparations from a variety of genuine vinegars and confirmed that the difference among them was very small (Masai et al., 1973). Some methodological improvements lead us to a conclusion that the method is sufficiently reliable for the practical inspection of commercial vinegars.

EXPERIMENTAL

Materials

Preparations guaranteed as a chemical reagent or as a food additive were used as synthetic acetic acid without any pretreatments. Those of biogenic acetic acid were prepared from authentic fermentation vinegars obtained from a cooperating source.

Carbon-14-toluene for the internal standard was purchased from Hakuto Co., Tokyo. This and the products of New England Nuclear Corp. (Mass.) and Packard Instruments Co., Inc. (Ill.) had consistent activities with a ± 2.2% error in our assay.

Isolation of acetic acid

The method of Simon et al. (1968), which was employed also by Mecca (1969), was modified to give a preparation containing less moisture. A 1,800-ml sample in most experiments, containing about 4% acetic acid, was distilled in a rotary evaporator with the aid of a −25°C coolant. Almost all distillate was pooled, with the first and last small fractions (20–30 ml each) being discarded. Approximately 75g of calcium carbonate was slowly added to the distillate. The mixture was boiled for 10 min, and the remaining carbonate removed by filtration. Calcium acetate in the solution was recovered as a hard block by concentration in a rotary evaporator followed by heating in a porcelain dish on a boiling water bath. It was finely ground in a mortar and dried in vacuo at 55°C for at least 20 hr. The white powder was then carefully mixed with three times as much warm (solubilized) pyrophosphoric acid. The mixture was placed in a distillation flask connected to a series of two traps, and preheated at 80°C for 30 min. For a short time after the vacuum pump was turned on, the distillate was passed through the first trap which was still left at room temperature. The trap was then placed in a dry ice-acetone bath and the distillate collected there, while the flask was heated at 100°C. For this step the pump had to work under excellent conditions and was protected from acids by keeping the second trap in dry ice-acetone. At the end of distillation, air was let in through the second trap, where the moisture was removed and the crystalline acetic acid was slowly warmed up before the apparatus was

Table 1—Radioactivity of some acetic acid preparations from genuine vinegars

Experiment in	Acetic acid from vinegar[a] No.	Made in	Counting efficiency (%)	Dpm per g carbon
Jan. 1972	12	Aug. 1970	60.6	19.8
	20	Aug. 1971	60.0	20.8
	22	Apr. 1970	59.4	20.3
	25	June 1971	60.4	20.7
	26	Sept. 1971	60.8	19.8
Mar. 1972	25	(see above)	58.6	20.8
	27	Oct. 1971	59.0	20.4
	29	Jan. 1972	58.5	20.7
	30	Dec. 1971	58.8	20.8
	31	Feb. 1972	59.4	19.9
Mar. 1972	26	(see above)	59.9	19.8
	30	(see above)	59.8	20.1
	31	(see above)	59.0	20.1

[a] Manufactured in Aichi Prefecture from ethanol and sake-cake.

Table 2–Radioactivity of biogenic ethanol

Sample	cpm	Counting efficiency (%)	Dpm per g carbon
Biogenic ethanol[a]	123.5	76.8	21.0
Biogenic ethanol[b]	124.2	78.9	20.6
Synthetic ethanol[c]	56.8	78.2	0

[a] Extra-pure reagent from Wako Pure Chemical Industries, Osaka

[b] Made from molasses; a gift from Chiba Alcohol Factory, Ministry of International Trade and Industries, Chiba

[c] A gift from Japan Synthetic Alcohol Co., Tokyo

Table 3–Effect of contaminants on the counting effciency[a]

Additions		Counting efficiency	
		Found (%)	% of control
None		61.26	100
Water	1% (v/v)	61.86	101.0
	2% (v/v)	62.20	101.5
	3% (v/v)	62.64	102.3
	6% (v/v)	64.48	105.3
	9% (v/v)	65.01	106.1
None		58.13	100
		61.18*	100*
Formic acid	1% (v/v)	58.64	99.2
	5% (v/v)	54.87	92.8
		56.68*	92.6*
	10% (v/v)	51.99	87.9
Propionic acid	1% (v/v)	57.68	97.6
	5% (v/v)	56.51	95.6
		60.37*	98.7*
Acetic anhydride	1% (v/v)	56.60	95.7
	5% (v/v)	50.44	85.3
		55.97*	91.5*
	10% (v/v)	44.55	75.3
Hydrochloric acid	10^{-3} N	58.97	99.7
	3×10^{-3} N	56.87	96.2
	10^{-2} N	54.87	92.8
		60.79*	99.4*
	3×10^{-2} N	45.58	77.1

[a] Synthetic acetic acid containing a given substance was counted and efficiency determined as in the standard experiments, in 2–5 replicates. Figures followed by an asterisk (*) were obtained with the use of twice as much PPO and POPOP.

taken apart. The acetic acid thus obtained, 30–40g, was pure enough for the present work.

Radioactivity determination

A liquid scintillation DPM-100 system (Beckman Instruments, Inc., Calif.) was used unless otherwise mentioned. Low-potassium glass vials were purchased from Hakuto Co. Tin reflectors generally used in their caps were covered with aluminum foil before use, for protection from corrosion.

A set of two vials was used for each preparation: one for ordinary counting and the other for the estimation of counting efficiency. Exactly 10 ml of acetic acid preparation and 5 ml of scintillator containing 8g 2,5-diphenyloxazole (PPO) and 0.2g of bis[2-(5-phenyloxazolyl)]benzene (POPOP) per liter of toluene were placed in every vial. Another 5 ml of either inactive toluene or radioactive toluene containing 2,000–3,000 dpm per ml were added, so that every vial contained 20 ml of the mixture.

A tritium channel window was utilized, because a carbon-14 window gave negligible counts from radiocarbon under the present condition. Counting was carried out for 50 min for a sample vial and up to a total of 1.6×10^4 counts for a standard vial, respectively. This cycle of counting was repeated at least four times.

In all experiments control synthetic and biogenic preparations were run in parallel; counts found with the former will be regarded as "background" in this paper.

The radioactivity of ethanol was determined similarly. However, a tritium radiocarbon channel window was used and the efficiency of counting was determined by re-counting after the normal counting and supplementing 0.5 ml of standard labeled toluene.

RESULTS & DISCUSSION

Carbon-14 content of biogenic acetate

Our preliminary work started in 1970 (Masai et al., 1973) suggested that the variation in carbon-14 content of biogenic acetate was much smaller than expected from earlier reports, although a few significantly higher values were found in our early experiments.

Table 1 summarizes data on the amount of carbon-14 in various biogenic acetate samples obtained after standardization of the method. The average and standard deviation were 20.32 and ± 0.28 dpm/gC, respectively.

Carbon-14 content of fermentation ethanol obtained from various sources is shown in Table 2. It is in good agreement with our data on acetic acid.

Experimental errors

The small deviation mentioned earlier stimulated us to attempt to make a quantitative analysis of a mixture of biogenic and chemical acetate. Before this attempt, it was necessary to consider how to minimize experimental errors.

Statistical errors. In this case, the statistical error in "counts" is not negligible, as a limited activity has to be observed.

Since a nuclear decay occurs according to the Poisson distribution, the observed cpm have a standard deviation of $\pm \sqrt{\text{cpm}/(\text{time in min spent for counting})}$. From this, a relative standard error of about ± 1.1% in the computed dpm was calculated, when one and five vials were used for fermentation and synthetic preparations, respectively, in our standard method.

Pipetting error. In this type of experiment an important human error is derived from pipetting, especially when small pipettes are used. A complicating secondary effect of this error is that the efficiency of counting is dependent upon the ratio of acetic acid to toluene, as described later. For these reasons we used larger pipettes and two vials per sample.

Purity of acetic acid preparation. Unlike most chemical experiments, the important conditions for a good preparation in this case are: (1) to contain the theoretical amount of carbon; (2) to be free from other radioactive elements; and (3) not to be contaminated with substances which appreciably cut-down the counting efficiency. Other radioactive atoms are eliminated during acetic acid isolation, except for natural tritium which gives less than 0.1 cpm under our conditions. The presence of materials which interfere with the efficiency will be found automatically in our standard procedure. Therefore, only condition (1) will be considered here.

We were afraid that contamination of water might be a difficult problem; however, it turned out to be groundless. First, acetic acid prepared by our method contained at most 1% water. Secondly, water contamination was easily detectable. Acetic acid containing more than a few percent of water produced momentary turbidity when mixed with toluene; more than 10% (v/v) of water in a preparation prevented the formation of a homogeneous 1:1 mixture with toluene.

Another simple test was to put a few

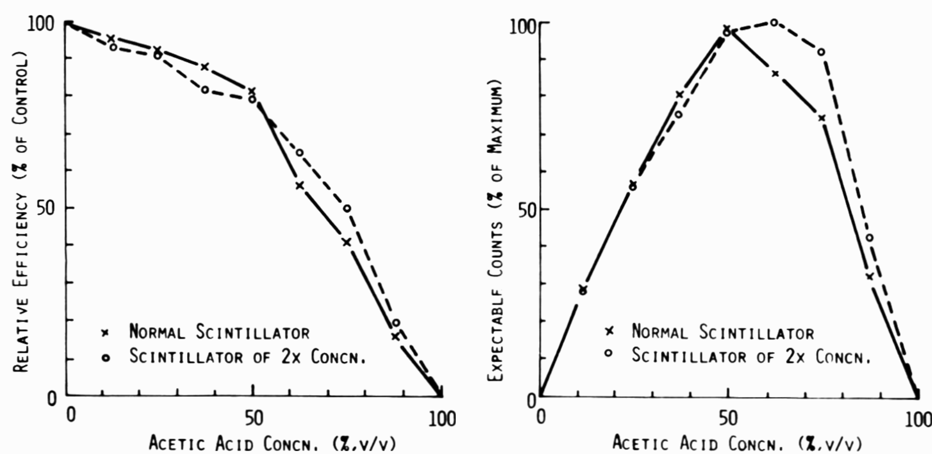

Fig. 1–Effect of acetic acid concentration on counting efficiency

mg of crystalline $CoCl_2 \cdot 6H_2O$ into a few ml of the preparation. When pure acetic acid (guaranteed grade, Wako Pure Chemical Industries, Osaka) was used, the pinkish-red color changed to violet within 1 min, and the solution became blue very slowly. In the presence of 0.2% or more water, the solution was almost colorless after 24 hr. When 4% water was present, the color of the crystals changed very slowly. Of course, determination of acidity, specific gravity, etc. will also be helpful. Finally, data given in Table 3 show that contamination with water increases the efficiency of counting. This is important, because it means that the effect of the contamination would be amplified in computing carbon-14 content of acetate in a careless experiment.

Acetic acid isolated by our method might be easily contaminated with some other volatile acids. But, their effects are limited or can be avoided. For example, a preparation containing 10% (v/v) formic acid, gives a 12% lower counting efficiency (Table 3), contains 2.4% less carbon and has 5.3% more acidity, when compared with a pure sample. Therefore, if one is careful, an effective amount of this contaminant could hardly be overlooked. The effect of propionic acid is even less important. Vinegars do not contain much of these anyway (Kodama and Maekawa, 1967).

Contamination with some neutral substances can be a more difficult problem. Our experiments suggested that over-heating (above 100°C) of calcium acetate with pyrophosphate produces some unknown substance(s), which is not or less acidic and decreases counts. In all our experience, however, there was no practical problem if only the standard procedures were followed.

The contamination of HCl, if any, is easily detected by the reaction with $AgNO_3$, and a preparation containing only 3×10^{-3} N of it produces a yellowish color when mixed with the scintillation cocktail.

Table 3 suggests that the efficiency is influenced by various impurities and can serve as a sensitive criterion for the purity of a preparation. Accordingly, it is recommended that the efficiency be determined with authentic acetic acid for comparison in all experiments. In one of ours, where four synthetic, five biogenic and 12 unknown acetate samples were compared, the efficiency in the presence of one of these groups was found to average 60.26, 60.13 and 60.25%, respectively.

Method of radiocarbon determination. Figure 1 represents the relationship between the acetic acid concentration in the counting mixture and the efficiency of counting with a wide open window. Expected counts (efficiency × amount of acetate) as a function of acetate concentration is also given, which suggests where the most sensitive counting would be possible.

It is fortunate that nearly maximal counts are expected with a 1:1 mixture of acetic acid and the scintillator, since we can prepare all counting mixtures without using any graduated pipettes.

Incidentally, the efficiency in the absence of acetate was 90–95%.

Some effects of concentration of PPO and POPOP are shown in Figure 1 and also in Table 3, indicating that either PPO or POPOP concentration is the limiting factor for counting efficiency, at least in some of our experiments. Data shown in Table 3 suggest that higher concentrations should be used to enhance efficiency, to improve the resistance to some of the impurities, and, hence, to obtain more reproducible results. However, this also means that the higher concentrations make it more difficult to detect some of the contaminants. Careful considerations should be paid to these factors, although a general suggestion may be that a beginner use lower concentrations.

Dioxane scintillation cocktail was useless in these experiments, because of the poor efficiency with it. A liquid scintillation counter of a different model gave essentially the same results: LSC-601 of Japan Radiation and Medical Electronics, Inc., Tokyo, Unilux II-A of Nuclear-Chicago Corp., Ill., and our DPM-100 registered consistent radiocarbon contents with the same series of vials with approximately ± 1.8% deviation.

As a summary, an actual example of our errors within a single experiment is presented in Table 4. In an earlier experiment, where six acetate preparations were made from the same fermentation liquor, the carbon-14 contents obtained for the individual preparations had a standard deviation of about ± 2.1% compared with the overall average.

Repeatability. Since this type of experiment need not be done in many laboratories, so-called repeatability or consistency of data obtained in different experiments carried out in the same laboratory, appears to be most important.

Table 1 includes data from some completely independent experiments on the same vinegar samples. They show a standard experimental error of about ± 1.2% of the average.

Because a commercial "standard" is not absolutely accurate, we keep our own standard toluene of appropriate radioactivity, so that a direct and accurate

Table 4–Experimental errors within a single experiment

	Average	Std dev (% of average)
Cpm of synthetic acetic acid[a]	28.41	±0.80
Cpm of biogenic acetic acid[b]	80.97	±1.08
Counting efficiency[c]	0.5895	±1.21
Dpm/gC of biogenic acetic acid[b]	21.37	±0.75

[a] Four authentic preparations
[b] Four preparations independently isolated from two genuine vinegars
[c] Data with eight vials

Table 5–Radioactivity of acetic acid in some vinegars[a] on the market

Experiment in	Acetic acid from vinegar manufactured: By	Located in	In	Counting efficiency (%)	Dpm per g carbon
Sept. 1971[b]	A Co.	Kyushu	(bought in June 1971)	59.9[b]	20.5
	B	Kinki	Apr. 1971		21.4
		(Australia)	(imported in Apr. 1971)		21.9
Jan. 1972	B	Kinki	June 1971	60.5	20.5
	C	Kinki	May 1971	60.6	20.0
	C	Kinki	Nov. 1971	60.0	20.2
	D	Kinki	July 1971	59.4	9.0
	E	Kinki	June 1971	60.7	5.0
Mar. 1972	C	Kinki	Dec. 1971	58.0	20.4
	E*	Kinki*	(not shown)*	60.4*	8.5*
	E	Kinki	Aug. 1971	58.7	21.0
	F	Shikoku	July 1971	56.9	19.9
	G*	Kyushu*	Dec. 1971*	61.1*	0*

[a] According to their representations, all vinegar samples should contain no synthetic acetic acid, except that 50% of acetate contained in those samples marked with an asterisk(*) should be nonbiogenic.

[b] In this particular experiment, counting efficiency was estimated from data on five representative vials.

determination of any new standards is made by a simple comparison.

Carbon-14 content of acetate from vinegars on the market

We have made acetic acid preparations from almost 100 vinegar samples purchased in the open market, and observed their radiocarbon contents. Typical examples of results, including some of the earlier ones, are presented in Table 5. They clearly show that acetate preparations from most samples contained enough carbon-14 as expected from the data shown in their formal representations. However, at the same time we found a small number of cases where acetate did not manifest the expected counts, strongly indicating an unfair representation. Another surprising finding is that a considerable amount of "vinegar" containing only the synthetic acetate had been imported for making a secondary product without a slight suspicion.

We found that acetic acid contained in some sauces, catsups and pickles may be examined essentially in the same way, and obtained very similar results as mentioned above (Masai et al., 1973).

CONCLUSIONS

EXTENSIVE STUDIES are available on the fate of carbon-14 created by nuclear experiments. They suggest that atmospheric CO_2 has become more and more homogeneous in its natural radioactivity and, it has been predicted that its radiocarbon content would be decreasing very slowly in these years (e.g., Nydal and Lövseth, 1970). Our data are consistent with this prediction.

Results presented here show that it is possible to estimate the carbon-14 content of acetate in a given vinegar within 1–2% error in a single experiment. Replicate and/or repeated assays naturally give more reliable data. Thus, the ratio of synthetic to total acetate may be determined with a few percent deviation.

Of course, more precise measurements are technically possible, if desired. However, the natural radioactivity of CO_2 is not exactly constant, being influenced by weather, by Suess effect, and so on, and is continuously decreasing. Actually Table 1 indicates that the different vinegars contained biogenic acetate of significantly different radiocarbon content. On the other hand, the ratio of nonbiogenic acetate to the total in a "synthetic vinegar" may be represented only at every 10% level, according to the present rules (Fair Trade Commission, 1970). Thus we may conclude that our method is reliable enough for the practical and routine examination of vinegars.

There is no technical difficulty in this method and no special equipment, except for the counter, is needed. It takes normally a few days for the preparation of acetic acid and a night for the counting. The reliability of the estimated ratio of synthetic to total acetate, however, depends on the quantity and quality of the data on authentic samples accumulated in the laboratory.

Our preliminary tests on lactic and glutamic acids suggested that a liquid scintillation counter is also useful for the determination of their carbon-14 contents, although some chemical pretreatments were necessary.

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RESEARCH NOTES

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A Research Note
RAPID ANALYSIS OF MOISTURE IN MEAT BY REFRACTOMETRY

INTRODUCTION

THE NEED FOR rapid, accurate methods for the determination of fat and moisture in meat and meat products has been documented (Everson et al., 1955). This need is based on the following factors: (1) government regulations control the proximate composition of nearly all meat products; (2) accurate determination of fat and moisture, combined with additive control, permits the determination of protein by difference; (3) proximate composition control of sausage formulation material, prior to extrusion and heat processing, combined with controlled moisture loss during heating, permits accurate control of final product composition; and (4) accurate proximate compositional analysis is a requirement for effective least-cost product formulation.

EXPERIMENTAL

A BAUSCH AND LOMB Abbe Refractometer, equivalent to the Abbe 3L (accuracy ± 0.0001 in refractive index), was used to determine refractive indices of isopropanol-water mixtures. A sodium D light source ($\lambda = 5892.62$ Å in air) is utilized. A standard curve of refractive index vs % water (v/v) was obtained which was linear over the range of % water (v/v) > 50. The method, as applied to meat, is as follows: (1) 20g meat is homogenized (Virtis, maximum speed, 1 min) with 40 ml anhydrous isopropanol; (2) the mixture is filtered and 1 ml is diluted with 1 ml distilled water; (3) mixture equilibrated to 20°C; and (4) the refractive index determined. Water dilution results in readings in the range of the straight-line portion of the standard curve. The % moisture is calculated as follows:

$$\% \text{ moisture} = \frac{100\,(2W - 100)}{100 - W}$$

Where W = % water (by volume) from standard curve and $\leq 66.7\%$.

Temperature control was accomplished by circulating water from a large (5 liter) reservoir through the refractometer and returning it to the reservoir. This system permits rapid adjustment of temperature when determinations are made.

The refractive index method was compared to oven drying (24 hr, 105°C) or toluene distillation for low and high fat products, respectively.

RESULTS & DISCUSSION

VERY GOOD agreement was obtained between refractometry and conventional methods (Table 1). Means for rapid and conventional methods are close and standard deviations of the rapid method approach those for conventional methods. They are within the limits required by quality control programs. The method requires 5–10 min to complete and, therefore, is the most rapid method developed thus far to our knowledge which does not require complex, expensive instrumentation. This method, together with those recently developed by Reineccius and Addis (1973) and Glass and Addis (1971), permits a wide choice of rapid moisture methods from the standpoints of speed (5–30 min), cost and degree of complexity. The method which best combines attributes of speed and simplicity is refractometry.

The determination of moisture in emulsions immediately prior to extrusion and heat processing would appear to be a most attractive application of this method. Where preblending (on the basis of fat content) is practiced, a single rapid determination of moisture on the preextrusion blend, combined with controlled shrink during heat processing, would permit the control of the final product proximate composition. Such an application would also reduce economic loss due to improper control of moisture content. Where preblending is not practiced, fat and moisture analysis at the time of extrusion would be necessary for adequate control of final product proximate composition.

Table 1—Moisture content of meat samples by conventional and refractive index methods

	% Moisture[a]	
Samples	Refractive index	Conventional
Emulsion	54.24 ± 0.20	54.28 ± 0.12[b]
Wieners	50.54 ± 0.23	50.52 ± 0.10[b]
Cheek meat	76.25 ± 0.17	76.44 ± 0.05[b]
Bull meat	73.58 ± 0.38	73.42 ± 0.28[c]
Turkey (light)	73.61 ± 0.42	73.80 ± 0.17[c]
Turkey (dark)	76.67 ± 0.40	76.73 ± 0.32[c]

[a] Mean % H_2O ± standard deviation (for six determinations)
[b] Toluene distillation
[c] Oven drying at 105°C for 24 hr

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A Research Note
RAPID ANALYSIS OF MOISTURE IN MEAT BY GAS-LIQUID CHROMATOGRAPHY

INTRODUCTION

ACCURATE MONITORING of fat and moisture in meat and meat products during the various phases of processing is an essential factor in quality control, insures compliance with governmental regulations and permits effective least-cost formulation. Furthermore, accurate determination of fat and moisture, combined with precise control of additives, permits the determination of protein by difference. Labuza et al. (1969) demonstrated that alcohol:water could be quantitated by gas-liquid chromatography (GLC). Glass and Addis (1971) devised a method for rapid (30 min) moisture analysis in meat and meat products, using the saponification reaction, which commenced with methanolic extraction of water from meat. Therefore, the present study was conducted to investigate the adaption of GLC to the problem of rapid water analysis of meat and to attempt to achieve a reduction of the time required by the saponification method of Glass and Addis (1971).

EXPERIMENTAL

PRIOR TO ANALYSIS, all meat samples were finely ground three times. A sample of ground meat (≤2.0g) was blended with 20 ml anhydrous methanol. The sample was covered, allowed to settle briefly, and 1 ml of the clear portion was decanted into a 2 ml serum vial for analysis. The moisture content of methanol was determined using GLC (Hewlett-Packard 7600A system). This system consists of a thermal conductivity gas chromatograph (Model 7620A), automatic sampler (Model 7670A), and an electronic integrator (Model 3370B). The methanolic meat extract (2 μl injection) was separated into water and methanol using a 1m × 0.32 cm OD stainless steel column packed with Poropak Q (Waters Assoc., Framingham, Mass.) operated isothermally at 110°C and helium carrier flow of 35 ml per min. The injection port and detector temperatures were 200°C and 225°C, respectively. A standard curve relating the ratio of methanol peak area to water peak area was prepared by adding water (0.1 ml increments up to 2 ml) to 20 ml of anhydrous methanol. The amount of water present in methanolic meat extracts could be read from a standard curve.

RESULTS & DISCUSSION

A COMPARISON of results obtained (Table 1) demonstrates good correlation between the GLC method and conventional methods for analyzing meat and meat product moisture. Only three of the 10 samples analyzed differed from the conventional method by more than 1%. When the same extract was analyzed repeatedly by the GLC method, the values obtained were similar to the degree that the standard curve could be read. Therefore, the variation that appeared within and between methods (Table 2) was probably due to sampling errors and differences in the efficiency of the methanolic extraction. Samples differed in the ease of blending with methanol and, therefore, extraction of the water may not have been uniform. The GLC method had a larger standard deviation than the oven method but smaller than the toluene distillation.

Table 1—Moisture content of meat samples obtained by conventional and GLC methods

Samples	% Moisture[a] GLC	Conventional
Fat	6.9	6.6[b]
Pork jowl	27.2	27.1[b]
Pork trim	36.6	37.2[b]
Navels	38.2	38.0[b]
Salami	40.5	39.3[b]
Emulsion, frankfurter[d]	51.1	51.0[b]
Cow meat	55.4	54.9[c]
Bull meat	69.2	70.6[c]
Cheek meat	71.4	69.9[c]
Turkey	75.9	76.3[c]

[a]Mean of five determinations
[b]Toluene distillation
[c]Oven drying at 105°C for 24 hr
[d]Emulsion obtained immediately prior to extrusion

Table 2—Comparison of means and standard deviations between conventional and GLC methods

Samples	% Moisture[a] GLC	Conventional
Pork trim	37.0 ± 0.2	37.1 ± 0.2[c]
Emulsion[b]	51.0 ± 0.1	51.0 ± 0.1[c]
Bull meat	67.3 ± 0.5	66.0 ± 0.1[d]
Cheek meat	71.4 ± 0.3	69.9 ± 0.2[d]

[a]Mean % H_2O (six determinations) ± standard deviations
[b]Emulsion obtained immediately prior to extrusion
[c]Toluene distillation
[d]Oven drying at 105°C for 24 hr

Therefore, it appears that gas chromatography may be used for rapid analysis of moisture in meats and is nearly as accurate as the standard procedures. The time required for analysis of meat (10 min sample) makes the method valuable for situations where time is critical and represents a reduction to one-third in the time required by the saponification method of Glass and Addis (1971). The method compares very favorably to the toluene distillation procedure for products possessing high fat contents. The GLC method will be useful to laboratories now possessing the required equipment. The method, as applied to quality control of sausage manufacture, would be extremely useful in instances where pre-blending (on the basis of fat content, several hours prior to manufacture) is practiced. Thus, a single, rapid determination of moisture on the pre-extrusion emulsion, combined with controlled shrink during heat processing, permits complete control of the proximate composition of the final product.

Addis and Chudgar (1973) have reported that refractometry of alcoholic extracts of water from meat is a method of equivalent speed and accuracy to the GLC method. It is significant that both the GLC and refractive index methods proved to be extremely effective at moisture analysis of pre-extrusion sausage emulsion. Comparing rapid with conventional mean values for moisture demonstrated agreement within ± 0.07% for both methods. Therefore, these methods permit absolute control of moisture in the manufacture of emulsified meat products.

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A Research Note
MODIFICATION OF COCKTAIL SHRIMP TEXTURE

INTRODUCTION

FIN FISH MUSCLES contain myofibrillar, sarcoplasmic and connective tissue proteins in the order of 65–75%, 20–30% and 2–10%, respectively, of the total proteins present (Connell, 1964). Textural deterioration during frozen uncooked storage of fin fish muscles is the result of reactions involving myofibrillar proteins. Aggregation and intermolecular bonding reactions of the myofibrillar proteins occurred upon storage of fin fish muscles at freezing temperatures (Connell, 1964; Love, 1968). These reactions resulted in toughing of the muscles. Cocktail shrimp toughens considerably during refrigerated storage (Ahmed et al., 1972). Softening of the toughened cod fish muscles was accomplished by treatment of fish fillets with polyphosphate solution (Love, 1968). Cooking the white muscle of Sacramento blackfish in boiling water for 20 min resulted in greater denaturation, crystallization, and cross-linking of myofibrillar proteins than freezing the same muscle (Chu and Sterling, 1970; Mao and Sterling, 1970a, b). These changes in the protein were enhanced upon storage.

The objectives of the present study were to: (1) determine the effect of fungal protease and sodium tripolyphosphate (polyphosphate) on the texture of cocktail shrimp; and (2) examine the effect of subsequent storage on the treated cocktail shrimp.

EXPERIMENTAL

Sample preparation

Peeled and deveined frozen shrimp (130–150 count) imported from India were stored at −29°C until used. The shrimp were thawed for 72 hr at 1°C prior to treatments.

Fungal protease (Protease G, Enzymes Development Corp., N.Y.) was dissolved in water to a concentration of 0.005% (w/v). A solution containing 5.0% sodium tripolyphosphate and 2.4% sodium chloride (w/v) was used for the polyphosphate dip. Distilled water was also used as a dip treatment. 1200g of the thawed shrimp were immersed in 12 liters of the dip solution for periods up to 60 min at temperatures of 25° or 65°C. The treated shrimp were cooked by immersion in 12 liters of boiling water for 2 min. The cooked shrimp were spread one layer thick and air cooled at 1°C. 20g of the cooled-cooked shrimp were placed in 160 ml Whirl-Pak bags (Nasco) and covered with 50 ml of cocktail sauce. The cocktail sauce used was a commercial preparation containing 35% soluble solids and with a pH of 3.90. Control samples were prepared in an identical manner without any dip treatment prior to cooking and packaging. Control and treated cocktail samples were stored at 1°C for periods up to 6 wk.

Texture measurement

The Instron Universal Testing Instrument Model TM equipped with the compression load cell Model CB was used to shear individual cooked cocktail shrimp muscles. Sample presentation to the instrument and shear measurements were similar to those used by Ahmed et al. (1972). Maximum shear force (g) was used to indicate the degree of toughness of shrimp muscle. Results are expressed as the average of 60 determinations per treatment.

A panel of 10 judges was selected for the organoleptic evaluation of cooked shrimp muscles. Each judge was presented with four shrimp muscles from each replicate. The judges were requested to chew the muscles and rate their responses for the texture on a scale ranging from 1 for extremely tender to 9 for extremely tough. There were three replicates per treatment and results are expressed as the average of 30 judgements per treatment.

RESULTS & DISCUSSION

COOKED SHRIMP MUSCLES which were soaked for 1 hr in either water or polyphosphate required larger forces to shear when the solution temperature was kept at 65°C than at 25°C (Fig. 1). It is probable that a temperature of 65°C is higher than the *'shrink temperature'* of shrimp muscle. Shrinkage of shrimp muscles may result in their toughening. Shrinkage and gelatinization temperature of fin fish skin and swim-bladder collagens are lower than mammalian skin collagens and presumably the same is true of the respective muscle collagens (Connell, 1964). Soaking shrimp muscles in either water or polyphosphate prior to cooking resulted in their tenderization in comparison to the no soak treatment (Fig. 1). The smallest shear force value was ob-

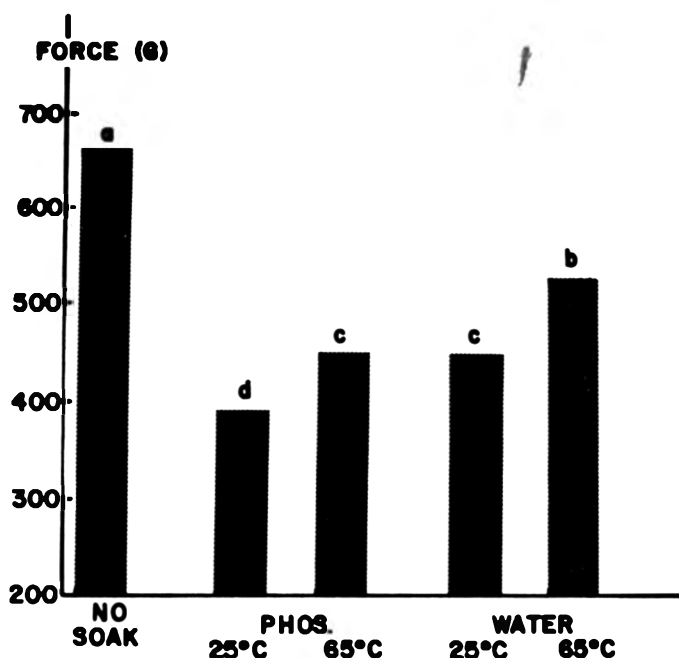

Fig. 1—Shear forces (g) of cooked shrimp muscles as influenced by the temperature of polyphosphate and water dip treatment for 60 min. Shrimp were stored for 6 wk at 1°C. Letters denote statistical significance at the 95% confidence level.

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Table 1–Instrumental and sensory texture measurements of cooked cocktail shrimp as influenced by water, Protease G[a] and polyphosphate[b] dip times at 25°C

Treatment	Dip time (min)	Storage (wk @ 1°C) 0	2	4	6	Mean
		Shear force (g)				
Water	60	553	865	874	971	816a
Protease G	15	502	542	543	862	612b
Protease G	30	457	397	345	450	412c
Polyphosphate	60	421	395	470	620	477c
Mean[d]		483y	550y	558y	726z	
		Sensory rating[c]				
Water	60	5.6	4.4	4.7	5.3	5.0a
Protease G	15	5.1	4.3	5.7	6.0	5.3a
Protease G	30	4.2	4.6	4.6	5.4	4.7b
Polyphosphate	60	2.1	2.6	2.9	2.0	2.4c
Mean[d]		4.2z	4.0z	4.5z	4.7z	

[a]0.005%
[b]5.0% polyphosphate and 2.4% sodium chloride
[c]Rating–1 = extremely tender; 9 = extremely tough.
[d]Means, within each group, followed by the same letter are not statistically different at the 95% confidence level.

tained using the polyphosphate treatment at 25°C. The tenderizing effect of polyphosphate on shrimp muscle was similar to that found with cod muscles (Love, 1968). It has been suggested that polyphosphate results in the weakening of cod muscle fiber structure and the swelling of its protein gel systems, thus increasing their water holding capacity. Possible similar mechanisms may also occur in the polyphosphate-treated shrimp muscle.

Lower shear force values were evident after treatment at 25°C with either Protease G or polyphosphate in comparison with the water dip (Table 1). Cooked shrimp which was treated with Protease G for 30 min exhibited similar degrees of shear resistance to those treated with polyphosphate. Cocktail shrimp stored for 6 wk required larger forces to shear than other storage treatments. However, the water soak treatment exhibited increased shear values after 2 wk of storage which was maintained throughout the remainder of the storage period. Sensory texture evaluation of the cooked shrimp indicated that the polyphosphate treated samples were more tender than other soaking treatments (Table 1). No differences were noted in the degree of toughness of cocktail shrimp treated with either water soak for 60 min or Protease G soak for 15 min. Shrimp muscles dipped for 30 min in the enzyme solution prior to cooking were more tender than the enzyme treatment for 15 min. No differences were found in the degree of toughness of the cooked shrimp due to storage treatments. Panelists described the texture of the polyphosphate treated shrimp as juicy or similar to fresh shrimp and that for the enzyme treated shrimp as mealy or crumbly. More chewing action was needed to masticate the enzyme treated shrimp than the polyphosphate treatment. It seems that factors contributing to increased juiciness of cocktail shrimp will favor a more organoleptically acceptable product.

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A Research Note
CONTINUOUS CONVERSION OF STARCH TO GLUCOSE BY AN AMYLOGLUCOSIDASE-RESIN COMPLEX

INTRODUCTION

ENZYMES CAN BE immobilized by binding to a water insoluble support such as an ion exchange resin, cellulose, charcoal, clay and other biopolymers. Thus, a continuous enzyme reaction with large amounts of substrate can be performed (Silman and Katchalski, 1966). A possible industrial application of an insolubilized enzyme is the use of amyloglucosidase for continuous conversion of starch into glucose. Wilson and Lilly (1969) reported that amyloglucosidase was chemically attached to DEAE-cellulose using the bifunctional reagent, 2-amino-4,6- dichloro-s-triazine, and that continuous conversion of maltose and dextrins into glucose was possible with enzyme-DEAE cellulose complex. Bachler et al. (1970) reported that amyloglucosidase was bound to DEAE-cellulose through an ionic bond, and the enzyme complex was used for continuous conversion of starch into glucose. Usami and Shirasaki (1970) reported that amyloglucosidase can also be adsorbed on acid clay and active charcoal.

Columns packed with DEAE-enzyme complex have the disadvantage that the DEAE-cellulose becomes compacted making it difficult to maintain a constant flow rate. We attempted to find a solution to this problem, examining various ion exchange resins in bead form; Amberlite IR-45 (OH) resin was found to bind a significant amount of enzyme. Further, the amyloglucosidase resin complex had fully demonstrated enzymatic activity.

This paper presents a method for the insolubilization of amyloglucosidase on Amberlite IR-45 (OH) resin for the continuous conversion of starch solution into glucose.

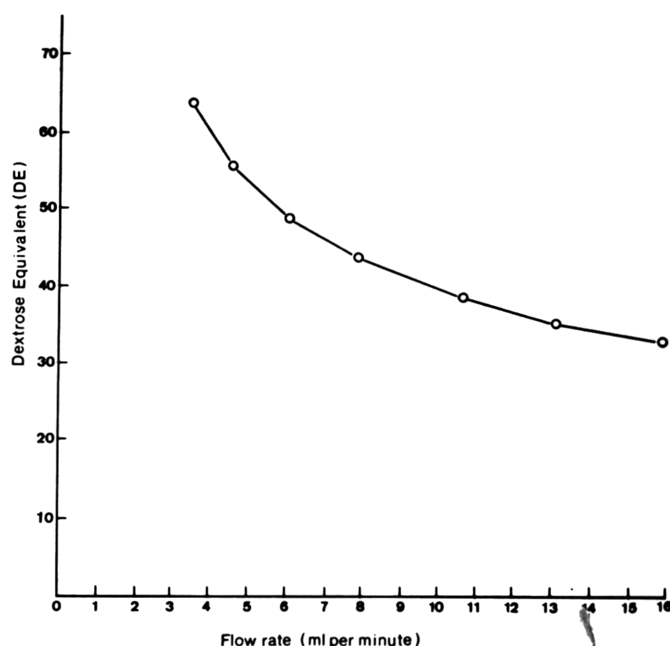

Fig. 1—Saccharification of 25 DE corn syrup plotted against various flow rates. An asymptotic relation is observed between extent of saccharification and flow rate.

MATERIALS & METHODS

Production of amyloglucosidase

Amyloglucosidase concentrate was obtained by ethyl alcohol precipitation of the enzyme from submerged fermentation filtrate of Aspergillus niger NRRL 3122.

Insolubilization of amyloglucosidase

Amberlite IR-45 (OH) resin 14-52 mesh, an anion exchanger, was used as an insoluble carrier for the enzyme. 100g of resin was washed by stirring with acetone and then with water. Following the water wash the resin was stirred with about three resin volumes of 1N NaOH, then washed several times with distilled water and finally with 1N HCl. After again washing the resin with distilled water, it was equilibrated with acetate buffer (0.05M, pH 4.0) by suspending it several times in about three resin volumes of buffer. The activated resin was then mixed with an enzyme solution prepared by dissolving 0.5g of amyloglucosidase concentrate in 200 ml of acetate buffer (0.05M, pH 4.0) and centrifuging to remove insoluble material. After agitating the resin and enzyme mixture for 30 min, the resin-enzyme complex was washed with buffer to remove unbound enzyme.

Preparation of the enzyme column

A jacketed column of a Bechman fraction collector with temperature control (Model 133 A), was packed with 2 x 5 cm of the resin-enzyme complex and the temperature of the column was kept at 45°C.

Preparation of substrate

Liquefied cassava starch solution and low DE (Dextrose Equivalent) corn syrup, were used as substrates. The liquefied cassava starch solution was prepared by treating a 20% suspension of cassava starch in water (w/v), with bacterial alpha-amylase at 80°C (pH 6.0) for 30 min. The liquefied starch solution was then boiled for 10 min to inactivate the alpha-amylase. The liquefied cassava starch solution had a DE of 10 and was adjusted to 20° Brix (pH 5.0) with 1N hydrochloric acid. A 25 DE of corn syrup was obtained from A. E. Staley Manufacturing Co., USA, and was also adjusted to 20° Brix (pH 5.0).

Determination of reducing substance

Reducing substance was determined by the method described by Somogyi (1945).

Amyloglucosidase assays

Amyloglucosidase activity was determined by the method of Miles Laboratory (Technical Bulletin, 5-125). One amyloglucosidase unit is the amount of enzyme required to form 1g of glucose from starch in 1 hr at 60°C.

Determination of dextrose equivalent (DE)

The extent of saccharification achieved is expressed as dextrose equivalent (DE). Total reducing substances (as dextrose) were calculated as percentage of total dry substance.

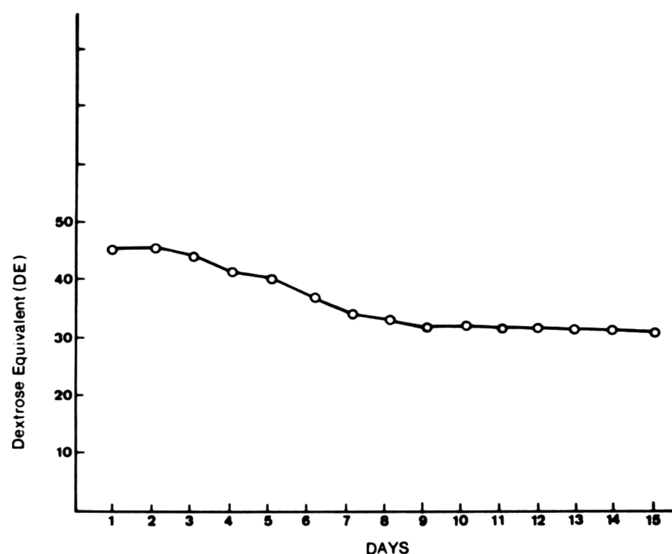

Fig. 2—Stability of amyloglucosidase-resin complex in column. The 10 DE of the liquefied cassava starch solution (20° Brix, pH 5.0) was continuously fed to the enzyme column 8 hr per day for 15 days. Flow rate was 3.5 ml per min, and temperature was 45° C.

RESULTS & DISCUSSION

THE 25 DE of corn syrup was fed into an enzyme-resin column containing 16 amyloglucosidase units of insolubilized enzyme. The amount of reducing substances in the starch solution was determined before and after it was passed through the enzyme column. The results for various flow rates are shown in Figure 1. An asymptotic relation is observed between the extent of saccharification and the flow rate. The 25 DE corn syrup was converted into 63 DE at a flow rate of 3.5 ml per minute. When liquefied cassava starch solution (10 DE, pH 5.0) was then fed through the enzyme column, the 10 DE liquefied cassava starch solution was converted into 46 DE after passing through the enzyme column at a flow rate of 3.5 ml per minute.

10 DE of liquefied cassava starch solution was then fed to the top of the enzyme column continuously for 8 hr per day for 15 days at a constant flow rate (3.5 ml per min). The constant flow rate was maintained by using a peristaltic pump. Determination of the reducing substances was made daily, and the DE value plotted against time (day). The results are shown in Figure 2. The 10 DE of the liquefied cassava starch solution was converted into 46 DE for at least two days. After 15 days, the 33 DE syrup obtained indicates that approximately 28% of the bound enzymes is lost.

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A Research Note

SOME NEWLY FOUND ORANGE ESSENCE COMPONENTS INCLUDING TRANS-2-PENTENAL

INTRODUCTION

AQUEOUS ORANGE essence has been analyzed qualitatively in several laboratories, and the major components and many of the minor ones have been identified (Schultz et al., 1967, Wolford and Attaway, 1967, and references therein). Recent work at this laboratory on Valencia orange essence oil has suggested that some volatile compounds not found to date in aqueous orange essence should be present in it and, if so, might contribute significantly to the odor and flavor of essence (Coleman and Shaw, 1971). Certain of these compounds have strong odors and are believed important to the odor of essence even if present as minor constituents (Shaw and Coleman, 1971).

The current study was undertaken to try to identify some minor essence components of possible flavor significance and to consider the effect that any newly found orange components might have on essence flavor and aroma. Reported in the present study are: the identification of 37 orange essence constituents including seven new components, and the flavor threshold of one new component (2-pentenal), that had not previously been found in citrus.

EXPERIMENTAL

2-LITER PORTIONS of 100-fold aqueous orange essence (Wolford et al., 1968) were either extracted with methylene chloride by the procedure used for grapefruit essence (Moshonas and Shaw, 1971), or with ethyl chloride by the procedure used by Scanlan et al. (1970) in a study of blackberry essence. The solvent was removed in either case to yield approximately 0.5g of anhydrous essence that was analyzed by gas chromatography (GLC).

GLC analyses were made on an F&M Model 810 gas chromatograph using a thermal conductivity detector and equipped with a 0.20 in. IDx 20 ft aluminum column packed with 20% Carbowax 20M on 60–80 mesh Gas Chrom P. The oven temperature was kept at 85°C for 12 min, then programmed to 220°C at 2°C per min with a helium flow of 50 ml per min. When separation of individual components was not complete, the mixture was collected and rechromatographed on a 0.20 in. IDx 20 ft stainless steel column packed with 10% of the nonpolar liquid phase UCW-98 on 60–80 mesh Gas Chrom P. For all runs, the injection temperature was 250°C and the detector temperature was 280°C.

Mass spectra were obtained with a Bendix Model 3012 (TOF) mass spectrometer, infrared (IR) spectra as oil films or in CS_2 with a Perkin-Elmer Model 137 spectrophotometer, and ultraviolet (UV) spectra in absolute EtOH with a Cary Model 14 spectrophotometer.

Individual components isolated were identified by comparing mass or infrared spectra as well as retention times with those of known compounds. The authentic sample of 2-pentenal was synthesized by the procedure of Hoaglin and Hirsch (1953) to afford a sample with b.p. 27–28°C at 0.4 mm. Just as reported earlier by Thomas and Warburton (1965), we found this distilled sample to be impure, but GLC separation on the Carbowax 20M column yielded in increasing order of retention times: 2-methyl-2-butenal (tiglaldehyde), UV (max) 225 nm (ϵ13,000), mass spectrum indistinguishable from that of 2-pentenal; 2-pentenal UV (max) 218 nm (ϵ12,500), IR (oil film) 2990(m), 2830(w), 2750(w), 1700(s), 1640(w), 1460(w), 1155(m), 1145(m), 1100(m), 1015(w), 980 cm^{-1}(m), m/e 27(86), 29(100), 39(57), 41(70), 55(98), 83(33), 84(70%); and 2-methyl-2-pentenal, UV (max) 227 nm (ϵ15,500).

Table 1—Flavor thresholds of trans-2-pentenal in water in triangular flavor tests.

Conc (ppb)	Correct judgments (48 presentations)	Confidence level (%)[a]
200	38	99%
150	32	95%
100	20	Not significant

[a] As reported by Krum (1955)

Table 2—Newly found orange essence components

1,1-Ethoxymethoxyethane
Ethyl vinyl ketone
trans-2-Pentenal
Heptanol
cis-2,8-p-Menthadiene-1-ol
cis-Carveol
Piperitenone

Flavor threshold

Flavor threshold determinations on trans-2-pentenal were made with the triangular comparison tests discussed by Boggs and Hanson (1949). Twelve trained panelists were given four presentations, two at each sitting, for a total of 48 judgments at each concentration. Samples for tasting were prepared by dissolving 8.2 mg of GLC purified trans-2-pentenal in 30 µl of abs. ethanol and diluting to 100 ml with distilled water. The ethanol aided in dissolving the sample. Aliquots of this solution were taken and diluted to 1 liter with water to achieve the concentrations shown in Table 1. The control solution was prepared each time using aliquots from a 100 ml solution of distilled water containing 30 µl of abs. ethanol.

RESULTS & DISCUSSION

EXTRACTION of orange essence with methylene chloride or ethyl chloride and GLC analysis of the concentrated extract has afforded some 37 identified compounds, seven of which are newly found orange essence components. Table 2 lists these seven newly-found orange essence components in order of their increasing retention times on a Carbowax 20M column.

One newly found orange essence constituent, 1,1-ethoxymethoxyethane, had previously been found at our laboratory in grapefruit essence oil (Coleman et al., 1972) and in tangerine essence (Moshonas and Shaw, 1972). In the present study, 1,1-ethoxymethoxyethane and 1,1-diethoxyethane (acetal) were both identified in a later season Valencia essence but not in a very early season Valencia essence. Finding 1,1-ethoxymethoxyethane in essence only where acetal is a constituent supports an earlier suggestion that the former compound is likely formed from acetal and methanol in the acidic juice medium (Coleman et al., 1971).

Ethyl vinyl ketone had been found earlier in Valencia orange essence oil volatiles (Coleman and Shaw, 1971) but had not been found in any citrus aqueous essences. Its strong acetylene-type odor could contribute significantly to the odor and flavor of essence (Shaw and Coleman, 1971).

Trans-2-pentenal had not previously been found in citrus. Because of its struc-

tural relationship to other α,β-unsaturated aldehydes with low flavor thresholds (Buttery et al., 1971), the flavor threshold of this new citrus component was determined. As seen in Table 1, the flavor threshold was approximately 100–150 ppb. Most tasters noted a fruity or apple-like aroma at these levels and a slightly astringent taste sensation. This flavor threshold value is in keeping with the trend reported by Buttery et al. (1971) for odor potency of α,β-unsaturated aldehydes to become progressively weaker in going from 2-nonenal (0.08) to 2-octenal (3) to 2-heptenal (13) to 2-hexenal (17ppb). Approximately 750 ppb of 2-pentenal was present in the aqueous orange essence, as estimated by integrating peak areas under the GLC curve.

The synthetic sample of trans-2--pentenal was prepared by the procedure of Hoaglin and Hirsh (1953). The product mixture was found to contain 2-methyl-2-butenal and 2-methyl-2-pentenal in addition to the desired 2-pentenal, and thus required GLC separation for adequate purification for flavor evaluation work. Assignment of the trans-configuration to the 2-pentenal isolated in this study is based on its IR absorption at 980 cm^{-1}, which was assigned to the trans C=C in trans-2-pentenal by Thomas and Warburton (1965), who also prepared the cis isomer.

Three alcohols identified in this study that had not previously been reported as orange essence components are heptanol, cis-2,8-p-menthadiene-1-ol, and cis-carveol. Heptanol had not been found in either grapefruit or tangerine essences (Moshonas and Shaw, 1971, 1972), although it has been identified as a cold-pressed orange oil component (Hunter and Moshonas, 1965). The trans isomers of both 2,8-p-menthadiene-1-ol (Moshonas et al., 1972) and carveol (Wolford and Attaway, 1967) had both been found previously in orange essence samples.

The last newly found orange essence component listed in Table 2, piperitenone, had recently been found at our laboratory in both tangerine essence (Moshonas and Shaw, 1972) and in orange essence oil (Coleman and Shaw, 1971).

The remaining 30 components identified in this study from both the methylene chloride and ethyl chloride extractions are: acetal, acetaldehyde, acetone, trans-carveol, carvone, ethanol, ethyl acetate, ethyl butyrate, ethyl propionate, hexanal, hexanol, 2-hexenal, cis-3-hexen-1-ol, isobutanol, isopentanol, limonene, linalool, 1,8-p-menthadiene-9-ol, trans-2,8-p-menthadiene-1-ol, methanol, 2-methyl-3-buten-2-ol, methyl butyrate, neral, nerol, octanal, octanol, 1-penten-3-ol, perillaldehyde, terpinen-4-ol, and α-terpineol. Since ethyl propionate had only been tentatively identified in orange essence (Wolford and Attaway, 1967), the present study confirms its presence. Use of the lower-boiling extraction solvent, ethyl chloride, was not advantageous in retaining a greater percentage of the more volatile essence components in the concentrated extract.

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Mention of brand names is for identification only and does not imply recommendation by the USDA.

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A Research Note
CRYOGENIC FREEZING OF TOMATO SLICES

INTRODUCTION

THE USE OF cryogenic fluids presents an alternative freezing method for foods whose freeze preservation was previously impractical. Reports of tomato slice cryogenic freezing is indicative of interest in the subject (Anon, 1964; Webster and Benson, 1966; Saray and Zackel, 1971) but scant information regarding techniques or methodology exists. A number of Florida breeding lines with firm texture and promising cultivation characteristics are being developed for machine harvest requirements (Crill and Burgis, 1970). It was our purpose to utilize some of these sturdier tomatoes in order to study freezing parameters and acceptability of frozen tomato slices.

EXPERIMENTAL

FLORIDA TOMATO breeding lines 1247, 1249, 1250 and 1104 were hand picked while mature green and ripened at 21°C. Stage of ripeness was expressed as days after color break. When the desired ripeness was reached, fruit were sliced 6 mm thick and precooled to 2°C. Slices were then either (1) aligned vertically, immersed into liquid nitrogen (−195°C) for 20 sec, equilibrated in the evolving nitrogen gas for 30 sec and immediately transferred to −34°C, or (2) dipped for 1 min into 0.08% calcium acetate (200 ppm Ca), drained, blotted and frozen as described. Controls consisted of similar precooled slices frozen at −34°C in the evaporator fan of a walk-in freezer and of freshly cut, precooled, unfrozen slices. All samples were evaluated within 24 hr of freezing after thawing at 25°C for 1 hr. Texture measurements employed an Instron Universal Testing Machine. Force was applied by a flat ended 4 mm diameter probe placed on the slice mesocarp. Force (g) required to penetrate slices 3 mm was considered indicative of tomato texture (two measurements on each of 20 slices). The sample was then blended, deaerated and analyzed by a Gardner Color Difference Meter using a "raw puree" reference tile (L=24.5, a_L=+27.6, b_L=+13.2). Similar samples were evaluated by 15 panelists for texture and color according to a 9-point hedonic scale for each attribute, using fresh unfrozen slices as the reference.

Table 1—Influence of freezing treatment on sensory texture scores[a] of tomato slices[b]

Texture[c]	Treatment	Color[c]
Fresh	2.57a	3.66a
LN	4.33b	3.57a
LN + Ca	4.80b	3.53a
−34°C	6.57c	3.07a

[a] 1 = excellent; 9 = extremely poor.
[b] Breeding line 1250, 6 mm slices, 8 days after color break
[c] Means followed by the same letter are not statistically different at the 0.05 confidence level.

Table 2—Influence of stage of ripeness (days) and freezing treatment on the texture (force, g) of tomato slices[a]

Treatment	Stage of Ripeness (days of color break) 4	6	8	10	12	14	Mean[b]
Fresh	360.30	235.27	189.58	125.03	115.24	140.98	194.39a
LN	174.98	84.33	42.84	24.59	28.11	22.40	62.87b
LN + Ca	146.09	75.92	43.54	37.50	37.40	27.70	61.35b
−34°C	56.73	25.78	13.29	12.09	10.64	11.16	21.61c
Mean	184.50x	105.30y	72.31yz	49.79z	47.85z	50.56z	

[a] 1104 Breeding line, 6 mm slices
[b] Means within the same group, followed by the same letter, are not significantly different at the 0.05 confidence level.

RESULTS & DISCUSSION

AN IMMERSION TIME of 20 sec in liquid nitrogen with ice crystal penetration of 2 mm was used because deeper freeze penetration resulted in slice cracking. This shattering phenomenon was the critical limitation in cryogenic freezing and dictated that slices be thick enough to possess rigidity but still sufficiently thin for adequate freeze penetration. Table 1 shows results of panel evaluation of thawed slice texture and color. Normal tomato color returned upon thawing but the texture was markedly affected by all treatments. Application of 200 ppm calcium (slice content 130 ppm) did not enhance slice texture significantly, but liquid nitrogen freezing was an improvement over slow freezing at −34°C. Table 2 indicates similar results over a range of fruit maturities. Whereas slices frozen 4 and 6 days after color break were quite firm, the attendant color and flavor were not satisfactory until the 8th day. Any manipulation by which tomato color and flavor could be developed without the noted softening would greatly improve the freezing prospects.

In an early study of tomato freezing only one of many cultivars examined was acceptable (Anonymous, 1964). Among the four Florida breeding lines there was no obvious relationship between fresh slice firmness and freezability, although in screening studies, fresh market tomatoes with fresh slice texture values about half those of the breeding lines reported were clearly unacceptable after freezing. It is clear that our tomato lines, treatments and techniques did not produce a frozen tomato slice which compared favorably with the fresh product. We feel, however, that with improved cultivars and greater attention to ripening stage, pretreatments and thawing procedure, a greatly improved frozen tomato slice can be developed.

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A Research Note
STORAGE STABILITY OF DEHYDRATED POTATO GRANULES PACKAGED IN CANS AND CARTONS

INTRODUCTION

POTATO GRANULES production has been growing steadily since 1951 and the growth is anticipated to continue (Boyle, 1967). At present, potato granules are packaged mainly in nitrogen-packed cans. The packaging and material costs are so high that other methods of packaging are needed. The use of cartons is economically superior to the cans in lowering packaging and shipping costs and a variety of products are now being stored in them (Anonymous, 1970). The purpose of this investigation is to compare the stability of potato granules packaged in nitrogen-packed cans and in air-packed foil-lined cartons stored at 75°F.

EXPERIMENTAL

DEHYDRATED potato granules (DPG) produced from potatoes of Russet Burbank variety were obtained from a commercial source in nitrogen-packed No. 10 cans. The cans were divided randomly into two lots. One lot was used as control and the DPG from the second lot were repacked into half gallon milk cartons ("Pure-Paks," polyethylene coated and foil lined). Each carton contained 2½ lb of DPG and was sealed with a hot melt glue gun.

The cans and cartons were stored under controlled environment of 75°F and 50% relative humidity (RH). Samples were withdrawn and analyzed at 4-wk intervals for 24 wk.

Moisture content of DPG was determined by drying the samples overnight in a vacuum oven at 70°C. The residual sulfite content in DPG was determined by the method of Potter and Hendel (1951) and the concentration of butylated hydroxytoluene (BHT) was determined by the procedure described by Schwecke and Nelson (1964).

A gas chromatograph (Varian Aerograph Model 1200) with a hydrogen flame ionization detector was used for hexanal determination according to the procedure of Boggs et al. (1964) with the following modifications. The column was packed with 5% SE-30 on VarAport 30 (Chromosorb W) and the column temperature was constant at 90°C. n-Hexaldehyde (K & K Laboratories) solutions at various concentrations were used as standards.

The color of DPG was determined with an Agtron color difference meter (Model F22). Color discs used for dark and light limits were shade numbers 5052.5 and 97, respectively. DPG samples were examined prior to rehydration.

A sensory panel consisting of 18 trained panelists was used to evaluate the flavor and aroma of DPG samples. The panelists were presented with a standard mashed potato sample made from the DPG in the can followed by coded samples, including a hidden control. The panelists were instructed to compare the test samples to the standard for aroma and flavor and score the difference on a 5-point scale ranging from five, "extremely large difference," to one, "no difference." The panelists were also asked to describe the off-flavor detected, if any. Analyses of variance were conducted and the calculated variance ratio (F value) was checked to determine if the difference between the samples is significant, as described by Larmond (1970).

RESULTS & DISCUSSION

TABLE 1 shows the changes in moisture content, concentrations of BHT, hexanal and sulfite in DPG stored at 75°F and 50% RH in nitrogen-packed cans and air-packed cartons. Results of color measurement and sensory evaluation are also presented in the same Table.

As shown in Table 1 the moisture content of DPG in both cans and cartons remained fairly constant throughout the 24-wk storage period. This indicates that the cartons are as effective as cans in protecting the DPG from absorption of moisture.

Oxidative deterioration is a major change in potato granules during storage, but it can be prevented effectively by nitrogen packing and/or by the use of antioxidants. Hexanal, a major product of autoxidation of linoleic acid, was used as an index of rancidification in the potato granules by Boggs et al. (1964). As shown in Table 1, hexanal was not detected in DPG samples until the 12th wk of storage. From the 12th to 24th wk of storage, only trace amounts of hexanal had been detected in the nitrogen-packed cans while the hexanal content in DPG air-packed in cartons increased from 0.26

Table 1—Changes in quality factors of dehydrated potato granules in nitrogen-packed cans and in air-packed cartons during storage at 75°F and 50% RH

Storage time (wk)	Moisture content[a] (%)		Hexanal content[a] (ppm)		Residual BHT (ppm)		Residual sulfite (ppm)		Agtron color value[a]		Differences detected by sensory panel (Significance)[b]	
	Can	Carton	Can	Carton	Can	Carton	Can	Carton	Can	Carton	Aroma	Flavor
0	5.9	6.0	0	0	10.9	11.1	385	380	44	44	–	–
4	6.2	6.2	0	0	11.0	9.1	309	364	–	–	NS	NS
8	5.8	6.0	0	0	8.8	5.1	282	247	–	–	NS	NS
12	6.0	6.2	0.07	0.26	8.9	3.0	251	303	44	43	NS	NS
16	6.2	6.3	0.09	0.32	8.1	1.0	235	212	–	–	*	*
20	5.8	6.2	0	0.30	7.7	2.0	218	246	–	–	NS	NS
24	6.3	6.4	0.05	0.49	6.7	1.7	209	122	46	46	*	*

[a]Average values of triplicate samples were taken.
[b]NS = not significant.
*Significant at $P < 0.01$ level

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to 0.50 ppm which is lower than the values reported by Boggs et al. (1964) for the slightly rancid potato granules.

The concentration of the antioxidant BHT in the control samples showed a slow, steady decline throughout the 24-wk storage period while the rate of BHT loss in carton-packed DPG was faster during the first 12 wk in storage and leveled off thereafter (Table 1). These data suggest that very little oxidation occured in the nitrogen-packed cans but a limited degree of autoxidation occurred in the air-packed cartons. The level of BHT added to the DPG during processing was fairly effective in preventing oxidative deterioration in the nitrogen-packed cans but less so in the air-packed cartons stored longer than 12 wk, as indicated by the trace amount of hexanal detected.

Results of sensory evaluation (Table 1) show that the differences in aroma and flavor detected by the taste panel between carton-packed and canned DPG samples were significant ($P < 0.01$) only at the 16th and 24th wk of storage. Off-flavors were mainly detected in the carton-packed samples by the panelists and were described in terms such as "oxidized, stale, green, and like hay." Rancidity of potato granules is probably a function of several compounds produced in fatty acid autoxidation and cannot be accurately measured by hexanal alone since the hexanal concentration in these samples was lower than 0.50 ppm (Table 1).

Sulfite was added at 400 ppm as SO_2 to the DPG during processing to retard nonenzymatic browning. As shown in Table 1, the rate of sulfite loss was approximately the same in the control cans and in the cartons. The Agtron color values remained essentially unchanged throughout the 24-wk storage period. These results indicate that cartons are as effective as cans in preventing the occurrence of browning reactions in the DPG stored at 75°F.

From these data we conclude that foil-lined cartons are as effective as cans in protecting the dehydrated potato granules from rehydration and against nonenzymatic browning when stored at 75°F and 50% RH for 24 wk. However, rancidity of potato granules developed to a limited extent in the cartons due to the presence of air.

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A Research Note
EXAMINATION OF FROZEN VEGETABLES BY TWO SAMPLE PREPARATION PROCEDURES

INTRODUCTION

MICROBIOLOGICAL studies on frozen processed vegetables provided information on the total aerobic plate counts and predominant types of microflora. (Smart, 1937, 1939; Splittstoesser and Wettergreen, 1964; Splittstoesser and Gadjo, 1966; Splittstoesser et al., 1965). Some of these studies of frozen vegetable products were based on the 'elution' type of sample preparation, i.e., the dislodging of bacteria from vegetable surfaces by shaking, while other studies were based on 'blending,' i.e., the release of bacteria by commutation of the vegetable products. Jones and Ferguson (1951) examined the comparability of these procedures and found that the use of the blender method reduced the bacterial count of dehydrated products, and that in other products greater variation existed using the blending method. They found in miscellaneous samples of food products that 32% of the samples showed no important difference between the two methods.

This study was undertaken to provide information concerning the comparability of two methods in the examination of frozen vegetable products.

MATERIALS & METHODS

IN CONJUNCTION with FDA regulatory work, eight types of frozen vegetable products were collected at 13 Northern California firms and examined for aerobic plate counts, coliforms, *Escherichia coli* and coagulase positive *Staphylococci*. The samples were transported and stored in the frozen state until analysis at the FDA laboratory in San Francisco. The basic analysis was by the AOAC method for Frozen Foods (1966) with the following modifications in sample preparations:

A. Shaking by hand vs. mechanical commutation. For each of the 234 samples a 50-g portion of the product was aseptically weighed into a sterile 1-qt blender jar to which 450 ml of Butterfield buffer (25°C) were added. The jar was shaken by hand 25 times in a 1-ft arc and set aside for 2 min to allow gross particulate matter to settle. Aliquots of the suspension were aseptically removed by pipette for inoculation and dilution preparations. Immediately

Table 1—Data from examination of eight vegetable types from various processors following parallel sample preparation procedures: Manual elution and mechanical blending

			APC/g 10^3				MPN/g Coliform		MPN/g E. coli		Indicated No./g Coag Pos Staph	
Product	Processor	No. of Samples	Arithmetic Blend	Avg Shake	Geometric Blend	Mean Shake	Blend	Shake	Blend	Shake	Blend	Shake
Carrots	L	16	3.3	2.7	3.7	2.3	6.7	–[a]	–	–	–	–
	F	10	1.9	0.8	1.6	0.6	–	–	–	–	–	–
Artichoke	B	10	47.0	23.0	31.0	21.0	17.0	9.7	–	–	–	–
hearts	C	15	1.0	0.7	0.9	0.3	–	–	–	–	–	–
Broccoli	F	18	2.2	2.0	2.3	1.0	–	–	–	–	–	–
spears	L	10	72.0	69.0	62.0	38.0	–	–	–	–	–	–
Asparagus	M	10	88.9	47.0	43.0	29.0	–	–	–	–	–	–
spears	A	8	25.0	8.5	25.0	9.5	3.6	–	–	–	–	–
	L	9	130.0	44.0	50.0	19.0	5.5	–	–	–	–	–
Spinach	E	6	5.8	1.5	3.8	1.5	–	–	–	–	–	–
Leaf	K	6	440.0	100.0	27.0	55.0	570.0	21.0	–	–	–	–
	I	6	6.4	1.5	5.8	4.3	70.0	41.0	16.0	–	10.0	10.0
	M	10	1800.0	22.0	1000.0	17.0	2100.0	140.0	1100.0	43.9	–	–
Spinach	M	5	1100.0	100.0	900.0	82.0	21.0	–	10.0	–	–	–
Chopped	I	5	26.0	0.6	15.0	0.4	19.5	3.6	–	–	10.0	10.0
	G	10	1000.0	92.0	950.0	86.0	1900.0	180.0	–	–	–	–
	H	10	960.0	70.0	940.0	68.0	90.8	–	–	–	–	–
	J	6	32.0	5.8	29.0	5.0	1000.0	260.0	–	–	–	–
Cauliflower	I	7	300.0	420.0	320.0	250.0	13.7	10.2	–	–	–	–
	D	10	1000.0	80.0	50.0	50.0	260.0	150.0	16.0	6.5	10.0	–
	H	10	780.0	170.0	600.0	130.0	290.0	280.0	13.0	–	–	–
Broccoli	A	10	1400.0	890.0	1400.0	340.0	160.0	27.0	–	–	–	–
Chopped	J	7	390.0	200.0	380.0	190.0	69.0	37.0	–	–	–	–
	D	5	60000.0	3700.0	47000.0	3200.0	820.0	240.0	23.0	23.0	0.6	10.0
	H	5	8800.0	1400.0	6300.0	1000.0	48.0	37.0	7.3	–	–	–

[a] "–" Means not found in 0.1-g portion

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Table 3—Effect of 2-methoxy-3-ethylpyrazine (V) on flavor of some potato containing products

Product	Conc (ppm)	N	Most potato flavor in[a] Control	Additive
Potato salad # 1	0.100	20	4	16**
Potato salad # 2	0.100	20	4	16**
Cream of potato soup	0.025	20	4	16**
Cream of potato soup	0.050	20	3	17**
Cream of potato soup	0.100	20	4	16**
Vegetable beef soup	0.015	20	9	11
Vegetable beef soup	0.025	20	5	15*
Vegetable beef soup	0.050	20	5	15*
Vegetable beef soup	0.100	20	5	15*
Scalloped potatoes	0.100	20	2	18***

[a]Paired comparison test. Judges asked to select sample in each pair with most potato flavor.
*P ⩽ 0.05; **P ⩽ 0.01; ***P ⩽ 0.001.

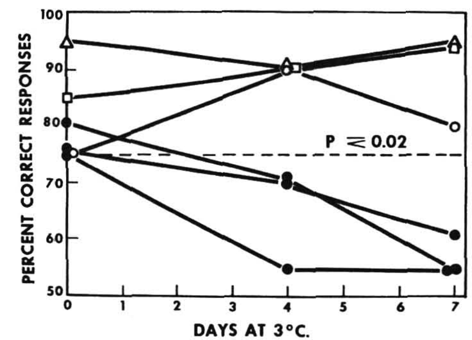

Fig. 2—Effect of storage on flavor enhancing effectiveness of various levels of 2-methoxy-3-ethylpyrazine in potato salad: □ = 1 ppm; △ = 0.4 ppm; ○ = 0.2 ppm; ● = 0.1 ppm. Paired Comparison Test, N = 20. Three different batches of product were tested at 0.1 ppm.

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

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ORANGE PEEL COLOR EXTRACT: ITS USE AND STABILITY IN CITRUS PRODUCTS

SUMMARY—Storage stability of the color was tested alone and in various citrus products using an extraction process which had been developed for obtaining a highly colored concentrated extract from orange peel. When concentrated color extract was added to single-strength orange juice in tin-lined or enamel-lined cans or clear glass bottles, color stability was good during storage at 35°F; except for some fading in glass-packed samples, no notable changes in color were observed after 10 wk. However, at 70°F and 85°F color changes occurred, mainly from darkening due to browning. These storage changes were more predominant in glass-packed juice and enamel-lined cans. Color in frozen concentrated orange juice remained stable more than 10 wk, showing no notable change in color during storage at −5°F. Stored in the concentrated form the color extract required protection from light and heat. Color was stable for more than 6 months when stored at −5°F in the dark.

INTRODUCTION

IMPORTANCE of color to acceptability of food products has long been recognized. This has been shown especially pertinent in orange juice products by a marketing research report (CECO Marketing Consulting Research, Inc., 1965) which indicated consumers prefer a dark orange color. Following recent public concern over the use of synthetic materials, food additives and the safety of foods being consumed by the general public, there is more interest than ever in the use of "natural" food sources.

Methods for extraction of coloring materials from citrus peel, treatment to remove color-interfering substances and flavor-influencing factors, and the incorporation of these color concentrates into citrus products have been described by Ting and Hendrickson (1968) and by Kew and Berry (1970). In the latter study, conducted on a relatively small laboratory scale, yields of color were found to vary depending upon the variety of citrus fruit used as a color source. Color concentrates from different sources also varied somewhat in degrees of redness or yellowness.

In order to be effective commercially, products treated with the color extract should be relatively stable. Thus in further development of the previously reported method, studies were made on the use of the color concentrates in commercial citrus products, particularly stability of the color-treated products stored at different temperatures and times. This paper reports these later developments.

EXPERIMENTAL

STABILITY during storage was determined both on products to which the color extract had been added and on the concentrated color extracts alone.

Storage tests on products

Color extracts from Valencia orange peel were checked for stability in concentrated and single-strength orange juice. Concentrated color extract prepared as described by Kew and Berry (1970) was added at 1 part/1000 to single-strength orange juice or at 4 parts/1000 to 45° Brix concentrated orange juice by blending the color into the product at about 120°F with gentle stirring. The color blended smoothly in a few minutes. Products were then canned and stored as follows: (A) 45° Brix concentrate in tin cans at −5°F and 35°F; (B) single strength in clear bottles at 35°F and 70°F; (C) single strength in tin-lined cans at 35°F and 85°F; and (D) single strength in enamel-lined cans at 35°F and 85° F.

Enamel-lined cans were included to check the possible effect of browning during high temperature storage and to avoid possible bleaching effects of tin. These samples were analyzed for Hunter citrus yellow (CY) and citrus red (CR) factors using the Hunter Lab D45 Citrus Colorimeter at 14-day intervals using the methods described by Kew and Berry (1970). Ranges and reproducibility of this method have been described by Huggart et al. (1966; 1969;) and Barron et al. (1967).

Storage studies on concentrated color extracts.

Samples of concentrated color extract prepared as described above were evaluated for storage stability using a Color Eye Reflectance Spectrophotometer Model D-1 (Instrument Development Laboratories, Kollmorgan Corporation, Attleboro, Massachusetts). Concentrated color extract was placed in small vials under nitrogen, 0.15 ml of color/vial. A standard method for reading the concentrated color on the Color Eye instrument was developed as follows: contents of the vial were diluted to 10 ml with hexane and a 4.25 cm dia disc of Whatman No. 1 filter paper was soaked in the vial for 10 min. The disc was air-dried for 2 min, placed in the instrument and read after 1 min. In the instrument, the disc was backed by two layers of Whatman filter paper and a white Vitrolite plate. A reference white Vitrolite plate was inserted in the other side of the instrument for comparative readings.

The Color Eye Spectrophotometer measures tristimulus color values X, Y and Z which are approximately degrees of redness, greenness, and blueness, respectively. Because there were slight variations in individual readings of a given sample, a range of experimental error had to be determined before evaluation could be effectively carried out. 30 samples of the color concentrate to be evaluated were prepared as described, and independent readings of X, Y and Z values made. Ranges were determined for each of the three values as follows: X = 102.0 − 100.5; Y = 93.7 − 90.2; Z = 36.4 − 25.9.

Stored samples of color concentrates were then evaluated at regular intervals until scores had changed to a degree that the probability of their being within the original mean was 1% or less. Samples of color concentrates were placed in storage and evaluated periodically as follows: clear vials of color concentrate prepared as above were placed at ambient temperature (about 80°F) on a laboratory bench about 3 ft from fluorescent light fixtures. Other samples were placed in the dark in a refrigerator at 35°F, and others in a freezer at −5°F. The samples at ambient temperature were evaluated daily until there was a color change. Then evaluation of 40°F stored samples was begun and they were evaluated at 4-wk intervals until a color change developed. Then samples stored at −5°F were evaluated at 30-day intervals until change developed.

RESULTS & DISCUSSION

RESULTS of storage studies of color stability in orange juice products are reported in Tables 1-4. Changes in CR and CY values of 45° Brix concentrates with added peel color extract and controls which did not contain added color, stored in tin cans at −5°F and 35°F, are shown in Table 1. After 10 wk at −5°F the experimental sample still had notably greater redness and yellowness values than controls although the color advantage had diminished some. After 10 wk at 35°F, which is above the temperature normally suggested for storing concentrated orange juice, the experimental sample had retained an advantage in yellowness, but had decreased in redness compared with the control sample.

Changes in color in single-strength orange juice with added color are compared with controls in Tables 2, 3, and 4 for samples stored in glass bottles, tin-lined steel cans and enamel-lined steel cans, respectively. Some browning occurred at the higher storage tempera-

On the basis of long-term use and lack of evidence of any deleterious effects of oral intake of natural carrageenan, it was included in the first GRAS (Generally Recognized As Safe) list by the Food and Drug Administration on November 20, 1959.

In 1961, FDA also authorized natural carrageenan as an approved food additive under the Food Additive Regulations (HEW, 1961). Seaweed species allowed as raw materials for the preparation of natural carrageenan included *Chondrus crispus, Chondrus ocellatus, Eucheuma cottonii, Eucheuma spinosum, Gigartina pistillata, Gigartina radula, Gigartina stellata,* and *Gigartina acicularis.*

Incriminating Evidence

Research nearly twenty years ago indicated that carrageenan could induce collagenous granuloma when injected subcutaneously into guinea pigs. However, **this form of administration is not considered to be appropriate** in assessing the safety of carrageenan as a food additive, since the high molecular weight (100,000) of the natural carrageenan precludes absorption in the gut of man when it is administered orally.

Several reports since 1969 (Marcus and Watt, 1969; Watt and Marcus, 1969; 1970a; 1970b) have linked ingestion of **hydrolyzed** low molecular weight carrageenan to ulcerative colitis and other tissue changes in some species of laboratory animals.

Absolving Evidence

Recent feeding studies (Bonfils, 1970; Benitz et al., 1972; Sharratt et al., 1971; Maillet et al., 1970) have shown that natural carrageenan (molecular weight greater than 100,000) used in food applications is not ulcerogenic in rhesus monkeys, rats, or guinea pigs. The hydrolyzed form of carrageenan used in peptic ulcer therapy does cause mucosal erosions and ulcerations in the intestines of rhesus monkeys, but not in rats and guinea pigs. The hydrolyzed form is, however, apparently absorbed and retained in the liver cells of rats, as well as rhesus monkeys.

It appears that the ulceration phenomenon is restricted to those animal species with the ability to absorb carrageenan. Herbivorous animals appear to have the ability to absorb natural carrageenan, while primates are able to absorb only the low molecular weight degraded form of carrageenan.

As part of the GRAS review, food-grade carrageenan was tested for the FDA by the Food and Drug Research Laboratories of Waverly, N.Y. and the Institute of Experimental Pathology and Toxicology of Albany Medical College, Albany, N.Y. (HEW, 1972). The results indicated that using the traditional 100-fold safety factor and extrapolating animal results to man, no evidence of human hazard exists at present levels of usage of food-grade natural carrageenan.

The chemically hydrolyzed form of carrageenan (C-16) has never been used as a food additive and should not be confused with the natural form of food-grade carrageenan used in the food industry as a beneficial food additive.

FDA's Response

As a result of these tests, the FDA discontinued carrageenan's GRAS status and strengthened the Food Additive Regulation for carrageenan to ensure continued use of only the natural, unhydrolyzed form in food.

The FDA concluded that an adequate margin of safety exists to continue safe use of food-grade carrageenan at any present or anticipated use levels in food (HEW, 1972).

Summary

There is a distinction between natural, undenatured carrageenan (molecular weight greater than 100,000) and chemically hydrolyzed carrageenan (average molecular weight 10,000).

Hydrolyzed low molecular weight carrageenan, which has been used as a drug in Europe, has been shown to cause ulceration in test animals and should not be used in foods based on present evidence.

FDA has recommended that carrageenan be dropped from the GRAS list in order to strengthen the Food Additive Regulation for carrageenan and will require molecular weight determination of carrageenan to ensure that low molecular weight carrageenan is not used in foods.

Natural, undenatured carrageenan can continue to be safely used at present or anticipated levels in foods.

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