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ABSTRACTS:
IN THIS ISSUE

PROTEIN HYDROLYSATE FROM FISH WASTE. W. TARKY, O.P. AGARWALA & G.M. PIGOTT. *J. Food Sci.* 38, 917–918 (1973)–Proposes a method to utilize fish waste (FW) from a filleting operation by recovering proteinaceous material of high functional properties. Pepsin was used to hydrolyze FW from English sole (*Parophrys vetulus*). 78% hydrolyzed protein was obtained when 0.2% pepsin was added to a 2:1 FW-water mixture. The hydrolysate was removed by centrifugation at 12,000G for 10 min. The supernatant was heated at 80°C to inhibit the enzyme, neutralized with 30% NaOH, filtered, concentrated to 30% total solids and spray dried. FPC from FW contained 80% protein and 22% ash. When mixed with casein, protein hydrolysate gave PER similar to that of pure casein, demonstrating that fish filleting waste can be upgraded by a simple nonsolvent process, yielding soluble, low-fat protein for supplementation of expensive proteins, reducing total cost without any loss in protein efficiency.

INFLUENCE OF NITRITE AND NITRATE CURING INGREDIENTS ON QUALITY OF PACKAGED FRANKFURTERS. S. SIMON, D.E. ELLIS, B.D. MacDONALD, D.G. MILLER, R.C. WALDMAN & D.O. WESTERBERG. *J. Food Sci.* 38, 919–923 (1973)–Triplicate all meat (beef and pork) and all beef frankfurters were prepared with 0, 1/16, 1/8 and 1/4 oz sodium nitrite per hundred pound meat block and 0 and 3/4 oz sodium nitrate. The frankfurters were packaged with and without vacuum and stored at 4.5°C for as long as 4 wk. At selected storage intervals the various products were evaluated organoleptically and analyzed for cured pigment conversion as well as general bacterial quality. The taste panel scores of all meat frankfurters without nitrite were low initially and became increasingly unacceptable with longer storage time. Raising the nitrite level improved acceptability throughout the storage period. The flavor of all beef frankfurters was not affected by lack of either nitrate or nitrite. Nitrates did not affect flavor or cured pigment conversion, although cured color formation was directly related to nitrite level. Total bacterial counts were not affected by nitrite levels or nitrate addition. Vacuum packaged frankfurters survived close to 2 wk longer than overwrapped frankfurters which spoiled in about 10 days.

HEAT TRANSFER, ORGANOLEPTIC QUALITY CHANGES AND MOISTURE EXCHANGE IN AIR-BLAST CHILLED POULTRY CARCASSES. A.A. VACINEK & R.T. TOLEDO. *J. Food Sci.* 38, 924–928 (1973)–A process for chilling poultry carcasses was developed where the birds were cooled in a blast of air at sub-freezing temperatures, followed by equilibration to cool the interior by conduction from the outer layer. This technique overcomes problems previously reported for chilling in air at 0°C in that exposure time is very short and a layer of ice immediately forms on the surface which protects the birds from dehydration and surface discoloration. A frozen crust about 9/16 in. thick is required at the time of removal from air blast and further temperature equilibration to attain the required final temperature in chilling of 4.5°C or lower. At 900 ft/min air velocity and −40°C only 13 min was required for chilling. Organoleptic evaluation of the crust-frozen birds showed no significant difference in quality when compared to an ice-slush chilled control. Moisture loss was negligible during air chilling and holding the birds in ice for 24 hr after air chilling resulted in a net gain averaging 3%.

USE OF ULTRAVIOLET LIGHT FOR EXTENDING THE RETAIL CASELIFE OF BEEF. J.O. REAGAN, G.C. SMITH & Z.L. CARPENTER. *J. Food Sci.* 38, 929–931 (1973)–30 samples of adipose tissue and 96 samples of longissimus muscle from beef ribs were subjected to one of three treatments to determine the feasibility of using UV light to increase retail caselife. Samples in Treatment 1 were not exposed to UV light, those in Treatment 2 were wrapped prior to exposure and those in Treatment 3 were wrapped after exposure to UV light. Samples of adipose tissue in Treatments 1 and 2 attained spoilage levels of bacteria ($\log_{10}$ = 6.5) after ≈ 2½ and 4 days, respectively, while samples in Treatment 3 had not attained the designated spoilage level after 6 days of retail display. Longissimus muscle samples from Treatment 3 displayed a decreased rate of bacterial growth which resulted in an approximate advantage of 1½ and 2 days in retail caselife when compared with samples from Treatments 2 and 1, respectively. Longissimus muscle samples in Treatments 1 and 2 were considered unacceptable from consumer desirability and muscle color standpoints after approximately 4½ days, while those in Treatment 3 were still desirable in appearance and color after 6 days of retail display. Significant increases in retail caselife were associated with exposure of muscle and fat surfaces to UV light for 2 min. Decreases in initial count and/or attenuation of the bacteria present on retail cuts via ultraviolet radiation resulted in higher muscle color ratings, increased consumer acceptability ratings and extended caselife.

A COMPARISON OF THE EFFECTS OF AGING, CONDITIONING AND SKELETAL RESTRAINT ON THE TENDERNESS OF MUTTON. P.E. BOUTON, P.V. HARRIS, W.R. SHORTHOSE & R.I. BAXTER. *J. Food Sci.* 38, 932–937 (1973)–Some muscles taken from mutton carcasses suspended from the Achilles tendon and conditioned at 0–1°C had greater shear force and adhesion values and shorter sarcomere lengths than those of corresponding muscles from carcasses conditioned at 15–16°C. Muscles restrained from shortening by skeletal attachments were not adversely affected by conditioning temperature. A comparison of the effects of conditioning at 0–1 or 15–16°C on muscles removed pre- and post-rigor from the carcasses showed that skeletal restraint was unimportant provided 15–16°C conditioning was used. More muscles were restrained from shortening when carcasses were hung, pre-rigor, from the pelvis instead of from the Achilles tendon and increased toughness associated with 0–1°C conditioning thus avoided.

CHEMICAL AND SENSORY EVALUATION OF IRON-FORTIFIED MILK. C.F. WANG & R.L. KING. *J. Food Sci.* 38, 938–940 (1973)–Feasibility of adding iron to milk intended as a dietary supplement was investigated. Ferric ammonium citrate, ferrous sulfate, ferric choline citrate and ferric ammonium sulfate were selected after screening many iron salts. Salts were added to raw milk followed by pasteurization and homogenization by both laboratory and commercial systems. Sensory evaluation was by expert and consumer panels and the TBA test. Chemical analyses included fat, iron, ascorbic acid, tocopherol, vitamin A and carotene. Milks containing up to 30 ppm iron as ferric ammonium citrate did not develop oxidized flavor after 7 days storage at 5°C and were indistinguishable from controls by both panels. Other iron salts caused lipid oxidation and losses of fat soluble vitamins.

ASSIMILATION OF IRON FROM IRON-FORTIFIED MILK BY BABY PIGS. C.F. WANG & R.L. KING. *J. Food Sci.* 38, 941–944 (1973)–The purpose was to evaluate the biological availability of iron in milk enriched with ferric ammonium citrate (FeAC). Fate of the iron was determined using five Hampshire-Yorkshire baby pigs maintained on modified cow's milk fortified with FeAC, vitamins and aureomycin. Hemoglobin, hematocrit, red blood cell (RBC) and body weight evaluations indicated maintenance of normal physiological conditions. Animals

were sacrificed 15 days after a single administration of ration labeled with ^{59}Fe as FeAC. Iron absorption was based on radioactivity in RBC, RBC plus body tissue and organs, and feces. Mean iron absorption by the three methods was about 30% and 90% was incorporated into RBC.

THE NATURE OF FATS AND FATTY COMPONENTS IN NONDAIRY IMITATION MILKS. M. FILSOOF, M. MEHRAN & F.V. KOSIKOWSKI. *J. Food Sci.* **38**, 945–948 (1973)–14 nondairy imitation milk powders and concentrates were analyzed for fat and fatty component characteristics. Fats of nondairy imitation milk products showed melting point ranges of < 5.0–44.5°C; refractive index 1.4482–1.4670; unsaponifiable matter 0.42–1.44%; and peroxide value 1.04–24.41. Sterols were mostly campesterol, stigmasterol and beta-sitosterol, but one nondairy imitation milk contained relatively high levels of cholesterol or its related compounds, 71.49 mg per 100g fat. The fatty acid composition was dominated by unsaturated forms, comprising 75–80% of the total sterols. Only one nondairy imitation milk powder contained fat resembling coconut.

A MODIFIED METHOD FOR AFLATOXIN DETERMINATION IN SPICES. J.I. SUZUKI, B. DAINIUS & J.H. KILBUCK. *J. Food Sci.* **38**, 949–950 (1973)–The usual fluorometric methods for the analysis of aflatoxins are not applicable to many spices because of naturally occurring interfering substances. These interfering substances have been removed successfully by chromatographing on a thin layer of silica gel using a different solvent system in a second dimension, after fixing the aflatoxins with phosphoric acid. The modified method was checked using spices containing known amounts of added aflatoxins.

INFLUENCE OF POST-HARVEST STORAGE AND SOAKING TREATMENTS ON THE YIELD AND QUALITY OF CANNED MUSHROOMS. R.B. BEELMAN, G.D. KUHN & F.J. McARDLE. *J. Food Sci.* **38**, 951–953 (1973)–Various post-harvest storage and water-soaking treatments were evaluated for their influence on the yield and quality of canned mushrooms. The canned product yield was most significantly increased when the fresh mushrooms were soaked 20 min in water, stored at 2°C for 18 hr and then soaked in water again for 2 hr prior to blanching. This set of treatments which was termed the PSU-3S process increased the canned product yield more than 9% compared to a conventional control process. The color and organoleptic quality of the canned product was not adversely affected by the new process but it did result in a greater number of mushrooms with exposed veils. The combined yield increases of the three operations of the PSU-3S process employed individually was 6.2% but when the three operations were employed in sequence a yield increase of 9.4% was obtained indicating a strong interaction existed among the storage and soaking operations of the process. Several alternatives to the process are presented and discussed.

QUALITY OF FROZEN GREEN VEGETABLES BLANCHED IN FOUR CONCENTRATIONS OF AMMONIUM BICARBONATE. M.S. EHEART & D. ODLAND. *J. Food Sci.* **38**, 954–958 (1973)–Fresh peas, limas, green beans, broccoli and Brussels sprouts were blanched in solutions of four concentrations of NH_4HCO_3 and freezer-stored at 0°F. Samples were analyzed after 2 wk and 6 mo storage for pH, titratable acidity, reduced and dehydro ascorbic acid, moisture and color difference and were panel tested after 5 mo for color, flavor, texture and acceptability. The alkaline additive effectively improved color of the vegetables as judged either instrumentally or by the taste panel. Texture was adversely affected by the higher concentrations of NH_4HCO_3 but in no case did the additive increase losses of ascorbic acid. Suggested concentrations of NH_4HCO_3 for optimum quality of each vegetable are given. Storage effects and reuse of blanch solutions are also discussed.

FLOW PROPERTIES OF SOME FOOD POWDERS. M. PELEG, C.H. MANNHEIM & N. PASSY. *J. Food Sci.* **38**, 959–964 (1973)–Some food powders were subjected to various physical measurements in order to study their flow properties and tendency to cake. Bulk density, tensile strength, shear stress, cohesion, angle of internal friction and flow function were measured and calculated. Results showed that compressibility, cohesion and flow function may be used as criteria for characterization of the powders according to their flowability and tendency to cake.

AN ELECTRONIC APPLE REDNESS METER. S.S. COLOSIE & J.E. BREEZE. *J. Food Sci.* **38**, 965–967 (1973)–An instrument capable of making an objective and reliable measurement of redness in the skin of apples was developed. Fiber optic light tubes were utilized both to illuminate the apple surface and to receive the reflected color. The Meter's electronic circuitry provides a meter reading of apple "redness." This portable instrument is immune to certain well-known shortcomings of the human eye. The effects of shape and size of an apple are negligible; it does not require a skilled technician for operation and use, and provides improved reliability in the color grading of most varieties of red apples by providing a basis for the establishment of the same minimum color standards from one growing area to another, year after year.

QUANTITATIVE DETERMINATION OF COMBINED HEMOGLOBIN AND MYOGLOBIN IN VARIOUS POULTRY MEATS. R.L. SAFFLE. *J. Food Sci.* **38**, 968–970 (1973)–A study was conducted to develop a spectrophotometric procedure for the determination of combined hemoglobin and myoglobin in poultry meat and to determine the concentration of pigment (mg/g of tissue) of various poultry meats. Max difference in O.D. for chicken and beef blood was 0.014 between unheated and heated (50 or 55°C) samples when heating time was extended to 30 min. Meat pigments were relatively stable to heat in the time periods used in this investigation. The amount of pigment increased greatly as the age of the bird increased. There was large variation within each type of poultry meat when pigment was expressed as mg/100g protein. Gizzards had highest values, followed by heart.

EFFECTS OF GAMMA IRRADIATION ON MYOGLOBIN. C. LYCOMETROS & W.D. BROWN. *J. Food Sci.* **38**, 971–977 (1973)–Purified deoxygenated sperm whale myoglobin solutions were exposed to gamma irradiation at levels up to 500 Krad. Following irradiation the solutions could be resolved by Sephadex chromatography into three fractions: monomer (molecular weight, M.W., estimate 17,000); dimer (M.W. 38,000); and polymers (M.W. 60,000 and greater). Dimer formation appeared to be intermediate to production of polymer fractions. All three fractions had the same spectral characteristics. Liganded derivatives of all three were less susceptible to change manifested by a lower Soret (409 nm) to protein (280 nm) absorbance ratio than was nonliganded metmyoglobin. The presence of excess ligand inhibited dimer and polymer formation, presumably due to the fact that such ligands may act as free radical scavengers. Irradiated myoglobin preparations showed many new bands on electrofocusing, underwent autoxidation at faster rates than controls, and were more susceptible to acid and thermal denaturation. Binding studies with n-butyl isocyanide suggested that irradiated myoglobin exhibits less steric hindrance than does control protein.

LIPIDS AND FATTY ACIDS OF CHICKEN BONE MARROW. K.E. MOERCK & H.R. BALL JR. *J. Food Sci.* **38**, 978–980 (1973)–Lipids and fatty acid compositions of broiler chicken bone marrow were determined. The lipid content of marrow from femur, tibia and ilium-ischium bones was similar. Average lipid content was 46.5%. Triglycerides were approximately 94.5% of the total lipid and contained primarily 16:0, 18:0, 18:1 and 18:2 fatty acids. Approximately 1.7% of the total lipids were phospholipids which had a relatively high percentage of 20:3 to 22:6 unsaturated fatty acids. Trace amounts of glycolipids were also found. Two components of the phospholipid fraction previously reported as 14:2 or 15:0 and 16:2 fatty acids were shown to be hexadecanal and octadecanal.

EFFECT OF POSTMORTEM AGING ON CHICKEN MUSCLE FIBRILS. J.D. HAY, R.W. CURRIE, F.H. WOLFE AND E.J. SANDERS. *J. Food Sci.* **38**, 981–986 (1973)–Electron micrographs of in situ muscle fibers and extracted myofibrils of chicken leg (red) and breast (white) muscle aged for 0, 3, 48 and 168 hr postmortem revealed Z line and M line degradation in in situ breast muscle but no such occurrence in leg muscle. However, sarcomere lengths appeared constant in the breast muscle, but increased with postmortem aging in leg muscle. Other more subtle changes are noted and discussed. Biochemical assays on the extracted myofibrils revealed little change in ATPase activity or sulfhydryl availability with aging.

ABSTRACTS:
IN THIS ISSUE

POLYACRYLAMIDE DISC GEL ELECTROPHORESIS OF FRESH AND AGED CHICKEN MUSCLE PROTEINS IN SODIUM DODECYL-SULFATE. J.D. HAY, R.W. CURRIE & F.H. WOLFE. *J. Food Sci.* 38, 987–990 (1973)–Chicken breast (*pectoralis major*) and leg (*adductor longus*) myofibrils, prepared at 0, 3, 48 and 168 hr postmortem have been examined using the technique of polyacrylamide disc gel electrophoresis. The patterns obtained revealed specific changes in the protein patterns during aging, with major changes involving a 44,000 Dalton component in breast muscle, and a 30,000 Dalton component in both breast and leg muscle. Other less obvious changes in the protein bands are noted and discussed. Disc gel patterns of sarcoplasmic proteins showed little change with aging.

EFFECT OF SODIUM CHLORIDE AND CONDENSED PHOSPHATES ON THE WATER-HOLDING CAPACITY, pH AND SWELLING OF CHICKEN MUSCLE. G.W. SHULTS & E. WIERBICKI. *J. Food Sci.* 38, 991–994 (1973)–The advantages of the addition of sodium chloride and food-grade phosphates to cooked chicken muscle were studied. A 15–20% reduction in meat shrinkage (reciprocal of water-holding capacity) resulted by the addition of 1% sodium chloride and 0.5% of sodium salts of tripolyphosphate, pyrophosphate or a commercial blend of polyphosphates, Kena FP-28. Tetrasodium pyrophosphate demonstrated the greatest effects on the pH, swelling and water-holding capacity of the chicken muscle. Small, but nonsignificant differences were found between pyrophosphate and Kena FP-28 for the water-holding capacity. The addition of hexametaphosphate, a commercial preparation of hexametaphosphate (Foodfos) and two commercial blends of polyphosphates (Curafos 22-4 and Curafos 11-2) resulted in significantly higher meat shrinkage values than found for pyrophosphate, tripolyphosphate and Kena FP-28. Water-holding capacity data for 10 combinations of sodium salts of tripolyphosphate, hexametaphosphate and pyrophosphate showed that pyrophosphate alone or a combination of 1 part tripolyphosphate, 1 part hexametaphosphate and 1 part pyrophosphate had the greatest effect on prevention of the loss of natural juices during cooking of white chicken muscle.

THE ASSOCIATION OF STRUGGLE DURING EXSANGUINATION TO GLYCOLYSIS, PROTEIN SOLUBILITY AND SHEAR IN TURKEY PECTORALIS MUSCLE. R.T-I. MA & P.B. ADDIS. *J. Food Sci.* 38, 995–997 (1973)–Glycolysis acceleration in avian pectoralis muscle may elevate shear properties and reduce water binding of proteins. Recent curtailment of effective electrical stunning permits increased muscular activity during exsanguination. This study was designed to determine the biochemical effects of struggling, as influenced by electrical stunning, and to further elucidate the mechanism of turkey breast muscle toughening. Struggling accelerated glycolysis ($P < 0.01$) and ATP loss ($P < 0.05$) and hastened loss of electrical stimulation response and rigor. Some birds displayed glycolytic rates exceeding those reported for extremely pale porcine muscle. Despite these differences, protein solubility was not significantly different, although correlations between shear value and 24 hr postmortem protein solubility were significant ($r = -0.54$, $P < 0.05$). Consistent trends in the data suggested that electrical stunning slightly retarded rates of postmortem changes and enhanced muscle properties. Results suggested that muscles which experience rapid pH decline and rapid loss of protein solubility exhibit increased shear values. Ultimate sarcomere length was related to shear value ($r = -0.79$, $P < 0.01$) indicating that both sarcomere length and protein solubility are factors related to tenderness. Studies in commercial facilities demonstrated that 75% of the variation in tenderness of the pectoralis was related to the time course of rigor mortis.

EFFECTS OF INTRAMUSCULAR COLLAGEN AND ELASTIN ON BOVINE MUSCLE TENDERNESS. H.R. CROSS, Z.L. CARPENTER & G.C. SMITH. *J. Food Sci.* 38, 998–1003 (1973)–Five muscles from each of 15 Hereford females, ranging widely in age (Group I = 305–642 days; II = 1382–1396 days; and III = 3635–5096 days), provided samples for use in determining the singular and combined effects of elastin and collagen on the tenderness of bovine muscle tissue. Sensory panel ratings for amount of connective tissue were significantly related ($P < 0.05$) to percentages of soluble collagen but were not associated with differences in elastin content. Regression equations which combined all of the chemical components measured in the present study accounted for 26.10% of the variation in panel ratings for amount of connective tissue. Total collagen concentration, percent soluble collagen and elastin concentration accounted for 12.45% of the variation in panel ratings for amount of connective tissue. Results of the present study indicate that quantitative measures of collagen and elastin content are significantly related to the connective tissue component of beef tenderness but that these chemical components are not significantly related to that portion of tenderness which is related to the myofibrillar component.

VIABILITY OF Staphylococcus aureus IN INTERMEDIATE MOISTURE MEATS. M. PLITMAN, Y. PARK, R. GOMEZ & A.J. SINSKEY. *J. Food Sci.* 38, 1004–1008 (1973)–Intermediate moisture strained chicken and pork dices were prepared using different concentrations of glycerol, 1,2-propanediol and 1,3-butanediol as humectants to achieve different levels of water activities. Based on the direction of water transfer during the moisture equilibration, IMF systems were prepared by desorption and adsorption methods. Results on the viability of *Staphylococcus aureus* in IMF systems, indicate that water activity of the environment is not solely responsible for controlling the availibility of water to microorganisms and total moisture content must be considered as well. Besides the water-binding properties of 1,2-propanediol and 1,3-butanediol, there is a bactericidal effect which depends on the chemical structure of the humectant molecules.

INTERACTION OF FORMALDEHYDE WITH FISH MUSCLE IN VITRO. E.A. CHILDS. *J. Food Sci.* 38, 1009–1011 (1973)–Experiments were undertaken to characterize the interaction of formaldehyde with fish muscle myofibrils and their constituent proteins. Exposure to formaldehyde was found to cause a reduction in extractable salt-soluble proteins in fresh and frozen Pacific true cod. SDS-acrylamide gel electrophoresis showed that tropomyosin and heavy chains of myosin were most easily insolubilized, and that actin and the 37,400 mol wt component were least reactive. Formaldehyde also decreased the quantity of extractable whole myofibrils in fresh and stored samples. Whole myofibrils extracted in the presence of formaldehyde had complete protein complements.

EFFECT OF TEMPERATURE ON LIPID EXTRACTION AND FUNCTIONAL PROPERTIES OF FISH PROTEIN CONCENTRATE (FPC). D.L. DUBROW, A. KRAMER & A.D. McPHEE. *J. Food Sci.* 38, 1012–1015 (1973)–Whole fish, red hake (*Urophycis Chuss*) was five-stage countercurrent extracted with isopropyl alcohol (IPA) at temperatures of 20, 40 or 50°C to determine the effect on lipid extractability and functional properties of the FPC's produced. Analyses were performed to determine residual lipid, lipid composition, protein solubility, suspendable solids, emulsifying capacity-stability, wettability, pH and reflectance. Results showed that five stages of extraction at 20°C reduced

the residual fish lipids to about 10%. At 40° or 50°C, extractability of lipids required four or three stages, respectively, to produce an FPC with 0.5% residual lipid or less. FPC's processed at 20°C had better functional properties than those processed at 40–50°C. All FPC's showed a decrease in protein solubility and suspended solids and an increase in pH with increased temperature of extraction. Wettability was poor in all samples regardless of extraction temperature.

IN VITRO DIGESTIBILITY OF PROTEIN IN YOGURT AT VARIOUS STAGES OF PROCESSING. E.S. BRESLAW & D.H. KLEYN. *J. Food Sci.* 38, 1016–1021 (1973)–Before digestion and after hourly intervals of in vitro pepsin digestion, curd particle size distribution was determined by screening and total nitrogen estimation. Supplemental analyses included nonprotein nitrogen and free amino acid fractions. Highly digestible samples contained most of the protein in the noncurd fractions, i.e., the precipitate and filtrate. Yogurt was found to be more digestible than raw mixture, which required twice the amount of time to reach an equivalent stage of digestion. No significant difference was observed in digestibilities of yogurt and various fruit-flavored yogurts. The free amino acid content of yogurt was twice that of the raw mixture. Little or no difference was shown in the digestibilities of nonstabilized and stabilized yogurts.

MOLECULAR SPECIES OF PHOSPHORYLASE IN POSTHARVEST POTATO TUBERS. J. IOANNOU, G. CHISM & N.F. HAARD. *J. Food Sci.* 38, 1022–1023 (1973)–The electrophoretic patterns on polyacrylamide gels and total activity of phosphorylase (α-1,4-glucan: orthophosphate glucosyltransferase, E.C. 2.4.1.1) from two varieties of potato tubers (*Solanum tuberosum* L. variety Kennebec and Monona) were examined after storage at 5°C and after subsequent conditioning at 20°C. The electrophoretic patterns of extracts of cold stored and conditioned potatoes were essentially the same for both varieties, although the minor bands appear to be more active in the Kennebec extracts. When the gels were assayed for starch degradation instead of starch synthesis, additional bands were seen, but the major band was the same for both assays. The total phosphorylase activity was essentially the same for both varieties.

CORN ODOR CLASSIFICATION FROM LOW-RESOLUTION GAS-CHROMATOGRAPHIC PROFILES OF HEAD SPACE VOLATILES. A. DRAVNIEKS & C.A. WATSON. *J. Food Sci.* 38, 1024–1027 (1973)–A simple process for a rapid pre-concentration and gas-chromatographic characterization of corn volatiles was devised. It was based on ambient-temperature collection of volatiles on high-surface-area polymeric adsorbents and resulted in low-resolution GC profiles which, in their application to a series of corn samples, were useful in discriminating corn samples with normal odors from those with odor defects at $p < 0.001$ for the group used in the development of the discriminants and at $p \approx 0.05$ in classifying additional samples.

CHEMICAL AND SENSORY PROPERTIES OF PIMENTO LEAF OIL. M.E. VEEK & G.F. RUSSELL. *J. Food Sci.* 38, 1028–1031 (1973)–Volatile compounds were separated and isolated from the essential oil of pimento leaf oil. Components were identified by retention indexes, infrared and mass spectrometry. Firm identification was established for the presence of α-pinene, sabinene, α-phellandrene, limonene, 1,8-cineol, cis-β-ocimene, γ-terpinene, trans-β-ocimene, p-cymene, terpinolene, α-copaene, linalool, α-gurjunene, 1-terpinen-4-ol, β-caryophyllene, alloaromadendrene, α-humulene, α-selinene, α-muurolene, δ-cadinene, eugenol methyl ether, eugenol and isoeugenol. Preliminary sensory screening of gas chromatographic fractions was undertaken to assess the contribution of individual components to the overall odor of the leaf oil.

THE NATURE OF CAROTENOID ESTERIFICATION IN TANGERINES. T. PHILIP. *J. Food Sci.* 38, 1032–1034 (1973)–The isolation and characterization of cryptoxanthin laurate, β-citraurin laurate and reticulataxanthin laurate from the pulp and peel of Dancy tangerines and Kinnow mandarins are described.

FLAVONOL GLYCOSIDES OF SOUR CHERRIES. A.J. SHRIKHANDE & F.J. FRANCIS. *J. Food Sci.* 38, 1035–1037 (1973)–Flavonoid pigments of sour cherries (*Prunus cerasus* L., var. Montmorency) were isolated and identified by paper chromatographic and spectrophotometric methods. The presence of quercetin-3-rhamnosylglucoside, kaempferol-3-rhamnosylglucoside, quercetin-3-glucoside and quercetin-4′-glucoside was confirmed. Three other flavonols were tentatively identified as kaempferol-3-rhamnoside-4′-galactoside, kaempferol-3-glucoside and kaempferol-4′-glucoside. Also discusses the merits of phenol in separating structurally close flavonol glucosides.

ISOLATION AND IDENTIFICATION OF SOME FLUORESCENT PHENOLIC COMPOUNDS IN CRANBERRIES. N.T. CHU, F.M. CLYDESDALE & F.J. FRANCIS. *J. Food Sci.* 38, 1038–1042 (1973)–A 95% ethanol extract from cranberries was concentrated in a rotatory evaporator under vacuum to remove ethanol and then extracted with ethylacetate. Compounds in both ethylacetate and aqueous extracts were separated and isolated by stepwise paper chromatography. Identification was accomplished by UV spectra, hydrolysis, methylation and IR spectra. A total of 11 fluorescent fractions were isolated, two of which later proved to be identical. This compound, identified as the 4-glucoside of caffeic acid, was recovered from both the ethylacetate and aqueous extracts. Tentative identification of six of the compounds are as follows: glucose derivatives of ferulic and gentisic acids; glucose esters of hydroxycinnamic and cinnamic acids; a glucose derivative of cinnamic acid; and a derivative of arabinose, glucose and ferulic acid. Three compounds remain unidentified.

EFFECT OF METALLIC IONS ON COLOR AND PIGMENT CONTENT OF CRANBERRY JUICE COCKTAIL. M.S. STARR & F.J. FRANCIS. *J. Food Sci.* 38, 1043–1046 (1973)–The effects of copper, aluminum, iron tin and a combination of iron/tin at low concentrations were studied for their effects on the total anthocyanin (ACY) content of cranberry juice cocktail. After appropriate levels of metals were established, based on the smallest concentration producing a measurable spectral change within 24 hr, a storage study was conducted using multiples of these levels to ascertain their effect on ACY content relative to each other, other metals and the control. Aluminum, iron and tin were added at 1, 5 and 10 ppm whereas copper was added at 5, 25 and 50 ppm. Each set of samples was prepared with and without the addition of ascorbic acid, and also at pH 2.2 and 2.7. Samples were assayed for total ACY content and spectral curves were obtained. ΔE values were developed from trichromaticity data. Results indicate a frequent protective effect of the metals on ACY content, a general interaction of metals and concentrations at the levels employed, and a protective effect at the lower pH on ACY. The predominent effect was the highly destructive effect of the added ascorbic acid.

ULTRAVIOLET ABSORPTION METHOD FOR EVALUATING CITRUS ESSENCES. J.M. RANDALL, W.L. BRYAN, O.W. BISSETT & R.E. BERRY. *J. Food Sci.* 38, 1047–1050 (1973)–Ultraviolet (UV) absorption was tested for evaluating strength of citrus essences (derived from juice) and aroma solutions (essence-like solutions derived from peel). UV measurements were correlated with total organic composition, as determined by chemical oxygen demand (COD) and with "less-volatile organics," i.e., total organics minus acetaldehyde, ethanol and methanol. UV absorbance at several wavelengths correlated well with an approximation of "less-volatile organics" as determined by bromate titration (measurement of unsaturated organics). Absorbance at 200 or 210 nm gave highest correlation coefficients and greatest sensitivity and is recommended as a rapid, nondestructive index of "organic" strength of citrus essences with potential as a simple process control procedure.

COMPARISON OF COLOR SCALES FOR DARK COLORED BEVERAGES. B.A. EAGERMAN, F.M. CLYDESDALE & F.J. FRANCIS. *J. Food Sci.* 38, 1051–1055 (1973)–Thirteen colorimetric scales, both currently in use and proposed, were tested to determine their usefulness for transmission colorimetry. Most of the scale parameters showed an area of confusion or nonlinearity and below this region, colorant scale values decreased, despite the presence of more pigment in the darker samples.

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Several dark colored commercial juices were tested to determine if these areas of confusion might interfere with the use of the scale. Several scale parameters did not have areas of confusion but were not linear with pigment concentration. The latter is desirable for predictive and quality control purposes.

DEVELOPMENT OF NEW TRANSMISSION COLOR SCALES FOR DARK COLORED BEVERAGES. B.A. EAGERMAN, F.M. CLYDESDALE & F.J. FRANCIS. *J. Food Sci.* 38, 1056–1059 (1973)–Color scales for transmission measurements on clear dark solutions, which are useful for relating instrumental measurements to pigment concentration were developed. The logic and mathematical approaches used in developing these scales is discussed. The scales proposed correlate sufficiently well with pigment concentrations to be used for predictive or quality control purposes. A different formula is necessary for each different colored system to achieve the desired level of accuracy. The significance of this work is that it shows the feasibility of developing chromaticity scales which are linear with concentration. This approach differs from the use of broad band filters in absorptimetry in that most of the visible spectrum is used. If the new scales are not suitable for tristimulus colorimetry, the same instrument could be programmed for both the new scales, and the conventional ones.

FACTORS INFLUENCING COLOR DEGRADATION IN CONCORD GRAPE JUICE. C. SKALSKI & W.A. SISTRUNK. *J. Food Sci.* 38, 1060–1062 (1973)–The total anthocyanins (T Acy) of Concord grape juice decreased significantly during 5 hr in a water bath at 43° and 75°C regardless of other treatments imposed. The addition of polyphenoloxidase (PPO) to the pasteurized grape juice resulted in a higher rate of loss of T Acy than other treatments. Oxygen is required for both PPO degradation of pigments and ascorbic acid (AA) oxidation. Cysteine and AA were effective inhibitors of T Acy degradation in grape juice although AA was readily oxidized under the conditions of this study. Glycine did not affect T Acy at 43°C but at 75°C there was a greater loss where glycine was added. The concentration of buffer system and pH apparently contributed significantly to the decrease of T Acy. These could be affected by maturity of grapes as well as other field factors. Catechol and caffeic acid were found to be good substrates for PPO from grapes. Higher concentrations of catechol (0.5%) had an inhibitory effect on the PPO. Apparently, certain of the predominant pigments of grape juice were good substrates for the PPO since T Acy decreased rapidly in the presence of the enzyme. Further research is in progress on characterization of the enzyme (PPO) and the application of the information to the manufacture of pasteurized grape juice and drinks.

Escherichia coli ON PECANS: SURVIVAL UNDER VARIOUS STORAGE CONDITIONS AND DISINFECTION WITH PROPYLENE OXIDE. L.R. BEUCHAT. *J. Food Sci.* 38, 1063–1066 (1973)–The survival of *Escherichia coli* on pecans and their destruction with propylene oxide (PO) was studied. Pecan halves were immersed in water containing *E. coli*, dried to 3.47, 4.54 and 6.18% moisture, and stored at −7, 0, 14, 21, and 30°C for up to 24 wk. Inoculated inshell pecans with nutmeats containing 2.72% moisture were stored at 0°C for up to 12 wk. Analysis for Most Probable Number (MPN) of *E. coli* revealed that the organism survives longest on low-moisture pecans stored at 0°C. Death was rapid in the nutmeat and inner tissue portions but not on the shell of inshell pecans. The lethal effects of 0, 40, 400 and 800 ppm PO at 20, 30 and 37°C were evaluated under relative humidities (RHs) of 0, 32, 67 and 97% for 4- and 16-hr exposure times. Disinfecting power of PO was demonstrated to increase with elevated temperatures and times of exposure. Relative humidities of 32, 67 and 97% were clearly more effectual than 0% when disinfecting pecans contaminated with *E. coli*. Concentrations of up to 400 ppm gaseous phase PO may be required to disinfect highly contaminated pecans.

NATURAL INHIBITORS OF NITROSATION REACTIONS: THE CONCEPT OF AVAILABLE NITRITE. T.Y. FAN & S.R. TANNENBAUM. *J Food Sci.* 38, 1067–1069 (1973)–The rate of nitrosation of morpholine, a model of a weakly basic amine, proceeds more slowly in foods and in certain model systems than in buffer at the same temperature and pH. Although this effect may be partially due to destruction of nitrite by competing reactions, there also appears to be an effect of combining nitrite in a form which can be measured by the Griess reaction but which is not available for nitrosation of morpholine. This effect is shown to occur at pH 6 in milk and over a range of pH with systems containing ascorbic acid.

IMMOBILIZATION OF MICROBIAL LACTASES BY COVALENT ATTACHMENT TO POROUS GLASS. L.E. WIERZBICKI, V.H. EDWARDS & F.V. KOSIKOWSKI. *J. Food Sci.* 38, 1070–1073 (1973)–Crude lactase (β-galactosidase, EC 3.2.1.23) preparations from yeasts, molds and bacteria were immobilized by covalent binding to diazotized porous glass particles. The protein/glass ratios of the washed lactase-glass complexes were estimated semiquantitatively by total nitrogen analysis, to range from 81–125 mg protein/g glass. Each immobilized lactase preparation was stored at 5°C; the lactase activity was periodically measured by determination of lactase hydrolysis in acid whey or skim milk after 5 hr contact of the immobilized lactase with fresh substrate adjusted to the optimum pH and temperature of the crude lactase preparations. The specific activites of immobilized lactases were usually at least several-fold less than the corresponding crude preparations, depending on the enzyme source and the glass carrier. Two types of porous 96% silica glass particles were studied: 200/400 mesh particles with mean pore diameters of 35.5 nm and 120/200 mesh particles with mean pore diameters of 86.5 nm. Equivalent protein loadings were obtained with both types of particles, but lactases immobilized on the 86.5 nm glass showed significantly greater retention of lactase activity during storage. In the case of one *Aspergillus niger* preparation, 74% of the initial lactase activity was present after 90 days of storage with periodic assays. Subsequent long-term studies with *A. niger* preparation packed in a column through which deproteinized whey is continuously pumped have shown still higher retention of lactose-hydrolyzing activity at 55°C.

GROWTH OF TWO GENERA OF PSYCHROTROPHS ON BEEF ADIPOSE TISSUE. B.W. BERRY, G.C. SMITH & Z.L. CARPENTER. *J. Food Sci.* 38, 1074–1075 (1973)–Adipose tissue samples from 10 beef carcasses were used to study the effects of carcass location (rib vs. brisket) and storage method (vacuum package, PVC film and petri dish) on the growth of inocula of *Pseudomonas* and *Flavobacterium* after 4, 8 and 12 days of storage at 2°C. Vacuum packaged samples inoculated with *Pseudomonas* exhibited significantly ($P < 0.05$) lower counts than counterpart samples packaged in PVC film or stored in petri dishes. *Flavobacterium* counts were significantly ($P < 0.05$) higher on brisket than on rib samples after each of the three storage periods. *Pseudomonas* counts were higher ($P < 0.05$) than *Flavobacterium* counts on both brisket and rib samples after all three storage intervals. These data suggest that both *Pseudomonas* and *Flavobacterium* can grow under the microaerophilic conditions which exist in vacuum packages. Swab samples obtained from the wholesale rib region should provide an accurate estimate of *Pseudomonas* growth on both moist and dry areas of the beef carcass, but may underestimate the extent of *Flavobacterium* growth on the brisket.

EFFECT OF ELECTRONIC, CONVECTION AND CONVENTIONAL OVEN ROASTING ON THE ACCEPTABILITY OF PORK LOIN ROASTS. D. DEETHARDT, W. COSTELLO & K. C. SCHNEIDER. *J. Food Sci.* **38**, 1076–1077 (1973)–Pork loin roasts from 30 animals were oven roasted, open pan method as specified for each oven used. Little difference was noted between the conventional oven and convection oven in cooking time, cooking losses and in acceptability as evaluated by a taste panel. Roasts done in the electronic oven with and without the browning unit were cooked in much less time, with about the same total cooking loss but significantly ($p < 0.01$) more drip loss. There was no significant difference in panel evaluations of texture, tenderness, juiciness and flavor. The overall appearance of the roast from the electronic oven was not as acceptable as those from the other two methods. Animal differences influenced the objective tenderness values.

ACCELERATED PORK PROCESSING: CURED COLOR STABILITY OF HAMS. R.W. MANDIGO & G.F. KUNERT. *J. Food Sci.* **38**, 1078–1079 (1973)–One ham from each of 48 pairs was assigned to either an accelerated or conventional processing method following separation from their respective sides at a commercial slaughter plant. Conventional treatment embodied a 24-hr chilling period prior to separation of the ham from the carcass. With accelerated treatment, the ham was removed immediately following completion of the slaughter procedure. Following separation from the carcass, the hams were skinned, outside fat removed, boned and pumped on a multiple needle pumping machine. The hams were then stuffed into fibrous casings, stockinettes and steel molds, and conveyed to the smoke house. A standard smoking and cooking schedule was utilized with final internal temperatures being 68°C. Conventionally processed hams were processed 24 hr later than the accelerated hams due to carcass chilling. After processing, the hams were transported to the University of Nebraska Meat Lab. and evaluated for nitroso- and total-pigment content under darkened refrigerated conditions (1.7°C) as well as color. Significant color fading due to time of exposure to 200 ft-c of light was shown in all studies. The greatest loss of nitroso-pigment was during the first 60 min of exposure. Total-pigments were extracted to measure the efficiency of the system. A significant influence of processing day on color stability was determined. Hot processed hams had higher nitroso-pigments in four of the six periods studied; total pigments followed the same pattern. Period differences may reflect day-to-day processing variables within the packing plant and were beyond the scope of the study. No apparent differences in color stability were attributable to the accelerated and conventional processing differences.

USE OF CHEMICAL COMPOUNDS AND A ROSEMARY SPICE EXTRACT IN QUALITY MAINTENANCE OF DEBONED POULTRY MEAT. J.H. MAC NEIL, P.S. DIMICK & M.G. MAST. *J. Food Sci.* **38**, 1080–1081 (1973)–The quality maintenance characteristics of several compounds–a rosemary spice extractive (RSE) at 0.01% and 0.05%, BHA + citric acid, and polyphosphates–were evaluated in use with simulated mechanically deboned poultry meat. Use of these compounds resulted in lower TBA values than in untreated samples, with RSE 0.05% and BHA + citric acid proving the most effective. Meat containing these additives also maintained lower total plate counts than untreated meat for up to 1 wk at 3°C. Meat containing RSE 0.01% was judged by sensory panelists to have a significantly ($P < 0.01$) better flavor than other treatments, including the untreated meat. Results from this study confirm similar tests conducted under industrial conditions with mechanically deboned poultry meat.

MOISTURE AND CALORIC CONTENT OF MALE TURKEY BREAST AND THIGH. R.J. PAULSON & W.W. MARION. *J. Food Sci.* **38**, 1082–1083 (1973)–Research was conducted to determine the heat of combustion for turkey breast and thigh, raw and cooked, with and without natural proportions of skin and subcutaneous lipid. Results show treatment (cooking), method of preparation and removal of skin and obvious deposited fat significantly influenced caloric and moisture contents. Moisture values were lowered by cooking thus increasing caloric values. Skin and deposited fat removal increased moisture values and decreased relative caloric values of the samples. Results generally confirm that caloric content of cooked turkey falls within the range of published data but differ somewhat on raw meat.

USE OF SODIUM ASCORBATE OR ERYTHORBATE TO INHIBIT FORMATION OF N-NITROSODIMETHYLAMINE IN FRANKFURTERS. W. FIDDLER, J.W. PENSABENE, E.G. PIOTROWSKI, R.C. DOERR & A.E. WASSERMAN. *J. Food Sci.* **38**, 1084 (1973)–The effect of $NaNO_3$, sodium ascorbate (NaAsc) and sodium erythorbate (NaEry) on N-nitrosodimethylamine (DMNA) formation in frankfurters made with 150 or 1500 ppm $NaNO_2$ was investigated. $NaNO_3$, NaAsc or NaEry were added to the emulsion at their maximum permissible levels and at 10 times these amounts. The frankfurters were cooked and smoked for either 2 or 4 hr. Frankfurters prepared with 150 ppm $NaNO_2$ alone or with $NaNO_3$, NaAsc or NaEry had no DMNA. With 1500 ppm $NaNO_2$, which induced DMNA formation, $NaNO_3$ had no effect; however, use of NaAsc or NaEry prevented formation of DMNA in frankfurters processed for 2 hr or reduced the amount formed after 4-hr treatment.

THE PARTIAL RECOVERY OF NITRITE NITROGEN BY THE KJELDAHL PROCEDURE IN MEAT PRODUCTS. J.G. SEBRANEK, R.G. CASSENS & W.G. HOEKSTRA. *J. Food Sci.* **38**, 1085–1086 (1973)–Nitrite in meat products is at least partially recovered by the conventional Kjeldahl procedure but the amount recovered depends on the amount present. Low levels of nitrite gave 80–90% recovery and high levels gave 25–30% recovery.

ULTRAVIOLET SPECTROPHOTOMETRIC DETERMINATION OF PROTEIN IN MEAT AND MEAT PRODUCTS. H.C. BEAVERS, H.W. OCKERMAN, V.R. CAHILL, N.A. PARRETT & R.J. BORTON. *J. Food Sci.* **38**, 1087–1088 (1973)–The spectrophotometric method of rapidly estimating the protein content of corned beef at a wavelength of 243 nm (Toma and Nakai, JFS 36:507) was modified and tested for fresh and cooked meat and meat products. The solvent system 8M urea in 0.86M sodium chloride was superior to all others evaluated. Highly significant positive correlations of 0.80, 0.90 and 0.89 were obtained for cooked beef at 280 nm, cured pork at 280 nm and cooked pork at 270 nm, respectively. Results of commercial products analyses revealed that a standard curve for calculation of protein for each meat product must be drawn separately.

PHENOLASES AND BLUE DISCOLORATION IN WHOLE COOKED DUNGENESS CRAB (Cancer magister). J.K. BABBITT, D.K. LAW & D.L. CRAWFORD. *J. Food Sci.* **38**, 1089–1090 (1973)–Blue discoloration of whole cooked crab was found to be related to the condition of the crab, handling and holding during processing, and under-cooking. Enzymes capable of oxidizing tyrosine, dopa and pyrocatechol to colored chromophores were isolated from crab. Antioxidants and chelating agents prevented the enzymatic oxidation of dopa and pyrocatechol. Heat denaturation of the phenolases did not prevent the oxidation of dopa or pyrocatechol to melanins, particularly under alkaline conditions and in the presence of metals (copper and iron). The rapid increase in phenolic content and pH in fresh crab held at 2°C suggests that the blue discoloration may be the result of the oxidation of polyphenols to melanins.

NUTRITIONAL STUDIES ON SOYBEAN CURD PRODUCED BY CALCIUM SULFATE PRECIPITATION OF SOYBEAN MILK. D.J. SCHRODER, J.I. ELLIOT & H. JACKSON. *J. Food Sci.* **38**, 1091–1092 (1973)–In view of the considerable variation in nutritive value that can result from processing, an evaluation was made of the soybean curd produced by calcium sulfate precipitation of heated soybean milk. Rats were fed a control diet containing casein as the sole source of protein and a test diet containing soybean curd as the sole source of protein. With either diet males consumed more feed, gained weight faster and were more efficient than females. All differences attributable to sex were significant ($P < 0.05$). The test diet meets all amino acid requirements for rats except methionine; however, the cost of supplementation with methionine would be very small.

UTILIZATION OF PLASTIC BAGS FOR CONCENTRATION OF DILUTE SOLUTIONS IN CONVENTIONAL FREEZE DRYERS. D. BERKOWITZ, S.J. BISHOV & L.R. FREEMAN. *J. Food Sci.* **38**, 1093 (1973)–The feasibility of using high density polyethylene

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bags for concentration of dilute solutions in conventional freeze dryers was investigated. A technique using bags and a simple suspension system was developed. The procedure was used routinely for a variety of extracts and proved fully satisfactory while providing a significant savings in time.

EFFECT OF pH ON THE THERMAL DESTRUCTION KINETICS OF PATULIN IN AQUEOUS SOLUTION. J. LOVETT & J.T. PEELER. *J. Food Sci.* 38, 1094–1095 (1973)–Patulin in McIlvaine's buffers of pH 3.5, 4.5 and 5.5 was heated at 105, 110, 115, 120 and 125°C. Ethyl acetate extracts of heated solutions were assayed for patulin remaining by TLC. Thermal destruction parameters (D and z values) were estimated to determine the effect of pH on thermal destruction kinetics. The classical thermodynamic estimates (ΔH, ΔF, ΔS and E_{exp}) were also made from the data obtained. These data show patulin to be resistant to thermal destruction at all pH's from 3.5 to 5.5. Both D and z values increased, however, as the pH decreased. Destruction times at all temperatures were greater for pH 3.5 than for 5.5, i.e., patulin is more stable in more acid solutions.

A CONTAMINANT IN N-NITROSODIMETHYLAMINE CONFIRMATION BY HIGH RESOLUTION MASS SPECTROMETRY. C.J. DOOLEY, A.E. WASSERMAN & S. OSMAN. *J. Food Sci.* 38, 1096 (1973)–The carcinogenicity of N-nitrosamines poses a potential health hazard and therefore its presence in foods must be carefully validated and evaluated. Current indications are that N-nitrosamines, when found in foods, are present only in the μg/kg range, requiring precise methods for recovery and identification. The paper reports the possibility of confusing the identification of N-nitrosodimethylamine with the ^{13}C and ^{29}Si isotopes of trimethylsilyl ion when using high resolution MS and suggests that false positive identification of DMNA could possibly be avoided by one or more of the following: (1) Utilizing GC column coatings other than Carbowax M to achieve separation of DMNA from the silicon-containing compound; (2) Use the alkali flame ionization detector to differentiate N-containing compounds; (3) Low resolution MS analysis of fairly pure preparations to obtain the entire fragmentation pattern; and (4) Monitor two specific ion peaks for DMNA in the high resolution mode.

WATER ACTIVITY DETERMINATION IN FOODS IN THE RANGE 0.80 TO 0.99. H.M. FETT. *J. Food Sci.* 38, 1097–1098 (1973)– Water activities (A_ws) of foods in the range 0.80–0.99 can be accurately measured based on equilibrium moisture adsorption of reference proteins at known A_ws at 25 ± 0.1° C. Equilibrium moisture adsorptions are determined for soy isolate, sodium caseinate and/or rennet casein at 0.01–0.02 A_w intervals over the specified range, using sulfuric acid or sodium chloride solutions as A_w standards. Reference curves of percent moisture adsorption vs. A_w are plotted and used for food A_w determinations. Accuracy is comparable or better than with an electric hygrometer in 0.80–0.90 A_w range; above 0.90 accuracy is greater than with an electric hygrometer.

ENZYMATIC GLUCOSYLATION OF SOLANIDINE. S.J. JADHAV and D.K. SALUNKHE. *J. Food Sci.* 38, 1099–1100 (1973)–A catalytic conversion of solanidine and UDP-glucose-U-C^{14} to β-glucoside by the enzymatic system in a suspension of potato slices and the enzyme preparation from sprouts demonstrated the presence of β-glucosyltransferase in *Solanum tuberosum* L.

JFS APPLIED SCIENCE and ENGINEERING

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PROTEIN HYDROLYSATE FROM FISH WASTE

INTRODUCTION

SOLID WASTE from fish processing plants constitutes a significant portion of the highly nutritional raw material which comes from the sea. The annual U.S. volume of solid wastes generated is about 1.2 billion pounds (Soderquist et al., 1970) and only 50% of this waste is recovered for animal feeds. Upgrading or recovery of the edible high-grade protein from these wastes (or more appropriately expressed, "secondary raw materials") will, directly or indirectly, assist in solving the world protein shortage and in reducing pollution problems.

The proteolytic activity of enzymes on fish flesh and viscera has been investigated as a means of solubilizing and extracting fish flesh by Freeman and Hoagland (1956), McBride et al. (1961), Sen et al. (1962) and Hale (1969). Pepsin was selected for the present investigation, owing to its low optimum pH, which controls undesirable bacterial degradation of the raw material. The purpose of this work was to develop a procedure for economical yield of high-grade protein from fish waste.

EXPERIMENTAL

Materials

The entire fish waste (FW) resulting from filleting English sole (*Parophrys vetulus*) was used in the study. It was ground and stored at −30°C until used. Pepsin (1:10,000) was purchased from the Sigma Chemical Co. and used in amounts calculated on the basis of total weights of substrate and water mixtures.

Methods

FW was thawed and homogenized with different proportions of water for 2 min, heated to 37°C, and incubated for periods of 1–12 hr for each type of hydrolysis. Initially, the pH was adjusted to 2.0 with concentrated HCl and enzyme was then added. In one set of experiments pH was adjusted back to 2.0 at 1-hr infugation, the protein hydrolysate was heated to 80°C to inhibit the enzyme. It was then cooled and neutralized with 30% sodium hydroxide, filtered, concentrated to 30% total solids, and spray dried.

For the desalting experiment, the protein hydrolysate filtrate before concentration was passed through a tubular ultrafiltration membrane (UF), type HFA-200 from Abcor, Inc. at 30 psig at a flow rate of 8 gpm. For second-stage desalting, the UF concentrate was diluted with twice the amount of water, and final sample was spray dried.

Analyses

Protein (Kjeldahl nitrogen x 6.25), fat, moisture, and ash were determined according to AOAC (1970). The nutritive value of the end product was determined by feeding tests on white male rats. The diet was based on 9% protein content (AOAC, 1970). Casein control from Shefield Chemical; commercial cottonseed

intervals. After hydrolysis, the samples were centrifuged at 12,000G for 10 min. The ratio of total Kjeldahl nitrogen in the middle layer (the solubilized protein layer) to that originally contained in the total substrate-water mixture was calculated as the yield of protein. After centri-

Table 1—Protein recovery by various proteolytic agents

Proteolytic agents	Protein recovery, % pH readjusted	pH not readjusted
Natural hydrolysis	–	38.6
Hydrochloric acid	51.9	43.7
Pepsin:		
Sterilization before pH reduction	58.2	49.5
Sterilization after pH reduction	64.4	50.1
No sterilization	76.4	66.6

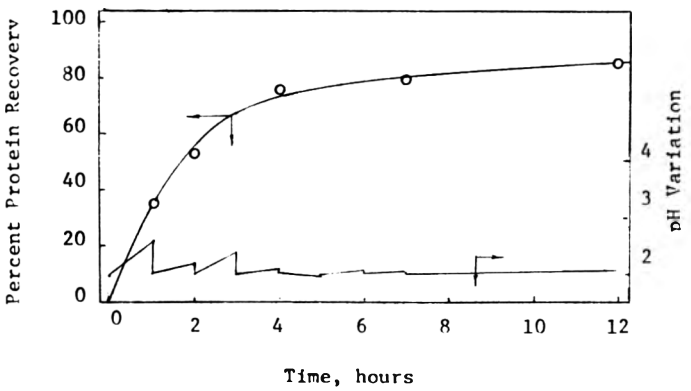

Fig. 1—Protein recovery and pH variation in FW hydrolysis by pepsin.

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Table 2—Proximate analyses and desalting by ultrafiltration

Characteristics	Fish waste	Product after hydrolysis	Final FPC & feed to ultrafiltration	Conc from ultrafiltration	After dilution feed to 2nd ultrafiltration	Conc from 2nd ultrafiltration
Total solids, %	20.6	16.6	12.8	14.9	4.8	5.4
Protein, % dry	62.6	56.5	68.0	74.0	74.0	80.0
Ash, % dry	12.0	18.7	30.5	27.0	27.0	22.0
Fat, % dry	26.2	12.7	0.7	–	–	–

Table 3—Protein efficiency ratios of protein hydrolysate only and that supplemented with amino acids

Assay group	Avg weight start (g)	Avg weight gain (g)	Avg food intake (g)	Avg protein consumed (g)	PER
ANRC reference casein	68.8	82.0	272	24.5	3.33
Protein hydrolysate diet	69.4	39.6	265	23.9	1.655
Hydrolysate plus valine, lysine, tyrosine and histidine	68.2	43.2	284	25.6	1.69
Hydrolysate plus valine, tyrosine, histidine, tryptophan and methionine	70.0	79.2	264	24.0	3.30
Hydrolysate plus casein 1:1, based on protein nitrogen content	78.4	87.8	288	25.9	3.39

oil, salt and vitamin mixture from Nutritional Biochemical Corp.; methyl cellulose from Mathison Colman; and dextrose from Merck, Inc. were obtained. 0.1% amino acid supplementation was used in some sets of feeding tests.

RESULTS & DISCUSSION

TABLE 1 shows the effect of pepsin, pH adjustment and heat treatment on the yield of protein hydrolsate. For this set of experiments, pepsin used was 0.1% (w/w) of the 1:1 (w/v) FW-water mixture. The results show that the adjustment of pH during hydrolysis, without previous sterilization, gives better yields. Figure 1 shows the protein recovery and pH variation for a 12-hr period hydrolysis, from which the optimum period of hydrolysis was chosen as 4 hr. Taking into consideration the factors like yield, cost of water removal and cost of enzyme, a 2:1 FW-water ratio and 0.2% pepsin concentration were selected as desired conditions.

The neutralization of hydrochloric acid by sodium hydroxide results in high salt content (chloride ions expressed as sodium chloride, determined by Mohr method, account for 89% of the ash content). Preliminary efforts at de-ashing by ultrafiltration are shown in Table 2. For a small increase in total solids concentration, initially protein content increased from 68 to 74% (dry basis), with some nitrogen losses of low molecular weights in the permeate. For further reduction in ash content, the concentrate from the first stage was diluted with tap water in 1:2 ratio and passed through an ultrafiltration membrane again. The final concentrate, with 80% protein and 22% ash, yielded a completely soluble, creamy white powder on spray drying. Commercially, of course, dilution will have to be avoided. Presently, the use of tighter ultrafiltration membranes and gel filtration techniques is being investigated for the purpose of desalting and characterizing the molecular weight distribution. The knowledge gained from this work will aid in optimizing the hydrolysis time period, thus minimizing the production of low molecular weight peptides, while maintaining maximum solubility.

The product obtained after spray drying of concentrated hydrolysate was creamy white, nonhygroscopic and soluble in water, with a salty and slightly bitter flavor. Low lipid content remaining in the product obviates the need for any further solvent extraction. Table 3 shows the protein efficiency ratio for protein hydrolysate, hydrolysate plus casein 1:1 mixture maintaining 9% content, and for hydrolysate supplemented with several amino acids. The hydrolysate alone showed poor nutritional quality, probably due to low level of tryptophan, demonstrated by the amino acid supplementation data presented here and by other data not presented in this paper. Poor recovery of tryptophan at optimal pH of pepsin has also been reported by Hale (1969). It is encouraging to note that when mixed with casein (and probably other proteins with excess tryptophan) the protein hydrolysate gave PER similar to that of pure casein, which should reduce the cost of total protein. Thus, it has been demonstrated that fish filleting waste can be upgraded by a simple, nonsolvent process, yielding soluble, low-fat protein for supplementation of expensive proteins, and thus reducing total cost without any loss in protein efficiency. A pilot plant utilizing this process has been constructed and will be operated to determine the commercial feasibility of the process, as well as improving the final product.

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INFLUENCE OF NITRITE AND NITRATE CURING INGREDIENTS ON QUALITY OF PACKAGED FRANKFURTERS

INTRODUCTION

THE USE OF sodium nitrite in pickling solutions to promote color fixation during the "corning" or curing of meat was authorized by the United States Bureau of Animal Industry in 1926 following investigations of Kerr et al. (1926) using sodium nitrite in place of sodium or potassium nitrate. Earlier work (Auerbach and Riess, 1919) indicated that the presence of sodium nitrite in the sodium or potassium nitrate as an impurity was responsible for the curing process. Other investigators (Lewis et al., 1925) also indicated the feasibility of using sodium nitrite in place of sodium nitrate for curing hams, bacon, tongue and beef.

In recent times, food additives have been questioned as possible potential health hazards. Since under certain acidic conditions, sodium nitrite can react with amines to form nitrosamines which have been shown to be carcinogenic in certain test animals (Magee and Barnes, 1956), the use of sodium nitrite in foods has been criticized. Investigations of various cured meat products (Fazio et al., 1971; Fiddler et al., 1971) have not confirmed the presence of nitrosamines to levels greater than 10 ppb. Recently, the presence of as much as 80 ppb N-nitrosodimethylamine has been reported to be found in commercial frankfurters in the United States (Fiddler et al., 1972). The occurrence of the N-nitrosodimethylamine appeared to be random with no adequate explanation for its formation.

The presence of nitrite in cured products also has been found to be important to deter the development of toxin by *C. botulinum* (Emodi and Lechowich, 1969; Johnson et al., 1969; Pivnick et al., 1969; Greenberg, 1972) and growth of other putrefactive organisms (Bulman and Ayres, 1952; Silliker et al., 1958).

The use of sodium nitrite in meat curing also has been reported to serve to develop a characteristic flavor. Brooks et al. (1940) studied the influence of nitrite on the curing of hams and bacon and reported the characteristic cured flavor was associated with the action of nitrite on the meat. A taste panel differentiated pork loins cured with sodium nitrite (Cho and Bratzler, 1970) from pork loins cured with the same pickle but not containing sodium nitrite. The panelists indicated those loins cured with sodium nitrite had a more cured pork flavor. Smoking did not mask this flavor difference.

Wasserman and Talley (1972) performed similar triangular taste test studies with frankfurters produced without nitrite, with 1/8 oz sodium nitrite per hundred weight of meat, and with 1/4 oz sodium nitrite per hundred weight of meat (the maximum nitrite level permissible in the U.S.A.). In this study cured product also was distinguished from product produced containing no nitrite. Smoking of the product did not interfere with the ability of the panelists to distinguish between frankfurters produced with and without nitrite.

This investigation was undertaken to determine if nitrite is necessary and the level of nitrite required to produce a frankfurter of acceptable taste quality. In addition, the importance of frankfurter formulation, nitrate, antioxidants, smoking and storage conditions were investigated.

EXPERIMENTAL

Frankfurter production

The frankfurters were prepared from a meat emulsion made using preblended lean and fat components obtained from a local packing plant. The lean and fat components were produced by grinding them through a 3/8-in. plate and mixing each in a blender. The amounts of each component used was calculated from the fat contents to produce a final frankfurter containing approximately 30% fat. This preblending procedure facilitated the addition of selected nitrite levels and other additives to a meat block of consistent composition. All meat and all beef frankfurters were prepared using customary commercial comminuting practices using the ingredients listed in Table 1. Sodium nitrite additions of 0, 1/16, 1/8 and 1/4 oz per hundred weight of meat were added to different batches of frankfurters (equivalent to 0, 39, 78 and 156 ppm, respectively, based on meat weight alone). The addition of 1/4 oz of sodium nitrite per hundred weight of meat is the maximum permitted addition in the U.S.A. (Code of Federal Regulations, 1970). Batches of these frankfurters also were prepared with additions of 0 and 3/4 oz sodium nitrate per hundred weight of meat.

The emulsion was stuffed into Size 25 NOJAX casing (Union Carbide Corp., Films-Packaging Div.) and linked to a length of 13 cm and 23 mm diameter. The frankfurters were smoked with kiln-dried maple sawdust at 80% density. They were cooked in accordance with commercial practice at 60–82°C (40% RH) for 30 min (Simon et al., 1966). The product was then held at 82°C for about 10 min until an internal temperature of 71°C was reached.

The frankfurters were either vacuum packaged in mylar/saran/polyethylene pouches or bulk packaged in VISTEN stretch bags (Union Carbide Corp., Films-Packaging Div.). Packages for storage tests were placed in a lighted commercial display case at 4.5–6.3°C. All frankfurter samples were evaluated immediately after production and for a storage period up to 4 wk. Commercial experience dictated that bulk packaged franks were unacceptable after approximately 2 wk storage. Consequently, 10 days was selected as an acceptable period for testing.

Table 1—All meat and all beef frankfurter formulations

Ingredient	All Meat	All Beef
Boneless chuck	26 lb	28 lb
Regular pork trim	24 lb	–
Beef plate	–	22 lb
Ice	13.75 lb	13.5 lb
Salt	1.5 lb	1.5 lb
Seasoning[a]	127.4g	127.4g
Sodium iso-ascorbate	12.5g	12.5g
Sodium nitrite	(0, 0.9, 1.8, 3.5g)	(0, 0.9, 1.8, 3.5g)
Sodium nitrate	(0 or 10.6g)	(0 or 10.6g)
BHA or BHT	0 or 2.2g	–

[a] Seasoning: 84.9g white pepper, 28.3g corriander and 14.2g mace.

The bulk packaged franks were visibly spoiled after 2 wk storage; the vacuum packaged franks after 4 wk storage.

Another series of tests was performed using all meat frankfurters produced as described above from the ingredients listed in Table 1 with the addition of either butylated hydroxytoluene (BHT) or butylated hydroxyanisole (BHA) at permissible levels (0.01%). These experiments were performed to determine if antioxidants would affect the taste of frankfurters produced containing no added nitrite. Comparison was made with frankfurters produced containing the maximum allowable level of sodium nitrite and 3/4 oz sodium nitrate per hundred weight of meat. In addition, frankfurters were produced using the all meat formulation smoked for 5 min using maple sawdust smoke (Simon et al., 1966). The frankfurter samples were vacuum packaged in mylar/saran/polyethylene pouches and evaluated initially and sampled weekly for a storage period up to 3 wk.

Table 2—Taste panel scores of vacuum or bulk packaged all meat frankfurters stored at 4.5°C

Total nitrite added/cwt meat (ppm)		Vacuum packaged (wk)										Bulk packaged (days)	
		Initial		1		2		3		4		10	
		With NO_3	W/O NO_3	With NO_3	W/O NO_3	With NO_3	W/O NO_3	With NO_3	W/O NO_3	With NO_3	W/O NO_3	With NO_3	W/O NO_3
0		2.6	3.3	3.5	2.6	3.6	3.1	5.0	2.3	3.9	2.0	4.0	2.0
		3.3	3.5	3.1	2.5	3.3	3.1	2.5	3.4	2.6	2.0	3.4	3.3
		2.9	1.6	2.9	2.6	3.4	3.5	2.1	3.3	2.4	2.9	2.5	2.5
	Avg	2.9	2.8	3.2	2.6	3.4	3.2	3.2	3.0	3.0	2.3	3.3	2.6
39		4.3	5.9	4.9	5.3	4.9	4.8	5.4	6.5	6.0	5.0	2.5	3.4
		5.9	5.0	5.1	4.3	4.6	3.8	3.1	2.4	3.3	3.3	4.0	6.0
		3.3	3.3	2.8	2.6	4.5	3.8	4.0	2.5	2.1	2.6	3.1	3.5
	Avg	4.5	4.7	4.3	4.1	4.7	4.1	4.2	4.7	3.8	3.6	3.2	4.3
78		5.3	6.0	5.8	5.1	5.4	6.4	4.9	5.0	5.1	5.6	4.5	4.8
		5.5	6.0	4.8	5.0	4.3	4.5	5.6	4.8	3.8	4.3	3.4	3.4
		4.0	4.8	4.4	4.8	6.3	6.0	3.4	3.4	2.4	3.0	3.5	2.9
	Avg	4.9	5.6	5.0	5.0	5.3	5.6	4.6	4.4	3.8	4.3	3.8	3.7
156		5.5	6.0	5.6	6.9	5.4	6.1	5.1	5.0	5.4	5.8	3.4	4.8
		6.0	7.0	6.3	6.4	5.6	5.1	5.1	5.0	5.1	5.8	6.4	5.6
		5.8	5.0	4.9	4.5	6.1	6.4	5.9	5.1	4.5	3.8	6.1	5.9
	Avg	5.8	6.0	5.6	5.9	5.7	5.9	5.4	5.0	5.0	5.1	5.3	5.4

Table 3—Residual nitrite and pigment conversion of all meat and all beef frankfurters

Total nitrite added/cwt meat (ppm)		All meat frankfurters		All beef frankfurters	
		Residual nitrite (ppm)	Pigment conversion (%)	Residual nitrite (ppm)	Pigment conversion (%)
0		3.6	3.2	0.8	3.5
		0.8	3.1	3.6	5.3
		2.0	4.3	1.2	5.7
	Avg	2.1	3.5	1.9	4.8
39		11.1	60.4	2.8	35.0
		11.5	75.1	7.8	57.2
		3.6	47.8	4.0	54.0
	Avg	8.7	61.2	4.9	48.7
78		18.9	66.0	18.5	61.1
		28.3	84.3	28.5	88.7
		16.6	78.1	12.0	65.0
	Avg	21.3	76.1	19.7	71.6
156		42.5	77.6	37.4	80.1
		40.5	90.6	35.0	91.7
		41.5	82.2	29.8	89.5
	Avg	41.5	83.5	34.1	87.1

Taste panel evaluation

Initial flavor quality and flavor after each storage period was determined using an eight-member taste panel. The evaluation was done using a 9-point hedonic scale in which 9 = like extremely, 5 = neither dislike nor like and 1 = dislike extremely (Amerine et al., 1965). Pieces of test frankfurters 1-in. in length were heated in a microwave oven for 60 sec and served immediately to the panelists. The taste evaluations were performed in individual booths located in a room separate from the sample preparation area. The judging area was lighted by green fluorescent light to eliminate visual color differences in lightness and darkness. The panelists were requested to judge the frankfurters on the basis of general flavor quality. Taste panel results were analyzed using analysis of variance procedures and individual means compared by Duncan's multiple range test (Steel and Torrie, 1960).

Chemical tests

The proximate composition of the frankfurters was determined using AOAC procedures for protein, moisture, fat and ash (AOAC, 1970). Residual nitrite was determined using the red complex formed between nitrites and Griess reagent (AOAC, 1970). The conversion of meat pigments to nitric oxide-heme pigments was determined using the method of Hornsey (1956).

Bacteriological testing

The aerobic bacteria populations of the sample frankfurter surfaces and cores were determined initially using trypticase soy agar with incubation at 32°C for 48 hr. Aerobic surface populations also were determined periodically for frankfurters stored in vacuum and bulk packages (APHA, 1958).

RESULTS & DISCUSSION

All meat frankfurters

The composition of the all meat frankfurters was found to be consistent from one replication to another containing about 30% fat as desired. The proximate composition determined was 11.5% protein, 30.2% fat, 54.6% moisture and 2.9% ash. The taste panel results (Table 2) indicated that for both vacuum packaged and bulk packaged product, significant differences ($p < 0.01$) in taste acceptance were associated with nitrite level, storage time and for the replicates produced on different production dates. It is not understood why differences were obtained in taste panel scores for replicate samples unless it was associated with slight quality differences in starting meat components. The inclusion of nitrate into the formulation was found not to affect taste acceptance. Flavor scores were significantly different ($p < 0.05$) between 0 and all other frankfurter nitrite levels, between 39 and 156 ppm nitrite levels, but not between 39 and 78 ppm and 78 and 156 ppm nitrite. However, a definite trend in taste panel scores was found as consistently higher taste panel acceptance was ob-

Table 4–Bacterial counts of vacuum or bulk packaged all meat frankfurters stored at 4.5°C

Total nitrite added/cwt meat (ppm)	Initial counts: Surface (log org/cm²) With NO_3	Initial counts: Surface (log org/cm²) W/O NO_3	Initial counts: Core (log org/g) With NO_3	Initial counts: Core (log org/g) W/O NO_3	Surface counts: Vacuum packaged (log org/cm²) 1 wk With NO_3	Surface counts: Vacuum packaged (log org/cm²) 1 wk W/O NO_3	Surface counts: Vacuum packaged (log org/cm²) 3 wk With NO_3	Surface counts: Vacuum packaged (log org/cm²) 3 wk W/O NO_3	Surface counts: Bulk packaged (log org/cm²) 1 wk With NO_3	Surface counts: Bulk packaged (log org/cm²) 1 wk W/O NO_3
0	0	0.48	4.11	4.11	0.30	1.00	5.70	6.30	0.30	0
	0	0	3.08	3.08	2.18	1.67	5.95	4.85	0	0
	0	0	3.00	3.30	1.52	0.30	6.18	6.00	2.00	2.00
39	0	0	4.15	3.90	0	1.04	6.00	6.00	0	2.74
	0	0	2.85	3.15	1.00	2.11	6.23	5.30	5.48	5.70
	0	4.19	3.00	3.00	1.60	1.60	6.08	6.08	3.00	2.60
78	0	0	4.08	4.18	3.48	3.48	5.90	6.00	0.78	0
	0.48	1.04	2.90	2.90	4.00	5.00	4.52	6.08	0.30	0
	0	1.11	3.00	6.08	0.30	2.08	4.36	5.90	0	3.70
156	1.79	0.30	4.04	3.90	3.00	0	6.00	5.81	1.43	2.00
	0	0	3.23	2.90	0.95	5.00	6.60	5.63	4.00	3.38
	0	0.60	3.18	3.00	0	0.48	6.00	5.25	2.48	3.60

tained as the nitrite content added initially was increased. These results compare with the triangular taste tests performed by Wasserman and Talley (1972).

The taste acceptance for both vacuum packaged and bulk packaged frankfurters decreased with time and was found not to be associated with nitrite level. These results indicated that to obtain an all meat frankfurter of acceptable quality (flavor and pigment conversion, Table 3), levels of nitrite close to the maximum permissible level were required.

Initial aerobic bacteria populations found on the surface and core of the all meat frankfurters were not associated with either nitrite or nitrate level (Table 4). The surface bacteria population of vacuum and bulk packaged frankfurters increased with storage time and may have contributed to lower flavor acceptance with storage time; however, no correlation could be established between nitrite/nitrate levels and bacteria count. Whether or not the same would have been true of core counts, remains to be indicated by another study.

Table 5–Taste panel scores of vacuum of bulk packaged all beef frankfurters stored at 4.5°C

Total nitrite added/cwt meat (ppm)		Vacuum packaged (wk) Initial With NO_3	Initial W/O NO_3	1 With NO_3	1 W/O NO_3	2 With NO_3	2 W/O NO_3	3 With NO_3	3 W/O NO_3	4 With NO_3	4 W/O NO_3	Bulk packaged (days) 10 With NO_3	10 W/O NO_3
0		6.9	6.1	5.5	5.3	5.3	5.8	4.4	4.1	2.8	3.6	4.5	5.1
		6.3	7.0	4.8	5.4	5.0	4.6	4.1	4.5	3.3	3.1	4.0	3.9
		5.5	6.8	5.3	5.3	4.0	6.3	4.0	4.0	6.0	5.3	2.3	2.8
	Avg	6.2	6.6	5.2	5.3	4.8	5.6	4.2	4.2	4.0	4.0	3.6	3.9
38		7.1	5.0	6.6	7.5	4.3	6.8	4.6	4.3	3.9	3.9	4.5	4.3
		6.4	5.5	6.3	5.9	4.9	3.6	3.9	4.3	2.9	3.0	3.3	3.6
		7.0	7.0	6.9	6.6	4.6	5.9	4.4	3.9	5.5	5.9	2.3	5.5
	Avg	6.0	5.8	6.6	6.7	4.6	5.4	4.3	4.2	4.1	4.3	3.4	4.5
78		6.1	5.9	6.9	6.8	5.0	5.1	3.4	4.1	3.5	4.3	4.1	6.1
		5.9	5.9	6.5	7.0	4.4	5.0	6.3	3.5	3.3	3.0	3.5	5.3
		6.3	6.3	6.1	7.0	5.8	5.0	5.1	4.5	4.9	5.5	4.0	4.3
	Avg	6.1	6.0	6.5	6.9	5.1	5.0	4.9	4.0	3.9	4.3	3.9	5.2
156		5.8	6.6	6.1	6.5	5.5	5.8	5.4	4.5	4.8	3.4	4.9	5.9
		5.6	6.6	7.5	5.5	6.1	5.3	4.1	5.0	2.6	3.4	5.8	5.9
		6.4	5.5	6.8	6.5	5.5	5.9	5.0	4.1	4.8	5.9	5.9	6.1
	Avg	5.9	6.2	6.8	6.1	5.7	5.7	4.8	4.5	4.1	4.3	5.5	6.0

All beef frankfurters

Frankfurters prepared from all beef components were found to contain 12.4% protein, 29.8% fat, 57.5% moisture and 2.6% ash.

On analysis of the taste panel results for both vacuum packaged and bulk packaged product, it was found that nitrite level did not affect the general flavor of the frankfurters initially or on storage (Table 5).

As observed with all meat frankfurters, the inclusion of sodium nitrate did not affect the taste acceptance significantly. Taste panel scores slowly decreased with time but were not associated with added nitrite level. The difference in taste panel scores associated with replicates indicated by analysis of variance were not significant for initial taste panel results but only significant for longer storage times.

The initial aerobic populations found on the surface and in the core of the all beef frankfurters also were found not to be affected by either nitrite or nitrate level (Table 6). As with the all meat frankfurters, surface bacteria populations of vacuum or bulk packaged frankfurters increased with storage time and also may have contributed to a lower flavor score on storage. However, no correlation could be established between bacteria counts and nitrite/nitrate level and remains to be established by another study.

Why all meat frankfurters and not all beef frankfurters produced product of unacceptable taste when either no nitrite or low levels of nitrite were added is not known at the present time. Possibly the differences were associated with the use of the pork fat component (more highly unsaturated than beef fat) in the all meat frankfurters.

Antioxidants and smoke

Since significant differences in taste quality resulted when all meat frankfur-

Table 6—Bacterial counts of vacuum or bulk packaged all beef frankfurters stored at 4.5°C

Total nitrite added/cwt meat (ppm)	Initial counts: Surface (log org/cm²) With NO_3	Initial counts: Surface (log org/cm²) W/O NO_3	Initial counts: Core (log org/g) With NO_3	Initial counts: Core (log org/g) W/O NO_3	Surface counts: Vacuum packaged (log org/cm²) 1 wk With NO_3	Surface counts: Vacuum packaged (log org/cm²) 1 wk W/O NO_3	Surface counts: Vacuum packaged (log org/cm²) 3 wk With NO_3	Surface counts: Vacuum packaged (log org/cm²) 3 wk W/O NO_3	Surface counts: Bulk packaged (log org/cm²) 1 wk With NO_3	Surface counts: Bulk packaged (log org/cm²) 1 wk W/O NO_3
0	0	0	2.30	2.48	3.38	0	6.08	4.51	3.11	1.72
	0	0	3.71	3.85	1.11	1.51	5.40	7.00	5.40	3.93
	0	0	2.30	2.90	3.00	3.90	5.72	7.00	2.00	2.00
39	0	0	2.00	2.00	2.26	1.00	2.00	5.53	0	4.70
	0	0.30	3.72	3.61	0.79	1.82	5.61	7.00	5.15	3.52
	0	0	2.60	3.18	4.08	3.78	6.11	6.90	3.86	2.00
78	0	0	2.30	2.00	3.11	0.30	6.30	5.90	2.96	1.81
	1.90	0.48	3.60	3.79	4.00	2.96	7.00	6.48	6.18	6.00
	0	0.48	2.30	2.60	2.78	2.30	6.11	5.85	2.00	2.00
156	0	0	3.00	2.00	3.57	5.00	6.30	6.30	5.00	0
	0	0	3.85	3.73	2.38	3.70	6.90	6.78	5.52	6.00
	0	0	3.26	2.48	1.15	0.78	3.00	3.00	4.78	2.00

Table 7—Taste panel scores of vacuum packaged all meat frankfurters stored at 4.5°C prepared to evaluate the effect of antioxidants

Total nitrite added/cwt meat (ppm)	Anti-oxidant	Smoke appln	Taste panel scores (wk stored) 0	1	2	3
0	No	Yes	3.4	3.6	2.0	2.5
		No	3.0	4.0	2.9	3.5
		No	3.8	2.8	3.4	2.1
0	BHA	Yes	5.3	4.6	4.0	3.3
		No	5.1	5.5	6.1	4.3
		No	6.6	4.9	4.9	4.6
0	BHT	Yes	3.8	4.4	2.8	3.3
		No	4.0	3.4	4.9	3.3
		No	4.6	3.9	3.5	4.0
156	No	Yes	6.6	7.0	6.9	6.1
		No	7.0	7.5	6.9	5.4
		No	7.8	7.3	7.5	6.8
156	BHA	Yes	5.9	7.3	6.8	6.9
		No	7.0	7.4	7.8	6.9
156	BHT	Yes	6.6	6.9	3.3	4.0
		No	6.4	4.8	5.9	4.8

ters were prepared containing no added nitrite, experiments were performed to see if these differences could be eliminated by either smoking the product or by incorporating legal limits of either BHA or BHT (0.01% by weight). The nitrite levels selected for this study were 0 and 156 ppm and all frankfurters contained permissible levels of nitrate. Examination of the taste panel results indicated that the 5 min smoke used in this study did not affect significantly the flavor of the frankfurters produced (Table 7). Only BHA of the two antioxidants studied improved significantly the flavor of uncured frankfurters. BHT produced a slight but not significant improvement in the flavor of uncured frankfurters. The flavor panel scores of the uncured frankfurters containing BHA began to decrease to an unacceptable level after about 1 wk of storage. Acceptable tasting frankfurters for the complete 3-wk storage period were obtained only when full cure was included in the formulation.

CONCLUSIONS

ALL MEAT frankfurters prepared without nitrite were scored low by a taste panel and remained low throughout the storage period up to 4 wk. Higher nitrite levels increased taste panel scores throughout the storage period but only frankfurters at higher nitrite levels had consistently good flavor. Nitrate was not found to be a significant factor in taste panel scores. The taste panel scores of all beef frankfurters were not affected by either nitrite or nitrate. Nitrite level was directly related to residual nitrite content and pigment conversion of both all meat and all beef frankfurters. The total bacteria counts obtained on the surface and core of all samples were not related to either nitrite or nitrate content. The use of BHA in uncured all meat frankfurters inproved taste panel scores but were still not as good as cured frankfurters. BHT was found to improve slightly taste acceptance of uncured frankfurters. Smoking for 5 min also did not improve significantly the flavor of uncured frankfurters.

Future investigations are planned to define the manner in which nitrite served to protect or provide the flavor of frankfurters of various formulations.

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HEAT TRANSFER, ORGANOLEPTIC QUALITY CHANGES AND MOISTURE EXCHANGE IN AIR-BLAST CHILLED POULTRY CARCASSES

INTRODUCTION

PREVIOUS STUDIES done on cooling of carcasses in an air blast were for the purpose of completely freezing the bird. Complete freezing resulted in significant loss in eating quality of the bird (Khan and Van den Berg, 1967) and bones of fryers were darkened by freezing and thawing (Brant and Stewart, 1950). Studies conducted where the birds were chilled in air at above freezing temperatures reported too long a chilling period, excessive weight loss and discoloration of the bird surface (Klose et al., 1960; Traver et al., 1956; Cook, 1939).

The present study was conducted to evaluate the requirements and merits of a process for chilling poultry carcasses using air at sub-freezing temperatures as the cooling medium. A dry-chilling process would have an advantage over presently used slush-ice chillers in that water consumption and sewage discharged is reduced and it could lead to labor savings since birds can be chilled while they are still retained on the conveying shackles and the rehanging operation after chilling can be eliminated with the appropriate modification of the weighing device now employed for sizing (Hale et al., 1973).

MATERIALS & METHODS

Chilling procedure

The crust-freezing process for chilling involved the exposure of wet birds to a cold air blast until a shell of frozen tissue was formed. The birds were then removed from the freezing medium and the interior was allowed to cool to the necessary chill temperature by conduction from the frozen layer while the birds were packed in ice.

Preliminary experiments were performed to establish the temperatures at various depths that would result in an average temperature below 40°F in the carcasses after temperature equilibration. These preliminary experiments showed that the temperature at a depth of 3/8 in. was not a reliable indicator of the final equilibration temperature. However, if the temperature at a depth of 9/16 in. was 32 to 40°F at the time cooling was stopped, an average carcass temperature below 40°F resulted after temperature equilibration.

Broilers averaging 2–2½ lb in weight were obtained from a local poultry processing plant after final washing and transported to the laboratory. There was a lag time of approximately 20 min from evisceration to the time the birds were subjected to the chilling procedure. The birds were wet by immersion in a tank of cold water to simulate wet carcasses leaving the final washer in a poultry processing plant, weighed and immediately introduced into a precooled wind tunnel. Temperature measurements were then started and when the temperature at the 9/16 in. depth reached 32–40°F, the access door to the test chamber of the wind tunnel was opened. The birds were removed, immediately transferred to a plastic tray and while they were covered with ice, the temperature was continuously monitored until a total of 30 min had elapsed from the time the birds were introduced into the tunnel. The birds were then weighed and the ice-packed birds stored in a walk-in cooler for at least 24 hr before evaluation. Each series of experiments conducted in 1 day was accompanied by a control which was obtained from the same lot at the poultry processing plant after chilling. The control was handled in the same manner as the experimental samples before evaluation.

Table 1—Treatment designation

Treatment no.	Carcass position	Tunnel air velocity (fpm)	Tunnel air temp (°F)
1	Horizontal	683	−40
2	Vertical	683	−40
3	Horizontal	683	−20
4	Vertical	683	−20
5	Horizontal	683	0
6	Vertical	683	0
7[a]	Horizontal	901	−40
8	Vertical	901	−40
9	Horizontal	901	−20
10	Vertical	901	−20
11	Horizontal	901	0
12	Vertical	901	0
13[a]	Horizontal	901	−40

[a] Treatment 13 is identical to treatment 7, except treatment 13 utilized broilers slaughtered in the laboratory.

The various treatments showing the air temperatures, velocities, and carcass positions in the tunnel are shown in Table 1. In the horizontal carcass position, air impinged against the neck and flow was parallel to the body of the bird. In the vertical position, air impinged against the breast and flow was perpendicular to the body of the bird.

Wind tunnel

A closed circuit wind tunnel was used in the experiments. The air after having passed through the test area, was returned to the blower by ductwork and recirculated. Figure 1 shows the tunnel construction. The tunnel consisted of 18 in. by 18 in. square ducts constructed out of 1/4 in. masonite painted with a moisture proof rubber-based paint. Surrounding the entire ductwork system was 3 in. thick styrofoam insulation overlaid with masonite.

The air circulating in the tunnel was cooled by liquid nitrogen which was sprayed into the tunnel through four nozzles located directly in front of the delivery side of the fan. Tunnel temperature was controlled by regulating liquid nitrogen application through solenoid valves

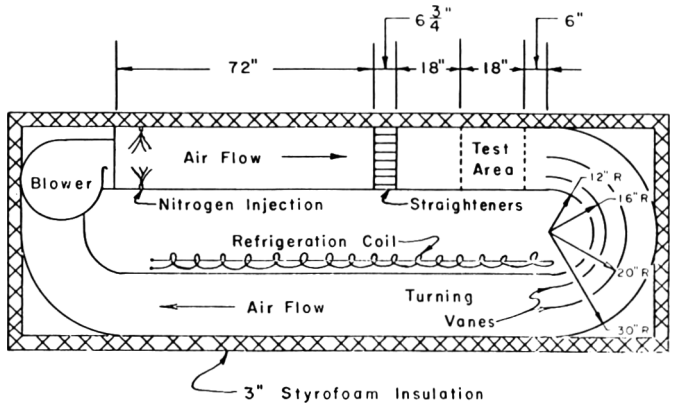

Fig. 1—Schematic diagram of wind tunnel.

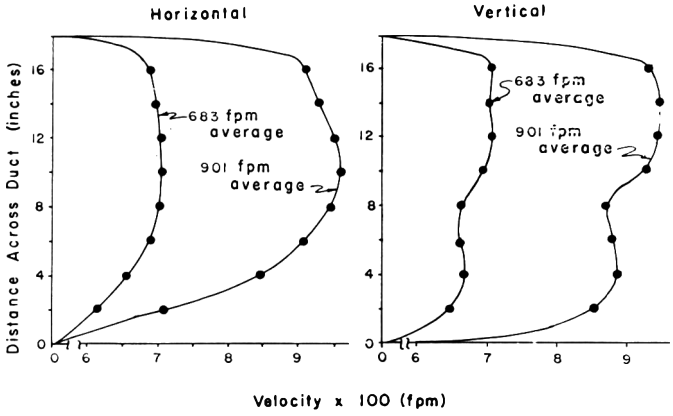

Fig. 2–Velocity profile of air flow inside wind tunnel.

activated by a Honeywell R7161B Versatronik indicating potentiometer controller. The sensing element of the controller was located at the test chamber a few inches downstream and slightly below the bird.

Air straightening vanes located downstream from the blower provided a more uniform velocity profile as the air entered the test chamber. The test chamber was 18 in. downstream of the air straightening vanes and this chamber was fitted with an 18 in. square plexiglass door for access.

A 15 in. diam by 15 in. long lightweight squirrel cage blower was used. Air velocities were adjusted by changing the speed of the blower by varying the ratios of the drive and driven pulleys. The system was constructed so as to enable operating at two speeds and the air flow through the tunnel was calibrated at each blower speed using a Hastings-Raydist Model B22-S hot wire anemometer. The velocity profiles of the air at the test chamber are shown in Figure 2. Since the duct has a square cross-section, the average velocity at a given speed was calculated by averaging all velocity readings.

Preliminary experiments on the tunnel have shown that excessive liquid nitrogen consumption occurs at the start of a run, when the liquid nitrogen has to precool the whole system. To reduce the liquid nitrogen requirements, a coil of copper tubing connected to a mechanical refrigeration system was installed at the dead space between the delivery and return ducts and this system was operated at least a day before the start of a series of experiments to precool the system.

Continuous monitoring of temperature while the birds were cooling inside the test chamber was achieved without air leaks to the outside by installing inside the tunnel female quick coupling thermocouple jacks that were connected to another set of male jacks outside. This system of quick connect thermocouple jacks enabled rapid introduction and removal of birds from the tunnel and rapid introduction into the crushed ice, while continuously monitoring the temperature.

Temperature measurement

Carcass temperatures were measured using copper-constantan thermocouples and were recorded on a Honeywell Electronik 16 potentiometer. At fixed distances away from the measuring junction, tape was wound around the thermocouple. Tape stopped penetration upon insertion of the thermocouple into the flesh and positioned the measuring junction at the desired depth. Temperatures were monitored at depths of 3/16, 3/8 and 9/16 in. The thermocouples were held against the carcasses by rubber bands to prevent them from pulling out while cooling in the tunnel, and during temperature equilibration in ice.

At the end of the equilibration period, there were differences of a few degrees between temperatures at the different thicknesses. The temperature at the 9/16 in. depth was always the highest. The arithmetic average of the three recorded temperatures was considered to be the final equilibration temperature.

The thermocouples were inserted into the breast muscle. The breast was chosen following the findings of May et al. (1961) and Connolly et al. (1954), who both showed that the breast took the longest of any part of the carcass to be cooled.

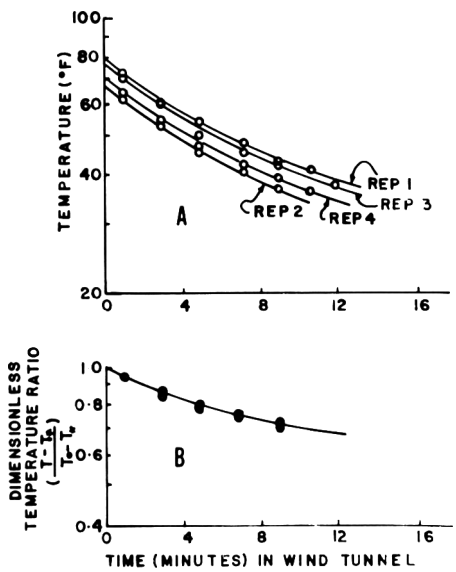

Fig. 3–Cooling curves of replicates of a treatment when carcass temperatures were plotted directly (A) and when plotted after conversion to a dimensionless ratio (B).

Evaluation of quality

24-hr ice-packed control and experimental samples were weighed, wrapped in aluminum foil and baked in an oven at 350°F until the internal temperature reached 185°F. Mickelberry and Stadelman (1960) have shown that this cooking procedure produced a more tender product than the other methods of cooking they studied. The foil wrapped cooked carcasses were allowed to cool at room temperature for about 3 hr before they were evaluated. The evaluation consisted of taste panel judgements of flavor, texture, juiciness, color and general acceptance and instrumental determinations of shear strength and expressed fluid.

Taste panelists were presented three samples at a time of which, one was the control. They were asked to rank the samples on a 7-point scale of which 1 was very desirable and 7 was very undesirable.

Since the panelists were comparing samples with a control at each judgement, the data that were analyzed for statistical differences between treatment, were the difference in scores between the treated samples and the control at each judgement, rather than the absolute scores given by the judges. Thus, a score of 0 means that the judges could not distinguish between the control and treated sample, a positive score means that the treated samples were preferred over the control and a negative score means that the treated samples were less preferred than the control.

Instrumental determination of shear strength was acheived using a Kramer shear cell mounted on an Instron Universal testing machine model 1130 equipped with a 1000-lb load cell and operated at 10 in. per minute crosshead speed. Previous investigators have found good correlations with tenderness scores and good reproducibility of the Kramer device when used on poultry meat (Dodge and Stadelman, 1959; Bele et al., 1966; Scholtyssek and Klose, 1967). Objective evaluation of juiciness was based on the observation by Bele et al. (1966), that when a constant load was applied on a given sample for a given period of time, the fluid expressed from the sample was an index of juiciness. 40-g samples from the sheared muscle after the determination of the shear strength were placed in a cylindrical cell equipped with a screen having 0.020 in. openings at the bottom and a movable piston 1.615 in. in diameter. A 1000-lb load was applied on the piston for 1 min and the expressed fluid was collected and measured.

For each bird, half of the breast muscle was diced into 1/2 in. cubes, mixed thoroughly with samples from the other replicate birds in the same treatment and served to the taste panel. From the other half of the breast was cut a 3/8 in. thick by 1-3/4 in. square sample which was individually sheared in a Kramer shear cell. The samples were cut and positioned in the shearing device such that shearing was carried out in a plane perpendicular to the muscle fibers.

RESULTS & DISCUSSION

Transient temperature curves of air blast cooled poultry carcasses

Analysis of the raw data showed that

when carcass temperature at the 9/16 in. depth was plotted against the time of exposure to the air blast (Fig. 3A), the four replicates of a treatment appeared as a family of curves which paralleled each other but were different because of different initial temperatures. Examination of the times of exposure required to achieve adequate chilling also showed wide variations between replicates in a treatment. Those carcasses showing lower initial temperatures required shorter residence times in the tunnel. Some cooling had occurred before the carcasses were introduced into the tunnel and the initial temperatures of 65 to 80°F measured were not representative of bird temperatures immediately after final washing at the poultry processing plant.

The raw data were treated in such a manner that differences in initial temperature could be taken into consideration in the analysis and the actual required exposure time to the air blast be based on a common initial temperature. When the temperatures were converted to the dimensionless temperature ratio $(T-T^*)/(T_o-T^*)$; where T is the actual temperature, T_o is the initial temperature and T^* the temperature of the air blast; and the dimensionless temperature ratio was plotted against time (Fig. 3B), points from all four replicates converged into a single curve. The basis for this treatment of the data is that solutions to the partial differential equation for unsteady state conduction heat transfer in solids (Bird et al., 1963; Kreyszig, 1963) usually assume the form:

$$\frac{T-T^*}{T_o-T^*} = F(h, x, \theta)$$

where: F is any function; h is the surface heat transfer coefficient; x is position in the solid; and θ is time. Thus, for replicates of the same treatment where the only difference is the initial temperature, the function F should be the same and for a fixed position and fixed heat transfer coefficient, data points expressed as the dimensionless temperature ratio when plotted against time should all fit in the same curve.

Figure 4 shows the dimensionless temperature plots against time for the various

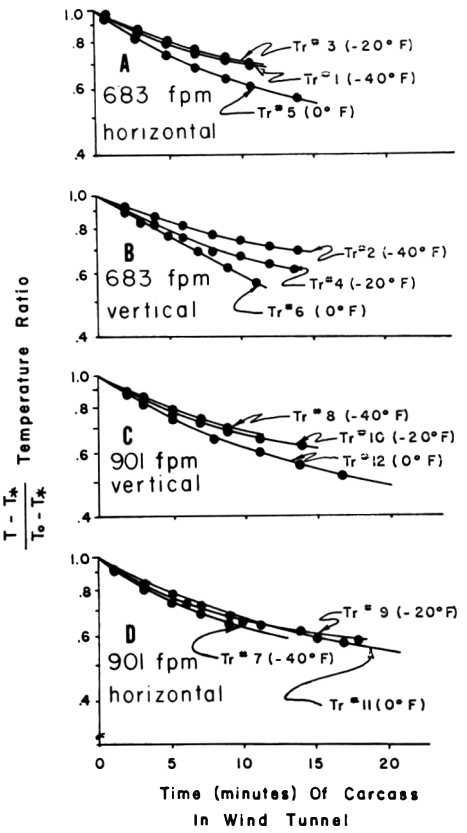

Fig. 4—Cooling curves of carcasses at different air temperatures and air flow with carcass temperature expressed as a dimensionless ratio.

treatments. If there are no differences in heat transfer rates and no change in phase, these curves should be independent of the temperature of the cooling medium. This can be seen at the 900 ft/min velocity (Fig. 4C and 4D). The curves for −20 and −40°F air temperature are very close to each other. There appears to be more differences in the extent of the change in phase between treatments with the birds in the vertical position as shown by the more divergent curves in Figure 4B.

The dimensionless temperature curves were used to correct the holding temperatures in the tunnel to take into account a uniform initial temperature of 100°F. The first recorded temperature (T) of the carcass during the chilling operation was determined and the time after introduction of the carcass into the wind tunnel when this temperature was recorded, was noted. The dimensionless temperature ratio corresponding to this temperature (T), the air temperature (T*) and an initial temperature (T_O) of 100°F was calculated. Referring to the appropriate temperature curve, the time required to achieve this temperature ratio was determined. This time was added to the difference between the actually observed holding time and the time of the first recorded temperature to obtain the adjusted holding time. Thus, if the first recorded temperature occurred at 1 min, the holding time observed was 15 min, and the time required to achieve the dimensionless temperature ratio corresponding to the first recorded temperature was 6 min, the adjusted holding time would be (15 − 1 + 6), 20 minutes.

Table 2 shows the adjusted holding times and the equilibrium temperature attained for the different treatments. Treatment 7 which was the −40°F air temperature, 900 ft/min air velocity, and the horizontal position gave the least average holding time of 12.8 min. All treatments that gave holding times less than 20 min used −40°F air temperature. At the −40°F air temperature the horizontal position required less time than the vertical position at both air speeds. However, at the 0°F and −20°F air temperature, the vertical position required less time than the horizontal position.

Moisture exchange

Moisture loss or gain during the chilling operation and the 24 hr ice-pack holding period are shown in Table 3. Moisture was absorbed in 10 out of the 12 treatments at the end of the 24 hr ice-pack holding period. During the actual chilling and temperature equilibration, moisture was gained in six of the 12 treatments.

Moisture loss or gain during the chilling and temperature equilibration process was never greater than ± 0.86%. Except for the −40°F, vertical, 680 ft/min treatment, all vertically positioned carcasses

Table 2—Average adjusted holding times in wind tunnel

	Horizontal position				Vertical position			
	901 ft/min velocity		683 ft/min velocity		901 ft/min velocity		683 ft/min velocity	
Air temp (°F)	Holding time (min)	Temp after equilibration (°F)	Holding time (min)	Temp after equilibration (°F)	Holding time (min)	Temp after equilibration (°F)	Holding time (min)	Temp after equilibration (°F)
0	35.1	34.8	25.6	36.3	25	31.5	22.8	38.5
−20	26.1	36.2	22.5	38.0	21.7	34.6	21.1	33.5
−40	12.8	38.6	17.1	37.9	15.8	34.3	21.1	37.9

lost weight during the chilling process. On the other hand, all horizontally positioned carcasses gained moisture during chilling except for the 0°F, 680 ft/min, horizontal treatment. This could be due to increased evaporation from the vertically positioned carcasses because of the larger surface area against which the air was impinging, and also due to the water draining out of the neck cavity. The moisture absorption could be due to the crust freezing of the film of water on the wet carcasses and this water was absorbed during temperature equilibration.

When both chilling and holding in ice for 24 hr are considered, except for two treatments which lost weight, all treatments gained 2.45–5.38% moisture.

Organoleptic and objective evaluation of quality

The forces required to shear samples of breast muscle from the different treatments and the control were comparable and an analysis of variance showed no significant difference. A similar result was obtained for the fluid expressed from the muscle.

Analysis of variance tables for the taste panel data shown in Table 4 show no significant differences in the response of various panelists, but showed significant differences among mean scores for juiciness, tenderness and flavor. There was no significant difference in color and general acceptance. Table 5 shows the ranking of the various treatments in taste panel preference. As previously discussed, zero indicates that the treated sample was equally preferred as the control. Negative values indicate the control was preferred over the treated samples. Although the mean scores indicate that all but four treatments were preferred over the control in juiciness, all but three treatments were preferred in tenderness and all but five treatments were preferred on flavor, only treatment 7 was statistically differ-

Table 3—Moisture exchange during chilling and holding

	Moisture gain in % of initial weight											
	Horizontal position						Vertical position					
	901 ft/min velocity			683 ft/min velocity			901 ft/min velocity			683 ft/min velocity		
Air Temp (°F)	Chilling and equilibration	24 hr ice pack	Total	Chilling and equilibration	24 hr ice pack	Total	Chilling and equilibration	24 hr ice pack	Total	Chilling and equilibration	24 hr ice pack	Total
0	0.74	2.67	3.41	−0.18	3.06	2.88	−0.85	6.23	5.38	−0.82	2.13	1.31
−20	0.39	3.69	4.08	0.03	−5.76	−5.69	−0.11	5.29	5.18	−0.86	5.07	4.21
−40	0.45	2.00	2.45	0.53	2.53	3.06	0.45	2.00	2.45	0.52	−7.28	−6.76

Table 4—Analysis of variance taste panel

		General Acceptance		Color		Tenderness		Flavor		Juiciness	
Source	DF	MS	F	MS	F	MS	F	MS	F	MS	F
Treatment	12	1.9821	1.52NS	1.6055	1.70NS	8.2811	4.69**	2.9251	2.30**	3.3962	2.29*
Panelists	13	0.7921	0.60NS	0.7185	0.76NS	1.7499	0.99NS	0.8030	0.66NS	1.1455	0.77NS
										1.4781	
Error	156	1.3031		0.9419		1.7620		1.2671			
Total	181										

* Significant at 5% level; **Significant at 1% level; NS = Nonsignificant.

Table 5—Duncan's new multiple range test, taste panel data

Juiciness*													
Treatment[a]	9	11	4	8	12	13	10	3	6	1	2	5	7
Treatment means[b]	−.5	−.321	−.250	−.035	0	.214	.214	.250	.285	.321	.678	.785	1.321
Tenderness**													
Treatment[a]	9	3	10	8	12	1	11	4	6	2	13	5	7
Treatment means[b]	−.357	−.071	−.035	0	.035	.071	.071	.535	1.107	1.250	1.357	1.428	2.071
Flavor**													
Treatment[a]	10	9	8	4	3	11	1	13	12	6	2	7	5
Treatment means[b]	−.714	−.464	−.357	−.357	−.107	0	.107	.250	.250	.321	.428	.642	.857

[a] See Table 1 for treatment designation.

[b] Any two means not underscored by the same line are significantly different; Any two means underscored by the same line are not significantly different.

* Computed on a 95% confidence level

** Computed on a 99% confidence level

ent and preferred in juiciness and tenderness. Treatment 7 (900 ft/min −40°F, horizontal) also had the least residence time in the wind tunnel and had positive moisture gains during chilling and 24 hr ice pack.

Treatment 13 was the same as treatment 7 except that the birds were slaughtered in the laboratory by hand and immediately introduced into the tunnel after evisceration. The results in Table 5 show that taste panel preference for samples from this treatment on flavor and tenderness was not statistically different from treatment 7 and was only slightly less preferred in juiciness. The effect of the time lag involved in transporting the carcass from the poultry processing plant before chilling did not appear to have a very significant effect on the organolpetic quality of the product.

CONCLUSIONS

THIS STUDY has shown that crust freezing in a cold air blast followed by equilibration of temperature by conduction from the frozen layer, is a feasible process for chilling of poultry carcasses.

Crust-frozen air-blast chilled birds were found acceptable by taste panelists based on judgements of general acceptance, color, juiciness, tenderness and flavor.

Objective measurements of shear strength and expressed juice were unable to detect differences between the experimental and control samples.

Moisture was gained (less than 1%) by broiler carcasses chilled in the horizontal position and moisture was lost (less than 1%) when chilled in the vertical position. Generally, moisture was gained during the 24 hr ice-pack holding period so that most (10 out of 12) treatments showed net moisture gains.

The choice of the air temperature for freezing and carcass positions significantly reduced holding time in the wind tunnel. The vertically positioned carcasses chilled in −40°F air at 900 ft/min velocity were found to be superior in terms of reduced holding time in the tunnel and organoleptic quality, over all the other treatments.

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USE OF ULTRAVIOLET LIGHT FOR EXTENDING THE RETAIL CASELIFE OF BEEF

INTRODUCTION

THE PHYSICAL appearance of a retail cut in the display case is the most important factor determining consumer selection of beef products (Danner, 1959; Dunsing, 1959). Jeremiah et al. (1972) reported that beef steaks ranging from pink to slightly dark red in color were most acceptable to consumers. Butler et al. (1953) determined the deleterious effect of bacteria on the color of beef steaks in the retail display case. A number of procedures have been investigated for reducing microbial contamination on cut surfaces (pretrimming, Gould, 1963; cleanliness of food handlers, Horwood and Minch, 1951; controlled sanitation in markets, Volz and Marsden, 1963) and for inhibiting microbial growth on cuts in the retail case (controlled incident heat from display lighting, Goeser, 1962; controlled display case temperatures, Roth, 1967; changes in packaging, Halleck et al., 1958). Bacterial spoilage of fresh beef at the retail level results in tremendous monetary loss to the retailer and higher prices for the consumer. Ultraviolet lamps have been extensively used in high-temperature aging of beef carcasses to control bacterial growth. The present study evaluated the use of UV light for reducing microbial growth and increasing the retail caselife of beef cuts.

EXPERIMENTAL

THREE GROUPS of beef tissue samples were selected for this study. Since bacteria grow and cause deteriorative changes on both fat and muscle, samples of adipose tissue (subcutaneous fat) and muscle tissue (longissimus muscle) from beef wholesale ribs were evaluated. Wholesale ribs were selected to be similar in color, firmness and marbling. Sample Group A consisted of 30 pieces of subcutaneous fat (surface area = 3 in.^{2}, thickness = 0.25 in.), sample Group B included 30 sections of longissimus muscle (surface area = 5 in.^{2}, thickness = 1.25 in.) and sample Group C included 36 sections of longissimus muscle (surface area = 5 in.^{2}, thickness = 1 in.). Sample Groups A and B were submerged in phosphate buffer with counterpart tissue samples (all fat samples in one solution, all muscle samples in another solution) for 45 min to equally distribute the initial bacterial load. Muscle sections in sample Group C were not subjected to the phosphate buffer bath. Upon completion of the phosphate buffer treatment, samples in Groups A and B were allowed to dry at room temperature for 15 min and then randomly assigned to one of three treatments. Muscle samples in Group C were randomly assigned to one of three treatments immediately after cutting. Since Treatment 1 served as the control, these samples were not subjected to UV light. Samples in Treatment 2 were wrapped for retail display prior to exposure to UV light (3660A maximal absorption wavelength) for 2 min at a distance of 15 cm from the exposed surface. Although the lamp utilized in these studies was maximal at 3660A, it is possible that the distribution of UV light was sufficient at 2537A to produce the effects reported in this study. Treatment 3 consisted of samples which were wrapped after exposure to UV light for 2 min at a distance of 15 cm from the exposed surface. All of the samples were wrapped in oxygen-permeable polyvinyl chloride film (Goodyear Choice-Wrap) and displayed in a retail display case at 0°C under 82 ft-c of incandescent light. Samples in Groups B and C were scored for consumer desirability at daily intervals by use of a 9-point scale (9 = extremely desirable; 1 = extremely undesirable). Samples in Group B were scored for muscle color at daily intervals by use of a 9-point scale (9 = very bright cherry red; 1 = black). Initial bacterial counts were obtained immediately prior to retail display for all of the samples. Subsequent counts were obtained for Groups A and B after 1, 2, 3 and 6 days of display and for Group C after 7 days of display. Bacterial counts were obtained by use of wet, sterile cotton swabs and sterile aluminum templates. The swabs were placed in phosphate buffer until the dilutions were made. Appropriate dilutions were made and aliquots were used to prepare pour plates on standard plate count agar. Plates were incubated at 5°C for 7 days and bacterial counts are reported as the $\log_{10}$ of the number of organisms per 2 in.^{2} of fat or muscle surface area.

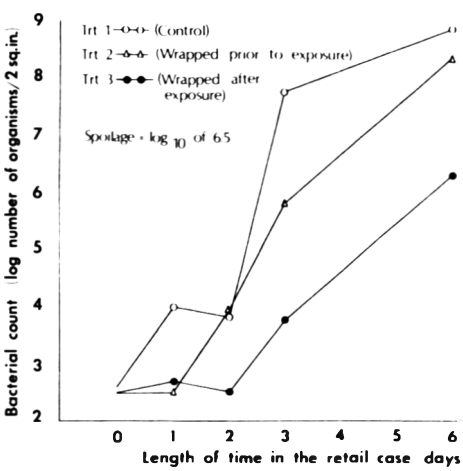

Fig. 1—Bacterial growth patterns for adipose tissue samples in Group A.

RESULTS & DISCUSSION

GROWTH PATTERNS for psychrotropic bacteria on adipose tissue samples in Group A are illustrated in Figure 1. The positive relationship between length of time in the retail case and bacterial count ($\log_{10}$) is in agreement with the findings of Reagan et al. (1971). Initial bacteria counts for samples in all three treatments were essentially equal, indicating that the use of the phosphate buffer bath treatment was effective in equally distributing the available bacteria among all samples. After 1 day in the retail case, bacteria on the control samples (Treatment 1) appeared to have entered the logarithmic phase, while those bacteria on samples in Treatments 2 and 3 were still in the lag phase. Bacteria on samples in Treatment 2 apparently entered the logarithmic growth phase between the first and second days of retail display while those bacteria on samples in Treatment 3 entered the logarithmic growth phase between the second and third days. Large differences in bacterial counts between treatment groups were evident after 3 days in the retail case, which is in agreement with Kaess and Wiedemann (1971). Bacteria on fat samples in all three treatments exhibited rapid growth during the third to sixth days of retail display, but the extent of spoilage was much greater in Treatments 1 and 2 than that observed in Treatment 3. Samples in Treatments 1 and 2 attained spoilage levels of bacteria ($\log_{10}$ of 6.5) after approximately 2½ and 4 days, respectively, while samples in Treatment 3 had not attained the designated spoilage level after 6 days of retail display. These data indicate an advantage of 3½ and 2 days for fat samples exposed to UV light prior to wrapping when com-

pared to those samples in Treatments 1 and 2, respectively.

Bacterial growth patterns for longissimus muscle samples in Group B are illustrated in Figure 2. Samples from the three treatment groups had essentially equal initial bacterial counts. After 1 day in the retail case, bacteria on samples in all three treatments appeared to be approaching the end of the lag phase. During the remainder of the retail display period, bacteria on samples in Treatment 1 remained in an accelerated logarithmic phase, while those on samples in Treatment 2 did not enter an accelerated growth phase until after the second day in the retail case. Samples exposed to UV light prior to wrapping (Treatment 3) exhibited a slower rate of bacterial growth than that observed in the other two treatments. The decreased rate of bacterial growth on samples from Treatment 3 resulted in an approximate advantage of 1½ and 2 days in retail caselife when compared with samples from Treatments 2 and 1, respectively.

Although the bacterial growth patterns on subcutaneous fat samples and longissimus muscle samples (Fig. 1 and 2, respectively) exhibited similar trends, the actual growth rates appeared to be more rapid on the fat samples. However, the relative effect of treatment with UV light appeared to be similar. These data suggest that bacterial studies can be conducted on subcutaneous fat samples and subsequently extrapolated to muscle tissue. The use of fat samples rather than muscle samples would result in substantially reduced costs.

Consumer desirability ratings for longissimus muscle samples in Group B are presented in Figure 3. Samples in all three treatments had identical ratings on the initial day of the study. During the first 3 days of retail display, samples from Treatments 1 and 2 displayed similar decreases in acceptability while samples which were wrapped after exposure to UV light (Treatment 3) did not change in consumer desirability. Samples in Treatments 1 and 2 decreased in consumer desirability at a rapid rate after the third day in the retail case and were considered unacceptable after approximately 4 and 4½ days, respectively. Throughout the caselife study, those samples in Treatment 3 maintained extremely high consumer desirability ratings and at the end of the 6-day period steaks in Treatment 3 were still "very desirable" in appearance.

Color ratings for longissimus muscle samples in Group B are presented in Figure 4. Initial color ratings were similar for all treatments; however, samples in Treatments 1 and 2 darkened at a more rapid rate than those in Treatment 3. Samples in Treatment 1 (untreated controls) and those wrapped prior to exposure to UV light (Treatment 2) exhibited undesirably dark color after approximately 4½ days in the retail case. However, steaks which were wrapped after exposure to UV light (Treatment 3) were "slightly bright cherry red" after 6 days in the retail case.

Consumer desirability ratings and bacterial counts for longissimus muscle samples in Group C are presented in Table 1. Samples in all three treatments displayed similar consumer desirability ratings during the first 3 days of retail display. Samples in Treatment 1 decreased in consumer desirability at a rapid rate from the third to seventh days of retail display. Samples in Treatment 3 exhibited the highest desirability ratings while those samples wrapped prior to exposure to UV light (Treatment 2) were intermediate in acceptability at the conclusion of the display period. These results may be partially explained by the reduction in the initial (day 0) bacterial counts associated with exposure to UV light. It is of importance to note the greater reduction in the initial bacterial counts on samples from Group C when compared with the reduction in initial counts on samples from Groups A and B. Two UV lamps were used in the present study. The maximal wavelength of both lamps was 3660Å but produced a distribution of light which included 2537Å which has been previous-

Table 1—Mean values for consumer desirability scores and bacterial counts for longissimus muscle samples in Group C.

Trait	Treatment 1	2	3
Consumer desirability score[a]			
Day 0 of retail display	9.0	8.5	8.3
Day 1 of retail display	8.4	8.5	8.3
Day 2 of retail display	8.5	8.3	8.4
Day 3 of retail display	8.0	8.1	8.1
Day 4 of retail display	7.4	8.0	8.0
Day 5 of retail display	6.1	6.8	7.6
Day 6 of retail display	5.0	6.3	7.0
Day 7 of retail display	4.3	5.6	6.6
Bacterial count[b]			
Day 0 of retail display	3.6	2.6	2.5
Day 7 of retail display	8.5	7.6	6.2

[a] Means based on a 9-point hedonic scale (9 = extremely desirable; 1 = extremely undesirable).

[b] Mean $\log_{10}$ psychrotrophic count per 2 in.^{2} of surface area.

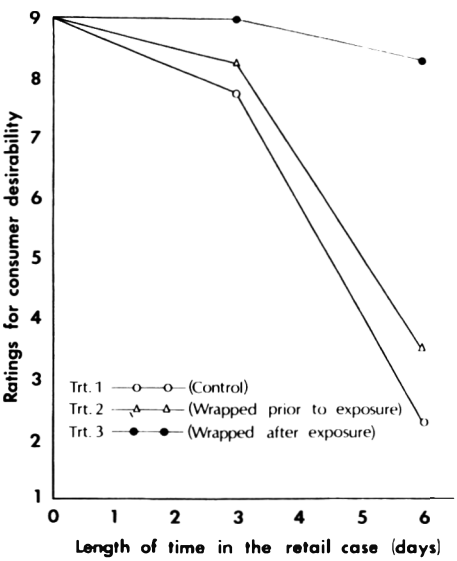

Fig. 3—Consumer desirability ratings for longissimus muscle samples in Group B.

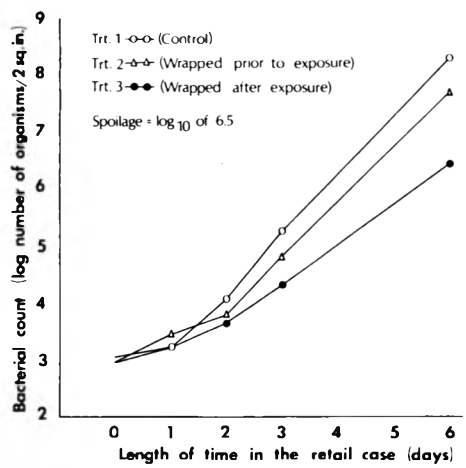

Fig. 2—Bacteria growth patterns for longissimus muscle samples in Group B.

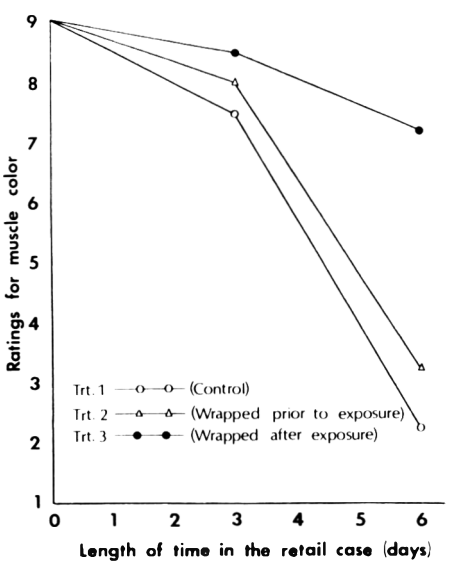

Fig. 4—Muscle color ratings for longissimus muscle samples in Group B.

ly recommended for decontamination of meat surfaces. However, the intensity of the lamp used on samples in Groups A and B was 80 microwatts/cm^2 while the light intensity of the lamp used on samples in Group C was 250 microwatts/cm^2. It appears that the difference in bacterial counts on treated vs. untreated samples is associated with light intensity as well as specific wavelength.

In summary, these data reiterate the importance of low initial bacterial counts in assuring acceptable retail caselife for fresh beef. While the importance of sanitation in preparation and handling cannot be overemphasized, significant increases in caselife may be obtained by exposure of muscle and fat surfaces to UV light. Decreases in initial count and/or attenuation of the bacteria present on retail cuts via the use of ultraviolet radiation resulted in increased consumer acceptability, higher muscle color ratings and increased caselife.

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A COMPARISON OF THE EFFECTS OF AGING, CONDITIONING AND SKELETAL RESTRAINT ON THE TENDERNESS OF MUTTON

INTRODUCTION

THERE IS a large body of evidence which shows that myofibrillar contraction state influences the toughness of meat (Herring et al., 1965a, b, 1967; Marsh and Leet, 1966; Davey et al., 1967). The extent of myofibrillar contraction postmortem can be modified by hanging carcasses in ways designed either to prevent muscles from shortening or to stretch them. Commercially the most feasible way of hanging carcasses in order to improve tenderness of a large proportion of the musculature is to hang them from the aitch bone or pelvis (Hostetler et al., 1970, 1972; Bouton and Harris, 1972a; Quarrier et al., 1972). The contraction state of muscles can also be modified by holding muscles or carcasses at 15–16°C until rigor has occurred (Locker and Hagyard, 1963; Smith et al., 1971). The aging process has been used for many years to improve the tenderness of meat but it has been shown that myofibrillar contraction state could affect the level of tenderness which could be achieved by aging (Davey et al., 1967).

The experiments reported in this paper were designed to determine which muscles on carcasses hung, pre-rigor, from the Achilles tendon, are most susceptible to the effects of cold or rigor shortening. These effects were assessed from measurements of shear force, adhesion and sarcomere length. The extent to which the deleterious effects of cold shortening could be offset by alternative methods of hanging the carcass or by conditioning the carcass at 15–16°C pre-rigor or by aging has also been investigated. A comparison of these methods of improving tenderness should enable a meat processor to select the methods most suitable to his purpose.

EXPERIMENTAL

Animals and experimental design

The term 'conditioning' has been used, throughout this paper, to describe the temperature-time treatment the muscles received while still nominally capable of shortening, i.e., pre-rigor. 2 days at 0–1°C or 1 day at 15–16°C was sufficient to allow the muscles to reach their ultimate pH (Cassens and Newbold, 1967). The temperatures chosen for conditioning were selected as likely to produce minimal (15–16°C) and maximal (0–1°C) contraction (Locker and Hagyard, 1963). Aging describes the treatment the post-rigor muscles received after completion of the initial conditioning.

In total 69 Merino X wethers, aged 3–4 yr, with a mean carcass weight of 23.9 ± 0.3 kg, were used in three separate experiments.

In Experiments 1 and 2 balanced incomplete block designs were used in which the animals were considered as blocks. The carcasses were split into sides and treatments allotted to sides according to the statistical plan for the experiment.

In Experiment 1, 45 animals (slaughtered 5 per day) were used. The hot carcasses were split and the 90 sides assigned to the 10 different conditioning and/or aging treatments listed below, according to the design of Plan 11.14 of Cochran and Cox (1957). All muscles were conditioned while still attached to the skeletal framework, viz. on the intact sides hanging from the Achilles tendon.

(1) 2 days conditioning at 0–1°C
(2) 2 days conditioning at 0–1°C followed by 7 days aging at 0–1°C
(3) 2 days conditioning at 0–1°C followed by 21 days aging at 0–1°C
(4) 1 day conditioning at 15–16°C
(5) 2 days conditioning at 15–16°C
(6) 1 day conditioning at 15–16°C followed by 7 days aging at 0–1°C
(7) 1 day conditioning at 15–16°C followed by 7 days aging at 15–16°C
(8) 1 day conditioning at 15–16°C followed by 21 days aging at 0–1°C
(9) 2 days conditioning at 15–16°C followed by 7 days aging at 0–1°C
(10) 2 days conditioning at 15–16°C followed by 14 days aging at 0–1°C

In Experiment 2, 12 wethers were used (4 slaughtered per day) and the 24 sides were assigned to 4 treatments (2 conditioning and 2 skeletal restraint) according to the design of Plan 11.1 of Cochran and Cox (1957). The whole design was replicated once. The treatments used were:

(1) 2 days conditioning at 0–1°C with the muscles still attached to the carcass, which was hung from Achilles tendon
(2) 1 day conditioning at 15–16°C with the muscles still attached to carcass, hung from the Achilles tendon
(3) 2 days conditioning at 0–1°C with the muscles dissected from the side within 1 hr of slaughter
(4) 1 day conditioning at 15–16°C with muscles dissected from the side within 1 hr of slaughter

Experiment 3 was designed to determine how successfully suspension of the carcass from the pelvis, immediately after slaughter, would counteract the cold shortening effects normally found in some muscles of carcasses hung from the Achilles tendon and conditioned at 0–1°C. 12 animals were used and the carcasses (unsplit) assigned at random, six to Achilles tendon and six to pelvic hanging treatments. All 12 carcasses were then conditioned for 2 days at 0–1°C. The animals were slaughtered four at a time.

The following muscles were used in the experiments: Mm. semimembranosus (SM); adductor femoris (A); biceps femoris (BF); semitendinosus (ST); vastus lateralis (VL); rectus femoris (RF); gluteus medius (GM); longissimus dorsi (LD); supraspinatus (SS); infraspinatus (IS); and triceps brachii (TB). The muscles were, with the exception of those used in treatments 3 and 4 in Experiment 2, dissected from the sides or carcasses after completion of the conditioning period. They were then, according to their designated treatment, either cooked immediately or vacuum packed in cryovac bags to be aged for the appropriate period of time. For Experiments 1 and 2 the three forequarter muscles (SS, IS and TB) were frozen at −40°C, after completion of their designated conditioning or aging treatments, and stored at −40°C until required. They were thawed at 5°C overnight before cooking. The IS and RF muscles were not used in Experiment 3. The LD muscles from Experiment 2 were split into two parts after completion of conditioning, one part cooked with the other muscles while the other part was sealed in cryovac and aged at 15–16°C for 7 days.

Cooking methods and pH measurements

The muscles were cooked for 90 min in polyethylene bags totally immersed in thermostatically controlled water baths at 75 ± ½°C. The method has been described fully by Bouton et al. (1971).

Measurements of pH were made at room temperature on the thoracic part of the LD muscle, removed after conditioning, using a Phillips C64/1 combined glass electrode with a Phillips PW9408 Digital pH Meter.

Objective measurements

All muscles were assessed after cooking, using a Warner Bratzler (W-B) shear device. The preparation of samples for these measurements has been described fully by Bouton et al. (1971) and Bouton and Harris (1972b, c). Sam-

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ples from each SM muscle were used for adhesion measurements as well as for W-B shear measurements.

Adhesion values were considered to be a quantitative measure of the strength of connective tissue and could be used to determine the effects of conditioning and aging on this important component of toughness (Bouton and Harris, 1972a, b, c).

Measurement of sarcomere length

Sarcomere lengths were determined on subsamples of each muscle obtained after completion of the conditioning and aging treatments and before cooking. The light diffraction method used has been described by Bouton et al. (1973).

Rate of chilling

Copper-constantan thermocouples were inserted into the center of the LD of each side at the last rib. Rates of cooling of the sides were expressed as the time required for the temperature at this location to reach 1° or 16°C. Carcass sides used in the first experiment were weighed both before and after conditioning at 0° or 15°C to determine weight loss in 24 or 48 hr.

Chiller conditions

Relative humidity (RH) was between 80 and 85% and air flow rates were 30–50m/min in the 15–16°C room and 120–160m/min in the 0–1°C room.

RESULTS & DISCUSSION

Conditioning and aging (Experiment 1)

In the sides conditioned at 0–1°C the temperature at the center of the LD reached 1°C in 383 ± 13 min and in the sides conditioned at 15–16°C the temperature at the center reached 16°C in 266 ± 16 min.

The effect of conditioning temperature on the state of myofibrillar contraction is illustrated by the mean sarcomere length values in Table 1. Aging had no significant effect on sarcomere lengths so that values for both unaged and aged samples were combined to show the effects of conditioning temperature on sarcomere length. The muscles have been, for convenience, considered in four groups, A, B, C and D. The group A muscles (SM, GM, BF, LD) have significantly shorter sarcomere lengths when conditioned at 0–1°C. Group B (A, VL) muscles showed no effect due to conditioning but sarcomere length values were less than 1.9 μm. Group C muscles (ST, RF) also showed no effect due to conditioning but sarcomere lengths were greater than 2.0 μm. The group D (TB, IS, SS) muscles were all frozen after conditioning and aging and had to be thawed before cooking. The results for the group D muscles were analysed separately.

Table 1–Mean sarcomere lengths (μm) of selected muscles from sheep carcasses initially conditioned at either 0–1°C or 15–16°C[a]

Muscle		Sarcomere lengths (μm) 0–1°C	15–16°C
SM	Group A	1.71	1.78
GM		1.61	1.70
BF		1.62	1.73
LD		1.65	1.77
A	Group B	1.78	1.78
VL		1.87	1.85
ST	Group C	2.32	2.34
RF		2.06	2.10
TB	Group D	2.01	2.22
SS		2.17	2.25
IS		1.83	2.00

[a] L.S.D. (5%) for comparing treatment effects is 0.06 for muscle groups A, B and C and 0.13 for muscle group D.

The mean W-B shear force values obtained for all of the selected muscles and for conditioning and aging treatments 1 to 6 are shown in Table 2. As none of the treatments 7, 8, 9 or 10 showed any significant improvement over treatment 6 they have been omitted from Table 2 for convenience. However, since there was no significant difference between treatment 6 and those treatments (viz 7, 8, 9 and 10) it can be stated that there is no benefit in aging mutton, conditioned at 15–16°C, for more than 7 days at 0–1°C.

The results in Table 2 show that the group A muscles conditioned at 0–1°C (treatment 1) have the highest shear force values. Aging for 7 or 21 days at 0–1°C gives significantly lower shear force values but even after 21 days aging the values are not significantly different from those obtained after conditioning at 15–16°C (treatment 4). Group B muscles are significantly tougher when conditioned at 0–1°C than when conditioned at 15–16°C. However 7 days aging at 0–1°C is sufficient to reduce this difference in toughness, related to conditioning, to nonsignificance. For the group C and D muscles, which are all restrained from large pre-rigor shortening by their attachment to the skeletal framework, there is no significant difference due to conditioning treatment. Aging of the group C and D muscles, as in treatments 2, 3 and 6 (N.B. the results from treatments 6, 7, 8, 9, 10 are not significantly different), gives equivalent shear force values for both conditioning at 0–1 and 15–16°C.

Adhesion values obtained for the SM muscles showed that muscles conditioned at 15–16°C had significantly ($P < 0.05$) lower values, i.e. 0.76 ± 0.03 than obtained for the 0–1°C conditioned sam-

Table 2–Mean shear force (kg) obtained for selected mutton muscles subjected to various post-slaughter aging and conditioning treatments[a]

Conditioning °C & time: / Aging °C & time:			Aging and conditioning treatments					
			0–1°C x 2d / 0d	0–1°C x 2d / 0–1°C x 7d	0–1°C x 2d / 0–1°C x 21d	15–16°C x 1d / 0d	15–16°C x 2d / 0d	15–16°C x 1d / 0–1°C x 7d
Muscle	SM	Group A	11.83	8.10	6.61	6.17	5.20	3.95
	GM		10.33	6.58	5.79	6.11	4.81	4.01
	BF		10.81	7.38	6.45	6.02	4.81	3.95
	LD		10.58	6.97	5.99	6.38	4.24	3.16
	A	Group B	9.22	6.86	5.15	6.24	5.49	4.00
	VL		6.40	4.02	3.41	4.86	3.75	3.00
	ST	Group C	6.42	4.56	4.47	5.81	5.15	3.86
	RF		5.24	3.68	3.02	4.93	4.47	2.75
	TB	Group D	3.90	3.38	3.61	3.95	3.72	3.70
	SS		3.45	3.56	3.34	3.59	3.31	2.91
	IS		4.15	3.93	4.22	4.13	4.49	3.77

[a] L.S.D. (5%) for comparing treatment effect is 1.27 for groups A, B and C muscles and 1.03 for the group D muscles.

ples (0.86 ± 0.03). It has been shown (Bouton and Harris, 1972a; Bouton et al., 1973) that muscles with long sarcomeres have lower adhesion values than muscles with shorter sarcomere lengths.

Conditioning thus affects fiber contraction state (as measured by sarcomere length measurements), shear force values and the adhesion between the meat fibers.

Sides conditioned at 0–1°C lost significantly less weight than sides conditioned at the higher temperature. These percentage weight losses at 0–1°C were 2.46 ± 0.08 at 24 hr and 2.86 ± 0.09 at 48 hr compared with 3.21 ± 0.09 and 4.28 ± 0.12 for 24 and 48 hr respectively at 15–16°C (least significant difference at P = 0.05 is 0.17). This increased weight loss is a distinct disadvantage of high temperature conditioning.

Measurements of pH before cooking showed that ultimate pH values lay generally in the range 5.4–5.8. The mean pH values obtained for the LD muscles cooled at 0–1°C, 5.82 ± 0.02 in the first experiment and 5.70 ± 0.02 in the second experiment, were significantly greater than those cooled at 15–16°C, 5.60 ± 0.01 for the first experiment and 5.54 ± 0.02 for the second experiment. Cassens and Newbold (1967) have reported that pieces of the sternomandibularis muscle of the ox chilled at 1°C had a significantly greater ultimate pH than when chilled at higher temperatures.

Effect of skeletal restraint (Experiment 2)

Table 3 shows the effects of skeletal restraint by comparing sarcomere lengths obtained for muscles taken from carcass sides which have been suspended from the Achilles tendon and conditioned at 0–1°C or 15–16°C, with the sarcomere lengths obtained for the corresponding muscles which have been removed pre-rigor (within 1 hr of slaughter) from the carcass and left free to contract for the same conditioning temperatures and times. The group C and D muscles which are tensioned when on the skeleton (when the carcass is hung from the Achilles tendon) contracted significantly when removed pre-rigor from the carcass but this contraction was not as severe for those conditioned at 15–16°C as for those conditioned at 0–1°C. Conditioning on the skeleton at 0–1°C generally gave shorter sarcomeres for the group A and B muscles than obtained for muscle samples, (regardless of whether they were conditioned on or off the skeleton) which were conditioned at 15–16°C. All muscles removed pre-rigor and conditioned at 0–1°C had significantly shorter sarcomeres than muscles subjected to any of the other three treatments.

The W-B shear force values obtained for muscle samples from the four different treatments are shown in Table 4. These results show that for the group C and D muscles conditioned on the carcass, and thus restrained from excessive shortening by the skeletal framework, the shear force values are the same for both 0–1°C and 15–16°C conditioning. For the group A and B muscles which are relatively free to contract, conditioning at 0–1°C produces greater contraction and hence appreciably greater shear force values than obtained for these muscles when conditioned at 15–16°C.

The results in Table 4 show that for sides conditioned at 15–16°C shear force values are unaffected by whether the muscles are attached to the skeletal framework or not. It is only at the lower temperature, 0–1°C, that those muscles not tensioned by the skeletal structure contracted. This result suggests that if, by using other hanging methods, more muscles could be stretched or prevented from shortening, then a much greater proportion of the musculature could be conditioned at any desired temperature without deleterious effects on tenderness.

The aging treatment (7 days at 15–16°C) carried out on samples of the LD from animals of the second experiment yielded the mean shear force values shown in Table 5. The 0–1°C conditioning treatments gave samples with significantly greater values than 15–16°C treatments. The samples removed pre-rigor and then conditioned at 0–1° had significantly greater values than those conditioned at 0–1°C and removed post-rigor. Aging significantly reduced the shear force values for the samples conditioned on the carcass and for the samples removed pre-rigor and conditioned at 15–16°C. The shear force values for the samples conditioned at 0–1° and then

Table 3—Mean sarcomere lengths (μm) obtained for selected muscles from sheep carcasses subjected to different post-slaughter treatments[a]

Muscles		Removed post-rigor[b] 2d 0–1°C	Removed post-rigor[b] 1d 15–16°C	Removed pre-rigor Stored 2d 0–1°C	Removed pre-rigor Stored 1d 15–16°C
SM	Group A	1.66	1.82	1.26	1.82
GM		1.62	1.72	1.27	1.81
BF		1.60	1.73	1.52	1.81
LD		1.56	1.79	1.31	1.75
A	Group B	1.78	1.78	1.51	1.79
VL		1.85	1.92	1.46	1.87
ST	Group C	2.32	2.43	1.64	1.90
RF		2.02	2.11	1.28	1.76
TB	Group D	2.09	2.12	1.29	1.82
SS		2.06	2.26	1.25	1.77
IS		1.82	1.92	1.21	1.78

[a] L.S.D. (5%) for comparing treatments is 0.14.
[b] Side hung from Achilles tendon

Table 4—Mean shear force (kg) obtained for selected mutton muscles subjected to different post-slaughter pre-rigor treatments and cooked at 75°C for 90 min[a]

Muscles		Treatments			
		Removed post-rigor 2d0–1°C	Removed post-rigor 1d 15–16°C	Removed pre-rigor Stored 2d 0–1°C	Removed pre-rigor Stored 1d 15–16°C
SM	Group A	11.67	4.97	15.03	4.11
GM		10.53	5.58	15.87	5.27
BF		10.65	5.52	13.28	5.20
LD		11.03	5.40	15.46	6.02
A	Group B	9.42	5.56	16.03	5.38
VL		7.57	4.97	13.17	5.02
ST	Group C	6.92	6.79	11.58	6.42
RF		5.86	5.18	10.26	5.63
TB	Group D	4.02	4.18	11.08	4.04
SS		3.59	3.54	5.63	3.20
IS		5.08	4.84	10.69	5.04

[a] L.S.D. (5%) for comparing treatment effects is 2.80 for groups A, B and C muscles and 1.37 for the group D muscles.

Table 5–Mean shear force (kg) and sarcomere lengths (μm) for LD muscle obtained after conditioning for 2 days at 0–1°C or 1 day at 15–16°C, and after a further 7-days' storage at 15–16°C in Cryovac[a]

Aging treatments	Treatments			
	Removed post-rigor 2d 0–1°C	Removed pre-rigor Stored 2d 0–1°C	Removed pre-rigor Stored 2d 0–1°C	Removed pre-rigor Stored 1d 15–16°C
Nonaged	10.85	6.17	15.37	5.54
7d aging 15–16°C	8.69	4.13	14.60	2.70
Sarcomere length (μm)	1.56	1.79	1.31	1.75

[a] L.S.D. (5%) for comparison of treatments is 2.61 and for aging effect 0.81.

Table 6–Mean adhesion values (kg/cm^2) and sarcomere length (μm) for SM muscles from sides subjected to various post-slaughter pre-rigor treatments[a]

	Treatments			
	Removed post-rigor 2d 0–1°C	Removed post-rigor 1d 15–16°C	Removed pre-rigor Stored 2d 0–1°C	Removed pre-rigor Stored 1d 15–16°C
Adhesion values (kg/cm^2)	0.84	0.72	1.13	0.58
Sarcomere length (μm)	1.66	1.82	1.26	1.82

[a] L.S.D. (5%) for comparing treatments is 0.21 for adhesion and 0.06 for sarcomere length values.

Table 7–Mean sarcomere length (μm) and shear force (kg) for muscles taken from sheep carcasses hung from Achilles tendon or from the pelvis immediately after slaughter for 2d at 0–1°C[a]

Muscle		Measurement			
		Sarcomere length (μm)		W-B shear force (kg)	
		Achilles tendon	Pelvis	Achilles tendon	Pelvis
SM	Group A	1.71	2.63	10.85	4.81
GM		1.71	2.48	8.29	4.47
BF		1.74	2.91	9.01	4.34
LD		1.72	1.80	10.17	6.36
A	Group B	1.81	2.78	7.76	5.24
VL		1.95	2.58	5.58	4.38
ST	Group C	2.34	2.85	6.67	7.11
TB	Group D	2.22	2.17	4.81	5.14
SS		2.05	1.98	5.22	5.14

[a] L.S.D. (5%) for comparing treatment effects on sarcomere length is 0.35 and for W-B shear force values is 2.58.

aged were still significantly higher than those obtained for the unaged 15–16°C samples. Aging had no significant effect on the shear values of the cold-shortened (pre-rigor removed) samples. This agrees with the results of Davey et al. (1967) on excised bovine sternomandibularis muscles.

Table 6 shows the adhesion values obtained for the SM muscles subjected to the designated post-slaughter pre-rigor treatments together with the sarcomere lengths which resulted from these treatments. Adhesion values were related to sarcomere length with the shortest sarcomeres having the greatest adhesion values. This agrees with results found for bovine muscle (Bouton and Harris, 1972a).

Effect of method of suspending the carcass (Experiment 3)

Table 7 shows the effects of the two different methods of suspending the carcass (the carcass being held at 0–1°C for two days) upon the mean sarcomere lengths and W-B shear force values obtained for some of the more important muscles. The results obtained show that pelvic suspension has produced much longer sarcomeres for the SM, A, ST, VL, GM and BF muscles, slightly longer for the LD and no difference for the forequarter (TB, SS) muscles. Pelvic suspension reduces the shear force values for most of the muscles studied, compared with those obtained using conventional Achilles tendon suspension. The shear force values obtained for the ST, TB and SS muscles, which had comparatively long sarcomeres for both suspension methods, were not significantly affected by the method of suspension. The results obtained for the LD muscles were not unequivocal since the sarcomeres were only slightly increased in length by pelvic suspension and the shear force values were not reduced to the same level as the SM, A, VL, GM and BF muscles.

Conditioning at 0–1°C produces cold-shortening effects which can reduce the tenderness of many of the larger muscles on the sheep carcass which are not prevented from shortening by their attachment to the skeletal framework. Subsequent aging of muscles which have shortened (because of ill-chosen processing temperatures) will partially compensate for the increased fiber toughness. However, if contraction of the muscle fibers has been too severe aging will not be effective. Pelvic hanging, however, can be used to make more of the major sheep muscles independent of processing temperatures, by restraining them from shortening.

Relationship between sarcomere length and shear force

A relationship between shear force and contraction, expressed as a percentage of the freshly excised length of the bovine sternomandibularis muscle, has been clearly demonstrated by Marsh and Leet (1966) and Davey et al. (1967). McRae et al. (1971) has shown a similar relationship with a number of lamb muscles. These authors have all used the percentage of shortening, and not a direct measurement of sarcomere length, as their criterion of myofibrillar contraction state. The mean shear force and sarcomere length results obtained for the muscle samples of Experiments 1, 2 and 3 cooked after the initial conditioning periods (and excluding the results for these samples which had been aged or frozen) are shown in Figure 1. The W-B shear force measurements have been shown (Bouton and Harris, 1972c) to be relatively insensitive to differences in connective tissue strength. Figure 1 shows that myofibrillar toughness is virtually independent of sarcomere length for muscles with sarcomere lengths greater than

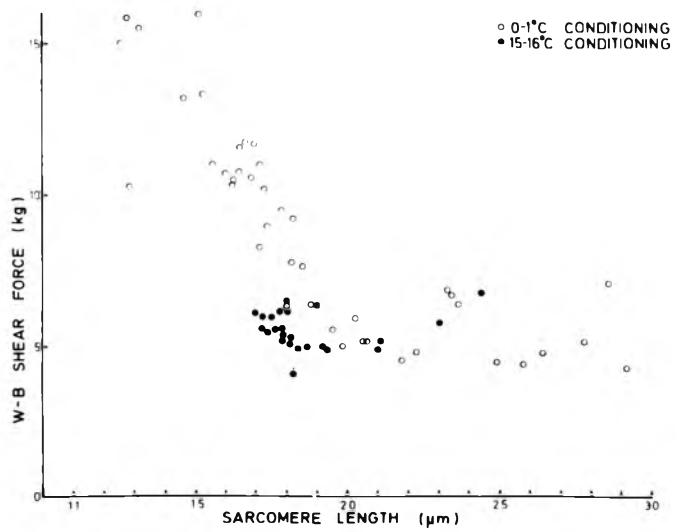

Fig. 1–Relationship between sarcomere length (μm) and shear force (kg) obtained for sheep muscles (neither aged nor frozen).

about 1.8 μm. However if only muscles with sarcomere lengths of less than 2.0 μm are considered then the relationship between shear force and sarcomere length is clearly a very good one.

Carcasses conditioned at 15–16°C have few muscles with sarcomeres much shorter than about 1.8 μm. Pelvic hanging of carcasses at 0–1°C gives, for virtually all the muscles studied, sarcomere lengths greater than 2.0 μm and shear force values are then effectively independent of myofibrillar contraction state. An exception to this is the LD muscle whose sarcomere lengths are about 1.8 μm – see Table 7 – and could show larger fluctuations in shear force.

Regression equation relating shear force with sarcomere length and temperature treatments

Figure 1 shows that shear force values decrease exponentially as sarcomere length increases. Table 2 suggests that shear force values of samples conditioned at 0–1 or 15–16°C also decrease exponentially with aging, viz either a longer period at 0–1°C or a longer period at 15–16°C. Therefore, the regression equation used to relate these variables was:

$$w=b_0+b_1\exp\left[-b_2(L-1\cdot2)-b_3T_1-b_4T_2\right]$$

where W is the Warner-Bratzler shear force in kg; L is sarcomere length in μm; T_1 is the no. of days at 0–1°C; and T_2 is the no. of days at 15–16°C.

The coefficient b_0 defines the lower limit for shear force, while the value of $b_0 + b_1$ is the shear force corresponding to a sarcomere length of 1.2 μm with no aging or conditioning. The coefficients b_2, b_3 and b_4 determine the contributions of sarcomere length, period at 0–1°C, and period at 15–16°C, respectively, to the rate of exponential decrease of shear force.

The equation was fitted to the combined data from experiments 1, 2 and 3 (a total of 882 muscles). Only the muscles common to all three experiments (and which were not subjected to a freezing and thawing treatment) were used. The least squares estimates of the coefficients and their standard errors (Table 8) were obtained using the procedure described by Nelder and Mead (1965).

The analysis of variance (Table 8) shows that the regression accounts for 76% of the total sum of squares. The sums of squares due to animals, sides, muscles, treatments and muscle-treatment interactions accounted for 87%, 93% and 82% of the total sum of squares for experiments 1, 2 and 3 respectively. Thus the above regression of the pooled data accounted for most of the variation in shear force that is due to muscles and to animals, so consequently may be used to anticipate the likely effect of various post-slaughter treatments on muscles of a given sarcomere length.

Comparison of methods

Since conditioning at 15–16°C and pelvic hanging at 0–1°C produced muscles with similar shear strength or myofibrillar toughness, the other relative merits of the methods must be considered. Conditioning at 15–16°C has the advantage that the carcasses can be hung normally, from the Achilles tendon, and abattoir handling is facilitated. However, it is necessary to keep carcasses conditioned at this temperature at a low relative humidity to reduce bacterial growth. The higher temperatures and low humidity result in a large increase in evaporative weight losses.

The altered shape of carcasses hung from the pelvis presents some problems when they are cut up but there are advantages which could counterbalance this. Connective tissue strength is lower in stretched than in contracted muscles (Bouton and Harris, 1972a; Bouton et al., 1973). The stretched muscles have a greater water-holding capacity which results in less moisture loss during subsequent aging (Bouton et al., 1972, 1973). Since a large proportion of the muscles are either stretched or restrained from shortening in carcasses hung from the pelvis, fast rates of chilling to 0–1°C can be used without causing undue toughening.

Table 8–Estimates of coefficients and analysis of variance for regression $w = b_0 + b_1 \exp[-b_2(L-1\cdot2) - b_3T_1 - b_4T_2]$

	Est. of coefficient	Std error
b_0	3.82	0.10
b_1	16.17	0.51
b_2	1.55	0.35
b_3	0.098	0.002
b_4	1.46	0.07

Analysis of variance

Source of variation	D.F.	S.S.	M.S.
Regression	4	6615.5	1653.9
Residual	877	2079.0	2.37
TOTAL	881	8694.5	

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CHEMICAL AND SENSORY EVALUATION OF IRON-FORTIFIED MILK

INTRODUCTION

IRON DEFICIENCY is usually the result of insufficient dietary intake of iron or poor utilization of iron from ingested food or a combination of both. In 1965, a U.S. Department of Agriculture survey of individual diets revealed that iron and calcium were in short supply among several population groups in the U.S.A., especially girls and women (ARS, 1969). Iron fortification of various foods such as flour, bread and cereals has been practiced as a means of correcting dietary iron deficiency. Milk is generally considered to be a rather complete food and provides a significant part of the dietary calcium but is a poor source of iron which cannot be significantly increased by oral administration of iron salts to lactating humans or animals (Henriques and Roche, 1930; Pond et al., 1965). Direct addition of iron to milk might be an effective means of increasing the dietary intake of iron to the general population. Such fortification of milk, however, might have detrimental effects on its quality and acceptability. Iron is known to catalyze the oxidation of milk lipids resulting in the development of an unpleasant odor and flavor (oxidized flavor) and may also cause loss of nutrients such as vitamin E, vitamin A and carotene. Because of the above well known effects, little research has been done concerning iron fortification of milk. The current concern over iron deficient diets is evidenced by two recent papers ralated to iron fortification of milk (Edmondson et al., 1971; Demott, 1971).

This paper reports the results of research to evaluate the feasibility of adding various forms and amounts of iron salts with regard to their effect on the sensory and certain nutritional properties of the milk.

EXPERIMENTAL

Selection of iron salts

Based on a review of literature (Harmon et al., 1967; Roberts et al., 1968) and preliminary work done in this laboratory, four iron salts were selected: ferric ammonium sulfate (FeAS), ferric ammonium citrate (FeAC), ferric choline citrate (FeCC), and ferrous sulfate (FeS). Selection was based on apparent biological availability, effect on lipid oxidation in milk and water solubility. Aqueous solutions were added directly to raw milk at the level of 10 ppm iron. The iron salt found most suitable was evaluated further at 10, 20 and 30 ppm of added iron. Iron salts found unsatisfactory in preliminary screening were ferrous ammonium sulfate, ferrous chloride, ferrous fumarate and ferrous gluconate.

Sources of milk

Milk used in this study was obtained from the University of Maryland dairy herd at College Park. The herd is predominantly Holstein but includes some Guernseys, Jerseys and Ayrshires and was barn fed on alfalfa hay, corn silage and grain concentrate.

Milk was taken directly from the holding tank immediately after milking was completed. In the preliminary screening of iron salts, milks were selected and used from individual cows on the basis of their susceptibility to oxidized flavor.

Milk processing

All samples were pasteurized by a high temperature-short time (HTST) system and homogenized using either a laboratory or commercial system (in one experiment both systems were used). The laboratory system employed glass coiled condensers for heat exchangers and a single piston electrically powered homogenizer (Erweka-Apparateban GMBH. Type Ho, Chemical & Pharmaceutical Industry Co., 260 West Broadway, New York, NY 10013). Heat treatment was 72–73°C for 15–16 sec followed by cooling to 7°C. Efficiency of pasteurization and homogenization was checked by the phosphatase test and the Farrall Index respectively. The laboratory system was cleaned by circulating detergent solution, hot water, chlorine solution, water, EDTA solution (for removal of trace metal contamination) and, finally, double distilled water.

The commercial system was located in the University dairy plant. Heat treatment was 75–76°C for 16 sec. Homogenization was at 500 and 2,000 psi. A vacuum treatment was not included.

Sample treatment

Aqueous solutions of the iron salts (10 mg Fe/ml) were added to freshly collected raw milk immediately before processing. The rate of addition was 10 ppm iron in two experiments and 10, 20 and 30 ppm in two experiments. In each experiment a control sample without added iron was initially processed. In the laboratory scale experiments 4 liters of milk were processed for each sample. For the commercial system the iron solution was added to 10 gallots of milk in stainless steel cans which were hand poured into the raw milk float tank of the operating system. Samples were packaged in 1 pint plastic coated milk containers and stored in the dark at 5°C.

Analytical

All samples were analyzed for fat by the Babcock procedure and for iron by a procedure developed in the study (Wang, 1972). The method employs bathophenanthroline (4,7-diphenyl-1,10-phenanthroline) to form a color complex with ferrous iron in an aliquot of the sample after dry ashing and reduction with thioglycolic acid. The relative standard deviation was 2.3%. Some samples were analyzed for ascorbic acid by titration with 2,4-dichlorophenolindophenol.

After 2 and 7 days storage the samples were analyzed for vitamin E, vitamin A, carotene and for oxidized flavor. Vitamin E was determined by a procedure reported by Erickson and Dunkley (1964) and modified in this laboratory (Tikriti, 1969), The procedure for vitamin A and carotene, developed in this study, is dual sprectrophotometric and employs trifluoroacetic acid as the chromogenic reagent. The relative standard deviation was 2.3% for vitamin A and 4.0% for carotene (Wang, 1972). Oxidized flavor was evaluated by a trained panel of four or more persons and by the 2-thiobarbituric acid (TBA) test (King, 1962). The sensory evaluation scale was: 0 - no oxidized flavor; 1 - questionable; 2 - slight, but definitely detectible; 3 - distinct or strong; 4 - very strong. The corresponding range of TBA optical densities is about 0.02–0.10. Correlation between the two tests based on 2752 observations was $r = 0.7$, $p<0.01$ (Oskarsson, 1968).

Consumer evaluation

The consumer panel consisted of 40 persons including men, women and children. The panelists were first presented with an identified sample of the standard (no iron added) followed by 4 or 5 samples to be evaluated against the standard, and always included an unidentified replicate of the standard. Presentation of samples was by a previously restricted random arrangement to eliminate treatment bias due to order of presentation. Evaluation was made after 7 days storage and samples were about 4°C. Each panelist recorded results on a form using five descriptive terms: better than (4), equal to (3), slightly below (2), definitely below standard in flavor (1) and unacceptable (0). The descriptive terms were converted to numbers (shown in parentheses) to allow statistical treatment of the data. Analysis of variance and the Student-Newman-Keul's test were used to detect significant differences among and between treatments.

RESULTS & DISCUSSION

THE OXIDATIVE stability of pasteurized, homogenized milk as influenced by the addition of the various iron salts is

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Table 1—Sensory and chemical evaluation of the oxidative stability of homogenized milk as influenced by the addition of various iron salts

		Storage time										
		2 days					7 days					
Trial no.	Iron salt added[a]	Flavor score[b]	TBA O.D.	Vitamin E	Vitamin A μg/g fat	Carotene	Flavor score[b]	TBA O.D.	Vitamin E	Vitamin A μg/g fat	Carotene	Consumer evaluation[c]
I	none	0.1	0.026	16.1	4.6	4.5	0.1	0.025	17.4	4.5	4.7	2.65[f]
	FeAS	1.3	0.050	12.9	4.4	4.5	1.1	0.055	11.6	3.9	3.8	1.98[g]
	FeAC	0.1	0.024	16.8	4.5	4.7	0.1	0.025	16.8	4.6	4.8	2.88[f]
	FeCC	0.3	0.035	14.2	4.4	4.7	0.6	0.042	12.9	4.2	4.2	2.15[g]
	FeS	3.0	0.092	9.0	3.7	3.6	3.6	0.089	6.5	3.5	3.0	0.93[h]
II	none	0	0.024	19.3	5.0	4.9	0	0.026	18.7	4.9	4.9	2.90[g]
	FeAS	1.2	0.046	16.1	4.9	4.8	3.3	0.058	12.9	4.2	4.3	1.08[f]
	FeAC	0.1	0.023	18.7	5.0	4.8	0	0.025	19.3	5.0	4.9	2.93[g]
	FeCC	1.6	0.048	14.8	4.8	4.5	3.3	0.065	11.6	4.0	4.1	1.23[f]

[a] Equivalent of 10 ppm iron. Control milks I, II contained 0.97 and 0.94 ppm iron respectively. Milks were processed by laboratory scale system and stored at 5°C. FeAS (Ferric ammonium sulfate); FeAC (Ferric ammonium citrate); FeCC (Ferric choline citrate); FeS (Ferrous sulfate).
[b] Mean flavor score of four or more trained panelists. Samples were evaluated for oxidized flavor on a scale of 0 (no oxidized flavor) to 4 (very strong).
[c] Mean score of 40 panelists. Increasing score indicates better flavor. Mean scores with common letter are not significantly different by Student-Newman-Keul's test of treatment means.

Table 2—Effect of processing system and various concentrations of ferric ammonium citrate on the oxidative stability of homogenized milk

		Storage time										
		2 days					7 days					
Processing system	Fe added ppm[a]	Flavor score[b]	TBA O.D.	Vitamin E	Vitamin A μg/g fat	Carotene	Flavor score[b]	TBA O.D.	Vitamin E	Vitamin A μg/g fat	Carotene	Consumer evaluation[c]
Laboratory	0	0	0.026	17.4	4.5	4.2	0	0.028	17.4	4.5	4.3	–
	10	0	0.027	16.8	4.5	4.3	0	0.026	17.4	4.5	4.3	–
	20	1.4	0.039	15.5	4.3	4.1	1.9	0.037	14.8	4.1	3.8	–
	30	2.1	0.068	12.3	3.8	3.7	2.6	0.059	10.3	3.5	3.3	–
Commercial	0	0	0.023	23.2	4.6	3.7	0	0.020	22.6	4.6	3.7	2.88
	10	0	0.023	23.2	4.7	3.8	0	0.023	22.6	4.6	3.7	2.98
	20	0.2	0.023	24.2	4.7	3.7	0	0.025	23.2	4.6	3.7	2.78
	30	0.4	0.028	21.6	4.6	3.7	0	0.026	20.0	4.5	3.6	2.80

[a] Laboratory and commercially processed milk contained 0.96 and 0.92 ppm iron respectively before fortification.
[b] Mean flavor score of four or more trained panelists. Samples were evaluated for oxidized flavor on a scale of 0 (no oxidized flavor) to 4 (very strong).
[c] Average score of 40 persons on the panel. There was no significant difference among the treatments.

shown in Table 1. Both the TBA evaluation and the average flavor score of the expert panel indicate that FeAC fortified milk was as stable as the control milk. FeS fortified milk was the most unstable and developed strong oxidized flavor after 2 days storage. FeS was not included in Trial II to eliminate its possible influence on the flavor panel and to allow a more critical evaluation of the other three iron salts. These results, also shown in Table 1, clearly indicate that both FeAS and FeCC fortification resulted in detectable oxidized flavor after 7 days storage. Again, the FeAC fortified milk was as stable as the control. Analyses for vitamin E, vitamin A and carotene complimented the flavor evaluations and clearly demonstrate that milk undergoing lipid oxidation also suffers losses of these essential nutrients. The greater losses of vitamin E probably reflect its role as an antioxidant in milk (King et al., 1966; and King, 1968) and may also serve to stabilize vitamin A and carotene as reported by Stuckey and Gearhart (1957). The consumer evaluation showed a highly significant difference among treatments ($p < 0.01$). There was no statistically significant difference in flavor between the control and FeAC fortified milk. The FeS fortified milk was highly significantly different ($p < 0.01$) than the other milks in the group and was considered to be unacceptable. In Trial II the control and FeAC milks were again evaluated as equivalent by the panel. There was no significant difference between the FeCC and FeAS fortified milks but they were clearly less acceptable than the FeAC fortified sample.

The results shown in Table 1 demonstrate that milk can be fortified with 10 ppm of iron as FeAC without impairing its consumer acceptability or certain nutrient properties. A useful product ought to provide a substantial part, if not all, of the daily requirement in a reasonable quantity of milk. The above level was adopted as a minimum goal and represents about 10 times the normal iron content of milk. The effect of increasing the level of iron and a comparison of the laboratory and commerical processing systems are shown in Table 2. Among the samples processed in the laboratory, again the level of 10 ppm added iron resulted in no detectable deterioration, but samples with 20 and 30 ppm were oxidized to a degree related to the quantity of added iron. Again, the chemical determinations complimented the flavor evaluations. When milks with these same levels of added iron were processed in a commercial system even the 30 ppm level was stable after 7 days storage; however, the sample containing 30 ppm iron showed a slight decrease in vitamin E. Only the commercially processed samples were evaluated by the consumer panel and there was no statistically significant difference among treatments. All three levels of FeAC fortified milk were as acceptable to the consumer as the control.

Results presented in this paper are perhaps inconsistent with respect to the

well-known oxidative instability of the lipid system in fluid milk. Homogenization is the most effective treatment currently employed to control oxidized flavor in commercial fluid milk and probably influenced the results presented in Table 2 comparing the laboratory and commercial processed samples. The commercial system included a multiple piston, two stage, high pressure machine as opposed to the single piston laboratory model. The higher temperature (75° vs. 72°) of the commercial system may have also contributed to the greater stability of these milks. In a recent report by Demott (1971), the iron-fortified milks were not homogenized, only pasteurized, and all of them showed development of off flavor. The flavor was reported as astringent, nct oxidized. Nonhomogenized fluid milk has essentially been eliminated as a commercial product due in large part to the high incidence of oxidized flavor, even without known metallic contamination. Several explanations have been proposed concerning the effect of homogenization and a reorientation or restructuring of the fat globule membrane probably influencing the association between metal catalysts and unsaturated lipids seems most plausible (Smith and Dunkley, 1962).

Processing factors do not alone explain the results here presented. Some of the iron salts did cause lipid oxidation. Ferrous salts have long been known to be more detrimental than ferric (Mulder et al., 1949; Swanson and Sommer, 1940). Demott (1971) used several ferrous salts and these milks were all more pronounced in off flavor than those containing ferric salts. Edmondson et al. (1971) reported that FeS addition at 10 mg/qt resulted in oxidized flavor, whereas FeAC containing milks developed rancid flavors, but not oxidized. Rancid flavors were not detected in the present study even though three times as much FeAC was used as compared to the Edmondson report. In neither of the studies referred to above were parameters other than sensory evaluation employed.

Ascorbic acid is known to be involved in milk lipid oxidation and if rapidly oxidized and the dehydroascorbic acid destroyed by heat treatment the milk becomes stable. The large addition of iron salts to raw milk might have this effect; however, analyses for ascorbic acid indicated that this mechanism was not involved.

The stability of the FeAC fortified milks must at least partially concern the structure of the iron salt itself. Perhaps the FeAC remains largely undissociated or is readily complexed to some component in milk thus inhibiting its lipid oxidative activity. The assumed association may be enhanced by heat treatment and homogenization. It has been demonstrated by Arrington and Krienke (1954) and King and Dunkley (1959) that metal chelators added to milk inhibit metal induced lipid oxidation.

Results presented in this paper demonstrate the FeAC is suitable for preparing an iron fortified milk by direct addition of an aqueous solution to raw milk followed by normal processing. After 7 days storage the iron fortified milks were as acceptable to consumers as the nonfortified control sample and the contents of vitamin E, vitamin A and carotene were not sacrificed. A normal serving would provide a substantial part or all of the RDA for iron in the diet. The biological availability of the iron is reported separately (Wang and King, 1973).

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ASSIMILATION OF IRON FROM IRON-FORTIFIED MILK BY BABY PIGS

INTRODUCTION

ENRICHMENT of food with iron to prevent or decrease the incidence of iron deficiency anemia has been of particular interest since the publication of results of the nutrition survey conducted by the U.S. Department of Agriculture (ARS, 1969). Selection of a food for enrichment should consider: the availability and usage pattern of the food; the compatability of the added iron with general or anticipated properties of the food; and the biological availability of the iron to the person or animal consuming the food.

Milk has been frequently suggested as a vehicle for introducing additional iron into the diet because of its general availability and rather routine inclusion in the American diet. However, because of the well known ability of heavy metals, especially iron and copper, to induce lipid oxidation in milk such proposals have been generally discouraged. While considerable research has been directed toward eliminating the effects of metal contamination only recently has the purposeful addition of iron to milk been investigated. Edmondson et al. (1971) reported that enrichment of milk with ferrous sulfate and ferric ammonium citrate resulted in off-flavors but concluded that the problem could be controlled by modifying processing techniques. The biological availability of the iron was not evaluated. Demott (1971) fortified milk with several iron salts all of which caused off-flavors, but ferrous salts were most detrimental. Absorption from two of the enriched milks was evaluated by rat feeding studies. Wang and King (1973) studied the feasibility of enriching milk with iron and reported that 30 ppm could be added in the form of ferric ammonium citrate without impairing certain nutritional and sensory properties.

The biological availability of iron in food is influenced by many factors including the oxidation state of the iron, type of iron compound, food to which iron is added, other foods in the diet, and physiological condition of the animal. Ferrous salts are better utilized than ferric presumably because the latter must be reduced before absorption can occur (Brown, 1963; Fritze et al., 1970). Fritze (1970) found that ferric ammonium citrate and ferric choline citrate were better absorbed than ferrous sulfate by the chick. Steinkamp et al. (1955) reported that iron absorption from enriched bread was the same for several forms and oxidation states of iron. Chodos et al. (1957) found natural iron in food utilized less than iron salts and that absorption was better from animal than vegetable foods (15–20 vs. 5–10%). Iron uptake from eggs was poor and their presence in the diet interfered with utilization from other sources (Elwood, 1968; Narula and Wadsworth, 1968). Schulz and Smith (1958) reported that natural and added iron in milk were equally absorbed by infants–about 10%. Fritze (1970) found the availability from ferrous sulfate not influenced by either skim or evaporated milk to anemic chicks. Del Mundo et al. (1964) reported increased iron absorption by infants fed an iron-fortified milk formula; however, Owen and Fomon (1963) reported no difference between the control and milk-iron supplemented groups using the same milk formula. Demott (1971) found greater body weight gains and hematocrit values in rats supplemented with ferrous sulfate in a dry ration than in groups fed iron-fortified milks.

The purpose of this study was to evaluate the apparent nutritional value of iron as ferric ammonium citrate (FeAC) in pasteurized, homogenized milk. The rate of absorption, distribution and excretion of iron was determined in physiologically normal baby pigs. The iron-fortified-modified-milk ration was labeled with ^{59}Fe ferric ammonium citrate and utilization was evaluated by three different techniques.

EXPERIMENTAL

Animal selection and management

Baby pigs were selected as test animals for this study because their circulatory and digestive systems more closely represent those of humans than do some of the more conventional research species. Also, suckling pigs have a substantial iron requirement and the modified milk ration closely approximated their normal diet. The intent was to evaluate iron utilization under "normal" physiological conditions, thus, no effort was made to develop an anemic state.

Five Hampshire-Yorkshire cross breeds from the same litter were provided by the Department of Animal Science, University of Maryland. They were 13 days old when received and had been fed only sow's milk. They were housed in individual stainless steel cages in a laboratory animal room with the temperature maintained at 29–30°C and received only the modified milk ration which was offered 4 times daily at 8 AM, 1 PM, 6 PM and 11 PM by self feeding from plastic containers. About 2 weeks were allowed for the animals to adjust to their new environment and ration. During this period ration consumption gradually increased until they were consuming 2–2.5 liter/pig/day. Feces and urine were separately collected and the cages were cleaned daily. Body weights were taken at 3-day intervals.

At the end of the adjusting period and after a 12-hr fast each animal received 20 ml of radio iron-labeled ration (50μci). The tracer dose was introduced directly into the stomach via a feeding tube. One hour after isotope administration each animal received about 50 ml of the experimental ration by self feeding. The feeding schedule established during the adjusting period was then resumed for the remainder of the experiment.

Experimental ration

The ration was based on the iron-fortified milk developed in the study and previously described (Wang and King, 1973). Whole, raw milk was standardized to 8.0% fat with 40% cream and fortified with FeAC (20 ppm iron) and vitamins (D, B_6, B_{12}, thiamine, nicotinic acid, pantothenic acid and folic acid) followed by pasteurization and homogenization in the dairy processing plant, University of Maryland. 40 gal were prepared at one time, packaged into 5 gal single service dispenser containers and stored at 5°C. Aureomycin was added to the ration at the time of feeding. The composition of the ration was based on the nutrient requirements of growing and finishing swine (Cunha et al., 1968) and the comparative composition of cow's milk (Macy et al., 1950) and sow's milk (Perrin, 1955).

The labeled dose was prepared by adding radioactive FeAC to a freshly prepared lot of fortified milk using the same source and relative amounts of components as in the experimental ration. The tracer was prepared by New England Nuclear, 575 Albany St., Boston, MA 02118 and described as follows: Fe-59 (ferric ammonium citrate) with high specific activity, 0.17 mci/mg iron; radiometric purity > 99% and no principal radioactive contaminants. It was added at the rate of 2.5 μci/ml milk (ration). The labeled ration was then processed

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Table 1—Change in body weight of baby pigs fed FeAC fortified-modified milk

Pig no.	Body weight (kg) Days 0[a]	4	14[b]	17	20	23	26	29	Avg wt gain (kg/day)
1	3.81	4.31	5.22	6.26	7.21	8.26	8.94	9.53	0.204
2	3.90	4.26	4.58	5.58	6.40	7.17	7.94	8.48	0.163
3	3.95	4.54	5.72	6.99	8.07	9.43	10.34	10.93	0.249
4	2.99	3.54	4.04	4.67	5.31	6.08	6.99	7.53	0.163
5	3.18	3.49	4.09	4.40	5.08	6.08	6.94	7.53	0.154

[a] 13 days old
[b] End of adjusting period, beginning of experimental period (labeled ration administered)

using a laboratory scale system (Wang and King, 1973). An aliquot was used to prepare a counting standard.

Sampling procedures

Blood. Samples from each animal were taken from the anterior vena cava (Carle and Bewhirst, 1942) at the beginning of the adjusting period, 90 min after administration of the tracer dose, and at 3-day intervals thereafter. The 10-ml samples were transferred directly from the syringe to heparin-treated centrifuge tubes for immediate analytical treatment.

Feces. The fecal collection pans were modified to allow continuous drainage of urine. Feces were collected on a 24-hr basis, weighed and stored under refrigeration until analyzed.

Tissue and organs. The baby pigs were sacrificed 15 days after administration of the radio iron. A plateau of radioactivity in blood had been reached by this time. The following organs and tissues were removed, placed in plastic bags, weighed and frozen: liver, heart, spleen, kidney (two), stomach, intestines (large and small), gall bladder, samples of ham and loin muscle and a sample of bone (tibia).

Analytical

The experimental ration was analyzed for fat by the Babcock procedure and for iron according to Wang (1972).

Blood was analyzed for hemoglobin by the cyanmethemoglobin method (Crosby et al., 1954), packed cell volume (hematocrit) by the micro-capillary centrifuge technique (Guest and Mier, 1934), and red blood cells (RBC) by use of a hemocytometer (American Optical Corp., Buffalo, NY 14215).

Radioactivity (gamma radiation) was measured in a deep-well scintilation counter (NaI crystal) and recorded as counts per minute (CPM). Duplicate samples of milk, plasma, red blood cells, feces and various tissues and organs were counted and the radioactivity was related to the activity of a counting standard. The counting standard was prepared by dilution of an aliquot of the radio iron fortified milk with the experimental ration. Because of the varied composition of the samples a preliminary study of the effect of different matrix on the counting rate was carried out. Results indicated that this factor did not affect the counting rate (Wang, 1972). A standard counting volume of 1.0 ml was adopted based on a normalization curve determined for the study. Blood was fractionated into plasma and red cells by centrifugation for 30 min using a clinical centrifuge. 24-hr collections of feces were blended with a measured amount of double distilled water using an Eberback stainless steel blending jar followed by homogenization in a Lourdes high speed blender. Organs and tissue were rinsed several times in isotonic saline solution to remove traces of blood, cut into small pieces and homogenized with measured quantities of double distilled water. Contents of stomach and intestines were removed by rinsing with distilled water prior to above treatment. Bone marrow was prepared by splitting the tibia and scraping the marrow cavity. The contents were wet-ashed in a 1:1 mixture of concentrated sulfuric and nitric acids. The volume of the digestion mixture was standardized with double distilled water prior to counting.

RESULTS & DISCUSSION

THE INTENT was to evaluate iron utilization under "normal" physiological conditions since absorption by an anemic animal is known to be abnormal (Bothwell et al., 1958). Evaluation of physiological condition was based on growth rate, hematological parameters and general observations during the adjusting and experimental periods. Consumption of the experimental ration increased from 0.6 to 2–2.5 liter/pig/day during the 4-wk feeding period. The overall average milk intake was 110–220 ml/kg body weight/day. Growth rate as indicated by increase in body weight is shown in Table 1. These data show weight gains among the 5 pigs of 0.154–0.249 kg/day. Hill (1966) reported average weight gains among 30 young pigs (8 wk old) fed 10, 15 and 20% protein ration for 16 wk of 0.077, 0.259 and 0.304 kg/day, respectively. He concluded that growth rate was very poor for the 10% group and normal for the others. In comparison to Hill's results the experimental ration used in this study produced normal rates of growth.

Hematological results are shown in Figure 1. Rather marked increases in hemoglobin and hematocrit were apparent after the adjusting period; however, the values for all three parameters were relatively constant during the experimental period. The ranges observed were 8.6–12.6 for hemoglobin, 21–43 for hematocrit and 5.0–9.9 × 10^6 for RBC. Hill (1966) reported that hemoglobin values of 9–10% and RBC of 5–6 × $10^6/mm^3$ were normals for 2–6 wk old pigs. Based on his results, the hematological condition of the test animals in this experiment was normal.

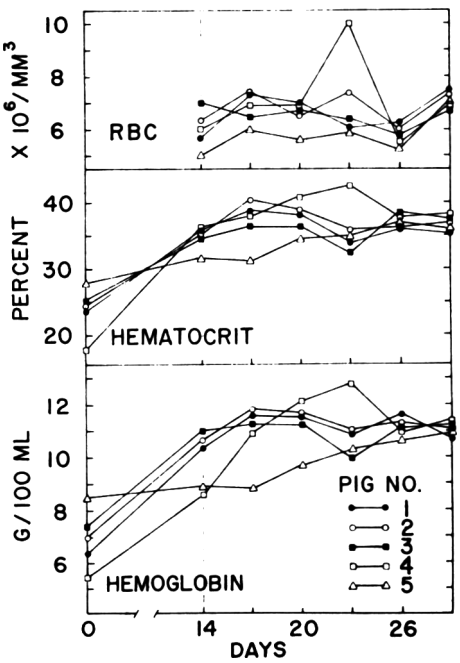

Fig. 1—Effect of the FeAC fortified-modified milk ration on values for hemoglobin, hematocrit and red blood cells of five baby pigs. Labeled ration was administered on day 14 (end of the adjusting period).

Iron absorption was determined by three different techniques, all based on the fate of the radioactivity in the tracer dose. The assumption that the fate of the radio iron was representative of the fate of the total iron in the ration was strengthened by using the same form of iron (ferric ammonium citrate) in the tracer dose as that used in the experimental ration. The composition and processing of the tracer dose were also the same as that used for the experimental ration.

The first method for estimating iron absorption (RBC radio iron method) is based on measurement of radioactivity of red blood cells from animals or humans with normal red cell utilization and is usually considered to be one of the more accurate methods (Bothwell and Finch, 1962). The rate of incorporation of ^{59}Fe into RBC, expressed as percent of administered dose, is shown in Figure 2. A plateau of constant radioactivity of RBC was reached 10–12 days after isotope administration. The plateau is interpreted as indicating maximum incorporation of ^{59}Fe in RBC and for the five baby pigs used in this study the value ranged from 25.38–30.97% as shown in Table 2. Mean iron absorption was 28.43 ± 2.2%. The transfer of ^{59}Fe from the GI tract to the blood was rapid. Significant radioactivity

was detected in the plasma (0.4% of dose) and in the RBC (0.3% of dose) only 90 min after the oral administration of the labeled ration. This method of estimating iron utilization assumes that (1) the iron in the red cell mass exists as hemoglobin and does not leave the cell nor exchange with iron in plasma and (2) that the total blood volume is accurately known and that all iron absorbed from the gut is rapidly incorporated into hemoglobin. The first assumption has been supported experimentally by the observation that radio iron incorporated into RBC is present as hemoglobin and no measureable exchange of such iron between red cells and plasma during the life span of the erythrocyte has been demonstrated (Miller and Hahn, 1940). The validity of the second assumption is related to the accuracy with which blood volume can be determined and the extent of iron absorption by tissue other than blood. This latter obviously results in lower values for absorption. Total blood volume was estimated to be 6% of body weight (Matrone et al., 1960).

The second method used for estimating iron absorption (corrected RBC radio iron method) considers the amount of ^{59}Fe deposited in tissue and organs as well as in RBC. These results are shown in Table 3 and ranged from 26.67–33.35% for the five pigs. Mean absorption of iron by this method was 30.59 ± 2.47%. The distribution of ^{59}Fe shows that more than 90% was incorporated into RBC. Among organs and tissue the percentage of absorbed iron was high in liver, spleen, small intestine, bone marrow and muscle. It was low in heart, kidney, large intestine and stomach. No radioactivity was detected in the gall bladder (bile). The high percentage of activity in the liver and spleen is consistent with the known iron storage activity of these organs. The difference in activities between the small and large intestine may indicate that greatest absorption is from the duodenum and progressively decreases in the more distal segments of the GI tract as previous findings have shown (Brown and Justus, 1958). Total activity of the bone marrow was based on total bone weight of the animal estimated to be 17.1% of the carcass weight for 6 wk-old pigs (McMeekan, 1940). The relatively high activity is consistent with RBC formation and iron storage associated with this tissue. The activity found in muscle is probably related to its myoglobin content. The total activity of muscle tissue was calculated on the basis of total lean body mass as described by Muldowney (1957). The absence of radioactivity in bile may indicate that no measureable ^{59}Fe was secreted to the GI tract during the short period of this experiment.

The third method used for estimating iron absorption (radio iron balance method) is based on the amount of the ingested dose recovered in the feces. Feces were collected until less than 0.5% of the

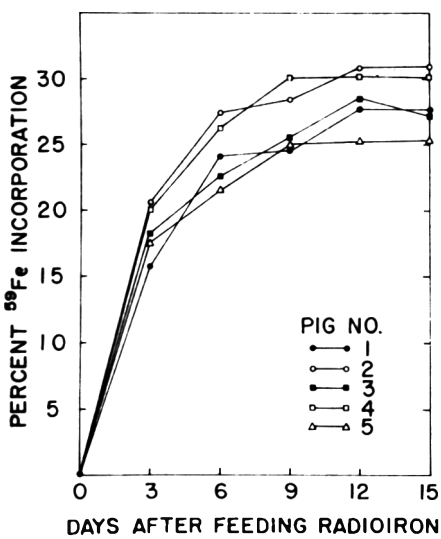

Fig. 2—Rate of incorporation of ^{59}Fe into red blood cells of baby pigs fed FeAC fortified-modified milk.

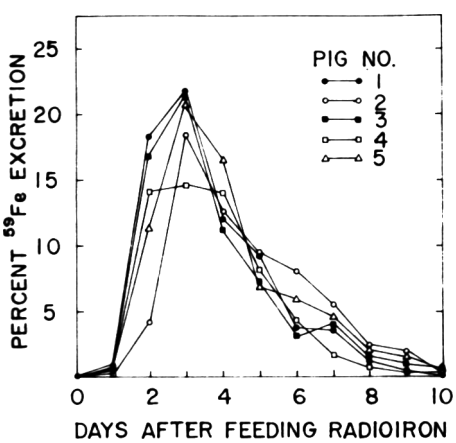

Fig. 3—Rate of excretion of ^{59}Fe in feces of baby pigs fed FeAC fortified-modified milk.

Table 2—Iron absorption from FeAC fortified-modified milk by baby pigs. Comparative results of three techniques

	Percentage[a] of iron absorption		
Baby pig no.	RBC radio iron	Corrected RBC radio iron	Radio iron balance
1	27.65	30.55	29.54
2	30.97	33.35	38.81
3	28.03	30.56	34.08
4	30.14	31.80	40.31
5	25.38	26.67	31.05
Mean ± std dev.	28.43 ± 2.20	30.59 ± 2.47	34.79 ± 5.80

[a] Percentage of administered dose (50μci ^{59}Fe)

Table 3—Distribution of absorbed radio iron in blood, organs and tissues of baby pigs fed FeAC fortified-modified milk

	Percentage[a] of radio iron absorption in baby pigs				
Sample description	1	2	3	4	5
Blood	27.65	30.97	28.03	30.14	25.38
Liver	0.72	0.50	0.39	0.41	0.34
Heart	0.13	0.16	0.13	0.16	0.07
Kidney	0.09	0.07	0.06	0.06	0.06
Spleen	0.13	0.19	0.13	0.18	0.12
Small intestine	0.21	0.22	0.23	0.18	0.16
Large intestine	0.03	0.03	0.02	0.02	0.03
Stomach	0.04	0.06	0.05	0.04	0.03
Gall bladder (bile)	0.00	0.00	0.00	0.00	0.00
Bone marrow[b]	0.35	0.32	0.25	0.32	0.28
Muscle[b]	1.20	0.83	1.27	0.29	0.20
Total absorption by corrected RBC radio iron method	30.55	33.35	30.56	31.80	26.67

[a] Percentage of administered dose (50μci ^{59}Fe)
[b] Data were estimated as described in Results & Discussion.

administered dose was present in a 24-hr collection. The rate of excretion of ^{59}Fe in the feces of the five pigs is shown in Figure 3. Most of the excreted radio iron appeared 2–5 days after the oral administration of the tracer dose. Less than 0.5% of the dose was observed in the 24-hr feces collection 10 days after the administration of radio iron. Total excretion of ingested radio iron was calculated from the data shown in Figure 3. Absorption ranged from 29.54–40.31 with a mean of 34.79 ± 5.80% as shown in Table 2. This method indicated greater iron absorption and larger variations among individual animals than the other two methods. Anything less than a complete recovery of feces, probably never achieved, would yield higher absorption values; however, this method is the most direct and does not involve assumptions and estimations associated with the other two methods. The radio iron balance method only yields information concerning gross absorption and was used here as a check on the other techniques. The results indicate that the estimations and calculations used in the corrected RBC method were sound and the results presented reasonably represent the fate and utilization of dietary iron present in FeAC-fortified milk.

In summary, baby pigs were maintained in a normal physiological condition on a modified milk ration containing iron in the form of FeAC. About 30% of the dietary iron was absorbed and 90% of that was incorporated in RBC and the balance was distributed among various organs and tissue. The percent absorption is considered very favorable as compared to other sources of supplemental iron and indicates that the FeAC-fortified milk developed in this study would provide a good source of iron for the human population. The level of iron fortification shown to be feasible (30 ppm) (Wang and King, 1973) and the favorable biological availability shown in this report indicate that about 1/2 pint of FeAC-fortified milk would provide the average daily iron requirement.

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THE NATURE OF FATS AND FATTY COMPONENTS IN NONDAIRY IMITATION MILKS

INTRODUCTION

IN A PREVIOUS paper, Kosikowski (1971) showed the nutritive and organoleptic qualities of 13 nondairy imitation milks, but not their fat characteristics. Earlier, the fatty acid composition of one nondairy imitation sample had been reported by Brink (1968). The present study deals with a comprehensive analysis of the fats and fatty components of available nondairy imitation milk powders and concentrates obtained country-wide over 2 yr.

METHODS & MATERIALS

Samples

A small nucleus of basic sources for nondairy imitation milks, continually contracting, limited the number of new types. Six nondairy imitation milks, 1, 2, 3, 4, 5, 6 and two cow's whole milk powders 7(c) and 8(c) (Table 1) were those from the previous report by Kosikowski (1971) listed there in order as 10, 5, 13, 6, 7, 4, 11 and 12. Nine other samples, many from the same sources, were obtained near the end of the present study and tested within 1–2 months of receipt. All lots were stored sealed, at 18–20°C, prior to testing.

Extraction

Fat was extracted from powders using Corning extra large size Soxhlet extractors with ethyl ether and from concentrate using Mojonnier extractors with ethyl and petroleum ethers. A minimum of 26g of fat for each sample was required for duplicate testing. After extraction, the solvents were evaporated gently and the remnants removed by heating in an oven at 50–60°C. The fat was filtered through No. 2 Whatman paper at 45–50°C and stored at 4°C. All steps were conducted under nitrogen.

Unsaponifiable matter

A 2.5-g portion of the filtered fat was weighted into an Erlenmeyer flask and saponified as described under methods of the American Oil Chemists Society (AOCS, 1967). The unsaponifiable matter was made ether free, dried under nitrogen and weighed.

Sterols

Thin-layer chromatography, as outlined in Standard Methods of the Association of Analytical Chemists (AOAC, 1970) was applied for separation of sterols from unsapifiable matter. The latter was dissolved in chloroform and transferred on a thin-layer sheet, Eastman Chromogen; 6060 Silica Gel with fluorescent indicator. After development, the sterol band was removed and the sterols extracted. Sterols were stored under nitrogen at −10°C.

For sterol identification, gas liquid chromaography (GLC) on a Varian Aerograph Model 2700 flame ionizer coupled with a Westronic Recorder, Model LD 11A was employed.

Peaks and retention times of standard mixtures of sterols were established on a 0.32 × 183 cm column packed with 1% OV-1, 90-100 Chromosorb W. Separation conditions were: Temperature: column 250°C; detector 275°C; flash 295°C. Flow rates: hydrogen 40 ml/m; air 320 ml/m; nitrogen 50 ml/m.

The flow rate of the carrier gas, nitrogen, was equivalent to a back pressure of 1.43 kg/cm^2 at the working temperature. The electrometer sensitivity was 10^{-10}; attenuation generally was 4 but in some instances 2 and 8. Recorder chart speed was 25.4 cm/hr. Retention times of the major sterols are given in Table 2.

To determine cholesterol and its related compounds (7-dehydrocholesterol and dihydrocholesterol), the sterols were brought to exactly 1 ml with chloroform and 2 μl of this mixture were injected. The relative sterol percentage was calculated from the surface areas of the peaks. Specific amounts of cholesterol were determined by dividing the surface area of the peak by the surface calculated for 1 mg of injected sterols from a standard mixture.

Oxidized fatty acids

The soap solution from the saponification step was treated to obtain free fatty acids as described in AOAC methods (1970). These acids were separated in petroleum ether by solubility as nonoxidized or oxidized.

Fatty acid composition

Nonoxidized fatty acids were methylated using the AOCS method (1967) and separated

Table 1—Identity of nondiary imitation milk powder or concentrates and qualities of their reconstituted forms

Product no.[a]	Identified as	Reconstituted to 12% T.S. Flavor Comment	Color	pH
1	Imitation whole milk powder	Malty, sl. oxidized	White	7.4
2	Imitation fluid milk powder	Sweet, malty, foreign	Light tan	6.9
3	Imitation whole milk conc	Beany, foreign	Tan	7.0
4	Imitation whole milk powder	Sweet, malty	White	6.9
5	Imitation whole milk powder	Sweet, malty, beany	Tan	7.1
6	Imitation whole milk powder	Sl. bitter, foreign, sl. oxidized	V. light tan	6.9
7(c)	Cow's whole milk powder	Oxidized	White	6.5
8(c)	Cow's whole milk powder	V. strong oxidized	White	6.5
9(c)	Cow's whole milk powder	Cooked-oxidized	White	6.5
10	Imitation fluid milk powder	Foreign	V. light tan	6.8
11	Imitation whole milk conc	V. sweet, beany	Light tan	7.2
12	Imitation whole milk powder	Beany	Tan	6.9
13	Imitation whole milk powder	Beany	Tan	7.2
14	Imitation whole milk powder	Sl. foreign-chalky-malty-lacks freshness	Light tan	7.0
15	Imitation whole milk powder	Sl. foreign, malty lacks freshness	Light tan	6.9
16	Imitation whole milk powde	Flat, foreign, malty lacks freshness	V. light tan	6.9
17	Imitation whole milk powder	Flat, foreign metallic	White	6.7

[a] Products No. 1, 2, 3, 4, 5, 6 and 7(c), were 18–24 months old and are listed in a previous paper by Kosikowski (1971) as products No. 10, 5, 13, 6, 7, 4 and 11, respectively. Products 9(c) and 10 to 17 were obtained and tested near the end of the study.

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Table 2—Retention times of major sterols under test conditions

Sterols	Retention time Initial (min)	Highest (min)	End (min)
Cholesterol	15.00	16.20	17.00
Ergosterol	17.00	19.10	21.00
Campesterol	20.00	21.00	22.30
Stigmasterol	21.00	23.00	24.15
b-Sitosterol	24.20	26.20	28.00

by GLC in a 0.32 x 244 cm SS column packed with 20% diethyleneglycol succinate 60-80 on Chromosorb W A/W. Separation conditions were: Temperature: column 200°C; detector 235°C; flash 255°C. Flow rates: hydrogen 30 ml/m; air 300 ml/m; nitrogen 40 ml/m (1.3 kg/cm²).

All fatty acids to C $_{20:1}$ were resolved under optimum conditions within 30 min in the above column at chart speeds of 25–250 cm/hr. The sensitivity was 10^{-10}, starting with attenuation 1 or 2 for very small peaks and often increased twofold to levels necessary to record all peaks on the scale.

Other fat tests

Other analyses performed on the extracted fat using AOCS methods (1967) were melting point by capillary tube, refractive index by Abbé refractometer and peroxide values. The acid number of fat was obtained by the AOAC method (1970) and the fat concentration of powders and concentrates by the method of Mohonnier and Troy (1925).

RESULTS

GENERAL and flavor characteristics of 14 nondairy imitation milk powders and concentrates and three cow's milk powders are listed in Table 1. Typical flavors of nondairy imitation milks, reconstituted to 12% T.S., were flat, foreign and metallic-oxidized, or sweet, malty and beany and of reconstituted cow's milk slightly to strongly oxidized (Table 1). Colors of samples were creamy white to tan. The pH of reconstituted nondairy imitation milks ranged from pH 6.8–7.4; and for each reconstituted cow's milk pH was 6.5.

Fat and fat characteristics

The fat concentration of 12 nondairy imitation powders averaged 26.25%, two nondiary milk concentrates 7.96% and three cow's whole milk powders 28.91% (Table 3).

Fats from nondairy imitation milk powders and concentrates had melting points of < 5.0–44.5°C and refractive indices of 1.4482–1.4670.

Unsaponifiable matter in fats of nondiary imitation milk powders and concentrates ranged from 0.45–1.44% with 11 of the powders displaying higher levels than found in fat from cow's milk powders (Table 3).

Table 3—Concentration of fat and cholesterol and fat constants in nondairy imitation milk powder and concentrate

Product no.	Fat (%)	Melting point (°C)	Refractive index at 40°C[a]	Unsaponifiable matter (%)	Cholesterol and related sterols (mg/100g fat)
1	26.40	29.5	1.4482	0.48	5.06
2	33.18	39.5	1.4624[a]	0.80	3.03
3	7.91	<5.0[b]	1.4665[a]	0.69	ND[d]
4	25.96	33.5	1.4613	0.66	1.70
5	22.08	28.0	1.4661	0.75	ND
6	28.54	37.5	1.4624	0.83	0.55
7(c)[c]	28.91	33.5	1.4541	0.50	393.84
8(c)[c]	28.91	34.4	1.4546	0.52	194.32
9(c)[c]	28.92	33.7	1.4544	0.42	277.51
10	16.90	42.5	1.4609[a]	0.84	1.43
11	8.00	<5.0	1.4670	0.50	ND
12	22.96	28.5	1.4617	0.60	10.80
13	22.44	<5.0[b]	1.4662[a]	0.77	ND
14	29.82	35.8	1.4616	1.44	13.62
15	26.06	36.0	1.4623	1.37	13.45
16	28.04	44.5	1.4597[a]	0.45	10.24
17	33.02	33.6	1.4591	0.96	71.49

[a] Exceptions to stated temperature are numbers 3 and 13 measured at 20°C and numbers 2, 10 and 16 measured at 50°C. Correcting factors for each °C were 0.000385 for oil and 0.000365 for fat.
[b] < 5.0 = liquid in refrigerator
[c] (c) = cow's whole milk powders.
[d] ND = not observable.

Table 4—Type and relative proportion of sterols in nondairy imitation milk powders and concentrates

Product no.	Sterols as % of total sterols Unknown[a]	Cholesterol and related sterols[b]	Campesterol	Stigmasterol	β-Sitosterol	Others[d]
1	25.71	5.54	6.24	8.99	48.	5.00
2	ND	1.36	20.52	10.21	53.78	5.04
3	ND	ND	21.66	20.49	49.66	8.08
4	ND	0.63	21.42	20.87	51.72	5.56
5	ND	ND	22.74	18.29	52.73	6.16
6	ND	0.60	21.23	18.21	53.09	6.88
7(c)[c]	ND	100.00	ND	ND	ND	ND
8(c)[c]	10.11	89.89	ND	ND	ND	ND
9(c)[c]	ND	100.00	ND	ND	ND	ND
10	ND	0.73	13.54	9.85	69.95	5.93
11	ND	ND	14.06	28.36	56.32	1.25
12	ND	4.21	20.62	19.40	51.41	4.34
13	4.90	ND	21.64	19.08	47.94	6.34
14	ND	2.98	19.91	16.38	48.34	12.30
15	ND	2.68	14.06	13.18	61.87	8.21
16	0.98	8.49	11.14	5.37	69.62	4.40
17	ND	18.16	10.21	8.07	55.29	6.67

[a] Retention time of these components is slightly less than cholestane, a derivative of cholesterol.
[b] Also includes perhaps some forms of activated ergosterol in very small amounts.
[c] Cows' whole milk powders
[d] Represents two different components, probably gamma and alpha sitosterol. The former appeared in all instances and comprises the major portion.
[e] ND = not detectable.

Sterols

Sterols are shown in Tables 3 and 4. Only cholesterol and related sterols were in the unsaponifiable matter of fat from cow's milk powders ranging from 194.32–393.84 mg/100g fat (Table 3). However, the 12-year cow's milk powder, 8(c), produced 10.11% of an unknown material near the cholestanol (a derivative of cholesterol) peak. One nondairy imita-

Table 5–Fatty acids of nondiary imitation milk powders and concentrates[a]

Product no.	Fatty acid carbon chain (% of total fatty acids)															
	$< C_6$	C_6	C_6^u C_7	C_8	C_8^u C_9	C_{10}	C_{10}^u C_{11}	C_{12}	C_{12}^u C_{13}	C_{14}	C_{14}^u C_{15}	C_{16}	C_{16}^u C_{17}	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	$> C_{18}$
1	ND	0.33	t	5.49	t	5.39	t	42.81	t	18.08	t	10.80	t	11.06	5.58	0.48	t	t
2	t	t	t	t	t	t	t	t	t	0.10	t	11.34	t	12.08	54.91	16.61	2.68	1.21
3	t	t	t	t	t	t	t	t	t	0.10	t	10.24	0.36	4.67	23.76	50.66	8.92	1.09
4	t	t	t	t	t	t	t	0.31	t	0.27	t	10.53	0.36	7.09	63.10	15.01	0.99	2.10
5	t	t	t	t	t	t	t	t	t	0.11	t	10.70	0.44	4.86	25.18	45.29	9.62	3.55
6	ND	t	t	t	t	t	t	0.13	t	0.29	t	9.77	1.44	9.29	58.38	16.74	1.03	2.96
7(c)	t	0.92	5	1.09	0.10	2.49	0.39	2.91	0.32	9.87	2.84	26.60	4.07	12.52	27.40	2.88	1.45	3.42
8(c)	0.18	1.25	0.21	1.18	0.25	2.03	0.40	2.69	0.88	11.06	5.17	22.74	5.50	16.02	23.90	2.14	1.60	2.80
9(c)	t	0.92	5	1.06	0.10	2.66	0.39	3.68	0.44	11.79	3.05	29.46	6.67	11.00	25.69	1.62	0.79	0.20
10	t	t	t	t	t	t	t	t	t	0.51	t	16.89	0.43	10.50	60.29	8.95	0.60	1.93
11	t	t	t	t	t	t	t	t	t	t	t	11.42	t	2.37	26.65	51.78	7.78	t
12	t	t	t	0.24	t	0.24	t	1.61	t	1.23	0.11	11.33	0.53	6.80	58.01	15.34	1.12	3.43
13	t	t	t	t	t	t	t	t	t	0.10	t	10.93	0.18	5.70	30.53	47.18	3.61	1.80
14	t	t	t	0.10	t	0.10	t	0.70	t	0.74	t	9.83	0.50	9.62	64.92	8.25	1.72	3.46
15	t	t	t	0.11	t	0.11	t	0.94	t	0.99	t	10.25	0.70	9.10	66.77	8.91	0.38	1.58
16	t	t	t	t	t	t	t	0.41	t	1.01	t	16.04	0.64	19.14	54.93	4.56	1.22	0.96
17	t	0.26	t	0.36	t	0.63	0.10	1.27	0.10	2.08	0.94	16.54	1.30	11.12	58.14	5.56	0.79	0.72

[a] t = trace; ND = not detectable; (c) = cow's whole milk powders; u = unsaturated forms.

Table 6–Oxidized fatty acids, acid number and peroxide values of fat in nondiary imitation milk powders and concentrates

Product no.	Oxidized fatty acids (%)	Acid no. (Mg KOH/g)	Peroxide value (meq/kg)
1	0.74	0.55	10.23
2	1.45	1.44	20.00
3	0.45	0.85	14.24
4	0.56	0.49	3.97
5	1.49	0.64	17.76
6	0.82	1.60	3.45
7(c)[a]	0.43	0.78	2.30
8(c)[a]	2.83	3.43	44.24
9(c)[a]	0.17	0.27	2.01
10	0.72	0.67	3.22
11	0.72	1.27	1.04
12	0.42	0.56	3.11
13	1.72	0.85	24.41
14	0.47	1.49	3.08
15	0.86	1.65	4.51
16	0.49	0.69	4.48
17	0.46	0.48	3.74

[a] (c) = cow's whole milk powders.

tion milk powder, No. 17, contained appreciable cholesterol and related sterols, 71.49 mg/100g fat. Nine other samples showed small amounts, 0.55–13.62 mg/100g fat and four had none (Table 3).

The nondairy imitation milk powders and concentrates mainly displayed campesterol, stigmasterol and beta-sitosterol with the latter comprising 4.74–69.95% of the total sterols (Table 4). Some gamma or alpha forms of sitosterol also were present. Unclassified plant sterols represented 1.25–12.30% total sterols.

Fatty acids

The fatty acid composition of nondiary imitation milk powders and concentrates was dominated by unsaturated forms (Table 5). In 12 nondairy imitation milks, unsaturated fatty acids comprised 60.71–80.09% of the total. Imitation powder No. 1 had a fatty acid composition like that of coconut fat and another, No. 17, suggesting a mixture of butter and vegetable fats.

Oxidized fatty acids, acid number and peroxide values

Analyses of the fats of 14 nondairy imitation milk powders and concentrates showed the following additional characteristics: oxidized fatty acids, 0.42–1.72%; acid number, 0.48–1.65; and peroxide values, 1.04–24.41 (Table 6). Two whole milk powders, 7(c) and 9(c) displayed significantly lower values while the very old cow's milk powder, 8(c), produced extremely high fat constants.

When nondiary imitation milk pow-

ders chosen at random were exposed to the atmosphere at room temperature for 2 months, the average acid number for eight samples rose from 1.0 to 1.8 and the average peroxide value for nine samples from 7.50 to 31.04.

DISCUSSION

PRIOR INFORMATION on the fats of nondiary imitation milks is almost nonexistent. The known analysis reported by Brink (1968) on one nondiary imitation milk for fatty acids suggested its fat was coconut. The present data on 14 nondairy imitation milks obtained during 1970–72 showed only one imitation powder containing fat resembling coconut while another apparently consisted of some butterfat. The majority of the nondiary imitation milks contained soybean or cottonseed fats or mixed fats made difficult to identify because of hydrogenation.

Nutritional labelling of foods as outlined in a recent U.S. Food and Drug Administration report (1972) will ultimately require the industry and regulatory officials to consider realistically the problems evident with nondairy imitation milks. One, noted from the present study, is the variability in their fats and fatty components. Presumably, these could be changed quickly and unpredictably, depending on availability and relative costs of certain fats.

Animal fats usually have low levels of unsaponifiable matter, generally not above 0.50%, while plant fats have, because of color compounds and other materials, higher values. Swern (1964) noted the highest unsaponifiable matter concentration from most plant fats as 1.5%. The vegetable fat, coconut, however, has a low level. In the present study several fats from nondiary imitation milks approached this unsaponifiable matter maximum.

Sterols in fats of nondiary imitation milks and in fats of cow's milk powders were markedly different as expected. Some difficulty was experienced in readily interpreting the nature of very small peaks residing near cholesterol. These may represent activated forms of ergosterol added to nondairy imitation milk powders for vitamin D fortification. The ergosterol peak on the gas chromatograph follows that of cholesterol but no ergosterol was observed in our samples, although it was in standard mixtures. Ergosterol has been reported in liver, milk, butter, egg yolk, yeast and lower plant forms by Wachs (1964).

In two nondiary imitation milk powders, No. 1 and 16, and a 12-year cow's milk powder, 8(c), unidentified sterol peaks representing 25.71, 0.98 and 10.11% of the total sterols appeared near cholestane and presumably may be related to it (Table 4). The old cow's milk powder was included in the study to observe the extreme degree to which butterfat can be decomposed.

The unsaturated nature of the fatty acids of most of the nondairy imitation milk powders and concentrates suggests that rapid flavor deterioration can be expected with these products. Oxidized and tallowy flavors were observed, but a number of these nondiary imitation milk products were relatively stable to change over 18 to 24 months at 18°C, indicating the presence of inhibitors.

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A MODIFIED METHOD FOR AFLATOXIN DETERMINATION IN SPICES

INTRODUCTION

A SEARCH of the literature fails to reveal any specific reports of aflatoxins in spices; nonetheless their possible presence is of concern for two reasons: (a) many spices are grown and processed in warm tropical areas where conditions favor fungal growth and (b) large numbers of colonies of *Aspergillus flavus* per gram of black pepper berries have been isolated by Christensen et al. (1967). The toxic metabolites of *Aspergillus flavus* apparently are produced under specific conditions and their presence is not necessarily a function of the number of colonies present, according to Goldblatt (1969).

A number of methods for quantitative estimation of aflatoxins have been reported for various agricultural commodities. In our early attempts to survey spices for aflatoxins, it became apparent that modifications of these methods were necessary to: (1) prevent the occlusion of aflatoxins in fixed and/or volatile oils, and (2) remove naturally occurring interfering substances, especially in black pepper.

MATERIALS & METHODS

SAMPLES OF ground spices from Indonesia, Malaysia and India were chosen for analysis. A 50-g sample of ground spice was weighed into a 400 ml beaker and mixed with 10g of Hyflo Super Cel and 70 ml of 0.1N HCl solution, as reported by Pons et al. (1970). The mixture was transferred into a Waring Blendor with 250 ml of methylene chloride and blended at high speed for 3 min. The slurry was filtered through #40 Whatman paper and 100 ml of the filtrate was collected. Solvent was removed on a hot water bath and the residue was dissolved in 10 ml of methylene chloride, this solution was ready for column chromatography.

A chromatographic column (22 mm × 300 mm) was packed with 5g of anhydrous Na_2SO_4, 15g of silica gel and an additional 5g of anhydrous Na_2SO_4 on top. The methylene chloride solution of the sample was transferred to the column and washed successively with 200 ml of benzene-acetic acid (9:1) solution, followed by 200 ml of diethyl ether-hexane (3:1) solution. Elution of aflatoxins was accomplished with 200 ml of methylene chloride-acetone (3:1) solution and the eluate was evaporated to near dryness on a hot water bath. The residue was dissolved in 20 ml of acetone, 60 ml of water and 20 ml of 20% lead acetate solution. According to Pons et al. (1970) the lead acetate treatment removes plant pigments, lipids, fatty acids, and other unknown materials which cause streaking on thin layer plates. This solution was filtered with the aid of 5g of Hyflo Super Cel and a 60 ml aliquot of the filtrate was extracted twice with 60 ml portions of methylene chloride. The combined methylene chloride extracts were evaporated to dryness and the residue was dissolved in 0.5 ml of benzene-acetonitrile (98:2) solution. This solution was ready for TLC analysis.

Silica gel thin-layer chromatographic plated of 0.25 mm thickness were used for this analysis. The plates were dried in an oven at 105°C for 3 hr and then stored in a desiccator over

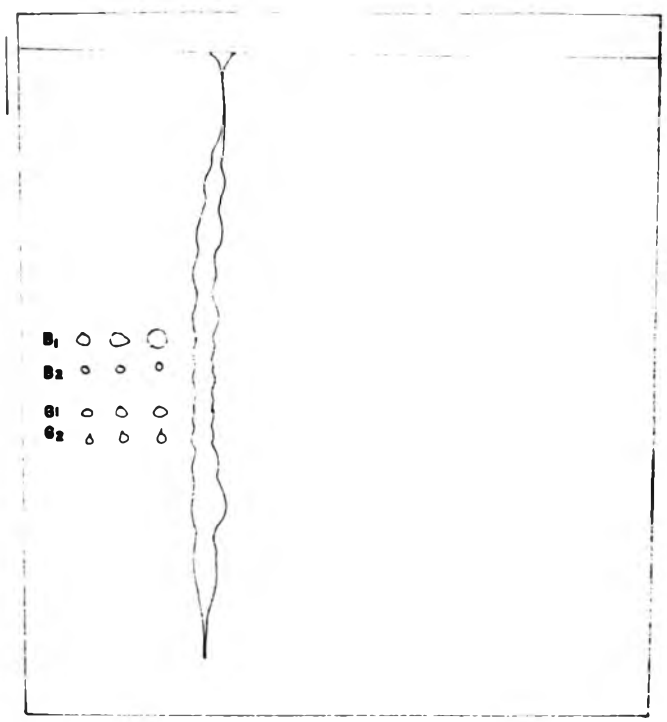

Fig. 1—TLC of aflatoxins: Schematic of 1st dimension development.

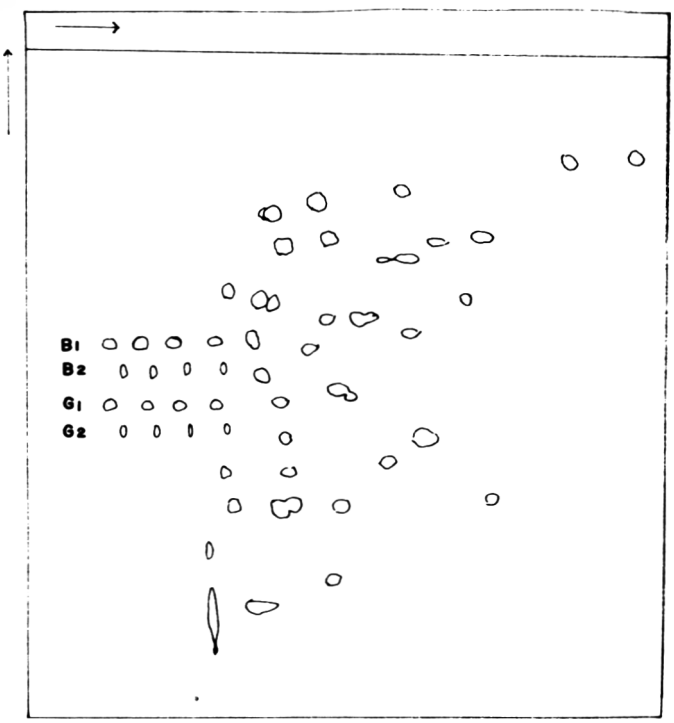

Fig. 2—TLC of aflatoxins: Schematic of 2nd dimension development.

Table 1—Aflatoxins in spices

Spice	Number of samples Analyzed	Aflatoxins found	Aflatoxin (ppb) B_1	B_2	G_1	G_2
Allspice	5	–	–	–	–	–
Anise	5	–	–	–	–	–
Black pepper	7	3	–	–	1.8–3.7	1.1
Cardamon	5	–	–	–	–	–
Caraway	5	–	–	–	–	–
Cinnamon	5	–	–	–	–	–
Celery seed	9	1	–	–	3.7	–
Coriander	5	–	–	–	–	–
Fennel	5	–	–	–	–	–
Fenugreek	5	–	–	–	–	–
Mustard	5	–	–	–	–	–
Nutmeg	5	3	2.5–5.5	0.75–1.1	–	–
Turmeric	5	–	–	–	–	–

anhydrous calcium sulfate until used.

A 20 μl aliquot of prepared sample solution was spotted on the thin-layer plate along with 3, 6 and 9μl of the standard aflatoxin solutions (SA 14-7) which were obtained from the USDA Southern Regional Research Lab. The concentrations of standard aflatoxin solutions used in this experiment were as follows:

Aflatoxin B_1 – 0.50 μg per ml
Aflatoxin B_2 – 0.15 μg per ml
Aflatoxin G_1 – 0.50 μg per ml
Aflatoxin G_2 – 0.15μg per ml

The developing tank was equipped with a stainless steel trough containing water as described by Pons et al. (1970) using chloroform-acetone (9:1) solution for 1st dimension development. The developed plate was air dried, sprayed with methanol-phosphoric acid (4:1) solution and dried in an oven at 60°C for 15 min. This plate was redeveloped in the 2nd dimension using a benzene-acetic acid-water (84.5:15:0.5) solvent system. Interfering substances which caused streaking on the thin-layer plate migrated with the 2nd dimension solvent, leaving the readily quantitizable aflatoxin spots behind (See Fig. 1 and 2.)

The confirmatory test for aflatoxins was performed as follows:

50g of aflatoxin-free ground pepper was spiked with standard aflatoxin solution and left to equilibrate overnight. The modified procedure was followed and the aflatoxins spots were found at the proper R_f values.

A 50-μl aliquot of the above extract was spotted on a TLC plate. Following first dimension development, the B_1 and G_1 spots were scrapped-off, extracted with chloroform-acetone (2:1) solution, filtered through anhydrous sodium sulfate and the solvent evaporated on a hot water bath. The residue was taken up with benzene acetonitrile (98-2) solution. On another TLC plate, 50 μl of standard aflatoxin solution was spotted and given identical treatment yielding the following solutions:

S_1 containing B_1 from the sample
S_2 containing B_1 from the standard
S_3 containing G_1 from the sample
S_4 containing G_1 from the standard

The above solutions were spotted on a new TLC plate, sprayed with methanol-phosphoric acid (4:1) solution, oven dried and developed with the 2nd dimension solvent system. With this developer, interfering substances migrated away and the aflatoxins remained where spotted. This plate was oven dried and redeveloped using the following developer: benzene, chloroform, methanol, acetic acid and water (65:15:8.5:10:1.5) with this developer the appropriate sets removed with identical R_f values.

When the plate was air dried and treated with ammonia the appropriate sets underwent identical color changes.

From the data we have concluded that B_1 from the sample is identical to B_1 from the standard, and likewise G_1 from the sample is identical to G_1 from the standard.

RESULTS

TO DATE about 80 samples of various spices were analyzed following the modified procedure. The results are presented in Table 1.

DISCUSSION

THE OCCLUSION of aflatoxins in resinous-fatty materials was one of the difficulties encountered in the recovery studies. When lead acetate solution treatment preceded column clean-up, the recoveries averaged about 25% of the theoretical. The recoveries became quantitative when lead acetate treatment was introduced after the column clean-up.

Successful analysis of aflatoxins in spice (black pepper in particular) depends on the ability of phosphoric acid to retain aflatoxin molecules on the TLC substrate (silica gel).

Aflatoxins have ether linkages, double bonds and a lactone group. According to Breitbeil (1973) a logical mechanism for fixation can involve acid hydrolysis of the lactone ring and formation of a biphosphate ester. Strong hydrogen bonding to the silica gel can then prevent movement with the solvent in the 2nd dimension development.

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

INTRODUCTION

A MAJOR PROBLEM encountered in commercial mushroom canning operations is the large loss of product weight generally referred to by the industry as shrinkage. This loss of canned product weight which occurs during blanching and thermal processing, usually ranges between 30–40% (McArdle and Curwen, 1962). Since mushrooms are an expensive commodity and the canned product is sold on a drained-weight basis, excessive shrinkage results in considerable economic loss to processors. In a recent economic study, Coale and Butz (1972) indicated that a 5% reduction in shrinkage in a mushroom canning plant processing a complete product line would increase revenues by approximately 20%. Thus, technological advances that can reduce shrinkage and thereby increase the yield of canned mushrooms are of great importance to mushroom processors.

McArdle and Curwen (1962) demonstrated that post-harvest cold storage for 24–48 hr prior to processing significantly reduced shrinkage or increased the yield of the canned product without adversely affecting quality. Yield increases of 2.9–4.4% were observed depending on the length of the cold storage period and whether the mushrooms had been cut or pulled. These reductions in shrinkage were shown to be independent of the 1.2–2.7% weight losses that occurred during storage due to evaporation of water and tissue respiration.

Bradley (1970) demonstrated that soaking mushrooms in water for 2 hr prior to blanching increased the yield of canned mushrooms about 2% depending on subsequent canning operations. Studies recently conducted at this laboratory indicated that an interaction existed between soaking and cold storage which was effective in increasing the yield of canned mushrooms during processing operations. Therefore, these studies were conducted to evaluate the influence of different combinations of soaking and cold-storage treatments on the yield and quality of canned mushrooms.

EXPERIMENTAL

CULTIVATED MUSHROOMS, *Agaricus bisporus* (Lange) Sing, were used in all studies. In each experiment they were selected from a uniform supply of cut mushrooms (strain D-26) from the same crop harvested at the same time at a commercial Pennsylvania mushroom farm. They were transported to the university laboratory within 4 hr after harvest. Mushrooms with cap diameters ranging from 1-1/8 to 1-5/8 in. of uniform maturity (closed veils) were selected and the stems were hand-trimmed to approximately 1/8 in. from the cap. The sized and trimmed button mushrooms were then pooled and accurately weighed; 1-kg samples were randomly selected and placed in Kraft bags. Four repetitions of each treatment were employed.

Processing conditions

An attempt was made to evaluate cold storage and soaking treatments individually and in combinations that would be commercially feasible and practical. Soaking was accomplished by placing the mushrooms in perforated plastic containers submerged in about 10 liters of cold (10–16°C) tap water for a specified time interval. Soaking prior to cold storage (Soak I) was conducted for 20-min intervals. When soaking was employed after cold storage or prior to blanching (Soak II) it was conducted for 2 hr unless specified otherwise. Cold storage treatments were conducted at 2°C and 85% RH.

All other factors affecting shrinkage were controlled by standardizing the processing conditions. A control treatment was included in each experiment; this included processing without any cold storage or soaking treatments. All sample lots were blanched by immersion in boiling water (96–100°C) for 5 min then cooled for 2 min in cold water, drained and weighed. The mushrooms were then filled into 4-oz plain-tinned cans (211 × 212) using a uniform fill weight of approximately 120g. One 20-grain NaCl tablet was added to each can, the cans were filled with boiling water and immediately closed. Thermal processing was conducted in a still retort at 117°C for 18 min. After retorting, the cans were held for 3 wk prior to opening for product evaluation.

Canned product evaluation

The percent canned product yield was determined by the following formula:

$$\%\ \text{Canned product yield} = \frac{\text{Total drained wt}}{\text{Initial sample wt}} \times 100$$

Drained weights were determined from a pooled sample of all cans within each experimental unit by draining the mushrooms for 2 min in a perforated stainless steel tray before weighing. Color of the canned mushrooms was determined using an Agtron M-30-A Reflectance Color Meter. Duplicate readings from each experimental unit were taken immediately

Table 1—Influence of various combinations of cold storage (C.S.) and soaking on the yield of canned mushrooms

Treatment	Canned product yield[a] (%)	Yield increase (%)	Total solids[a] (%)
Control	75.7 A	–	9.79 A
Soak II	76.9 A	1.2	9.56 B
C.S. 18 hr	77.8 A	2.1	9.50 BC
C.S. 18 hr + Soak II	80.9 BC	5.2	8.86 DE
C.S. 48 hr	78.1 A	3.4	9.40 BC
C.S. 48 hr + Soak II	80.6 BC	4.9	8.92 DE
Soak I + C.S. 18 hr	80.2 B	4.5	9.55 B
Soak I + C.S. 18 hr + Soak II (PSU-3S-process)	85.4 E	9.7	8.81 E
Soak I + C.S. 48 hr	82.5 CD	6.8	8.99 DE
Soak I + C.S. 48 hr + Soak II	84.2 DE	8.5	8.82 DE
C.S. 18 hr + Soak I + C.S. 18 hr	81.3 BC	5.4	9.11 DE
C.S. 18 hr + Soak I + C.S. 18 hr + Soak II	82.3 BCD	6.6	8.81 E

[a] Means having the same letter are not significantly different ($\alpha = 0.05$)

after the drained-weight determinations by filling the sample cup halfway, covering the cup with the metal lid, and reading the meter standardized with the M00 and M44 discs using the green mode. Total solids analysis was accomplished by freeze drying a 30-g sample of chopped mushrooms to a constant weight and determining solids by weight difference. The incidence of exposed veils in the canned mushrooms was determined by counting the number of mushrooms with over one-half of the veil exposed. The data were analyzed statistically using a single classification analysis of variance and the significant differences between means were determined using an unpaired "Student's" t-test.

RESULTS & DISCUSSION

VARIOUS storage and soaking treatments employed individually and in combinations were evaluated for their influence on yield of canned mushrooms in the first experiment. The results of these tests are reported in Table 1. A yield of 75.7% was observed using the control treatment. Treatments involving only cold storage for 18 and 48 hr or soaking for 2 hr prior to blanching resulted in small yield increases similar to those reported previously for storage (McArdle and Curwen, 1962) and soaking (Bradley, 1970). However, when soaking (Soak II) was employed following 18 and 48 hr of cold storage, significantly larger increases in yield of 5.2 and 4.9% respectively were observed. When the mushrooms were soaked (Soak I) prior to the 18- or 48-hr cold storage treatments the increase in yield was 4.5 and 6.8% respectively. The highest yield, 85.4%, was observed when the mushrooms were soaked (Soak I) prior to 18 hr of cold storage followed by soaking (Soak II) prior to blanching. This combination treatment was later termed the 3S-process since it involved soaking, storage and soaking again. A similar treatment which involved 48 hr of cold storage between the two soaking operations caused a similar increase in yield (8.5%) compared to the control treatment. However, it would be of less practical value on a commercial basis since it would involve extra time and expense. The addition of an 18-hr cold storage operation prior to the 3S-process also reduced the benefits obtained by the 3S-process.

The range of total solids observed among the different treatments was from 9.79% for the control treatment to 8.81% for mushrooms canned using the 3S-process or a difference of 0.98% solids between these treatments where the difference in yield was 9.7%. The solids decreased in the mushrooms processed by the 3S-process by approximately 10% relative to the control treatment. Therefore, it appears the near 10% increase in weight retention due to the 3S-treatment was probably due to increased water retention in the tissue. Dommel (1964) reported that the total solids content of canned mushrooms was 1.3% greater than fresh mushrooms and attributed this observation to water loss and salt uptake during processing. In this experiment, the 3S-process resulted in much lower solids increase than those reported by Dommel (1964), probably due to greater water retention. Bradley (1970) demonstrated that mushrooms soaked in water for 2 hr increased about 32% in weight. He speculated that soaking caused swelling of protein due to the absorption of water and thereby increased water retention during blanching and thermal processing. Hamm (1960) reported that a relationship exists between the swelling capacity of meat tissues and their water-holding capacity. He reported that water is mechanically immobilized in meat tissue by the network of cellular protein filaments by capillary condensation and when swelling occurs a loosening of this network of protein filaments increases the potential space for immobilized water. Since mushrooms contain approximately 25% protein on a dry-weight basis (Dommel, 1964) it is possible that soaking increases the water retention in processed mushrooms by a similar mechanism.

Table 2—Influence of individual operations of the PSU-3S-process and their combinations on the yield and quality of canned mushrooms

Treatment	Canned product yield[a] (%)	Yield increase (%)	Agtron color[a] (% Reflectance)	Exposed veils[a] (No./kg)
Control	68.5 A	–	28.4 BCDE	2.3 A
Soak I	69.1 AB	0.6	29.5 A[b]D	2.5 AB
Soak II	71.8 C	3.3	31.3 A	4.3 AC
Soak I + Soak II	73.2 D	4.7	32.3 AB	7.5 C
C.S. 18 hr	70.8 BC	2.3	27.8 CDF	3.3 AB
Soak I + C.S. 18 hr	74.7 E	6.2	27.7 EF	8.5 CD
C.S. 18 hr + Soak II	75.2 E	6.7	31.8 A[b]	9.0 BCD
Soak I + C.S. 18 hr + Soak II (PSU-3S-process)	77.9 F	9.4	30.7 AC	12.0 D

[a] Means having the same letter are not significantly different ($\alpha = 0.05$)
[b] Means are significantly different ($\alpha = 0.05$) despite letter designation.

A second experiment was conducted to determine the effect of each operation of the 3S-process singly, and in combination, on the yield and quality of canned mushrooms. The influence of these treatments on total shrinkage, color and incidence of exposed veils is reported in Table 2. Except for the treatment which involved a 20-min soak, each individual operation or combination of operations significantly increased the canned product yield. However, the yield increases relative to the control were more than additive when employed in any combination, indicating that interactions existed among the soaking and storage operations. As found in the previous experiment, the sequence used in the 3S-process resulted in the greatest yield—9.4% greater than the control. The combined yield increases of the three operations of this process employed individually was 6.2%. This interaction between soaking and storage seems to indicate that some reactions occur in mushroom tissue during post-harvest storage that increases their ability to retain natural water, water added prior to storage, or even water added after storage just prior to blanching. Hamm (1960) speculated that the increased water-holding capacity of meat tissue due to aging might be due to proteolytic cleavage of muscle proteins. He reported that subtle splitting of only a few peptide bonds could result in considerable loosening of the protein structure thereby increasing the space for immobilization of water. It is possible that such reactions might occur in mushrooms during post-harvest storage resulting in increased water-holding capacity. Swelling of the protein network due to soaking either before or after these reactions could then be synergistic with them in regard to increasing water-holding capacity.

Agtron color readings demonstrated that the 3S-process produced mushrooms with a lighter, more desirable, color compared to the control. Apparently when soaking (Soak II) was employed color deterioration was prevented by the replacement of tissue oxygen with water thereby decreasing the oxygen catalyzed browning-reactions that occur during blanching and thermal processing.

Some of the operations of the 3S-process also influenced the incidence of

Table 3–Influence of variations in the soaking operations of the PSU-3S-process on the yield and color of canned mushrooms

Treatment	Canned product[a] yield (%)	Yield increase (%)	Agtron color[a] (% Reflectance)
Control	71.9 A	–	38.2 A
Spray + C.S. 18 hr + Soak II	83.7 B	11.8	37.5 A
"Water-cool" 18 hrs.	81.7 B	9.8	40.8 A
Soak I + C.S. 18 hr + Soak II (PSU-3S-proc.)	82.2 B	10.3	37.5 A

[a] Means having the same letter are not significantly different ($\alpha = 0.05$).

Table 4–Influence of length of Soak II operations of the PSU-3S-process on the yield and color of canned mushrooms

Treatment	Canned product[a] yield (%)	Yield increase (%)	Agtron color[a] (% Reflectance)
Control	64.0 A	–	37.4 A
Soak I + C.S. 18 hr + Soak II (1/2 hr.)	69.0 B	5.0	30.8 B
Soak I + C.S. 18 hr + Soak II (1 hr.)	69.7	5.7	32.0 B
Soak I + C.S. 18 hr + Soak II (2 hr) (PSU-3S-process)	71.1 C	7.1	32.6 B

[a] Means having the same letter are not significantly different ($\alpha = 0.05$)

canned mushrooms with exposed veils. The two soaking operations employed individually and 18-hr cold storage treatment did not cause significant increases in the incidence of exposed veils. However, when the two soaking operations were employed together or in combination with storage, a significant increase in the number of mushrooms with exposed veils occurred. The greatest number of exposed veils occurred when the 3S-process was employed. In most cases, the mushrooms opened during blanching. Therefore, it appears that if such a process were adopted commercially, it would necessitate a careful inspection after blanching and prior to filling when fancy buttons or sliced buttons were packed. In this case, mushrooms with exposed veils could be diverted during inspection for use in the "stems and pieces" pack without a great loss of revenue.

In order to determine the influence of the 3S-process on the sensory quality, mushrooms from this experiment processed by the 3S-process and the control process were subjected to organoleptic evaluation. A triangle test was conducted with 10 experienced judges on two different dates. No significant differences in sensory quality were observed between the treatments as determined by Chi Square analysis. In addition, the preferences of the judges for the mushrooms was evaluated using a 9-point hedonic rating test. The analysis of variance of the data indicated that the judges showed no significant preference for the mushrooms from either treatment. Therefore, it appeared that the 3S-process had no detrimental influence on the sensory quality of the mushrooms.

Additional experiments were conducted to determine possible variations in the 3S-process which could either improve the process or make it more practical for adoption on a commercial basis. The first study, reported in Table 3, indicated that results comparable to the 3S-process could be attained by replacing the Soak I operation with a 20-min water spray treatment or by replacing the entire process by a "water-cooling" process which involved holding mushrooms submerged in water at 2°C for 18 hr. The results of this experiment indicated that processing mushrooms by these methods reduced shrinkage relative to the control in a similar manner and produced mushrooms with Agtron color values equal to the control treatment. Since both of these variations might be more practical or offer additional advantages to the processor, they represent alternatives for the adoption of this process in commercial mushroom canning operations. For instance, the 20-min spray procedure (to replace the Soak I operation) might be more practical since it might also serve as a washing operation. The 18-hr "water-cooling" operation could be more practical since it might eliminate the additional handling necessary with the 3S-process.

Another experiment was conducted to determine if the time of the Soak II operation of the 3S-process could be reduced below the 2-hr interval normally employed. The results reported in Table 4 indicated that Soak II intervals of 1/2 and 1 hr were not as beneficial in reducing shrinkage as the normal 2-hr soaking interval. Therefore, it appears that a Soak II operation approaching 2 hr is necessary to obtain the full effect of the 3S-process. However, this time-consuming operation could possibly be replaced with a vacuum-soaking operation which would require much less time. Studies with vacuum soaking are now underway in this laboratory.

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QUALITY OF FROZEN GREEN VEGETABLES BLANCHED IN FOUR CONCENTRATIONS OF AMMONIUM BICARBONATE

INTRODUCTION

IN A PREVIOUS STUDY (Eheart and Odland, 1973) the use of alkaline additives to preserve chlorophyll in processed green vegetables was reviewed. The advantages of using ammonium compounds for this purpose were stated and two ammonium compounds were studied. In the first study, a 0.24M solution of NH_4OH was used in microwave blanching or precooking broccoli prior to freezing. In the second, frozen green beans were conventionally blanched and/or cooked in a 0.1% (0.013M) solution of NH_4HCO_3. Results of the latter study were particularly encouraging and indicated that further study was justified.

The present research was conducted to determine the concentration of NH_4HCO_3 in the blanching operation which would optimize quality of five frozen green vegetables when conventionally cooked in water. The project was designed to simulate commercial operation on a small scale.

EXPERIMENTAL

FIVE REPLICATIONS of five field-harvested vegetables were blanched in four concentrations of NH_4HCO_3 solution and frozen. After 2 wk and 6 mo storage (0°F), the samples (a total of 200) were cooked and analyzed. The same sample design was used for panel testing except all samples (10 reps) were stored 5 mo before evaluation.

The vegetables studied were: Allsweet peas, Olympia green beans, Thaxter limas, Waltham-29 broccoli and Brussels sprouts (variety unknown). All vegetables were grown in Maryland except the Brussels sprouts which were air-freighted from Long Island, N.Y.

The harvested vegetables were transported to the laboratory and placed in refrigerated storage. The temperature of refrigeration was maintained at 32, 55, 48, 40 and 40°F for peas, beans, limas, broccoli and sprouts, respectively. Samples used for chemical analyses were processed within 30 hr after storage and those for panel testing within 54 hr.

Blanching treatment

Each vegetable was washed, then prepared for blanching as required: beans were mechanically snipped and cut into 1½-in. pieces; broccoli was trimmed and cut into 5-in. radial sections; and sprouts were trimmed. Peas and limas had been shelled in the field.

Vegetables were blanched in 0.0 (control), 0.1, 0.2 and 0.3% NH_4HCO_3 solutions, except broccoli which was blanched in 0.0, 0.05, 0.1 and 0.2% NH_4HCO_3 solutions. It was found in preliminary work that 0.3% NH_4HCO_3 caused sloughing of buds in broccoli. The ratio of vegetable to blanching medium was 1 lb to 1 gal.

For each treatment, sufficient vegetable for 10 lots of 300g each (250g for sprouts) was prepared. Two groups of five lots each were blanched consecutively (blanch 1 and blanch 2) in the same solution contained in an 8 gal stainless steel steam-heated kettle.

Vegetables were immersed in the blanching medium in galvanized steel blanching baskets. Temperature was readjusted between blanches and samples of the blanch water were taken before and after each blanch for pH determination.

Blanching times at recommended temperatures (Feinburg et al., 1968) were determined by negative peroxidase tests (Masure and Campbell, 1944.) Times and temperatures for each vegetable are given in Table 1.

Blanched vegetables were plunged into cold water for several minutes, then allowed to drain. The 300-g samples (250-g for sprouts, due to limited supply) were packaged in conventional freezer bags and waxed cartons, plate-frozen at −10°F overnight, then freezer stored at 0°F.

Cooking methods

Each frozen sample was added to 150 ml boiling tap water containing 0.63g NaCl (125 ml water and 0.52g NaCl for sprouts) in a covered pyrex saucepan. Vegetables were cooked to doneness according to times which had been predetermined subjectively. Cooking times for samples treated with NH_4HCO_3 were less than those for the control samples (Table 1).

Chemical analyses

Two raw samples of each vegetable were

Table 1—Blanch temperatures and blanch and cooking times used for vegetables

Conc of NH_4HCO_3 in blanch water (%)	Blanch temperature (°F)	Blanch time (min)	Cooking time (min)
Peas			
0.0	205	2	8
0.1			6
0.2			6
0.3			6
Green beans			
0.0	200	4	10
0.1			9
0.2			7
0.3			7
Lima beans			
0.0	200	2	19
0.1			19
0.2			18
0.3			18
Broccoli			
0.0	205	3	12
0.05			10
0.1			9
0.2			8
Brussels sprouts			
0.0	205	5	10
0.1			8
0.2			7
0.3			5

drawn for moisture and reduced ascorbic acid (RAA) analysis at the time of blanching. After 2 wk of storage (0°F), three samples of blanch 1 and two of blanch 2 for each treatment were cooked for analysis. The remaining samples (two of blanch 1 and three of blanch 2) were cooked and analyzed after 6 mo storage.

Titratable acidity, pH and RAA were determined by methods which have been previously described (Eheart and Gott, 1965). Dehydro ascorbic acid (DAA), analyzed by the method of Roe and Oesterling (1944), was determined for limas, broccoli and sprouts. Moisture determinations were made by drying samples to constant weight at 65°C in a forced-draft oven.

Color difference

Color differences on whole pieces of the cooked vegetables were determined by use of a green standard (L 52.3, a_L -26.1, b_L 6.6) on a Gardner XL-10 Digital Color Difference Meter (Gardner, 1969). Duplicate determination for each sample were averaged and values were expressed as L and the ratio a/b.

Panel tests

Samples cooked as described above were panel tested after 5 mo frozen storage. A panel of six trained members evaluated 10 replications of each vagetable for flavor, texture, color and acceptability. A 7-point rating scale was used with 7 the maximum score (Eheart and Odland, 1973). A hedonic scale was used only for acceptability. A total of eight samples (2 reps x 4 treatments) was evaluated at each session. The first replication served was from blanch 1 and the second from blanch 2 in all cases. Flavor and texture were judged under blue light to disquise color, which was thought in a previous study (Eheart and Odland, 1973) to introduce bias in the evaluation of these qualities.

Statistical analyses

Data were analyzed by variance and by Newman-Keul's Studentized range (Sokal and Rohlf, 1969). Differences were considered significant at the 5 and 1% levels. Judge consistency was determined by dividing mean square values for treatment by mean square values for replication for each judge (ASTM, 1968).

RESULTS & DISCUSSION

Effect of NH_4HCO_3 concentration

Chemical and physical tests. The pH of all vegetables (Table 2) was increased significantly by NH_4HCO_3. The use of 0.1% solution did not increase the pH to 7.0 in any of the vegetables. However, the 0.2 and 0.3% solutions caused pH to go above 7.0 in five out of nine cases. Alkaline additives have been found (Sweeney and Martin, 1961) to have no adverse effects on ascorbic acid or flavor when the pH of the vegetable is maintained between 6 and 7.

Both the extent of pH change and the rate of change due to increments in NH_4HCO_3 differed with vegetable. The difference between the pH of limas blanched in 0.0 and 0.3% NH_4HCO_3 was 0.16, a small increase compared to 0.99 for sprouts. This difference could have been due to buffer systems, i.e., proteins, since limas contain 7.4% protein and sprouts 3.2% (Watt and Merrill, 1963). The rate of change in pH due to increasing concentrations of alkali approached linearity in the case of peas whereas in sprouts, 0.1% NH_4HCO_3 increased pH by 0.46 over the control but 0.3% solutions effected an increase in pH of only 0.23 over the 0.2% NH_4HCO_3.

Titratable acidity was decreased by NH_4HCO_3 additions except for limas where control and treated samples did not differ significantly.

Lightness of color (Gardner L values) decreased significantly (although differences were small) for peas and beans when NH_4HCO_3 blanches were used, but there were no changes in broccoli, limas or sprouts. Greenness (Gardner a/b values) was found to increase with NH_4HCO_3 addition for all vegetables except limas. The failure of the additive to improve the color of limas was thought to be due to the minimal change in pH produced in this vegetable and to the fact that the chlorophyll in limas is concentrated in the cotyledons which were not exposed to reflectance measurements in samples of whole beans. In the four vegetables which were improved in color by NH_4HCO_3, the improvement in color was not linear with increasing concentrations of NH_4HCO_3, the largest increment effecting the least improvement. Similar results were noted above for pH changes.

Ammonium bicarbonate had no effect on RAA or DAA content of peas, beans, limas and broccoli. Brussels sprouts blanched in all concentrations of NH_4HCO_3 were higher in RAA ($P < 0.05$) than control samples and the same trend was noted in total ascorbic acid (TAA). It must be remembered that it was necessary to cook control samples longer than NH_4HCO_3 blanched samples in order to attain a similar degree of doneness. In the case of Brussels sprouts the increased ascorbic acid retention in alkali-treated samples was due to an interaction between blanching solution and storage (Table 3). Samples blanched in 0.2 and 0.3% NH_4HCO_3 retained more RAA and TAA during storage than those blanched in 0.0 or 0.1%. Furthermore, samples blanched in 0.2 and 0.3% solutions did not lose significant amounts of TAA during the 6 mo storage period. It is obvious that in this study an increase in pH of vegetable tissue even to 7.32 was not

Table 2–Effect of concentration of NH_4HCO_3 in blanch water on the composition of cooked frozen vegetables[a,b]

Conc of NH_4HCO_3 in blanch water (%)	pH	Titratable acidity (meq/100g)[c]	Color (Gardner) L	Color (Gardner) a/b	Ascorbic acid (mg/100 g)[c] RAA	DAA	TAA
Peas							
0.0	6.71^{w}	6.5^{z}	44.4^{z}	−0.69^{w}	26	–	–
0.1	6.85^{x}	5.8^{y}	43.2^{yz}	−0.75^{x}	31	–	–
0.2	7.01^{y}	5.1^{x}	42.8^{y}	−0.81^{y}	32	–	–
0.3	7.17^{z}	4.6^{w}	42.1^{y}	−0.84^{z}	27	–	
Green beans							
0.0	6.07^{w}	11.2^{z}	38.4^{z}	−0.57^{x}	32	–	–
0.1	6.39^{x}	9.4^{y}	37.3^{yz}	−0.76^{y}	36	–	–
0.2	6.71^{y}	7.6^{x}	36.2^{y}	−0.91^{z}	38	–	–
0.3	6.86^{z}	7.3^{x}	37.0^{yz}	−0.90^{z}	48	–	–
Lima beans							
0.0	6.51^{x}	9.8	57.9	−0.49	19	5	25
0.1	6.53^{x}	9.6	56.3	−0.48	17	5	22
0.2	6.60^{y}	9.2	56.3	−0.52	21	5	26
0.3	6.67^{z}	8.7	57.0	−0.51	20	5	25
Broccoli							
0.0	6.52^{w}	11.3^{z}	39.1	−0.68^{y}	473	84	557
0.05	6.78^{x}	10.1^{yz}	37.5	−0.79^{z}	463	88	550
0.1	6.93^{y}	9.1^{xy}	37.5	−0.82^{z}	490	70	560
0.2	7.10^{z}	7.9^{x}	38.4	−0.85^{z}	444	70	514
Brussels sprouts							
0.0	6.33^{w}	21.7^{z}	49.0	−0.43^{y}	365^{y} *	103	468^{y} *
0.1	6.79^{x}	18.5^{y}	46.7	−0.57^{z}	467^{z}	92	559^{yz}
0.2	7.09^{y}	14.8^{x}	47.2	−0.62^{z}	491^{z}	81	572^{yz}
0.3	7.32^{z}	11.6^{x}	44.6	−0.67^{z}	507^{z}	94	601^{z}

[a] Means of 10 samples

[b] Means with different superscripts are significantly different ($P<0.01$) unless indicated by * ($P<0.05$).

[c] Reported on dry basis

deleterious to ascorbic acid stability.

Panel evaluation. The six trained panelists were found to be about equally discriminating when their scores for consistency were evaluated. When treatment differences were large, all judges had significant F ratios and when differences were small, no one judge was consistently better than another in discerning the difference.

Panel members were able to discern a significant increase in greenness of color for all vegetables blanched with NH_4HCO_3 (Table 4). The extent of this color change, however, differed with vegetable and tended to be related to changes in pH and a/b value of the vegetable. Changes in color scores and a/b values due to NH_4HCO_3 were greatest in beans and least in limas (Tables 3 and 4).

Flavor was not affected by NH_4HCO_3 except in the case of peas where flavor scores were decreased by the highest concentration of the additive. This decrease seemed to be due to a detection of off-flavor by the judges.

An adverse effect of the alkaline additive on texture was found for all vegetables except peas. For the most part, however, this adverse effect was found only at the highest concentration of NH_4HCO_3 used for each vegetable. A study of individual scores revealed that vegetables blanched at the highest NH_4HCO_3 concentrations were judged "too soft" in a majority of cases.

Overall acceptability of peas and limas was unaffected by the additive. This was probably due to relatively small differences in color scores for these two vegetables. In beans, broccoli and sprouts differences in acceptability probably resulted from improvement in color on the one hand and adverse textural changes on the other as a result of using NH_4HCO_3. All treated samples of beans rated higher than controls in acceptability but there was no difference due to concentration of NH_4HCO_3. Broccoli blanched in 0.1% NH_4HCO_3 rated higher than controls in acceptability. At this concentration color was improved but texture was not impaired. Adverse effects on texture probably accounted for the decreased acceptability of sprouts blanched in 0.3% NH_4HCO_3.

Effect of freezer storage

The pH of peas, beans, broccoli and sprouts was found to decrease during storage (Table 5). Limas increased significantly in pH during storage. Decreases in pH due to storage of frozen vegetables have been reported previously (Van den Berg, 1961). Increases in total acidity were significant only for beans and sprouts. Peas, limas and sprouts decreased significantly in lightness of color (L values) and all vegetables except sprouts decreased in greenness during storage. There were no significant interactions between treatment and storage for pH, acidity and color.

Losses in RAA and TAA and increases in DAA during 6 mo storage were highly significant for all vegetables. The magnitude of these losses is shown by calculations based on analyses of the raw samples. Losses of RAA in cooked samples stored 2 wk (0°F) were 41, 44, 59, 64 and 67% for broccoli, sprouts, peas, beans and limas, respectively. After 6 mo storage (0°F) these losses, in the same order, were 64, 66, 91, 91 and 83%. Although excessive in all cases, losses were greatest in vegetables which do not belong to the *Brassica* genus.

A possible explanation of these large ascorbic acid losses is thought to be copper contamination of the blanching waters by worn blanching baskets. Fluctuation in storage conditions cannot be

Table 3–Storage (0°F) × treatment interaction for reduced and total ascorbic acid in Brussels sprouts[a,b,c]

Conc of NH_4HCO_3 in blanch water (%)	RAA (mg/100g)		TAA (mg/100g)	
	2 wk	6 mo	2 wk	6 mo
0.0	517^{xy}	212^{w}	597^{y}	340^{x}
0.1	641^{z}	293^{w}	698^{z}	420^{x}
0.2	555^{yz}	427^{x}	598^{y}	546^{y}
0.3	571^{yz}	443^{x}	627^{yz}	574^{y}

[a] Means of 5 samples
[b] Means with different superscripts are significantly different ($P < 0.05$).
[c] Reported on dry basis

Table 4–Effect of concentration of NH_4HCO_3 in blanch water on panel scores[a] of cooked frozen vegetables[b,c]

Conc of NH_4HCO_3 in blanch water (%)	Color	Flavor	Texture	Acceptability
Peas				
0.0	3.7^{x}	4.1^{z}	4.8	4.1
0.1	4.3^{y}	4.2^{z}	4.6	4.0
0.2	5.0^{z}	3.8^{yz}	4.7	3.8
0.3	5.3^{z}	3.1^{y}	4.4	3.6
Green beans				
0.0	2.3^{w}	4.1	5.7^{z}	4.0^{y*}
0.1	4.4^{x}	4.6	5.7^{z}	4.6^{z}
0.2	5.9^{y}	4.7	4.8^{y}	4.6^{z}
0.3	6.3^{z}	4.7	4.8^{y}	4.5^{z}
Lima beans				
0.0	4.1^{x}	4.1	5.5^{z}	4.3
0.1	4.4^{xy}	3.9	5.6^{z}	4.3
0.2	4.8^{y}	3.8	5.4^{z}	4.3
0.3	5.2^{z}	3.9	4.6^{y}	4.0
Broccoli				
0.0	3.3^{y}	4.8	5.9^{z}	4.6^{y*}
0.05	5.4^{z}	4.8	5.3^{yz}	5.1^{yz}
0.1	5.7^{z}	5.2	5.7^{z}	5.3^{z}
0.2	6.1^{z}	4.5	4.9^{y}	4.9^{yz}
Brussels sprouts				
0.0	3.1^{y}	4.7	5.0^{z}	4.3^{z*}
0.1	5.7^{z}	4.4	5.3^{z}	4.5^{z}
0.2	5.9^{z}	4.2	4.5^{yz}	4.4^{z}
0.3	6.2^{z}	3.9	3.9^{y}	3.9^{y}

[a] Judged on a 7-point scale with 7 considered optimum
[b] Means of 60 judgements
[c] Means with different superscripts are significantly different ($P < 0.01$) unless indicated by * ($P < 0.05$).

cited as a cause since storage temperature was maintained constant at 0°F. The use of NH_4HCO_3 as a cause can also be eliminated since the only significant interaction between storage and treatment, discussed above, showed that treated samples of sprouts were higher in RAA after storage than control samples.

Effect of blanch order

Reuse of blanch water through a continuous blanching procedure is a common practice in industry. Both from the standpoint of effluent pollution and economics, reuse of the blanch solution becomes even more important when an additive is used. The effect of blanch order (batch procedure) on the composition of vegetables is reported in Table 6. Small but significant decreases in the pH of peas and broccoli occurred in the second blanch. Total acidity in no case differed between blanches 1 and 2. Sprouts from blanch 1 were greener (a/b) than those from blanch 2 but the opposite was true for limas. Since there was no consistent effect on color when the blanch water was reused, this procedure can be recommended for at least two batches. The fact that ascorbic acid content was not affected by blanch order, except for peas, further substantiates the practicality of reusing the blanch water. The lower ascorbic acid values for peas from blanch 2 cannot be explained.

Table 5—Effect of storage (0°F) on the composition of cooked frozen vegetables[a,b]

Storage period	pH	Titratable acidity (meq/100g)[c]	Color (Gardner) L	Color (Gardner) a/b	Ascorbic acid (mg/100g)[c] RAA	DAA	TAA
Peas							
2 wk	6.97[z]	5.4	43.8[z]	−0.82[z]	48[z]	–	–
6 mo	6.89[y]	5.5	42.4[y]	−0.73[y]	10[y]	–	–
Green beans							
2 wk	6.55[z]	8.4[y]	37.6	−0.82[z]	62[z]	–	–
6 mo	6.47[y]	9.4[z]	36.8	−0.75[y]	16[y]	–	–
Lima beans							
2 wk	6.54[y]	8.9	57.6[z]	−0.53[z]	26[z]	3[y]	28[z]
6 mo	6.61[z]	9.7	56.2[y]	−0.47[y]	13[y]	7[z]	21[y]
Broccoli							
2 wk	6.87[z]	9.7	37.9	−0.80[z]*	577[z]	28[y]	605[z]
6 mo	6.79[y]	9.5	38.3	−0.77[y]	357[y]	128[z]	485[y]
Brussels sprouts							
2 wk	6.95[z]	15.2[y]	49.8[z]	−0.57	571[z]	59[y]	630[z]
6 mo	6.81[y]	18.1[z]	44.0[y]	−0.57	343[y]	127[z]	470[y]

[a] Means of 20 samples
[b] Means with different superscripts are significantly different ($P < 0.01$) unless indicated by * ($P < 0.05$).
[c] Reported on dry basis

Table 6—Effect of blanch order on composition of frozen vegetables[a,b]

Blanch order	pH	Titratable acidity (meq/100g)[c]	Color (Gardner) L	Color (Gardner) a/b	Ascorbic acid (mg/100g)[c] RAA	DAA	TAA
Peas							
1	6.96[z]	5.3	42.7[y]*	−0.77	36[z]	–	–
2	6.92[y]	5.4	43.6[z]	−0.78	17[y]	–	–
Green beans							
1	6.49	8.9	37.0	−0.78	39	–	–
2	6.49	9.4	37.2	−0.79	40	–	–
Lima beans							
1	6.58	9.7	57.0	−0.48[y]	21	5	27
2	6.58	9.3	57.0	−0.51[z]	20	5	25
Broccoli							
1	6.87[z]	9.7	37.7	−0.79	455	78	533
2	6.79[y]	9.7	38.6	−0.77	459	73	531
Brussels sprouts							
1	6.95	15.9	45.5	−0.60[z]	500	89	588
2	6.85	17.1	48.4	−0.53[y]	455	104	559

[a] Means of 16 samples
[b] Means with different superscripts are significantly different ($P < 0.01$) unless indicated by * ($P < 0.05$).
[c] Reported on dry basis

TENTATIVE RECOMMENDATIONS

SINCE VEGETABLES differ in pH, buffer systems and textural properties, it was anticipated that the optimum concentration of NH_4HCO_3 would vary with different types of vegetables. Data in Tables 2, 3 and 4 support this view.

Although the data in this study are limited, they are being used to make the following preliminary recommendations until such time that further research either substantiates, modifies or rejects them:

(1) 0.2% NH_4HCO_3 for peas and limas. Color improvement was noted at this concentration with no adverse effects on ascorbic acid retention, flavor, texture or acceptability. Higher concentrations adversely affected flavor of peas and texture of limas.

(2) 0.1% NH_4HCO_3 for green beans. Color stability during cooking of this vegetable is known to be difficult due to its low pH. Although increased retention of color was attained at the 0.2 and 0.3% concentrations of NH_4HCO_3, texture was adversely affected at these levels. Since softening was accompanied by sloughing in this vegetable, it was thought that a concentration of additive should be used which improves color without incurring any textural changes.

(3) 0.1% for broccoli and Brussels sprouts. No further color improvement was found at higher concentrations and texture and/or acceptability decreased at levels above 0.1%.

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FLOW PROPERTIES OF SOME FOOD POWDERS

INTRODUCTION

FLOW DIFFICULTIES and caking are common problems in industries producing or utilizing food powders. Several powders, such as powdered fruits and vegetables are known for their tendency to cause flow problems, while other powders like flour and starch under normal storage conditions are known to be relatively free flowing. High relative humidity, compaction pressure and small particle size are some of the causes for flow difficulties. Various ways of overcoming, to some extent, the effect of these parameters are applied commercially and include: drying to low moisture content, instantization processess and addition of anticaking agents.

There is little fundamental knowledge of the flow phenomena of food powders. Most of these powders are nonhomogenous and anisotropic, so that determination of parameters for defining flowability and cohesiveness poses a difficult problem. A quantitative way to assess physical powder characteristics regarding their interparticle forces is to measure their bulk density. Its relation to compaction might be employed to characterize a powder qualitatively and quantitatively.

Kawakita and Ludde (1969) suggested a general equation claimed to fit the behavior of powders under vast ranges of pressure. Williams and Birks (1967) suggested that under relatively small pressures, up to a few kg/cm^2, the specific volume of a powder was dependent on log pressure.

A power law dependency between the latter parameters was found by Ashton et al. (1965), and an exponential relationship by Jenike et al. (1960). The cohesive forces acting between particles might be van der Waal's forces, interfacial forces, liquid and solid bridging and interlocking (Rumpf, 1961). The forces are dependent on the distance between particles and number of contact points. When compressing a powder the number of contact points increases; solid bridges are formed which cause the powder to harden and cake (Rumpf, 1961).

Another quantitative way of evaluating powder flow characteristics was developed by Ashton et al. (1964) based on an idea by Tideswell and Tolleyfield (unpublished). In this method the tensile strength of a powder compact was measured and gave a good indication of forces acting between particles. Tensile strength was affected by the bulk density, moisture and particle size distribution of the powder (Peleg and Mannheim, 1973).

A general theory for the determination of flowability of solid particles was developed by Jenike (1967) and extended by Williams and Birks (1967). This theory is based on failure properties of a compacted powder bed and thus treats inclusively both free flowing and cohesive powders. Their experimental technique was based on compaction of a powder specimen under a determined consolidation load followed by a shear operation. The stresses acting on a powder are represented by a yield locus in a system of coordinates where the S axis is the shear stress and V the applied compressive stress (Fig. 1). The solid will fail when stresses, as determined by a Mohr semicircle, are tangential to the yield locus. The yield locus terminates at a point M which is the point where the specimen fails without change in volume.

The intersection of the yield locus with the shear stress axis gives the value of cohesion (C), which is a function of the powders' property as well as of the preconsolidation pressure. The inclination of the line connecting the ends of the yield loci of the powder, (performed under various preconsolidation loads) with the normal stress axis, is the angle of

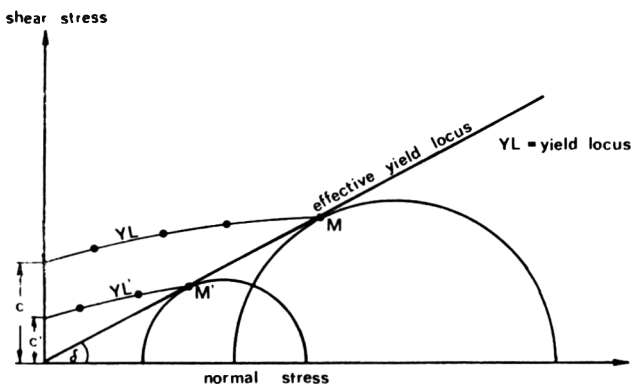

Fig. 1—Mohr semicircle for stresses description.

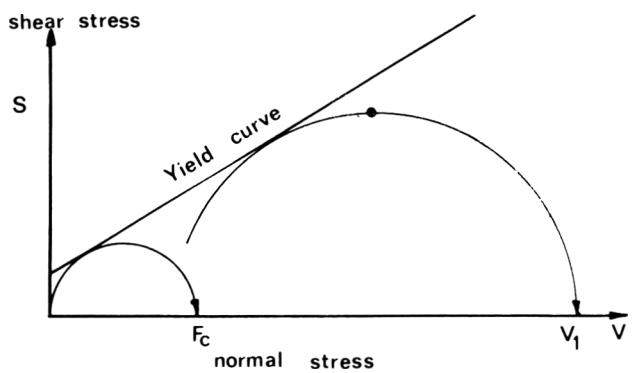

Fig. 2—Calculation of flow function.

internal friction (δ). This angle is a measure of the resistance to flow when the powder is on the move and is therefore an important characteristic.

Another important property of a powder is the flow function (FF) according to Jenike (1967). This is derived from a family of yield loci, using two Mohr semicircles. The first semicircle tangential to the yield curve at its end point (Fig. 2) gives the maximum principal stress under which the specimen is consolidated $-V_1$. The second Mohr semicircle tangential to the yield curve and passing through the origin, gives the point on the axis corresponding to zero external force called the unconfined yield locus $-F_c$ (Fig. 2). This flow or failure function describes behavior of a powder at a given bulk density over a wide range of pressures, which might occur in storage.

A quantitative characterization of flow properties of some food powders with and without anticaking agents as affected by physical properties such as pressure and moisture was looked for. A Jenike Flow Factor Tester and a tensile strength apparatus were used to establish criteria for cohesiveness and flowability of various food powders.

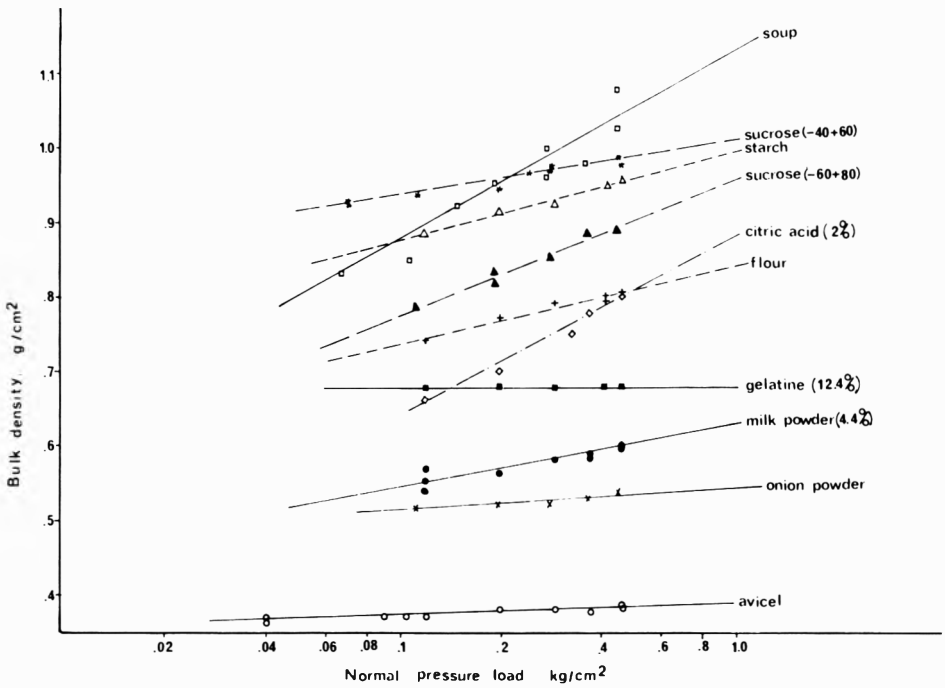

Fig. 3—Relationship between bulk density and normal pressure loads for various food powders.

Table 1—Bulk density and compressibility of various food powders[a]

Material	Moisture content (%)	Initial bulk density (g/cm²)	Constants of regression eq	
			a (g/cm³)	b Compressibility
Avicel	6.3	0.345	0.388	0.017
Onion powder	dry[b]	0.509	0.545	0.032
Onion powder	5.2	0.510	0.566	0.051
Instant coffee	1.6	0.240	0.293	0.040
Instant coffee	9.3	0.378	0.585	0.155
Gelatine	12.4	0.675	0.678	~0
Milk powder	4.4	0.426	0.629	0.089
Sucrose +60–80	dry[b]	0.622	0.960	0.152
Sucrose +60–80	R.H=52%	0.498	0.961	0.185
Flour	11.8	0.597	0.842	0.106
Corn starch	dry[b]	0.814	0.995	0.119
Corn starch	18.5	0.687	0.885	0.149
Citric acid	2.0	0.506	0.883	0.244
Soup mix	5.4	0.700	1.135	0.268

[a] Six to 13 measurements were made of each powder. In all cases the data fit the semilog equation to the 1% significance level.
[b] Vacuum dried at 70° C for 16 hr

EXPERIMENTAL

A NUMBER of food powders covering a wide range of flow properties were tested in this work. Citric acid, sucrose, gelatine and onion powder were ground in a laboratory mill (Junior Lab. Mill, Christy and Morris Ltd.) and were sieved to the desired size fractions, prior to use. Sucrose, gelatine and onion powder were used in their +60–80 mesh fraction while citric acid was used in the +120 mesh fraction since it was hard to compact at the larger particle sizes. Soup mix, Avicel, spray dried instant coffee and milk powder were tested in their regular commercial form.

Anticaking agents employed were aluminum silicate and calcium stearate (B.D.H., England). The anticaking agents were admixed with the powders in a domestic type kitchen mixer for 15 min. When effect of moisture was studied, samples of powders were allowed to equilibrate in vacuum desiccators containing saturated salt solutions as suggested by Rockland (1960).

Tensile strength measurements

The apparatus used for these measurements consisted of a horizontally split lucite cell with cross sectional area of 11.95 cm² and 4.5 cm high as described previously (Peleg and Mannheim, 1973). The powder was poured into the cell using a top ring and consolidated in a method similar to that described by Jenike (1967). From the measured loads the force per unit area needed to split the sample was obtained. The bulk density at different consolidating values was also determined.

Shear strength measurments

All materials were subjected to shear analysis using a Jenike Flow Factor Tester (Jenike, 1967). The apparatus consists of a horizontally split circular cell. The lower half is fixed and the upper is moveable. A powder specimen is placed inside the cell and consolidated to a desired state of compaction. The shear force is applied at a constant rate to the upper part of the cell and recorded until failure occurs. To obtain a yield locus for a given powder a set of specimen were prepared at the same state of compaction. The specimen were then subjected to different normal pressure loads and the shear force needed to cause failure was measured. When the shear force at failure was plotted versus the normal load, the yield locus curve was obtained. Extrapolation of the line to zero normal load (Fig. 1) enabled calculation of the cohesion value at a particular bulk density. Angle of internal friction and flow function were calculated as described by Jenike (1967).

RESULTS & DISCUSSION

Consolidation curve under normal compaction pressure

Figure 3 and Table 1 show the bulk density for cohesive and for fairly free flowing powders under normal compressive stresses.

The relationship between the bulk density and the applied normal pressure up to 0.6 kg/cm² was found to fit highly significantly a semilog curve:

$$B.D. = a + b \log P$$

where: B.D. = bulk density (g/cm³); P = applied normal pressure (kg); and a, b = constants of the equation, with the fol-

lowing physical meaning: a is the bulk density at unit pressure (1 kg/cm^2) and b is the slope of the line which describes the compressibility.

Compressibility may be used to characterize food powders in regard to their cohesive properties. This concept, by definition, describes the influence of pressure on the bulk density, or in other words shows in what manner pressure reduces the volume of a powder bed. In the case of noncohesive powders like Avicel, gelatine or dry onion powder, there are almost no interparticle forces or of a weak nature only. Therefore, these powders will occupy the volume of a container leaving only random voids, due to random arrangements and friction (Fig. 4). The original structure will be relatively dense, the pressure applied will affect mostly these random voids, and will have small effect on the bulk density (Table 1) resulting in very low values of compressibility (b). It was assumed and microscopically observed that in the range of compaction pressures used, no particle shape deformation occurred.

In the case of cohesive powders like citric acid and instant coffee, strong interparticles forces enable the formation of an open structure (Fig. 4). Pressure, when applied will have greater effect by causing the collapse of the open structure. In this case high values were obtained for compressibility.

The compressibility (b) was increased by addition of moisture to the powder. For cohesive and compressible powders, like instant coffee, the change in b was very high, as compared to the noncompressible powders like Avicel and dry onion powder. This behavior supports the suggested mechanism, where strong interparticles forces cause the open structure to collapse under pressure, thus increasing the values of compressibility.

These results show that the compressibility of powders may be used as means of characterizing food powders in respect to their cohesiveness, with the assumption that the density of the solid in the food powders is about 1.6; therefore, by definition, compressibility has the same meaning for all powders.

Tensile strength curves

The tensile strength for a number of cohesive and noncohesive powders as a

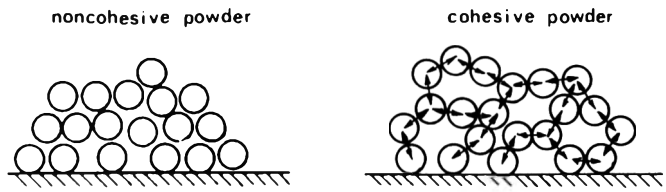

Fig. 4—Particles arrangement in cohesive and noncohesive powders.

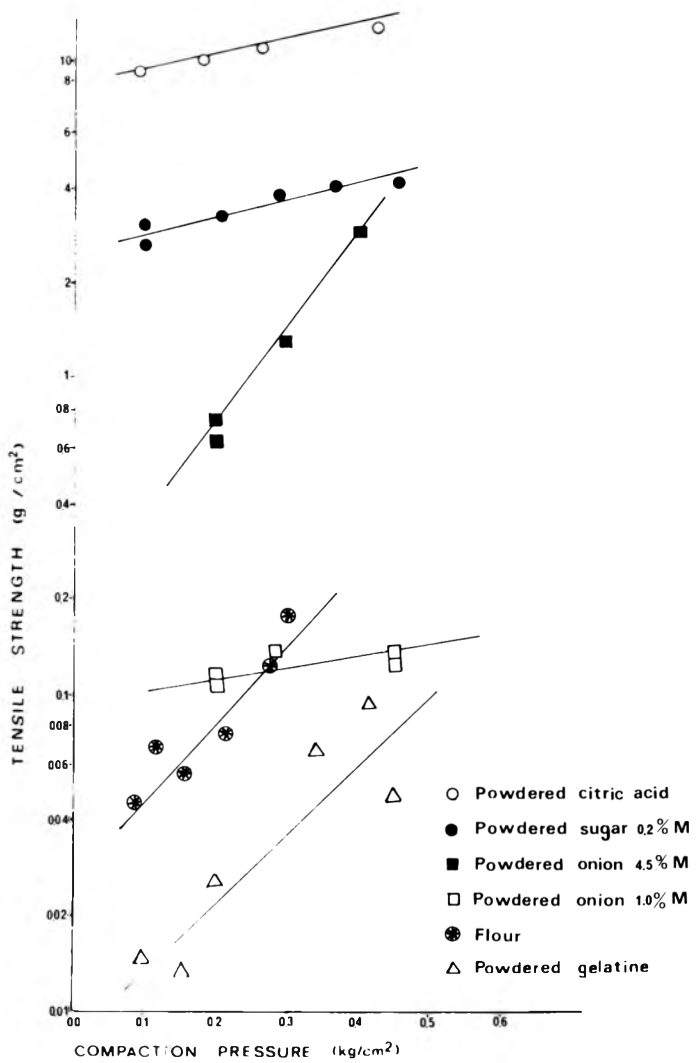

Fig. 5—Tensile strength of various food powders.

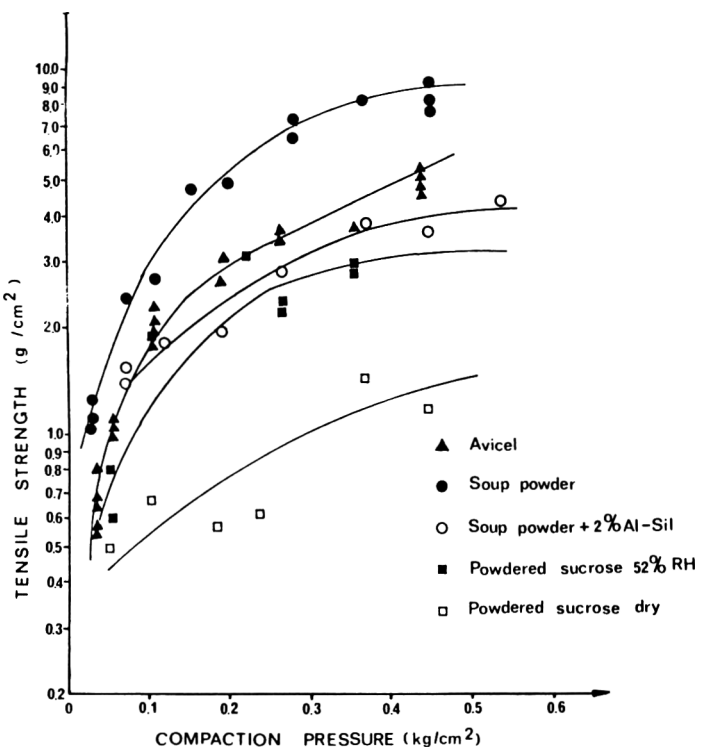

Fig. 6—Influence of moisture content and anticaking agent on tensile strength of food powders.

Table 2—Flow properties of sucrose powder and starch at 6.5 kg consolidation load[a]

Material	Moisture content (%)	Anticaking agent Material[b]	Anticaking agent %	Cohesion C (g/cm²)	Angle of internal friction δ (deg)
Sucrose +60–80	dry[c]	–	–	12.2	38.8
Sucrose +60–80	dry[c]	AS	1	7.9	39.0
Sucrose +60–80	dry[c]	CS	1	6.3	34.0
Sucrose +60–80	R.H=52%	–	–	14.2	34.7
Sucrose +60–80	R.H=52%	AS	1	12.5	41.3
Sucrose +60–80	R.H=52%	AS	2	7.3	42.8
Sucrose +60–80	R.H=52%	AS	3	7.6	42.5
Sucrose +60–80	R.H=52%	CS	1	7.8	35.8
Sucrose +60–80	R.H=52%	CS	2	4.0	35.6
Sucrose +60–80	R.H=52%	CS	3	8.6	34.8
Corn starch	10.9	–	–	4.6	36.2
Corn starch	18.5	–	–	13.0	31.0
Corn starch	9.3	AS	1	1.8	37.4
Corn starch	8.7	CS	1	5.5	31.3

[a] Six measurements at least were made of each powder. In all cases the data fit the linear equation to the 1% significance level.
[b] AS – Aluminum silicate; CS – Calcium stearate
[c] Vacuum dried at 70°C

function of the compaction pressure is presented in Figures 5 and 6.

In compacts like Avicel, where the powder is thought to be isotropic and homogenous, the scatter of results using the above described apparatus was fairly low. In other powders, the high scatter of results might be explained by the following difficulties in the compaction procedure:

(a) Nonhomogenous compaction due to random orientation of particles;
(b) The exposed failure plane being different in each experiment; and
(c) The failure rate was not constant.

Results show that the range of tensile strength for cohesive powders like powdered citric acid, powdered sucrose and powdered onion (at 4.5% moisture content) was 0.5–15g/cm². For noncohesive powders like powdered gelatine, flour and dry onion powder the range was 0.01–0.2g/cm² for the same compaction pressures which ranged from 0.1–0.5 kg/cm².

Tensile strength may be used as a measure of the forces acting between particles; therefore, it might be concluded that powders like flour and gelatine, which have low tensile strength, will not cause caking or flow problems. Regarding powders with high tensile strength it was difficult to draw conclusions. Under normal storage conditions the compaction may not be as high as the one in the test; therefore, a material which is free flowing under normal storage conditions might show high tensile strength under compaction. An example for this phenomenon was Avicel (Fig. 6). Under normal storage conditions, this material does not cake and flows easily, but under compaction the mutual interparticle attraction of the crystalline particles, causes the powder to have a high tensile strength.

Moisture increases the tensile strength of powders as demonstrated in Figure 5 for onion powder and in Figure 6 for sucrose powder, by forming a liquid film on the particles. The addition of anticaking to soup powder decreased its measured tensile strength distinctively. This might be due to the fact that aluminum silicate separates the powder's particles mechanically, and therefore decreases its tensile strength.

Shear yield locus and flow function

Results of cohesion, angle of internal friction and flow function of various food powders, as tested with Jenike's Flow Factor tester are given in Tables 2, 3 and 4 and Figures 7, 8 and 9.

The yield locus of a set of specimen, obtained under different normal compaction loads was found to fit, highly significantly, a straight line:

$$S = m + nV$$

where S = the shear stress (kg); V = the normal compaction load (kg); m = is the intersection of the yield locus with the shear stress axis, from which cohesion (C) expressed in units of g/cm² was calculated and n is the slope of the yield locus

Table 3—Flow properties of various food powders[a]

Food powder	Moisture (%)	Consolidation load (kg)	Cohesion C (g/cm²)	Angle of internal friction δ (deg)
Milk powder	1.05	6.5	7.0	39.4
Milk powder	4.4	6.5	9.6	38.9
Grapefruit juice powder	1.8	6.5	7.9	38.1
Grapefruit juice powder	2.6	6.5	10.4	37.2
Red beets	3.3	6.5	8.6	42.8
Sodium chloride	0.32	6.5	2.0	36.4
Potato starch	14.95	6.5	2.0	36.8
Gelatine	–	4.75	0.8	39.4
Soy flour	–	6.75	0.9	34.3

[a] At least six measurements were made of each powder. In all cases the data fit the linear equation to the 1% significance level.

Table 4—The effect of increased pressure (i.e. bulk density) on flow properties[a]

Food powder	Moisture (%)	Consolidation load (kg)	Cohesion C (g/cm²)	Angle of internal friction δ (deg)
Milk powder	4.4	4.5	1.6	41.0
Milk powder	4.4	6.5	9.6	38.9
Milk powder	4.4	8.0	10.9	38.1
Milk powder	4.4	10.5	16.9	36.7
Sucrose powder +60–80	R.H=52%	4.5	9.0	38.3
Sucrose powder +60–80	R.H=52%	6.5	4.4	38.4
Sucrose powder +60–80	R.H=52%	8.0	14.2	34.7
Sucrose powder +60–80	R.H=52%	10.5	17.9	36.0
Corn starch	dry[b]	4.5	5.5	35.5
Corn starch	dry[b]	6.5	5.9	33.5
Corn starch	dry[b]	8.0	5.4	33.1
Corn starch	dry[b]	10.5	4.3	31.0

[a] At least six measurements were made of each powder. In all cases the data fit the linear equation to the 1% significance level.
[b] Vacuum dried at 70°C

(Fig. 1). (C = K·m, K is a conversion factor which equals 14.1 in this case).

Cohesion (C) in all powders increased significantly when the materials were subjected to increased moisture (Tables 2, 3 and 4 and Fig. 7). Onion powder served as an example of a hygroscopic powder. Its cohesion was measured at various moisture contents under a preconsolidating load of 6.5 kg (Fig. 7). The cohesion changed gradually up to about 5% moisture and above this value a steep increase in cohesion was observed. No yield point of the onion powder in the Jenike apparatus could be obtained, under these moisture and operational conditions.

By increasing the bulk density of powders, i.e., using higher preconsolidation loads, cohesion usually increased, as was demonstrated for milk powder, and sucrose (Table 4). This observation was found to be true mostly for powders which were compressible. Powders like starch did not change their cohesion solely by applying pressure in the range tested, but the combination of increasing moisture and applying pressure was required to affect this parameter.

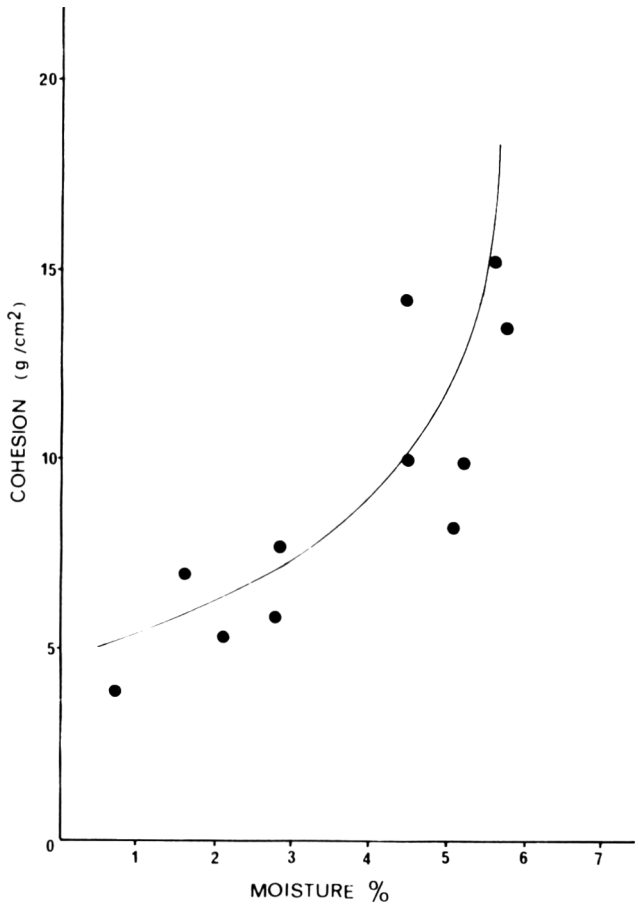

Fig. 7–Cohesion vs. moisture content of onion powder.

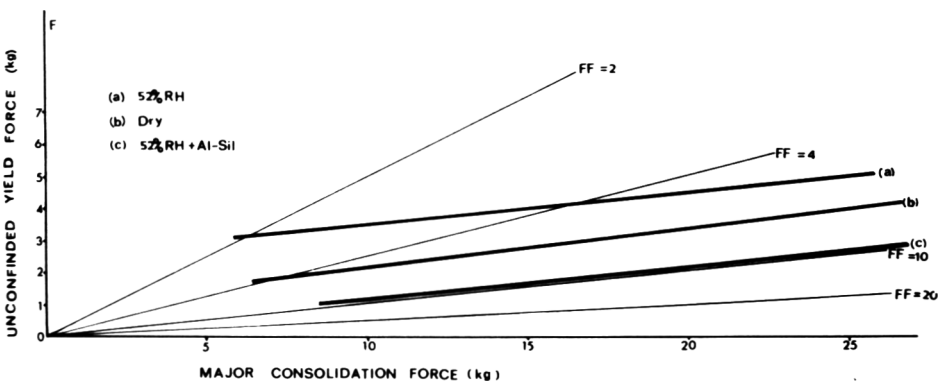

Fig. 8–Flow function of sucrose under various conditions.

The angle of internal friction, as mentioned before, is a measure of the resistance to flow when the powder is on the move (Williams and Birks, 1967). This angle was affected very little by experimental variation, i.e., change of compaction pressures and its deviation was usually 2 degrees from the average. Results show, in some cases, a decrease of up to 3 degrees with increasing moisture content (Tables 2 to 4). This fact suggests the existance of a liquid film on the particle's surface acting like a lubricant. Water adsorption may also cause dissolution of sharp edges of water soluble materials like sucrose, thus smoothing the surfaces in addition to the lubricating effect.

Flow functions show how the strength of a free surface in the powder depends on the stress under which the specimen was consolidated. It was assumed that during the test, temperature and humidity were constant, while the time of consolidation at rest was zero, otherwise this function might be temperature, humidity and time dependent which complicates the situation.

Results of failure or flow function for sucrose and starch are shown in Figures 8 and 9.

The degree of flowability of these powders (i.e., flow function) is shown to be affected by increased moisture. The values of these flow functions can be used to classify the powders according to Jenike's classification (1967).

Regarding the sucrose sample, the powder changed from "easy flowing," with FF values of 5–10 (depending on consolidation) while dry, to "cohesive" after moisture adsorption, with FF values of 4–5 (Fig. 8).

The classification for starch changed from "free flowing" while dry to "easy flowing" after moisture adsorption (Fig. 9).

The effect of anticaking agents on flow properties

Effect of two anticaking materials, (aluminum silicate and calcium stearate) on flow conditions of sucrose powder, was investigated and results are shown in Tables 2 and 3 and Figures 8 and 9. From these tables it was found that both agents generally decreased the cohesion. Aluminum silicate decreases the interparticles attraction forces by separating physically the powder particles and breaking the continuity of the liquid layer, thus de-

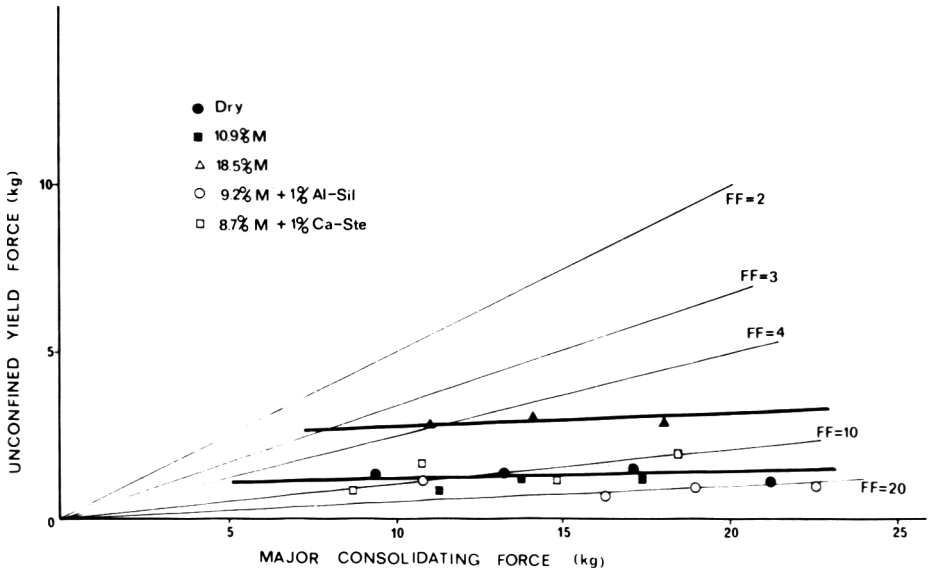

Fig. 9—Flow function of starch under various conditions.

creasing the cohesion (C). With the powders tested, this flow conditioner was less effective than calcium stearate and under the same compaction loads (Table 2) had no effect. On the other hand, due to the reduction of the liquid's lubricating effect, a growth of the angle of internal friction was demonstrated (Table 2). Calcium stearate, due to its fatty structure acts as a lubricant by itself and also reduces the interparticle attraction forces, and as such decreases the cohesion more drastically and the angle of internal friction by 2–4 degrees.

The flow function of the sucrose powder as affected by anticaking agents are presented in Figure 8. This measure gives the overall effect of flowability.

Aluminum silicate improved only slightly the flowability of moist sucrose powder while calcium stearate brought about a significant increase in flowability (FF = 10–20) which was found to be even higher than that for dry sucrose. The flowability of starch did not improve with addition of the above mentioned anticaking agents (Fig. 9) under the test conditons.

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AN ELECTRONIC APPLE REDNESS METER

INTRODUCTION

TODAY, apples are merchandized by "color" as much as by taste, size or shape. It has been established that the skin color of fruits bears a direct relationship with fruit maturity and with its overall eating quality (Brearley et al., 1964; Brearley and Breeze, 1966; Lott, 1943, 1944; Birth and Norris, 1958; Kvåle, 1967). Hence, grading of apples on the basis of color has become a key factor in the sorting of the fruit on its way to market.

MATERIALS & METHODS

Color grading and color sorting

In the packing house, fruit is graded in terms of its skin color. To help the fruit industry in maintaining uniform grades from year-to-year, Inspectors of the Fruit & Vegetable Div. of the Federal Dept. of Agriculture assign minimum color standards as representative of the different grades of the fruit. In this paper, therefore, color grading refers to the selection of individual fruits as representative of a grade. The grades thus defined are used as a basis for color sorting during the packing of the crop.

Color grading by eye

In the past, color grading has been done visually with not too satisfactory results. The judgment of color is an opinion which is susceptible to the influence of both environmental and personal factors which affect the human ability to perceive color with day-to-day accuracy. Such factors may include health, fatigue, eye strain, color sensitivity of the eyes and sample conditions such as surface gloss and glare, color distribution, quality of the ambient illumination, location and size of other objects in view, the effects of metamerism and visual field, etc.

Results of extensive field tests with three types of visual color comparators (Brearley and Breeze, 1966) developed by B.C. Research revealed that although such color aids were of some help to the grading inspectors, they were far from being satisfactory since a uniform color interpretation to which several observers would agree could not be achieved.

Color instruments

"Color" is an important marketing index of the quality of fresh apples and other agricultural products. Several different techniques have been tried for measuring color with each new approach claiming certain advantages over previous ones. Numerous workers in the field of color instrumentation derived their inspiration from the tristimulus colorimetry of the C.I.E. system.

The Lovibond Tintometer (Lovibond, 1887) which is a subtractive type colorimeter was developed in 1887 and found useful for color measurement of materials that transmit light, such as lubricating oil, vegetable oil, beer, etc. In this instrument the single light beam providing the comparison field is modified on being passed successively through three filters (red, yellow and blue), each of which "subtracts" at every wavelength a certain fraction of the light incident upon it. This was followed by the Jones Subtractive Colorimeter (Jones, 1920) in 1920 which used cyan, magenta and yellow filters. In 1953, Desrosier (1954) reported the development of the Purdue Color Ratio Meter which was capable of measuring the surface color of specimens in terms of two spectrum color bands (red/yellow ratio).

In 1948, Hunter reported his Color and Color Difference Meter incorporating three filters which approximated the X, Y, Z functions of the C.I.E. System (Hunter, 1942). Francis (1952) reported an attachment device for use with the Hunter Color and Color Difference Meter or the Photovoltaic Reflection Meter, to measure the skin color in apples. In 1961, Hunter reported his Direct-Reading Tomato Colorimeter based on similar principles. In 1965, Birth and Norris of USDA's Instrumentation Research Laboratory, developed their Abridged Spectrophotometer which works by measuring optical density difference between two wavelengths, i.e., $\Delta OD\ (\lambda_A - \lambda_B)$.

The shortcomings of visual comparators and the fact that none of the color meters available could be classed as simple, portable, direct reading instruments, opened the way for consideration of the development of an electronic instrument which would be capable of making a more "objective" measurement of the skin color of apples.

Desired features. There was a pressing need for an instrument of the following features.

1. It should be adaptable to a wide range of fruit types and sizes.
2. Instrument readings should have good correlation with visual subjective grade evaluation of colors.
3. It should be capable of making quick measurements.
4. Color grade readings should be direct and unambiguous. (A tristimulus instrument is not considered direct reading because three separate readings have to be taken and the color value computed therefrom.)
5. Size, shape and curvature of an apple should not affect color measurement to any significant extent.
6. Environmental conditions, such as ambient lighting, humidity and temperature, should have no significant effect on meter readings.
7. It should be capable of making color measurement of any specifically marked skin area of an apple's surface.
8. It should be simple to operate.
9. It should not damage the specimen apple in any way.

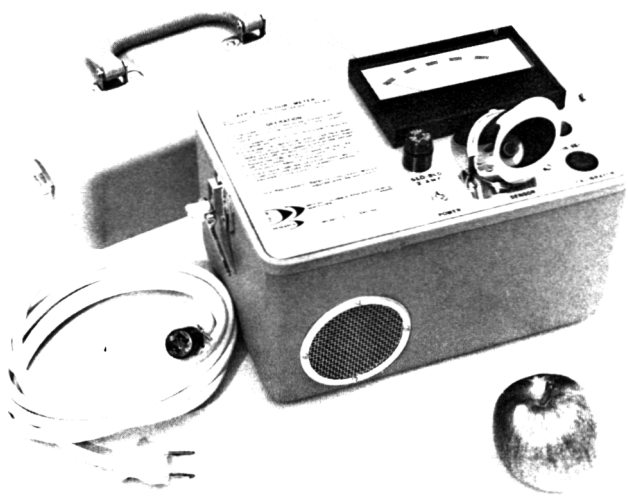

Fig. 1—Apple Redness Meter.

10. It should be of a rugged and reliable design.
11. It should be portable in size and weight.

Initial spectrophotometric studies

A selection of red apples was sorted visually into four to five different grades of redness. Using a "Beckman" Model DB spectrophotometer, readings of diffused reflectance (percent) against a Vitrolite reference (100% absolute reflectance) were then taken for a uniformly colored red section of the skin of each apple. These spectral reflectance readings were taken over the visible range of 380–760 mμ at wavelength intervals of 30 mμ. It was found that a good correlation existed between the visually assigned grades and the corresponding ratio of radiant flux reflected in the red region (640–760 mμ) to the radiant flux reflected throughout the visible range (380–760 mμ) as shown below:

$$\text{Visually assigned grade} \propto \frac{\sum_{640}^{760} \lambda R(\lambda) E_{\lambda(C)} \Delta\lambda}{\sum_{380}^{760} \lambda R(\lambda) E_{\lambda(C)} \Delta\lambda}$$

where $R(\lambda)$ is the spectral reflectance of the apple's surface; $E_{\lambda(C)}$ is the spectral distribution of the flux (in terms of C.I.E. source C) irradiating the apple's surface.

Development of the Apple Color Meter

An Apple Color Meter (Fig. 1) based on the above stated ratio principle has been developed under the joint sponsorship of the Fruit & Vegetable Div. of the Canada Dept. of Agriculture and the British Columbia Fruit Growers' Association. It incorporates a constant light source consisting of a quartz-iodine projection lamp plus a fiber-optic light guide which provides suitable illumination for the apple surface being viewed.

The instrument is equipped with a specially-designed illuminator/sensor head consisting of a central fiber optic light guide as illuminator surrounded by a concentric ring of 46 fiber-optic light tubes which receive the reflected light from a 1-3/8 in. diam area of the apple surface. These receptor fibers are coupled alternately to two spectrally matched photo sensors, one of which is provided with a red filter which essentially filters out all light radiation below 640 mμ. The photo sensors feed the color information into the transistorized circuitry of the Apple Color Meter which immediately produces an analog indication of the "Redness" of the viewed area. The physical design and dimensions and orientation of the component parts of the illuminator/sensor head are such that the masking effect of specular reflectance is effectively eliminated.

The principle of the design of this instrument will allow much larger surface areas to be viewed making possible the design of instruments for measuring the color of other fruit and vegetable products.

RESULTS & DISCUSSION

Evaluation of the instrument

To evaluate the agreement of meter reading with visual grading a panel of 20 persons (17 males and 3 females) was asked to sort apples visually into 10 grades of increasing redness, from very pale to very dark red. The arrangements for sorting were such that each sorter worked alone with all apples being returned to a certain bin prior to sorting by the next panel member. Three varieties of British Columbia grown red apples were sorted separately.

Table 1 shows averages of visual and instrument readings derived from the raw data collected where x designates the grade indicated by the meter and y the visual grade. The computed coefficient of concordance (Shannon, 1968) for the panel was 0.99 indicating that a good degree of agreement existed between the chosen members of the panel. A straight line provided an excellent fit between visual and meter grades, the squared correlation coefficient being 0.99 for the three apple varieties tested (Delicious, Spartan and McIntosh). Hence, very good agreement existed between the subjective visual grades and the grade values obtained by means of the Apple Redness Meter. In view of the close agreement of the values for the three varieties of apples tested, a common regression equation was derived using the data given in the last two columns of the top section of Table 1. A plot of this equation is shown in Figure 2 indicating the very close correlation between visual observations and the values obtained with the Apple Redness Meter.

Five Apple Redness Meters were supplied for field evaluation to the Fruit & Vegetable Div. of the Canada Dept. of Agriculture in the provinces of British Columbia, Ontario, Quebec and Nova Scotia. The results reported from all areas were gratifying. The field users reported favorably on the instrument's weight (5 1/2 lb), its rugged design and compact size (6 in. × 9 in. × 6 in.), the ease and speed of operation and the reproducibility and general freedom from ambiguity of measurement results.

CONCLUSION

IN USE, the instrument displays a sensitivity to changes in "redness" closely equivalent to that of the human eye. It has been found suitable for defining the minimum acceptable color for three varieties of red apples, namely, Red Delicious, McIntosh, and Spartan, without the necessity for separate color calibration for each red variety.

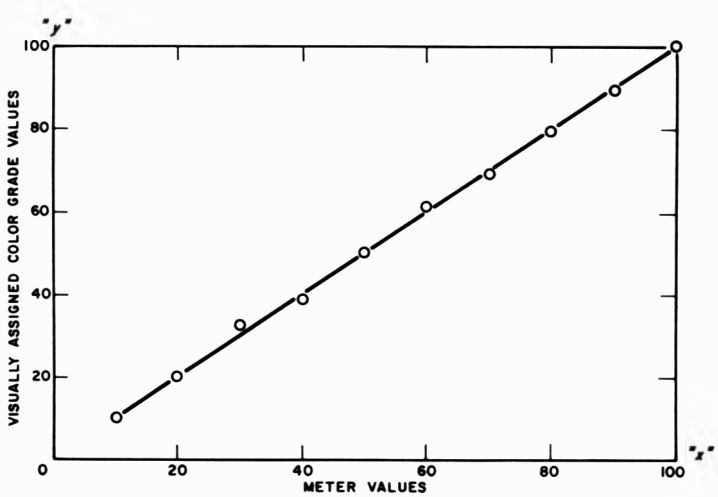

Fig. 2—Relationship between visually assigned redness grades and Apple Redness Meter values for the same apples.

Table 1—Computed average visual grades "y" obtained by a panel of 20 people and instrumental color grades "x"

Red Delicious		Spartan		McIntosh		Σ		
y	x	y	x	y	x	y	x	
10	10	10	10	10	10	10	10	Pink
20	20	20	20	20	20	20	20	
30	30	31	30	34.5	30	31.8	30	
40	40	39	40	35.5	40	38.2	40	Medium Red
50.5	50	51	50	50	50	50.5	50	
64.5	60	59	60	60.5	60	61.3	60	
66	70	70	70	71	70	69	70	
79	80	81	80	78	80	79.5	80	Dark Red
90	90	89	90	90	90	89.7	90	
100	100	100	100	100	100	100	100	

	Regression equation	Squared correlation coefficient
Red Delicious	: y = 0.992x + 0.433	0.996
Spartan	: y = 0.996x + 0.200	0.992
McIntosh	: y = 0.992x + 0.433	0.994

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QUANTITATIVE DETERMINATION OF COMBINED HEMOGLOBIN AND MYOGLOBIN IN VARIOUS POULTRY MEATS

INTRODUCTION

THE ONLY INFORMATION concerning the quantity of hemoglobin and myoglobin in various poultry tissues which could be found in the literature was that reported by Froning et al. (1968) for turkey thigh and breast meat. Color of muscle when poultry meats are sold as whole or cut-up carcasses is not considered a major concern as with red meats. With the present interest in further processing of poultry and especially with the use of chicken meat in emulsion-type products, color of the meat becomes important. Extracts of poultry meats are cloudy and cannot be cleared by lowering pH, filtering or "salting out" with ammonium sulfate. Consequently, the most commonly used spectrophotometric procedure for determining pigment in red meats cannot be directly adopted for the quantitative determination of pigment in poultry meat.

Snyder and Ayres (1961) stated that beef myoglobin was not precipitated by heating at 55° for 5 min and partial purification of the pigment could be accomplished by this method. They did not present data to show whether all of the pigment was unaffected nor the effect of longer heating periods on the stability of myoglobin. Bernofsky et al. (1959) reported that denaturation of myoglobin in meat is considerable at temperatures at which the thermal denaturation of the pigment is negligible in pure solution. They stated that this may be a result of enzyme action or co-precipitation. Draudt (1969) also states that the temperatures at which the myoglobin derivatives precipitate are much higher in the purified systems than in ground meat systems. Satterlee and Zachariah (1972) reported no significant differences among bovine, ovine and porcine metmyoglobins in their heat stability properties.

This study was conducted to develop a spectrophotometric procedure for the determination of combined hemoglobin and myoglobin in poultry meat. In addition, the concentration of pigment (mg/g of tissue) of various poultry meats was determined.

MATERIALS & METHODS

IN PRELIMINARY STUDIES, 50g of skinless, boneless, leg and thigh meat were extracted by the method described by Fleming et al. (1960), except 0.01N acetate buffer solutions at pH 4.0, 4.5, 5.0 and 5.5 were used. All solutions were cloudy. Aliquots of each extract were brought to 50, 60, 70, 80, 90 and 100% saturation with ammonium sulfate using the method of Brown (1961); however, all extract remained cloudy. The third method, in the preliminary studies, used to attempt to obtain a clear solution was the procedure described by Fleming et al. (1960), in which cold distilled water was used to extract the pigment.

Groups of three 12-1/2 ml aliquots of extract were drawn for each of the following time periods, placed in test tubes and heated at 55°C in a constant temperature water bath for 5, 10, 15, 20, 25, 30 and 60 min. Each group of three tubes was removed after the appropriate time interval and placed in an ice bath for approximately 10 min. The extract was then treated with 1/2 ml of potassium ferricyanide and 1/2 ml of potassium cyanide to provide final concentrations of 0.6 and 0.8 μl per liter respectively, of the compounds after dilution. Each of the extracts was filtered through Whatman No. 2 paper. When it was found that a clear solution containing the cyanmet-pigments could be obtained by heating, a study was made to determine the optimum temperature and time of heating for obtaining a clear solution without loss of pigment. Chicken blood, beef blood and beef muscle extract were used for this section of this study. The chicken and beef blood samples were citrated, centrifuged and the serum decanted. The red blood cells were washed three times with physiological saline. After the third decanting of the saline, distilled water was added and the samples placed in a cold room overnight to permit lyophilizing of the cells. The solution was centrifuged and diluted with distilled water to obtain an O.D. value at 540 mμ of between 0.20 and 0.30. The beef muscle extract was obtained using the procedure of

Table 1—Optical density of chicken blood, beef blood and beef muscle extract after heating at 50, 55 and 60°C for various time intervals

Time	Chicken blood			Beef blood			Beef muscle extract		
(min)	50°C	55°C	60°C	50°C	55°C	60°C	50°C	55°C	60°C
0	0.21	0.20	0.28	0.21	0.20	0.19	0.33	0.30	0.36
5	0.21	0.20	0.28	0.20	0.21	0.19	0.34	0.32	0.37
10	0.22	0.20	0.28	0.21	0.20	0.19	0.35	0.31	0.37
15	0.21	0.20	0.29	0.21	0.21	0.19	0.33	0.31	0.37
20	0.21	0.20	0.29	0.21	0.21	0.20	0.33	0.30	0.38
25	0.21	0.20	0.29	0.21	0.20	0.20	0.33	0.30	0.37
30	0.22	0.20	0.29	0.21	0.20	0.20	0.33	0.30	0.37
60	0.22	0.20	0.28	0.21	0.20	0.21	0.32	0.30	0.37

Table 2—Percent protein and milligrams of hemoglobin and myoglobin in light and dark meat of chickens

Carcass No.	Protein (%)		Pigment (mg per g) Heated		Pigment (mg per g) Centrifuged		Pigment-protein ratio (mg per 100g)	
	Dark	Light	Dark	Light	Dark	Light	Dark	Light
8 week old broilers								
1	18.87	22.40	0.50	0.01	0.50	0.01	2.65	0.05
2	17.28	22.69	0.37	0.01	–[a]	–	2.14	0.04
3	18.50	21.83	0.38	0.01	0.37	0.01	2.05	0.05
4	19.29	22.27	0.38	0.01	–	–	1.97	0.05
5	18.59	22.36	0.36	0.01	0.37	0.01	1.94	0.05
6	17.71	22.85	0.33	0.02	–	–	1.86	0.09
Mean	18.37	22.40	0.39	0.01	0.41	0.01	2.10	0.06
26 week old light females								
1	20.06	24.79	0.97	0.08	–	–	4.84	0.32
2	21.44	24.79	0.99	0.05	0.98	0.06	4.62	0.20
3	20.10	24.57	1.06	0.07	–	–	5.27	0.28
4	21.72	24.99	1.10	0.08	1.10	0.08	4.60	0.32
5	21.54	25.17	1.28	0.10	1.26	0.10	5.94	0.40
6	21.96	24.43	1.30	0.90	–	–	4.55	0.37
Mean	21.14	24.79	1.12	0.08	1.11	0.08	4.97	0.32
26 week old males								
1	21.39	24.57	1.32	0.11	1.31	0.11	6.17	0.41
2	21.34	25.30	1.51	0.12	–	–	7.08	0.47
3	21.04	25.52	1.60	0.10	–	–	7.60	0.39
4	20.44	25.14	1.53	0.09	–	–	7.49	0.36
5	20.86	24.65	1.62	0.10	1.63	0.10	7.77	0.41
6	21.59	25.10	1.50	0.10	–	–	6.95	0.40
Mean	21.11	25.04	1.51	0.10	1.47	0.10	7.18	0.41

[a] No determination

Table 3—Percent protein and milligrams of hemoglobin and myoglobin in muscle of 26 wk old turkeys

Carcass No.	Sex[a]	Protein (%)		Pigment (mg per g)		Pigment-protein ratio (mg per 100g)	
		Dark	Light	Dark	Light	Dark	Light
1	M	18.50	22.20	1.36	0.11	7.35	0.50
2	M	19.01	22.28	1.39	0.13	7.31	0.58
3	M	18.27	22.65	1.59	0.16	8.70	0.71
4	F	18.64	24.18	1.59	0.16	8.53	0.66
5	F	19.77	23.59	1 47	0.14	7.44	0.59
6	F	18.44	24.33	1.58	0.11	8.57	0.45

[a] M = male; F = female.

Fleming et al. (1960). The extract was diluted with distilled water to obtain an O.D. value at 540 mμ of between 0.300 and 0.375. Each solution was clear at the start of the test.

12-1/2 ml aliquots of each of the solutions were heated at 50, 55 and 60°C for 0, 5, 10, 15, 20, 25, 30 and 60 min. Each tube was removed after the appropriate time period and placed in an ice bath. The pigments were converted to the cyanmet-derivative, filtered and read at 540 mμ on a Bausch & Lomb "Spectronic 20."

Six separate samples containing 50g of boneless, skinless, leg and thigh meat and six samples of boneless, skinless, breast meat were taken from 8 wk old broiler chickens, 26 wk female chickens, 26 wk male chickens, 28 wk turkeys and two samples of leg, thigh and breast meat of young ducklings. In addition, six samples of 50g of six completely boneless and skinless duckling carcasses were obtained. Six samples of 50g each were obtained from hearts and gizzards of 8 wk broiler chickens.

The extraction procedure for each sample was the same as that described by Fleming et al. (1960). Duplicate 12-1/2 ml aliquots of each of the extracted solutions were placed in test tubes and heated at 55°C for 20 min in a constant temperature water bath. Immediately after heating, each tube was placed in an ice bath for approximately 10 min. The solutions were converted to the cyanmet-derivative, filtered and read at 540 mμ on a B & L "Spectronic 20" as described previously for the beef muscle extract and for the beef and chicken blood.

Duplicate samples from the extracted solutions of both dark and light meat of three birds from the 8 wk broilers, 26 wk females and 26 wk males and from beef muscle extract were centrifuged at 184,200 × G for 1 hr in a Spinco Model L ultracentrifuge. The clear solution containing the pigments was withdrawn from the centrifuge tube through an 18 gauge needle which was inserted through the cellulose nitrate tube 4 mm from the bottom. Care was excercised that none of the white loosely packed material which was at the top of the tube after centrifuging the poultry samples was removed. Duplicate 12-1/2 ml aliquots of each of the samples were converted to the cyanmet-compounds as described previously and the O.D. read at 540 mμ. The O.D. values were converted to mg of pigment per gram of tissue as described by Drabkin (1950) and Ginger et al. (1954) and as modified by Fleming et al. (1960).

Percent total protein was determined for all of the tissue samples by the Kjeldahl procedure (AOAC, 1970).

RESULTS & DISCUSSION

THE MAXIMUM DIFFERENCE in O.D. for chicken blood and beef blood was only 0.014 between unheated and heated samples (50 or 55°C) when heating time was extended for as long as 30 min (Table 1). In all cases, when beef muscle extract was heated for either 5, 10 or 15 min at any of the three temperatures a heavy precipitate of protein was obtained and some of the precipitate was small enough to pass through the filter paper resulting in a cloudy solution with a higher O.D. When the beef extract was heated for 20 or 25 min at 50 and 55°C the O.D. readings were the same as those obtained in the untreated control. Apparently the additional heating caused the smaller denaturated protein particles to form larger aggregates which precipitated and could be filtered out. The results presented in Table 1 indicate that the meat pigments are relatively stable to heat in the time periods used in this study, especially those at 50 and 55°C. For the remaining work in this study a heating schedule of 55°C for 20 min was selected; however, there appeared to be other time intervals and temperatures which would be equally good.

It is evident that the amount of pigment in chicken increases greatly as age of the bird increases (Table 2). It also appeared that 26 wk male chickens had more pigment per g of tissue than females of the same age. This is in agreement with work reported for red meats (Saffle, 1973) in which bull meat was higher in pigment content than cow meat. Froning et al. (1968) also found for turkeys more pigment in males than females and an in-

Table 4—Percent protein and milligrams of hemoglobin and myoglobin in duckling meats

Carcass no.	Protein (%)		Pigment (mg per g)		Pigment-protein ratio (mg per 100g)	
	Leg	Breast	Leg	Breast	Leg	Breast
1	18.95	17.99	1.16	0.53	6.12	2.95
2	18.43	17.48	1.14	0.54	6.19	3.09
Mean	18.69	17.79	1.15	0.53	6.15	3.02
Sample no.	Boneless meat composite[a]					
3	18.35		0.80		4.36	
4	16.00		0.63		3.94	
5	18.19		0.64		3.52	
6	17.93		0.51		2.84	
7	17.36		0.56		3.23	
8	15.99		0.73		4.57	
Mean	17.30		0.64		3.74	

[a] Meat from entire carcass combined

Table 5—Percent protein and milligrams of hemoglobin and myoglobin in hearts and gizzards from 8 wk old broiler chickens

Sample no.	Protein (%)		Pigment (mg per g)		Pigment-protein ratio (mg per 100g)	
	Heart	Gizzard	Heart	Gizzard	Heart	Gizzard
1	13.87	17.48	3.79	3.90	27.48	22.31
2	13.77	18.50	3.47	3.98	25.20	21.51
3	14.08	17.13	3.74	3.90	26.56	19.90
4	13.84	17.54	3.84	4.30	27.75	24.52
5	14.28	17.54	3.55	4.54	24.86	25.88
6	14.04	17.42	3.49	5.28	24.86	30.98
Mean	13.98	17.60	3.65	4.32	26.09	24.18

crease in the amount of pigment as age increased. The centrifugation and the heating methods for determining mg of pigment per g of tissue gave almost identical results (Table 2).

Saffle (1973) expressed his results as mg of pigment per 100g of protein for various red meats. This has permitted the use of this value in a linear program for determining the potential amount of color in finished processed products by simply multiplying the constant values by the percent total protein for each specific meat ingredient. The values for red meat have been within a relatively narrow range for each specific meat ingredient. In the various poultry meats there is a large variation within each type of meat (Tables 2, 3, 4 and 5) when the pigment is expressed as mg per 100g of protein. Because of this large variation within samples of the same type of meat this method would not appear to be useful.

Duckling meat is generally considered to be all dark meat. The age of the ducklings used in this study was not known but was probably approximately the same as the broiler chickens. It is interesting to note that duckling meat from the leg had approximately three times more pigment than meat from the breast even though both are considered to be dark meat (Table 4). The breast meat of ducklings had more pigment than the dark meat of broilers, but less than the dark meat of the older female and male chickens and turkeys (Tables 2, 3 and 4).

The amount of pigment in broiler hearts and gizzards is given in Table 5. Of all the poultry tissues studied, gizzards had more pigment than any other with hearts having the second highest amount. It was interesting to note that all of the gizzard extracts were clear without heating. Therefore, all of the gizzard extracts were not heated. One-half of the extracts from the hearts was clear and the other half was cloudy. Heating the clear extracts from the hearts did not increase the O.D. values.

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EFFECTS OF GAMMA IRRADIATION ON MYOGLOBIN

INTRODUCTION

CHANGES in the physicochemical properties of hemoproteins upon irradiation have been investigated for a variety of reasons. Myoglobin in particular, being responsible for the color of meats, has been extensively studied because of color problems that may arise when irradiation is used for preservation.

It has been observed that fresh meat upon irradiation develops a brown color that can be attributed to metmyoglobin; at higher dose levels this may be converted to a bright red pigment (Tappel, 1956). Several workers have attempted to identify this pigment, which exhibits spectral characteristics similar to those of oxymyoglobin. Some accept that it is a form of oxymyoglobin (Brown and Akoyunoglou, 1964; Giddings and Markakis, 1972; Satterlee et al., 1972), while others doubt this on the basis of small differences in the position of the absorption maxima from those of oxymyoglobin (Clarke and Richards, 1971).

In addition to changes in color, which presumably have to do with the iron configuration, the modifications of the globin part of the molecule resulting from irradiation are also of importance. In the case of the hemoproteins, globin plays an important role in influencing reactivity. Small peptides and ammonia have been found in solutions of irradiated proteins (Brown and Akoyunoglou, 1964); dimerization has been reported for serum bovine albumin; and degradation of some amino acids following irradiation has been reported for several proteins (Rosen, 1971).

In the present work some effects of gamma-irradiation on sperm whale myoglobin solutions have been examined. This commercial myoglobin preparation has been lyophilized, a treatment that may introduce some cross-linking (Van den Oord et al., 1969). However, this disadvantage is compensated for by the fact that aspects of structure of the whole protein are known in great detail (Edmunson and Hirs, 1961; Kendrew et al., 1961). Furthermore, our experimental procedure was designed to remove any aggregated material in the commercial preparation by chromatography.

EXPERIMENTAL

Purification and irradiation of myoglobin

Sperm whale myoglobin was obtained from either Sigma or Calbiochem. Both of these commercial products were of similar purity and contained mostly metmyoglobin though different batches had varying concentrations of oxymyoglobin. Myoglobins were dissolved in the appropriate buffer and the insoluble material was removed by filtration (Whatman No. 1 filter paper), or by centrifugation at 10,000 rpm for 10 min.

Additional purification was done by one of the following procedures:

(1) DEAE chromatography as described by Brown (1961) using DEAE-cellulose purchased from Bio-Rad Laboratories, Richmond, Calif. The exchange capacity of this material was 0.94 meq/mg.

(2) Sephadex CM-50 essentially as described by Fosmire (1970) using a carboxy methyl Sephadex (Pharmacia) column (2.5 × 30.0 cm) and eluting with 0.015M phosphoric acid neutralized to pH 7.35 with Trizma base (Sigma).

There are reports that myoglobin eluting from the DEAE columns can be resolved by other means into three or more fractions (Edmundson and Hirs, 1961). For this reason wherever the purity of the sample was considered critical, the CM-cellulose purified myoglobin was used.

Aliquots of purified material were placed in vials which were flushed with nitrogen (which had been freed of oxygen by passing the gas through an alkaline solution of pyrogallol) and then sealed.

Vials other than controls were irradiated in a swimming-pool type research irradiator with a cobalt-60 source of approximately 17,000c. The irradiation dose was 500 Krad, unless otherwise stated. All irradiations were done at ambient temperature (19–23°C).

Chromatography

Both the native and the irradiated samples were subjected to gel filtration column chromatography. The column used was a Pharmacia K15/90 (1.5 cm diam × 90 cm length). The gel was Sephadex G-75 Superfine which excludes globular proteins with molecular weights larger than 70,000 (manufacturer's specifications). The eluant was a 0.2N potassium chloride solution, previously degassed. Ascending chromatography was the standard mode for each fractionation.

The bed volume of the column was 154 ml and the void volume 51 ml. The flow rate was kept constant using an LKB 10020 Perpex peristaltic pump (∿ 7.5 ml/hr or 0.07 ml/cm²/min). The eluant was monitored by an LKB-Uvicord UV monitor at 280 nm and fractions were collected in an LKB-Ultrarack fraction collector.

In order to determine the approximate molecular weights of the fractions obtained from the irradiated myoglobin by gel filtration chromatography, a variation of Whitaker's method (1963) was used.

SDS gels were prepared as described by Weber and Osborn (1969). The staining was done as described by Rice and Frankel-Conrat (1973) with Coomassie blue.

Electrofocusing

An LKB Model 8101 electrofocusing apparatus was used. The ampholyte solutions used were from pH 7–9 for the native myoglobin, and from pH 5–9 for the irradiated sample (achieved by mixing the appropriate ampholytes). The density gradient was a sucrose gradient prepared by mixing a dense solution of sucrose (28g of sucrose to 55 ml) with a water solution of the myoglobin sample. The ampholyte solution was equally distributed in the two solutions. The cathode solution was prepared by dissolving 15g sucrose to 15 ml and adding 0.5 ml ethylendiamine. The anode solution was water acidified with a few drops of concentrated sulfuric acid. The column was maintained at 6°C throughout the run by a refrigerated water bath. The potential was increased from 400 volts initially to a final 800 volts for the last half of the run. After focusing the column was emptied by pump, being monitored at 410 nm with a Gilford 300 spectrophotometer; 4 ml fractions were collected. The pH of each fraction was then measured with a Radiometer PH28 pH-meter at room temperature. The pH of each component was calculated by fitting the pH reading to the graph obtained by monitoring at 410 nm.

Myoglobin derivatives

Four ligands were used to block the sixth position of the heme: nitric oxide, cyanide and nitrite ions, and carbon monoxide. These derivatives were prepared as follows:

$Mb^+(CN)^-$. Potassium cyanide was added to a solution of metmyoglobin (DEAE-purified). The final concentrations were approximately 10^{-3}M for Mb and 10^{-2}M for KCN. The solution so obtained was dialyzed against water to remove excess salt, then flushed with nitrogen and finally irradiated.

$Mb^+(NO_2)^-$. The procedure was the same as that described for $Mb^+(CN)^-$ except sodium nitrite was the salt used.

$Mb^+(NO)$. To prepare this derivative nitric

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oxide was flushed through the myoglobin solution. Since the nitric oxide easily reacts with oxygen to form nitrogen dioxide, care was taken to exclude any oxygen from solution. Also, in order to eliminate any nitrogen dioxide already formed, the gas was passed through an alkali solution. Since the pH of the protein solution drops considerably during this operation, care was taken not to flush the myoglobin solution longer than necessary; a few minutes was usually enough. The sample so treated was then immediately dialyzed versus water to raise the pH towards the neutral region. Any precipitate was removed by centrifugation and the supernatant was flushed with nitrogen.

Mb(CO). The procedure described for $Mb^{+}(NO)$ was employed, the only difference being that myoglobin was previously reduced to the ferrous state (Brown and Mebine, 1969) and carbon monoxide gas was used.

All these derivatives were exposed to 500 Krad. The identity of the desired derivatives was established spectrophotometrically.

Acid denaturation

A 4-ml protein sample was placed in a test tube immersed in a vat where water of controlled temperature was circulating from a Lauda refrigerated water bath. A slow motor (1 rpm) was used to drive the shaft of a graduated micropipette containing the acid solution. The time required for the titration was around fifteen minutes. The pH changes were followed with a Radiometer PH28 pH-meter equipped with a combined glass electrode. This pH-meter was also equipped with a device for compensating for the pH dependence on temperature, this being necessary since readings at different temperatures were made. The reaction was monitored by a Gilford 300 spectrophotometer at 409 nm using a flow-through cuvette (light path 10 mm). The absorption at this peak is known to decrease by a factor of four upon denaturation (Acampora and Hermans, 1967; Shen and Hermans, 1972). The solution was circulated from the titration vat to the cuvette by an LKB peristaltic pump (200 ml/hr). The protein solution was continuously stirred by a magnetic stirrer. Changes in optical density were recorded and on the same chart paper the values of pH were marked manually.

Temperature denaturation

The technique used was the one described by Fosmire (1970). A thermostatable cuvette holder (Cary Instruments) was connected to a Lauda K/2R water bath. A motor with a speed of 1 rpm was connected by a pulley to the top of the thermoregulator of the water bath. The thermoregulator was driven so as to increase the water bath temperature approximately 1°C per minute. The temperature was measured with a Mettler TM15 digital readout thermometer using a probe inserted in the outflow of the cuvette holder. The readings were recorded on the chart of the spectrophotometer (Cary 15) where the reaction was monitored at 409 nm.

Autoxidation

Rates of oxidation of the native, the "monomer" and the "crude" irradiated myoglobin were determined by following the rate of formation of metmyoglobin from oxymyoglobin. Oxymyoglobin was prepared as described by Brown and Mebine (1969). A 5-ml sample from the solutions was kept at constant temperature 35°C and the spectrum from 600 nm to 500 nm was recorded from time to time. The lowering of the 580 peak of oxymyoglobin was used as a measure of autoxidation.

Determination of the velocity constant

In the literature the following symbols are used by convention for the binding reactions of hemoproteins. Suppose we have a reaction with n-butyl isocyanide (BIC):

$$Fe + BIC \underset{i}{\overset{i'}{\rightleftharpoons}} FeBIC$$

where Fe represents a hemoprotein, i' is the rate constant for binding and i the rate constant of dissociation.

The "on" rate constant, i', for the binding of BIC with myoglobin was determined using a Durrum-Gibson stopped-flow spectrophotometer. The light path of the observation tube was 2 cm. Equal volumes of BIC and deoxymyoglobin from two gas tight syringes were mixed in the observation tube with the help of a hydraulic driven shaft. The formation of the complex was monitored at 435 nm (Olson and Gibson, 1971). The value of the rate constants was calculated by determining the reaction half-time (assuming that all myoglobin in solution is converted to the BIC derivative).

n-Butyl isocyanide was obtained from Aldrich. The working solution was prepared as follows: 0.1 ml was added to 100 ml deoxygenated buffer and was thoroughly mixed in a stoppered flask. Finally a small amount of dithionite was added to remove any trace of oxygen from solution.

Since dilute solutions of deoxymyoglobin are difficult to prepare by physical methods due to the high oxygen affinity of myoglobin, dithionite is frequently used to prepare the deoxygenated form. Control experiments have shown that dithionite can be used without significant effect on the kinetics (Antonini et al., 1965). Both native and irradiated myoglobin were diluted to a final concentration $\sim 10^{-6}$ M with a 1/15M phosphate buffer at pH 7. The deoxyform was prepared by adding dithionite until the color change indicated deoxymyoglobin formation.

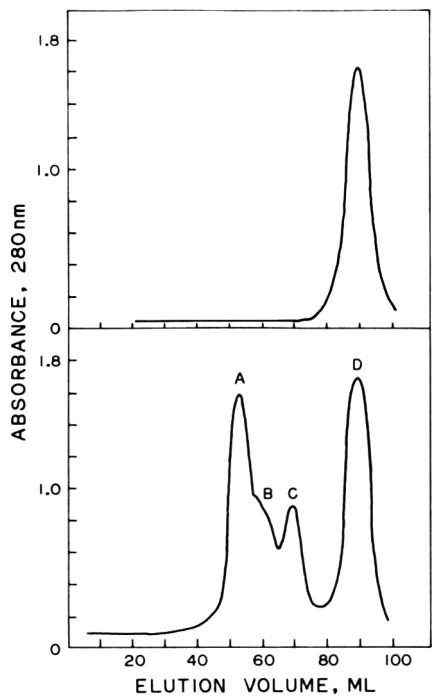

Fig. 1—Chromatography of native (top) and irradiated (bottom) sperm whale myoglobin. Sephadex G-75 superfine column, 1.5 cm × 90 cm at room temperature. Effluent 0.2N KCl monitored at 280 nm. Flow rate 0.07 ml/cm² /min.

RESULTS & DISCUSSION

Polymerization

The native unirradiated myoglobin, when passed through the G-75 Sephadex column under the conditions described in detail earlier, showed only one peak (Fig. 1, top) suggesting homogeneity, as expected. The irradiated myoglobin, however, was resolved into three main fractions (Fig. 1, bottom). These fractions are labeled as "polymers" (peak A), "dimer" (peak C) and "monomer" (peak D). The "polymers" eluted with the void volume for the column; this fraction is so-labeled because there is evidence for more than one component having varying molecular weights which can not be separated effectively by G-75 gel filtration (note the shoulder in Figure 1 marked as peak B).

The molecular weights determined chromatographically for these fractions were found to be: fraction A, over 70,000; fraction B, around 60,000; fraction C, around 38,000; and fraction D, around 17,000.

This method has been found to work relatively independently of protein concentration and column size but to depend somewhat on temperature, and on whether the protein is globular or fibrous. The fact that the dimer has an apparently larger molecular weight than twice the monomer, then, may be evidence of unfolded helices.

Identical samples were exposed to varying dose levels of 100, 300 and 500 Krad. The results indicated that the dimer was the first new component to be formed and that it is an intermediate to higher polymers. Increasing the dose level increased the extent of polymerization.

Samples with the same concentration of myoglobin but of different ionic strengths (0.0, 0.2, 1, 2N of potassium chloride) were exposed to 500 Krad. Apparently increasing the ionic strength of the solution enhanced the extent of the polymerization, but only up to a certain point (no change beyond 1N potassium chloride).

The pH of the solution as a factor in the polymerization process was investigated by exposing myoglobin solutions to 500 Krad at three different pHs: 6.0, 7.5 and 9.0. Myoglobin is known to be stable within this range. There were no detect-

able differences at least from the point of view of polymerization. This does not, however, exclude the possibility of minor changes which are not detectable by this criterion of polymerization, since it has been reported that radiation-induced changes in some oligopeptides show a large dependence on pH (Garrison and Weeks, 1962).

Myoglobin solutions at different concentrations were exposed to 500 Krad. There was a considerable depression of the polymerization process in the more dilute samples. When two myoglobin solutions with concentrations of 1 mg/ml and 10 mg/ml were irradiated the polymerization products were almost three times as much in the latter.

The results were similar for all the myoglobin derivatives (i.e., with various ligands bound to the sixth position) as judged from the extent of polymerization. The polymerization pattern was the same as for irradiated metmyoglobin. Some myoglobin derivatives were irradiated with the excess (unbound) ligand present in solution (dialysis omitted). In these cases no polymerization products were observed after irradiation. These observations suggest that the sixth ligand has little effect on the polymerization process. The effect that the excess ligand has can be explained on the basis that these ligands, being electron rich, may act as free radical scavengers.

Nature of the dimer bonds

The presence of the "dimer" (and other polymerization products) was consistent after irradiation. It would be expected that the formation of polymers is a result of the reaction of amino acid residues. However, since the dimer exhibited spectral characteristics similar to those of oxymyoglobin and since Fe-O-O-Fe bonds have been suspected in other iron proteins (Alben et al., 1968) it was considered worthwhile to evaluate whether two myoglobin molecules might be held together with such a bond.

To determine if such a possibility exists, the heme group was removed from the protein moiety by the procedure described by Lewis (1954). This was done on both monomer and dimer fractions. SDS gels were then run with both the heme-free and intact molecules of monomer and dimer. Each gel showed only one band and the mobilities were the same for the pairs: monomer:heme-free monomer and dimer:heme-free dimer.

These results support the rather obvious conclusion that the bonds responsible for the polymerization are covalent bonds between the peptide chains.

Spectra; heme modification

All three fractions from column chromatography ("monomer," "dimer" and "polymers") showed the same spectral characteristics. The "polymers" fraction showed broader peaks especially at 540 and 580 nm. This is probably due to a slight turbidity that this fraction showed. The ratio of the absorbance at the Soret compared to the 280 nm peak gives a descriptive criterion of the extent of damage, since the one decreases and the other increases upon irradiation. These ratios are tabulated in Table 1 together with those for the myoglobin derivatives named earlier. It is obvious from these results that the ratio changes more in the case of MetMb, where it is reduced to half. This increased vulnerability to radiation-induced polymerization may be due to the fact that MetMb has a more open configuration than do liganded ferrous derivatives and therefore has more exposed amino acid residues. The spectra of these derivatives showed the same pattern as in the case of polymerization previously discussed, that is when excess ligand is present in solution the spectra are virtually unchanged.

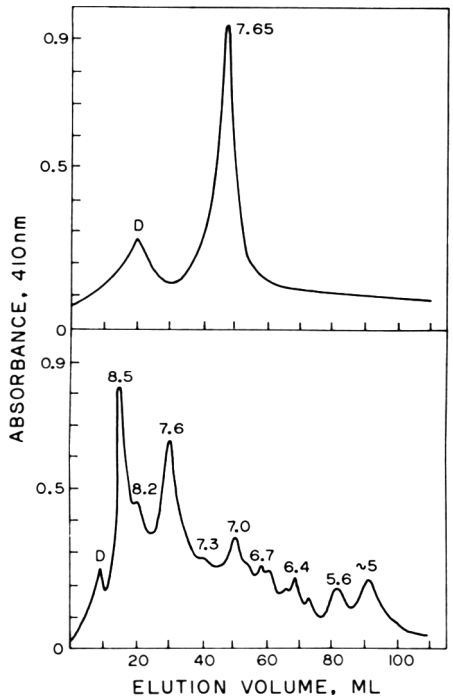

Fig. 2—Elution pattern of unirradiated (top) and irradiated (bottom) metmyoglobin from the pH 7–9 electrofocusing column. Temperature 6°C, voltage 400v initially to 800v, time 3 days. The peak labeled D is an artifact due to abrupt change in density between cathode solution and gradient ampholyte mixture.

In order to detect any changes in the heme ring during irradiation, the heme was removed by the procedure of Lewis (1954). Ultra violet and visible spectra of acetone solutions of heme were recorded; no difference was found.

From the overall appearance of the spectra it seems that the heme group does not suffer major changes. Hence differences in spectra observed for the whole molecule are probably due to changes in the protein moiety of the molecule.

Electrofocusing

The results of electrofocusing are presented in Figure 2. Native myoglobin shows only one main peak (top). The small peak labeled "D" is an artifact due to an abrupt change in density between the cathode solution and the gradient ampholyte mixture. Irradiated myoglobin, however, shows a large number of minor peaks (Fig. 2, bottom). Some diffusion of the irradiated sample during shorter runs was observed. In the beginning it was thought that the heme iron was somehow more exposed after irradiation and, as a result, sharp focusing was inhibited (the manufacturer of the ampholytes states that poor results may be found at times with metal proteins). This

Table 1—Ratio of absorbance of Soret (409 nm) to protein (280 nm) peak of control and irradiated myoglobin fractions

	Derivative	Ratio
MetMb:	native	5 or more
	irradiated (monomer)	2.4
	irradiated (dimer and polymers)	2.0
$Mb+CN^-$:	native	3.8
	irradiated (salt present)	3.5
	irradiated (dialyzed)	3.3
$Mb+NO_2^-$:	native	5.3
	irradiated (salt present)	5.0
	irradiated (dialyzed)	4.4
Mb+NO:	native	5.6
	irradiated	5.1
MbCO:	native	6.3
	irradiated	5.2

appears at least partly true because if the irradiated sample is treated previously with nitrite to block the sixth ligand position, focusing of the main bands, at least, was achieved in relatively shorter periods of time (∿ 20 hr); there was still some diffusion along the column. It should be mentioned that native myoglobin could have been considered to be "focused" in a shorter time, but it was run for the same period as the irradiated sample for comparative purposes.

Acid denaturation

The reversibility of the assumed two-stage process has been thoroughly discussed by Shen and Hermans (1972). In control experiments we obtained results similar to those of the workers just cited. A dramatic effect of ionic strength on the transition pH was observed; thus it was necessary to use the same solution (0.2N potassium chloride) for all experiments. A salt solution was used mainly to avoid adsorption of the sample by the gel during the chromatographic fractionation. In order to avoid dialysis after this procedure a solution of a neutral salt was chosen instead of a buffer which would interfere with the titration of the sample. Neither of the ions of potassium chloride binds with myoglobin (Breslow and Gurd, 1962). Titrations of the native myoglobin and the monomer of irradiated samples were carried out at different temperatures. These results are plotted in Figure 3 for native (top) and irradiated (bottom) samples, as lnK versus pH. They are plotted in this fashion in order to allow calculation of the number of protons bound per molecule (Acampora and Hermans, 1967) using the equation:

$$\Delta v = d \ln K / d \ln a_{H^+}$$

where Δv is the apparent difference of protons bound (Hermans and Acompora, 1967).

The value of the equilibrium constant K, was calculated through the equation:

$$K = y/(1-y)$$

where y is the fraction of molecules in the denatured state. The value of y can be determined through the relation

$$y = \frac{A_o - A_t}{A_o - A_\infty}$$

where A_o, A_t and A_∞ are the absorbancies in the beginning, at a given midpoint and at the end of the process.

Repeated titrations of native myoglobin showed good reproducibility. This was not the case, though, for irradiated myoglobin. There were differences even for different samples of the same batch. Nevertheless, the transition pH for the irradiated myoglobin was consistently higher than that of the native. Also the slope from plots of denaturation of irradiated samples were smaller.

This lack of reproducibility with irradiated myoglobin samples is not surprising because there are so many factors affecting the results of irradiation, some of them demonstrated by the gross criterion of polymerization. Moreover, the manner in which irradiated myoglobin was fractionated (Sephadex G-75) gave a number of components, versus the single one of native myoglobin as revealed by electrofocusing.

Examination of the slopes of these curves suggests that the native material apparently has a larger number of titratable groups in this range. As reported by Breslow and Gurd (1962), six of the imidazole groups are buried towards the hydrophobic center of the molecule and thus are titrated only after the helices have been unfolded. This leads then to the conclusion that in the case of the irradiated myoglobin some of these groups are no longer buried because some of the helices have unfolded during irradiation.

Using the data presented in Figure 3 we can calculate the enthalpy of denaturation using the equation $k \ln K/d(1/T) = \Delta H/R$. This can be done graphically by plotting ln K versus 1/T for a certain pH, the value of the enthalpy being given as the slope. In addition to the lack of reproducibility there are other limitations to using this technique, the most important being the reading of the pH-meter. Since the titration has to be slow the pH meter tends to drift, especially around the transition point; it is difficult to obtain a reading without some error.

Of the results obtained, the best show a certain dependence of enthalpy on temperature (solid lines in Fig. 4). A straight line was drawn using Askowitz's method (1957) for the best straight line according to the criterion of least squares, assuming that enthalpy is little affected by temperature in the narrow range under consideration. Doing so we obtained values in the vicinity of 20 Kcal/mole for the native and about 16 Kcal/mole for the irradiated myoglobins.

Since we are making these calculations close to the transition point we can assume that $\Delta F = 0$ and thus $\Delta S = \Delta H/T$. From the results given above we can con-

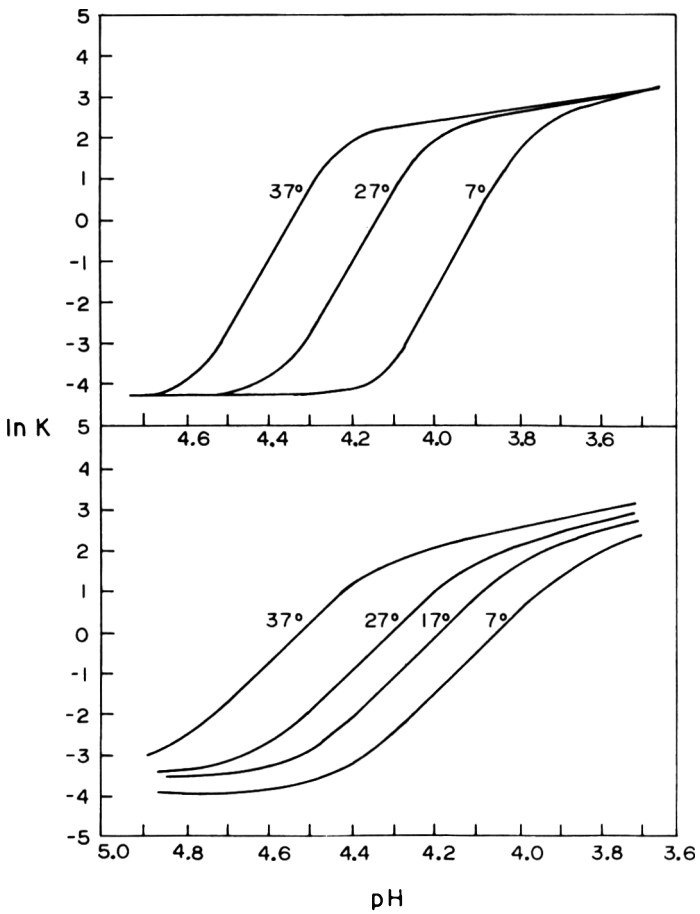

Fig. 3—Acid denaturation of unirradiated (top) and irradiated (bottom) metmyoglobin plotted as ln K versus pH at different temperatures. Reaction monitored at 409 nm.

clude that the entropy change is less for the denaturation of the irradiated than for the control myoglobin. Making a final assumption that the denatured forms of both the irradiated and the native are equally disordered, less entropy change suggests that the irradiated is more disordered than the native. This is in line with the general knowledge that irradiated proteins have a more disordered structure and are more susceptible to stress than are their nonirradiated counterparts.

Another point related to the reproducibility is the history of the sample. If it is true that some helices unfold, at least partly upon irradiation, then some if not most should refold with time. In this case, different results might be expected depending on how long the sample was stored after irradiation and its treatment; that is how long it was kept at room temperature, how long did it take to pass through the fractionating column, etc. Following the same reasoning, any deviations from the native folding might then be attributed to modifications of some amino acids along the chain, provided of course that the molecule had time to refold. Under our experimental conditions this should be very possible since renaturation may only take a few hours.

Heat denaturation

The reversibility of heat denaturation was tested and the results are presented in Figure 5. The denaturation of the irradiated myoglobin appears to be less readily reversible. It was found that the reversibility depends on the time the sample remains in the denatured form and to what temperature above the transition point it was exposed.

Again, the ionic strength has some effect on heat denaturation, so it is necessary to keep the ionic strength of the solution used the same.

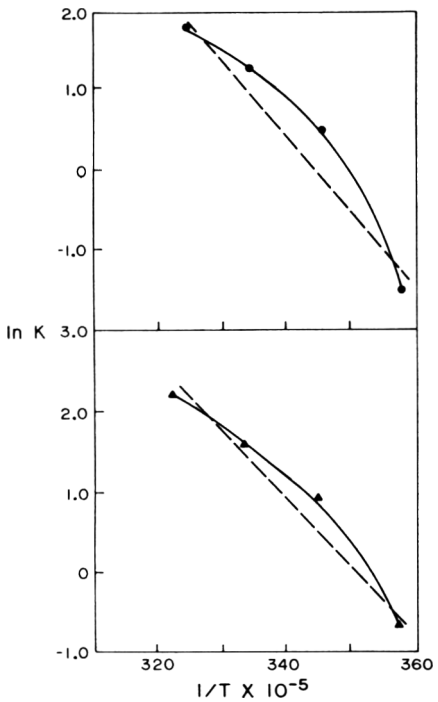

Fig. 4–Plot of Van't Hoff equation for unirradiated (top) and irradiated (bottom) myoglobin.

Figure 6 shows comparative heat denaturation of native myoglobin and the "monomer" of the irradiated myoglobin. The denaturation temperature is clearly lower for the irradiated myoglobin. The behavior of the irradiated sperm whale myoglobin in this case is different from that observed for the irradiated bovine myoglobin as previously determined by the same procedure (Satterlee et al., 1972). Initially it was thought that this might be due to the presence of other components in the bovine myoglobin solution. To test this, "crude" irradiated sperm whale myoglobin was heat denatured; the denaturation pattern was quite similar to that of the monomer. Thus the only apparent explanation is that the two myoglobins suffer different degrees of damage upon irradiation.

Autoxidation

The redox potential for the couple ferroheme-ferriheme is conditioned by the basicity of porphyrin (electron attracting power of the porphyrin side chains), and the influence of side chain groups in the protein. The redox potentials of such ion couples can be determined by potentiometric titrations. However, electron exchange is a slow reaction in metalloporphyrins and there may also be interference by atmospheric oxygen. Therefore instead of determining the redox potentials directly, indirect evidence about the oxidative state was obtained by determining the autoxidation rates.

These results are plotted in Figure 7 as the disappearance of the peak at 580 nm (difference of optical density from the optical density at zero time) versus time.

It is clear that the autoxidation rates are higher for the two samples of irradiated myoglobin, i.e., the "monomer" and "crude" than for unirradiated myoglobin. The fact that the "crude" sample has the highest rate may be due to the fact that the heme pocket of the polymerization products is damaged and thus less effective in maintaining the iron ion in the ferrous form. It may be that the environment of the heme group is considerably

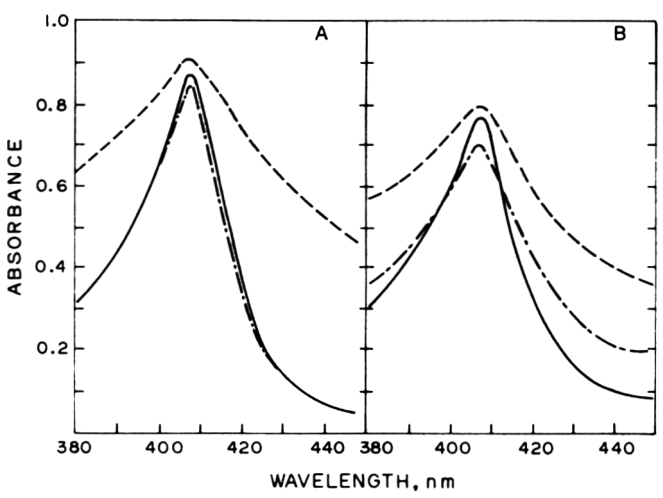

Fig. 5–Reversibility of thermal denaturation. A, unirradiated myoglobin. B, monomer of irradiated myoglobin. Visible spectra of native (———), denatured (– – – –) and renatured (– · – · –) samples.

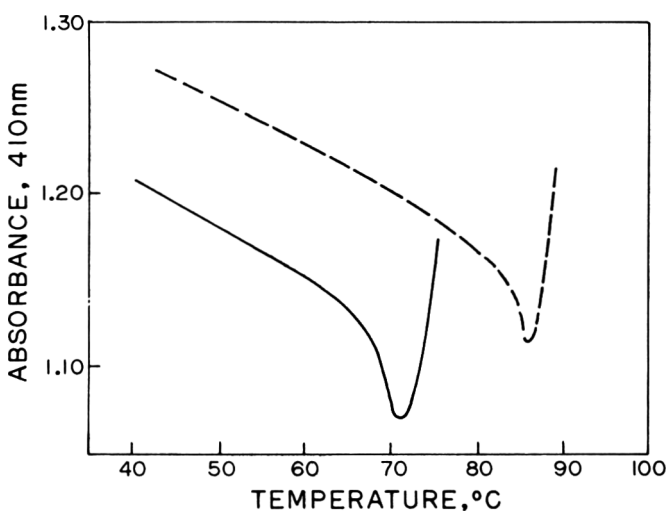

Fig. 6–Thermal denaturation of unirradiated (– – – –) and irradiated (———) metmyoglobin.

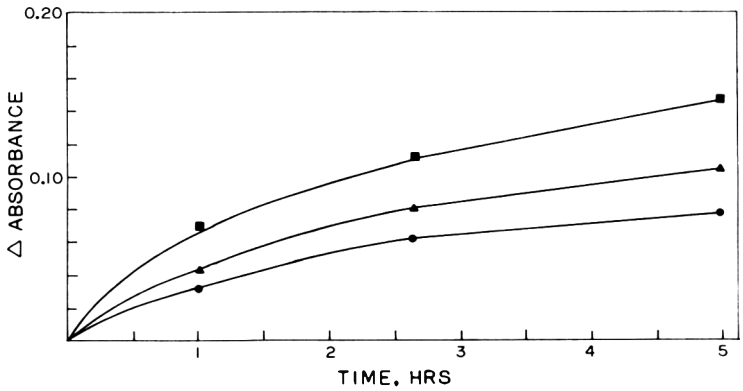

Fig. 7—Autoxidation rates of unirradiated (—●—●—), monomer of irradiated, (—▲—▲—) and crude irradiated myoglobin solutions (—■—■—) as estimated by observing the lowering of the 580 nm peak of oxymyoglobin.

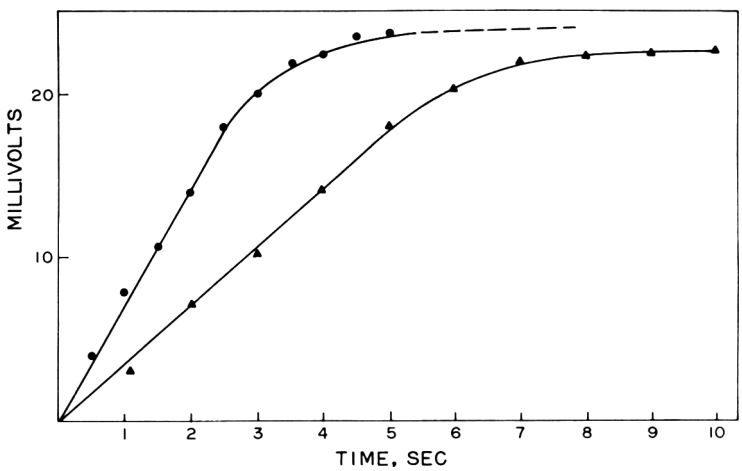

Fig. 8—Rates of binding of n-butyl isocyanide on unirradiated (—▲—▲—) and irradiated myoglobin (—●—●—). Reaction monitored at 435 nm using a Durrum-Gibson stopped flow spectrophotometer.

less hydrophobic since transfer of electrons occurs more easily. This can be attributed to the loss of some part of the peptide chain or modification of the amino acid residues that are responsible for the hydrophobic environment of the heme pocket.

Ligand binding

Isocyanides with varying sizes of R-group (methyl-, ethyl-, propyl-, t-butyl, phenyl, etc.) have been used to determine their reactivity towards the heme iron of several hemeproteins (Lein and Pauling, 1956; St. George and Pauling, 1951; Talbot et al., 1971). Early work by Pauling (Lein and Pauling, 1956; St. George and Pauling, 1951) showed that the affinity of free heme for alkyl isocyanides is independent of the size of the R-group. It was found, however, that for hemoglobin and myoglobin the affinity decreases as the bulkiness of the R-group increases. This clearly indicates a steric hindrance exercised by the globin part of the molecule. This might be used to demonstrate structural differences between the monomer of the irradiated myoglobin and native deoxymyoglobin.

Since rate constants and equilibrium constants are available in the literature, only the reaction with the n-butyl isocyanide (BIC) was examined; this substance reacts fairly slowly and hence the reaction can be easily followed.

In Figure 8 the results are plotted as mV (corresponding to the change in optical density at 435 nm) versus time. From the slopes of these curves it is obvious that the rate that BIC binds to the irradiated myoglobin is at least double the rate that it binds to the native. The value of i' depends on the BIC concentration and the pH (Talbot et al., 1971). Under the conditions of this experiment, i' has a value around 3.5 M^{-1} sec^{-1} for the native and 7.0 M^{-1} sec^{-1} for the irradiated Mb. These values have no absolute significance, but indicate that irradiated myoglobin does exhibit less steric hindrance to ligands binding in the sixth position. This can only be attributed to a protein moiety that has been altered considerably.

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LIPIDS AND FATTY ACIDS OF CHICKEN BONE MARROW

INTRODUCTION

THERE HAVE BEEN many reports on the lipid and fatty acid composition of chicken tissues (Widmer and Holman, 1950; Chang and Watts, 1952; Miller et al., 1962; Marion and Woodroof, 1963 and 1965). However, very little is known about the lipid composition of chicken bone marrow with the exception of Siegel and Latimer (1971) who reported that chicken bone marrow contains high levels of unsaturated fatty acids. Seitz (1969) has reported a very high lipid content for human bone marrow that varied according to bone source and age. The major fatty acids for swine bone marrow were oleic, stearic and linoleic (Seitz, 1969).

In the past few years the mechanical deboning process has been used extensively to remove poultry meat from bones. This process incorporates unknown quantities of bone marrow in the deboned meat. Because oxidative rancidity is believed to be the major cause of flavor deterioration, the lipid composition of bone marrow could have a significant influence on the stability of finished products containing deboned meat.

The objective of this study was to identify and quantitate the lipids and fatty acids of chicken bone marrow.

EXPERIMENTAL

LEGS AND BACKS from 8 to 9 wk old broilers were randomly selected at a local processing plant. All samples were obtained within 12 hr of slaughter and were packed in ice. Soft tissue and skin were removed from the tibia, femur and ilium-ischium bones. The bones were split lengthwise and the bone marrow was removed. 2-g samples were used for determining percent moisture and total lipids.

The method of Bligh and Dyer (1959) was used to extract and purify the lipids for further studies.

Separation into lipid classes

A 100/200 mesh Unisil (Clarkson Chemical Co., Williamsport, Pa.) silicic acid column was used to separate the neutral lipids, glycolipids and phospholipids. 7g of Unisil were mixed with a small volume of chloroform and added to a 1x15 cm column. 1g of lipid material was transferred onto the top of the column. The lipids were eluted from the column using in sequence 300 ml each of chloroform, acetone and methanol. The chloroform eluant contained the neutral lipids, the acetone eluant contained the glycolipids, and the methanol eluant contained the phospholipid fraction. All fractions were tested for purity using thin-layer chromatography. Glycolipids and phospholipids were identified by spotting on silica gel G plates and developing with a solvent mixture of chloroform-methanol-H_2O (65:35:5, v/v/v). Phospholipids were visualized by spraying with molybdenum blue reagent (Applied Science, State College, Pa.) and glycolipids were detected by spraying with diphenylamine (Applied Science, State College, Pa.). The neutral fraction was tested for purity by spotting on silica gel G plates, developing with a solvent mixture of petroleum ether-ethyl ether-acetic acid, (90:12:1, v/v/v) and visualizing with iodine vapors.

The lipids were recovered by evaporating the solvents to 10 ml using a rotary evaporator at room temperature and then evaporating the remaining solvent under a stream of nitrogen.

Separation of individual neutral lipids

Individual neutral lipids were separated utilizing a 14-gram Unisil column. Dry Unisil was added to a 1 × 15 cm column containing dried hexane. Approximately 600 mg of neutral lipids were placed on the column in 1 ml of hexane and eluted with increasing percentages of diethyl ether in dried hexane (Table 1).

The eluants were tested by collecting 10 ml fractions. A portion of each 10 ml fraction was spotted on silica gel G plates and developed using solvent mixture of petroleum ether-ethyl ether-acetic acid (90:12:1, v/v/v). Lipid content of the eluants were quantitated gravimetrically.

Preparation of fatty acid esters

The fatty acids of the triglyceride and phospholipid fractions were esterified by the saponification-transesterification method as described by Metcalfe et al. (1966). 5 ml of a 0.5N methanolic NaOH solution were added to approximately 30 mg of either phospholipid or triglyceride samples and heated over a steam bath for 3 min. 5 ml of 10% boron trifluoride in methanol were added to this mixture and boiled for 2-½ min. Thin-layer chromatography was used to determine the completeness of the transesterification. Silica gel G plates were spotted with the reaction product and a known standard and developed with petroleum ether-ethyl ether-acetic acid as previously described. A comparison of the R_f values of the reaction products to trioleate, methyl oleate, and oleic acid indicated that the conversion of the triglyceride and phospholipid fractions to methyl esters was complete.

Table 1—Separation of chicken bone marrow lipids by Unisil column chromatography

Fraction	Eluting solvent	
Cholesterol ester	50 ml	1% ether in hexane
Triglycerides	150 ml	6% ether in hexane
Free fatty acids	80 ml	9% ether in hexane
Cholesterol and diglycerides	150 ml	20% ether in hexane
Monoglycerides	100 ml	100% ether

Determination of fatty acid composition

A Barber-Coleman model 500 gas chromatograph equipped with a 1.83m × 3 mm glass column and employing a flame ionization detector was used to separate the methyl esters of the fatty acids. The column was packed with 100/120 mesh Gas-Chrom Q (Applied Science, State College, Pa.) coated with 10% EGSS-X (Applied Science, State College, Pa.) and operated isothermally at 130°C for 2 min, then programmed to 190°C at 3°C/min. The injector temperature was 220°C and the detector temperature was 205°C. The percentage composition of a known mixture of methyl esters of 16:0, 18:0, 18:2, 18:3 (Applied Science, State College, Pa.) was confirmed with approximately 1% error. Peaks were identified by comparing their retention times from semi-logarithmic plots of the fatty acid standards. Gas chromatography-mass spectrometry (Bendix Time-of-Flight Spectrometer) and gas chromatography on polar and nonpolar columns were used to indentify various unknown peaks.

RESULTS & DISCUSSION

THE LIPID CONTENT of chicken bone marrow was approximately 46.5%. The total lipids, polar lipids and neutral lipids are reported in Tables 2 and 3.

Approximately 98.4% of the total lipids were neutral lipids with triglycerides the predominate lipid in chicken bone marrow. The phospholipid fraction comprised about 1.7% of the total lipid. Only trace amounts of glycolipids were found. On a weight percentage basis, bone marrow contained a slightly higher percentage of phospholipids than most other tissues. Katz et al. (1966) reported that white chicken meat and dark chicken meat contained 0.48% and 0.52% phospholipids respectively. There was little variation in the triglyceride or phospholipid content of different bone marrow samples from various carcass parts. In contrast to these data, the lipid characteristics of human bone marrow varied according to bone source (Seitz, 1969).

Table 2—Lipid content (percent) of chicken bone marrow from various carcass parts[a]

	Total[b] (%)	Phospholipids[c] (%)	Neutral[c] (%)	Glycolipids[c] (%)
Femur	46.4 ± 0.1	1.6 ± 0.1	98.4 ± 0.2	Trace
Tibia	46.6 ± 0.2	1.6 ± 0.1	98.4 ± 0.2	Trace
Ilium-Ischium	46.6 ± 0.2	1.6 ± 0.1	98.5 ± 0.2	Trace

[a] Each value a mean and deviation of triplicate determinations from three independent samples
[b] Percentage of raw bone marrow
[c] Percentage of total lipid

Table 3—Lipid fractions (percent) of chicken bone marrow from various carcass parts[a]

Lipid fraction	Femur (%)	Tibia (%)	Ilium and Ischium (%)
Triglycerides	94.6 ± 0.5	94.6 ± 0.3	94.6 ± 0.2
Phospholipids	1.7 ± 0.1	1.6 ± 0.1	1.7 ± 0.1
Cholesterol	1.4 ± 0.1	1.3 ± 0.1	1.3 ± 0.2
Diglycerides	0.8 ± 0.2	0.8 ± 0.2	0.8 ± 0.1
Free fatty acids	0.7 ± 0.1	0.7 ± 0.2	0.7 ± 0.1
Monoglycerides	0.6 ± 0.1	0.7 ± 0.1	0.6 ± 0.1
Cholesterol ester	0.3 ± 0.1	0.2 ± 0.1	0.2 ± 0.1
Glycolipids	Trace	Trace	Trace

[a] Expressed as a percentage of total lipids. Each value a mean and deviation of duplicate determinations from three independent samples

Table 4—Fatty acids (percent) of chicken bone marrow[a]

Fatty acid[b]	Triglyceride (%)	Phospholipids (%)
14:0	0.90 ± 0.2	0.17 ± 0.1
hexadecanal	----------	2.36 ± 0.2
16:0	21.75 ± 0.5	20.50 ± 1.3
16:1	3.55 ± 0.2	1.45 ± 0.3
octadecanal	----------	1.60 ± 0.2
17:0[c]	0.10 ± 0.0	0.10 ± 0.0
17:1[c]	----------	0.10 ± 0.0
18:0	9.34 ± 0.4	15.10 ± 0.8
18:1	34.90 ± 2.1	12.20 ± 0.5
18:2	25.18 ± 1.5	18.20 ± 0.3
18:3	1.56 ± 0.1	0.61 ± 0.2
20:0	0.25 ± 0.1	0.17 ± 0.1
20:2	0.10 ± 0.0	0.55 ± 0.1
20:3	0.10 ± 0.0	1.45 ± 0.2
20:4	0.81 ± 0.2	13.07 ± 0.5
20:5	0.46 ± 0.1	2.00 ± 0.2
22:4	0.10 ± 0.0	2.05 ± 0.1
22:5	0.10 ± 0.0	1.40 ± 0.3
22:6	0.23 ± 0.1	4.25 ± 0.3
24:0	----------	0.70 ± 0.2
24:2	----------	0.27 ± 0.1
24:4	----------	0.83 ± 1.1

[a] Each fatty acid expressed as a percent of total fatty acids. Each value a mean and deviation of duplicate determinations from three independent samples
[b] Carbon chain length: number of double bonds
[c] Tentatively identified

The fatty acid content of the triglyceride and phospholipid fractions are reported in Table 4. The triglyceride fraction was very low in polyunsaturated fatty acids. Approximately 91.3% of the fatty acids were comprised of 16:0, 18:0 and 18:2 acids. Marion and Woodroof (1965) and Katz et al. (1966) reported similar results in the triglyceride fractions of breast muscle, thigh muscle and skin tissue. Bone marrow triglycerides were slightly lower in linoleic than these soft tissue samples, but were higher in 20:3 to 22:6 unsaturated fatty acids. There were no apparent variations in the triglyceride fatty acids between femur, tibia and ilium-ischium bones.

Widmer and Holman (1950) found that phospholipids in poultry were more unsaturated than in red meat. The phospholipid fractions for all bone marrow samples also contained high levels of polyunsaturated 20 to 24 carbon fatty acids. The 20 to 24 polyunsaturated fatty acids comprised approximately 27% of the total phospholipid fatty acids as compared to 1.9% for the triglyceride fractions. The polyunsaturated bone marrow phospholipids could be susceptible to autoxidation. Holman (1954) points out that each double bond in a polyunsaturated fatty acid could increase the rate of autoxidation by a factor of 2. The heme pigments present in bone marrow could also accelerate the oxidation of phospholipids in bone marrow. Hemoglobin has been reported to be as much as 90% of the total pigment of bone marrow (Froning and Johnson, 1973).

The phospholipids of bone marrow contained high levels of arachidonic acid (13.1%). Miller et al. (1962) reported that most of the arachidonic acid found in soft tissue was found in the phospholipid fraction. Miller et al. (1962) also reported that arachidonic acid found in poultry meat was synthesized from linoleic. This may explain why the phospholipid fractions were higher in arachidonic acid and lower in linoleic acid than the triglyceride fractions. Marion and Woodroof (1965) also found that the greatest differences between the phospholipid and triglyceride fractions were the high levels of arachidonic acid and the low levels of oleic and linoleic acids in the phospholipids. Bone marrow phospholipids contained lower levels of oleic acid and higher levels of arachidonic acid as compared to phospholipids from skin, breast and thigh tissues.

Trace amounts of glycolipids were also found in the bone marrow lipids. Since complex amphiphilic lipids, phospholipids and glycolipids, are associated with membrane structures, these lipids are probably from erythrocytes or leucocytes found in the bone marrow.

Marion and Woodroof (1965) and Marion et al. (1967) reported the presence of a tetradecadienoic acid in the phospholipid fraction of broiler breast muscle. Katz et al. (1966) reported the presence of a pentadecanoic acid in chicken breast phospholipids. Marion and Woodroof (1965), Marion et al. (1967) and Katz et al. (1966) also reported the presence of a hexadecadienoic acid in their phospholipid fractions. Gas chromatography-mass spectrometry, however, indicated that there was no tetradecadienoic, pentadecanoic or hexadecadienoic acids present in the chicken bone marrow phospholipids. Instead, two compounds were found that exhibited large mass peaks at 75 m/e, P-28 m/e, and had molecular weights of 286 and 314. The mass units produced by these compounds were consistently two mass units higher than fragmentation peaks produced by fatty acid methyl esters. To produce such peaks, oxygenated hydrocarbons must contain two oxygen atoms and no unsaturated bonds (Friedel and Sharkey, 1956). Dimethyl acetals (DMA) produce such peaks. The molecular weights of these two compounds corresponded to C-16 and C-18 dimethyl acetals. Gas chromatography on polar and nonpolar stationary phases also indicated that these two compounds were less polar than fatty acid methyl esters.

DMA's have retention times similar to dienoic fatty acid methyl esters on polar stationary phases. Since Marion and

Woodroof (1965), Katz et al. (1966) and Marion et al. (1967) used polar stationary phases for the separation of fatty acid methyl esters, it appears that these DMA's were mistaken for fatty acids. Gardner et al. (1972) using infrared spectroscopy also reported the possible presence of hexadecanal and octadecanal in chicken breast phospholipids.

These long chain DMA's appear to be derived from plasmalogens. Plasmalogens have been found in a variety of animal tissues (Peng and Dugan, 1965; Neudoerffer and Lea, 1967; Webster, 1960). Methanolysis of plasmalogens with boron trifluoride-methanol yields a mixture of methyl esters and dimethyl acetals (Mahadeven et al., 1966).

Farquhar (1962) reported the presence of dimethyl acetals in the membranes of human erythrocytes. DMA's found in chicken bone marrow could be derived from erythrocyte or leucocyte membranes. Gardner et al. (1972) found that phospholipids from raw broiler muscle contained 6% hexadecanal. A lower level of hexadecanal was found in bone marrow phospholipids.

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EFFECT OF POSTMORTEM AGING ON CHICKEN MUSCLE FIBRILS

INTRODUCTION

IN A PRIOR study (Hay et al., 1972), we showed that postmortem aging of chicken carcasses had little effect on the representative physical and chemical properties of the natural actomyosin extracted from both the leg and the breast muscle. These results suggested that marked changes in the interaction of actin and myosin are not an essential prerequisite in the process of rigor resolution and post-rigor tenderness development. Composed principally of the contractile proteins, actin and myosin, natural actomyosin probably does not contain several of the proteins now recognized as "regulatory" in the processes of contraction and relaxation, in the same spatial relationships nor in the same weight ratios as exist in vivo. As well, the extraction procedures undoubtedly disrupt the uniform array of the proteins within the myofibrils.

Myofibrils, however, do contain the regulatory proteins in addition to the actin and myosin, and moreover possess a structural arrangement which closely resembles the contractile apparatus as it exists in vivo. For these reasons, the myofibril as a unit should reveal more information regarding changes accompanying postmortem aging than actomyosin alone. Various morphological and biochemical changes have already been noted in myofibrils from several species as a result of postmortem aging. These changes, which have been reviewed by Fukazawa and Briskey (1970) and Goll et al. (1970), include the deterioration or removal of the Z line and M band, an increased susceptibility of the myofibrils to mechanical fragmentation and finally alterations in NTPase activity.

This paper presents the results of the continuation of our work regarding the effect of aging on chicken muscle and focuses on the morphological and some biochemical properties of myofibrils from typical white (pectoralis superficialis) and red (adductor longus) chicken muscle. The electron microscope was employed to monitor gross morphological changes, while Ca^{++} and Mg^{++} mediated ATPase activities and sulfhydryl group availability were examined to provide information regarding the biochemical changes in the myofibril during the aging process.

EXPERIMENTAL

Muscle source

Commercial broilers ranging in age from 8–12 wk were used in this study. The birds were killed as before (Hay et al., 1972) and the carcasses skinned, eviscerated and washed in cold running water. The carcasses were aged at 2°C in humidity controlled cabinets and wrapped in paper towels soaked in 10 mM sodium azide to minimize microbial growth. Muscle samples were removed at 0, 3, 48 and 168 hr.

Preparation of myofibrils

The myofibrils were prepared essentially according to the procedure described by Goll and Robson (1967). Approximately 20g of excised muscle were minced in a precooled meat grinder, transferred to a Waring Blendor and homogenized in 5 volumes of the extracting solution (0.25M sucrose, 1 mM disodium (ethyl dinitrilo) tetraacetate (EDTA), 0.05M Tris, pH 7.6) by three separate bursts of 15 sec duration with a 45 sec interval between each burst. The extract was stirred gently for 1 hr at 2°C and sedimented at 2,500 × G for 10 min. The sediment, consisting mainly of myofibrils, was resuspended in 5 volumes of extracting solution and stirred for another hour. The sedimented myofibrils were resuspended in 5 volumes of 0.05M Tris, pH 7.6, 1 mM EDTA and passed through three layers of cheesecloth to remove connective tissue. Purification of the myofibrils was achieved by washing the myofibrils successively with (1) 0.15M KCl, 0.03M Tris, pH 7.6; (2) 1 mM EDTA, pH 7.6; (3) double-deionized distilled water; and (4) 0.15M KCl, 0.03M Tris, pH 7.6. Following each washing the myofibrils were sedimented at 2,500 × G for 10 min. The rather densely packed myofibrils were resuspended after each centrifugation by gently stirring for 10–15 min in the cold. The myofibrils were finally suspended in 0.15M KCl, 0.03M Tris, pH 7.6.

Electron microscopy

Small pieces (0.5 mm × 4–5cm) of muscle were dissected out at the appropriate time postmortem and immediately immersed in 3.5% glutaraldehyde in 0.1M phosphate buffer, pH 7.4, for 4 hr at 4°C. Examination of segments of this size excised after attachment to glass rods after the method of Stromer et al. (1967) revealed no significant differences in sarcomere length regardless of the excision technique employed. Following a buffer wash overnight, the samples were post-fixed in 2% phosphate buffered osmium tetroxide for 2 hr at room temperature. The material was dehydrated with graded ethanol solutions and propylene oxide and then embedded in araldite.

The myofibril samples were obtained as a loosely packed pellet which was resuspended in buffered 3.5% glutaraldehyde and processed as above. The myofibrils were gently centrifuged between the preparative steps to facilitate the exchange of solutions.

Sections were cut and mounted on Formvar coated copper grids and stained using uranyl acetate and lead citrate (Reynolds, 1963) for up to 1 hr with each reagent. The sections were viewed using a Philips 300 electron microscope.

Estimation of protein concentration

The protein concentrations were determined using the biuret procedure described by Gornall et al. (1949) as modified by Robson et al. (1968). These values were further confirmed by a micro-Kjeldahl method, with a value of 16.7% N being used for converting NH_3 into protein. Tris N was accounted for by suitable blank determinations.

pH measurements

pH was determined by inserting a glass electrode (Beckman combination electrode #39511) into the cut surface of the muscle to be sampled.

ATPase and sulfhydryl measurements

Ca^{++} and Mg^{++} ATPase activities and SH groups were determined according to the procedures described previously (Hay et al., 1972). The reaction mixture for the ATPase activities consisted of 20 mM Tris-acetate buffer (pH 6.8), 1 mM $CaCl_2$ or $MgCl_2$, 0.02M KCl and 1 mM ATP. The reaction mixture for the SH group determination (Sedlak and Lindsay, 1968) consisted of 0.75 ml of 0.2M Tris buffer and 0.2M EDTA (pH 8.2), 0.67 ml of 0.15M KCl myofibril solution (protein concentration = 2.5–5 mg/ml), 0.05 ml of 0.01M 5,5′-dithiobis-2-(nitrobenzoic acid) (DTNB) dissolved in methanol and the volumes were made up to 5 ml with H_2O or 8M urea in duplicate samples and blanks.

RESULTS

Breast muscle in situ

The electron micrographs of longitudinal sections of in situ chicken breast muscle are shown in Figures 1a-d. The typical ultrastructural features and banding patterns of skeletal muscle are clearly defined (Fig. 1a). The thick and thin filaments are visible and muscle shortening is indicated by the short I bands and the narrow H zone. Careful scrutiny reveals the typical transverse striations of the Z line, elements of the sarcoplasmic reticulum and T-system are also apparent. For convenience the distinguishable morphological features have been appropriately

labelled in Figure 1a alone. There is little observed alteration in the morphological appearance of the chicken breast muscle after aging for three hours (Fig. 1b). Minor changes include the disappearance of the H zone and some loss of definition at the Z line and A-I junction. Gross morphological changes have occurred in the breast muscle after aging for 48 hr (Fig. 1c). The Z lines have lost their prerigor appearance, become diffuse and ruptures have occurred at several points (see arrows). The A regions and I bands are now only vaguely discernible and there is marked deterioration of the sarcoplasmic reticulum, T-system and mitochondria. Aging for 7 days has resulted in a further deterioration in the structure of the myofilaments as seen in Figure 1d. The Z lines have broadened and become very jagged. The M line has been partly displaced and appears in some instances to have divided into two bands. There is also an impression of coarse granularity associated with the myofilaments.

Breast myofibrils

The 0-hr breast myofibrils shown in Figure 2a bear little resemblance to their in situ appearance. The usual structural features are not evident and the actin and myosin filaments, instead of being in parallel array, are distributed haphazardly. Only the Z lines are distinguishable and appear for the most part to be distorted. The denser regions on either side of the Z line are probably contracture zones. The M line is totally absent. The appearance of the myofibrils from muscle aged 3 hr (Fig 2b) does not differ markedly from the 0-hr preparation except for a greater degree of parallel alignment of the actin and myosin filaments. The myofibrils obtained from the muscle aged 48 hr (Fig. 2c) reveal a partial integrity of the banding pattern, similar to that observed in in situ muscle. The A and I bands and H zone can be clearly observed though the M line is not discernible and the Z line is diffused and disrupted. Ridges of electron dense material appear on either side of the H zone, probably as an effect of thin filament overlap, resulting from sarcomere shortening. Additional deterioration of the Z line is evident in the seven day preparation (Fig. 2d).

Leg muscle in situ

The 0-hr in situ leg muscle (Fig. 3a)

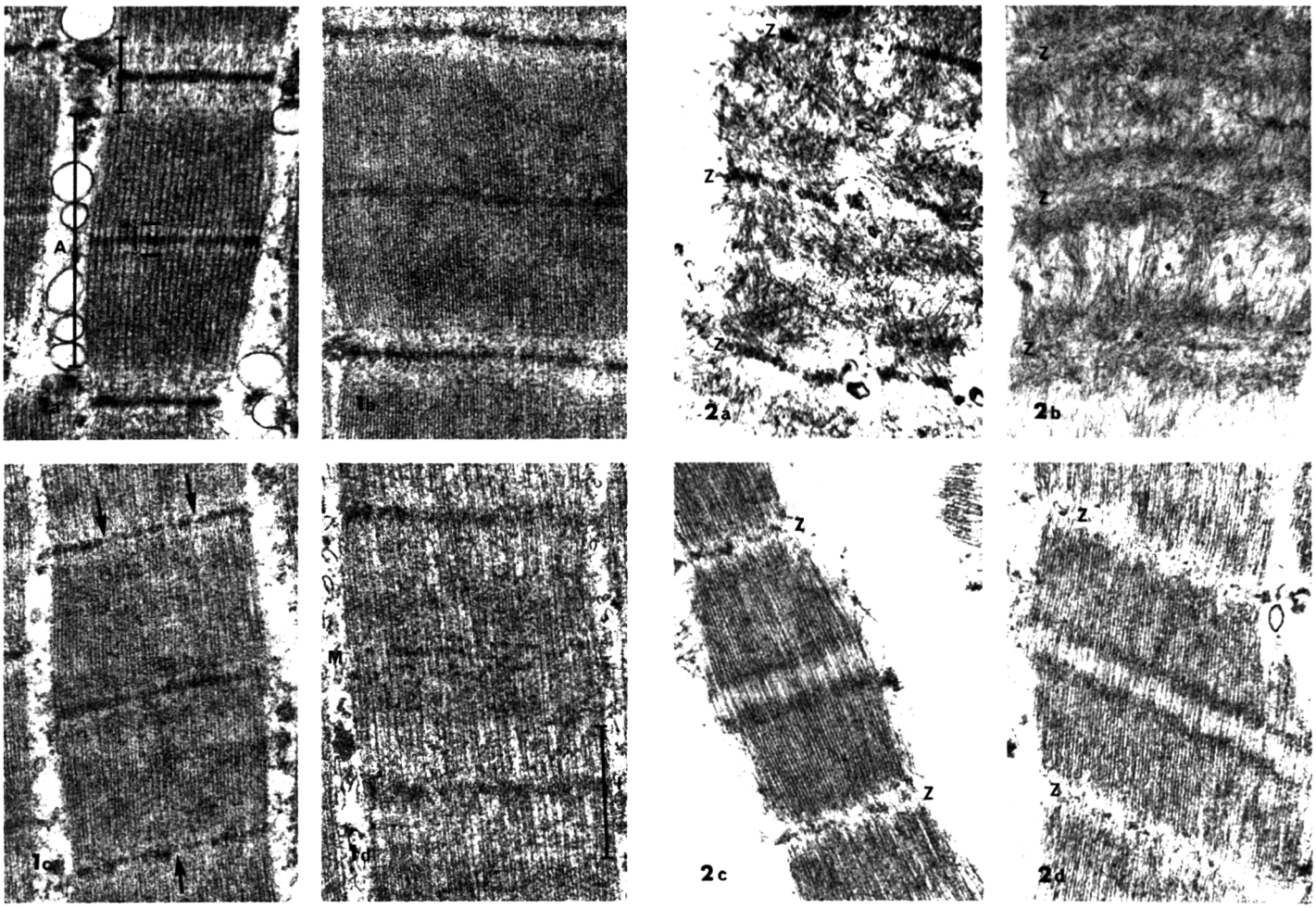

Fig. 1—Chicken breast muscle (pectoralis superficialis) fixed in situ. (a) 0 hr postmortem. All the typical features of skeletal muscle are present and labelled according to convention (× 25,300); (b) 3 hr postmortem. Note the disappearance of the H zone (× 25,300); (c) 48 hr postmortem. The Z line appears to be ruptured at several places (arrows) (× 25,300); and (d) 168 hr postmortem. The I band has widened and the M line is indistinct and may be divided into two separate lines in some areas. The Z line is highly diffuse (× 25,300).

Fig. 2—Myofibrils extracted from chicken breast muscle (pectoralis superficialis). (a) 0 hr postmortem. There is extreme disorganization of the fibrils and the only clearly recognizable features are the Z lines (× 19,900); (b) 3 hr postmortem. In comparison with Figure 2a there is slightly more ordering of the fibrils (× 19,900); (c) 48 hr postmortem. The parallel array of fibrils is now fully evident; all components are recognizable with the exception of the M line (× 25,300); and (d) 168 hr postmortem. Similar to Figure 2c but with further degradation of the Z line (× 25,300).

differs in several respects from the 0-hr in situ breast muscle. Compared to the breast muscle, the I band and Z lines of the leg muscle are wider, the H region and M line are less clearly delineated and glycogen granules, concentrated principally in the vicinity of the T-system, are relatively abundant. The leg muscle also contains large mitochondria. Apart from an appreciable disintegration of the cellular elements adjacent to the myofilaments, no marked changes are apparent in the 3-hr in situ leg preparation (Fig. 3b) when compared to the 0-hr sample. Aging for 48 hr (Fig. 3c) causes structural alterations in the in situ leg preparations. There is an increase in sarcomere length (see Table 1) seemingly due to a stretching of both the A and I band. The I bands are poorly defined and show regions of irregular electron density (see small arrows). A denser region (see large arrows) appearing on either side of the Z line approximates the size of the I band region seen in the 3-hr preparations. The A band is more uniform in density though less dense regions appear towards the middle of the band. Aging for this period of time causes no apparent deterioration in the Z line. In the 7-day preparation (Fig. 3d), the denser regions on either side of the Z line appear again in the banding pattern. There are fewer glycogen granules in this sample.

Leg myofibrils

The haphazard arrangement of the thick and thin filaments characteristic of the breast 0-hr myofibrils are observable in the 0-hr leg preparation (Fig. 4a). Apart from the barely discernible Z line, the banding pattern characteristic of the 0-hr in situ preparations is absent. Though still in disarray, the 3-hr myofibrils (Fig. 4b) appear to be arranged in a more orderly fashion than the 0-hr sample. Z lines and in some cases H zones can be discerned. The myofibrils from the leg muscle stored 48 hr (Fig. 4c) reveal many of the banding features observed in the 0-hr in situ state. However, within the one preparation, three distinct types of

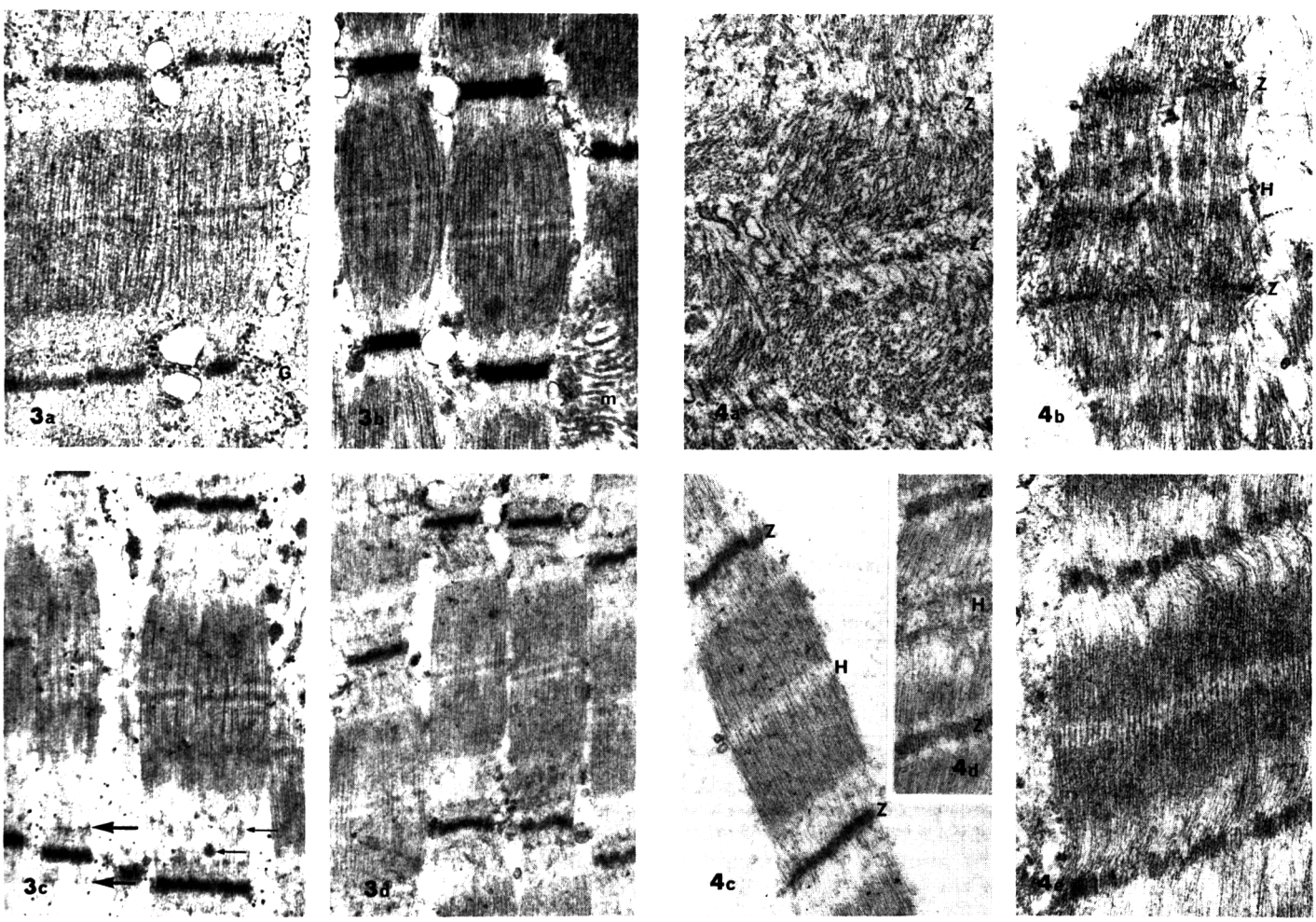

Fig. 3–Chicken leg muscle (adductor longus) fixed in situ. (a) 0 hr postmortem. All features of skeletal muscle are evident. Note the increased quantity of glycogen (G) in comparison with the breast muscle, Figure 1a (× 19,900); (b) 3 hr postmortem. Similar to Figure 3a but showing a deteriorated mitochondrion (m) (× 19,900); (c) 48 hr postmortem. The sarcomere length has increased in comparison with Figures 3a and 3b. Between the large arrows can be seen a distinct region which approximates the size of the I band of Figure 3b. Note the irregular electron density of the I band (small arrows) (× 18,400) and (d) 168 hr postmortem. The Z lines are very evident when compared with the breast muscle at the same time postmortem, Figure 1d (× 18,400).

Fig. 4–Myofibrils extracted from chicken leg muscle (adductor longus). (a) 0 hr postmortem. The fibrils are in disarray with the Z lines barely discernible (× 19,900); (b) 3 hr postmortem. Some parallel alignment of the filaments has been restored. The H zone is now evident (× 19,900); (c) 48 hr postmortem. Parallel alignment completely restored; M line is absent (× 19,900); (d) (inset) 48 hr postmortem. In this example the I band is narrow and the H zone is disrupted in comparison with Figure 4c (× 19,900); (e) 168 hr postmortem. Note the integrity of the Z line compared to the breast myofibrils at this time postmortem, Figure 2d (× 19,900).

Table 1—Z-Z Distances and A band widths of sarcomeres from in situ chicken breast and leg muscle[a]

	Hours postmortem							
	Z-Z Distance				A Band width			
Muscle	0	3	48	168	0	3	48	168
Breast	1.80 ± .04	1.76 ± .04	1.73 ± 0.6	1.56 ± .12	1.38 ± .10	1.37 ± 0.5	1.54 ± .08	Not Measurable
Leg	2.40 ± .24	1.88 ± .03	2.96 ± .22	2.78 ± .13	1.33 ± .11	1.36 ± .07	1.62 ± .18	1.56 ± .11

[a] Figures are expressed in μ ± S.D. representing the average of five measurements each from five separate microscopic fields.

Table 2—Postmortem pH measurements of chicken breast and leg muscle[a]

	Hours postmortem			
Muscle	0	3	48	168
Breast	6.5 ± .4	6.0 ± 0	5.6 ± .2	5.4 ± 0
Leg	6.6 ± .3	6.0 ± 0	5.9 ± 0	5.9 ± .2

[a] Figures are expressed as the average of four measurements per aging period.

Table 3—ATPase activity of myofibrils isolated from chicken breast muscle[a,b]

	Time of postmortem storage			
Activator	0 hr	3 hr	48 hr	168 hr
1 mM Mg^{++}	0.14 ± .01	0.15 ± 0	0.14 ± .01	0.16 ± 0
1 mM Ca^{++}	0.11 ± .01	0.12 ± 0	0.11 ± .01	0.12 ± 0

[a] Conditions of assay: 0.2 mg myofibrils/ml; 1 mM ATP; 20 mM KCl; 20 mM Tris-acetate (pH 6.8); 25°C.
[b] Figures are μmoles Pi/min/mg protein expressed as means plus or minus the standard deviation from determinations on separate muscle preparations from four different birds.

Table 4—ATPase activity of myofibrils isolated from chicken leg muscle[a,b]

	Time of postmortem storage		
Activator	0 hr	3 hr	48 hr
1 mM Mg^{++}	0.09 ± .02	0.11 ± .01	0.07 ± .02
1 mM Ca^{++}	0.06 ± .01	0.07 ± .01	0.05 ± .01

[a] Conditions of assay: 0.3 mg myofibrils/ml; 1 mM ATP; 20 mM KCl; 20 mM Tris-acetate (pH 6.8); 25°C.
[b] Figures are μmoles Pi/min/mg protein expressed as means plus or minus the standard deviation from determinations in separate muscle preparations from four (0 hr) and three (3 and 48 hr) different birds.

sarcomere can be observed. The predominant type (Fig. 4c) has thick, clear striated Z lines and well defined wide I and A bands. The H region is indistict. The second type (Fig. 4d) is similar to the previous one except that the H zone region has completely broken up and the I bands appear to be much narrower. The third type resembles the white breast muscle type (Fig. 2c), showing narrow I bands, absence of M bands and a Z line which has lost some definition. Little further change in the myofibrils extracted from 7-day muscle (Fig. 4e) can be observed apart from a slight disruption of the Z lines and a small reduction in the sarcomere length as compared to the in situ sarcomeres (Table 1). An M band may also have reappeared.

Postmortem pH measurements are shown in Table 2. Both leg and breast muscles show a similar reduction in pH at rigor. However, differences occur throughout the remaining part of the aging period. The leg muscle remains constant at this pH level (5.9–6.0) while the breast muscle continues to fall, from 6.0 to 5.4.

The results of calcium and magnesium ATPase activity measurements and sulfhydryl analyses are shown in Tables 3 and 4 and 5 and 6, respectively. Little change was observed in these parameters, although absolute differences were apparent between the leg and breast muscle samples.

DISCUSSION

COMPARING the 0-hr in situ breast muscle and the corresponding leg muscle, two differences are apparent. First, the breast muscle sarcomere lengths are shorter, and second, the breast muscle is deficient in glycogen granules.

Factors affecting the short sarcomere lengths in breast muscle may include changes in pH as a result of anaerobic glycolysis (de Fremery and Pool, 1960, 1963; de Fremery, 1966), cold shortening (Smith et al., 1969; Locker and Hagyard, 1963) and the degree of mechanical restraint imposed by the muscle attachments (Bouton and Harris, 1972; McCrae et al., 1971). Our observation that pH in both leg and breast muscle are quite similar at 0 and 3 hr would tend to minimize the significance of pH in shortening. Wood (1973), measuring isometric tension development in chicken breast muscle, showed that cold shortening is virtually instantaneous, but is completely abated within 15–30 min postmortem. The short sarcomere lengths of breast muscle observed in the present study probably reflect this rapid onset of cold shortening. A combination of both cold shortening and the resultant mechanical stresses exerted by the muscle attachments offers perhaps the best explanation for the differences between breast and leg muscle sarcomere length, and may be due to differences in muscle type. The depletion of glycogen in the breast muscle is probably caused by the excessive utilization of energy in the death struggle together with the high glycolytic rate which is characteristic of the muscle type (de Fremery and Pool, 1960, 1963; de Fremery, 1966).

Apart from the visible shortening of the leg muscles, the appearance of the in

Table 5—Sulfhydryl group analyses of myofibrils isolated from breast muscle[a]

Diluent	Time of postmortem storage			
	0 hr	3 hr	48 hr	168 hr
H_2O	3.9 ± 1.0	3.8 ± .4	3.6 ± .2	3.4 ± .3
Urea	8.6 ± 1.7	8.9 ± .5	8.9 ± .9	8.6 ± .2

[a] Figures are moles SH/10^5 g protein expressed as means plus or minus the standard deviation from determinations on separate muscle preparations from four different birds.

Table 6—Sulfhydryl group analyses of myofibrils isolated from leg muscle[a]

Diluent	Time of postmortem storage		
	0 hr	3 hr	48 hr
H_2O	3.9 ± 1.0	3.6 ± 0.2	3.0 ± .3
Urea	9.4 ± 0.3	9.6 ± 0.2	9.2 ± .4

[a] Figures are moles SH/10^5 g protein expressed as means plus or minus the standard deviations from determinations on separate muscle preparations from four (0 hr) and three (3 and 48 hr) different birds.

situ muscle samples after 3 hr aging (at a time when rigor mortis is known to occur (de Fremery and Pool, 1960) is virtually identical to the 0-hr case.

The electron micrographs of breast and leg myofibrils extracted from 0-hr muscle samples closely resemble the disheveled structures obtained by Stromer and Goll (1967), Greaser et al. (1969) and Weidemann et al. (1967) in their respective studies on bovine, pork and ox muscle myofibrils. The thick and thin filaments are completely disorganized and clearly lack a fixed relationship to each other. Contracture bands are visible on either side of the Z line, probably caused by the release of Ca^{++} from the sarcoplasmic reticulum during homogenization. The amount of EDTA added to the medium to sequester Ca^{++} is apparently insufficient to maintain the relaxed state. Supercontraction of the type described by Hoyle et al. (1965) may have occurred but it is difficult to discern whether the thick filaments have passed through the Z lines or are pushed against them and crumpled. Interpretation of these micrographs must be taken cum granis salus.

There is little apparent change in the myofibrils prepared from 3-hr muscle samples, although a number of thick filaments are beginning to arrange themselves parallel to each other. This is probably caused by the depletion of ATP, which in turn increases the actin and myosin interactions, and is manifested by the appearance of more ordered arrays of thick and thin filaments, particularly in the leg samples.

After 48 hr of aging, major changes occur in the morphology of both the leg and the breast muscle in in situ preparations and extracted myofibrils. Since these changes are most meaningful when referred to the 0-hr micrographs respectively, and since the structural changes are unique for each sample, each will be discussed separately.

Regarding the in situ breast muscle after 48 hr, the most obvious change is a marked degeneration of Z line and M band structures, reminiscent of the observations of Fukazawa and Yasui (1967), Takahashi et al. (1967), Fukazawa et al. (1969) and Sayre (1970) on chicken breast muscle. Z line degeneration is also apparent in the myofibrils prepared from 48-hr breast muscle. It is easy to speculate upon the factors which may cause Z line degradation in muscle. From our studies there may be some significance in the observation that pH decline appears to accompany Z line degradation. In breast muscle the fall in pH after rigor from 6.0 to 5.4 coincides with Z line degradation. In leg muscle there is no fall in pH and no Z line degradation throughout the aging period following rigor mortis. The view that pH fall may be an instrumental factor in causing Z line degradation is supported by Fukazawa et al. (1969), who demonstrated a marked reduction in the fragmentation of myofibrils when the postmortem pH was maintained at a high level by insulin injection.

The myofibrils isolated from the 48-hr samples differ markedly from their 0-hr counterparts. The regularity of the banding patterns and relative absence of tangled filaments might be explained by invoking the argument that cross bridges between the thick and thin filaments as a result of ATP depletion resist the homogenization treatment which caused so much disorganization in the myofilaments of the prerigor muscle. Alternatively, the trauma suffered by the muscle during homogenization may have caused a sudden release of Ca^{++} ions which, in the 0-hr case, might have resulted in a type of convulsive contraction in the presence of ATP, and a resulting loss of structural integrity. After depletion of ATP, no such convulsive disorganization would be possible, and the myofibrils might retain a more uniform appearance.

Studies of this nature have never been published for a red muscle from the chicken; therefore, these results may be of special interest. It is apparent that the effects of aging on longus adductor are quite different from the effects on pectoralis superficialis. This difference is manifested mainly at the Z line. In both the 48-hr and 7-day leg muscle in situ samples, the Z line was little affected by aging. By way of comparison, in the breast muscle the Z line was only vaguely apparent at 7 days.

It is thought that increases in muscle tenderness with aging can be related in part to degradation of the Z line and in part to changes in actin and myosin interactions (Goll et al., 1970). However, since the Z line of leg muscle is hardly affected by aging, it might be speculated that this muscle disposes of its rigor tensions by what could be termed a reversal of shortening coupled with a certain degree of stretching. It is noticeable that the widths of the electron dense regions on either side of the Z line (see Fig. 3c; large arrows) in the 48- and 168-hr aged in situ leg muscle equal the widths of the I band in the rigor state. The remainder of the I band in the aged in situ samples therefore appear to consist of filaments which have been extruded from the A band. A reversal of shortening with aging has been observed in previous studies (Weidemann et al., 1967; Takahashi et al., 1967; Gothard et al., 1966; Stromer et al., 1967). It is presumed not to be a relaxation in the physiological sense since this would require ATP, which is known to be absent in this system.

One feature which distinguishes the post-rigor from the prerigor relaxed state may explain this phenomenon. From the 48- and 168-hr aged muscle micrographs, there appear spots of irregular electron density throughout the unusually wide I band region (see Fig. 3c; small arrows). Stromer et al. (1967) suggested that an autolytic cleavage of the cross bridges uniting actin and myosin could cause the actin to slide out of the A band in the manner described by Huxley and Niedergerke (1954) and Huxley and Hanson (1954). This idea was abandoned when Stromer discovered that heavy meromyosin was not present in water soluble extracts. However, autolysis could occur at the junction of the heavy and light meromyosin fragments (trypsin sensitive region) and this could allow the actin combined with the heavy meromyosin to be released from the A band. Thus we speculate that the regions of irregular density in the I band might represent the actin combined with the heavy meromyosin.

In addition to the broadening of the I band, a broadening of the A bands may have occurred in the 48- and 168-hr aged muscle samples. Davey and Gilbert (1967) also observed a broadening of the A band in aged bovine muscle samples. It is interesting to observe that the sarcomere lengths of the 48- and 168-hr myofibrils are approximately 0.5μ shorter

than their in situ counterparts (Table 1), and this is probably due to the release of these tensions brought about in the preparation of the myofibrils.

The Ca^{++} mediated ATPase activities of myofibrils for both breast and leg samples are lower than the corresponding values obtained in our actomyosin studies. This is probably due to the decreased percentage of myosin in the myofibrils as opposed to actomyosin. The Ca^{++} ATPase activities do not appear to change with aging, a result in keeping with our previous study and also those of Wu and Sayre (1971); Khan and van den Berg (1964) and Jones (1972), suggesting that the site of this activity does not deteriorate with aging.

Though there is a slight increase in Mg^{++} ATPase activity at rigor in leg myofibrils there is comparatively little change throughout the aging process in the breast myofibrils. Our previous studies (Hay et al., 1972) indicated that the Mg^{++} ATPase activity of actomyosin was closely allied with the degree of interaction of actin and myosin. This relationship is partly borne out by one's observations of the sarcomere lengths of both breast and leg muscle. Comparing leg muscle in the 0-hr case to the 4-hr case, the muscle in rigor has shorter sarcomere lengths, which indicates a relatively strong interaction between actin and myosin, and relatively high Mg^{++} ATPase values. Leg muscle after 48 hr has elongated sarcomeres, which indicates a degree of dissociation of the actin from the myosin and low Mg^{++} ATPase values. Breast muscle, which shows little variation in sarcomere length, also shows no change in Mg^{++} ATPase activity. Ward et al. (1965) have demonstrated a similar relationship between sarcomere length and ATPase activity in frog sartorius muscle, though the results of Goll and Robson (1967) did not confirm this finding.

Similar to our prior study (Hay et al., 1972), no evidence of a marked reduction of total SH groups implies that there has been no oxidation of SH groups throughout the aging process. There may be a very slight reduction in SH groups with aging, a trend which was barely perceptible in our previous study (Hay et al., 1972). It would be misleading to attach much importance to this result.

CONCLUSIONS

ON THE BASIS of the data reported here, it is possible to draw several conclusions regarding the effects of postmortem aging on chicken muscle. Firstly, a lack of change in the biochemical parameters of Ca^{++} and Mg^{++} mediated ATPase activity in breast myofibrils confirms the fact that the sites of this activity undergo little change during aging. Secondly, the uniformity of sarcomere lengths in breast muscle strongly indicates that contraction and relaxation phenomena are not occurring with post-rigor aging.

Z line degradation is apparent only in the case of breast muscle. Leg muscle in situ preparations and myofibrils are refractory to the factors which result in the disruption of this important structural feature in the sarcomeres of breast muscle. However, in contrast to the breast muscle in situ sarcomeres, the leg muscle in situ sarcomeres show increases in length, and some slippage or stretching between the actin and myosin filaments seems apparent. That this stretching is likely related to the tensions on the muscle while still attached to the muscle web on the carcass is very likely, in light of the fact that the myofibrils prepared from this muscle and freed from the muscle fibers revert back to a shorter sarcomere length.

Myofibrils prepared from freshly killed birds are highly disordered, but postmortem aging diminishes the tendency of these structures to become disrupted.

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POLYACRYLAMIDE DISC GEL ELECTROPHORESIS OF FRESH AND AGED CHICKEN MUSCLE PROTEINS IN SODIUM DODECYLSULFATE

INTRODUCTION

THE USE OF sodium dodecylsulfate (SDS) has gained wide acceptance in recent years by many workers in the protein field. SDS, in combination with agents that reduce disulfide bridges, e.g., β-mercaptoethanol or dithiothreitol (DTT) brings about the disruption of the forces maintaining the secondary and tertiary structures of the proteins, and resolves them into single polypeptide chains. The electrophoretic mobility of these SDS treated proteins in polyacrylamide gels is closely correlated to their molecular weight (Shapiro et al. 1967; Weber and Osborn, 1969; Dunker and Rueckert, 1969). The correlation is due to the constant weight ratio of SDS to protein regardless of protein size (Reynolds and Tanford, 1970).

This technique has recently been applied to a study of the muscle proteins. Sender (1971) separated the protein constituents of rabbit leg myofibrils treated with SDS using a 4% polyacrylamide gel, and found that the molecular weights of the myofibrillar proteins calculated from electrophoretic mobility agreed closely with previously reported values calculated by a variety of physical methods. Scopes and Penny (1971) achieved similar success with this technique. They employed polyacrylamide slabs in their experimental procedure and included in their study an examination of the sarcoplasmic as well as the myofibrillar proteins. Jones (1972) included gel electrophoresis in his examination of chicken muscle actomyosin.

Our previous studies of the biochemical and morphological properties of muscle protein systems at various postmortem aging periods have provided us with considerable insight into the many factors which appear to be associated with the development of rigor mortis and the post-rigor resolution leading to tenderness in chicken muscle (Hay et al., 1972; Hay et al., 1973a, b). While subtle changes have been observed in the morphological properties of both in situ and extracted myofibrils during the aging process, the complexity of the myofibrillar system renders it refractory to most modern methods which might be used to evaluate these changes. Z-line degradation and changes in actin and myosin interaction have been observed during postmortem aging of muscle (reviewed by Goll et al. 1970), although these observations have not been adequately characterized. The demonstrated ability of polyacrylamide gel electrophoresis to separate the proteins of myofibrils treated with SDS provides a new and powerful technique to study any changes in these proteins which might accompany postmortem aging.

The objective of the present study is to define the myofibrillar protein constituents in both chicken breast (pectoralis major – white) and chicken leg (adductor longus – red) muscle according to electrophoretic mobility in SDS-gels, and to evaluate changes in these proteins resulting from the postmortem aging of muscle.

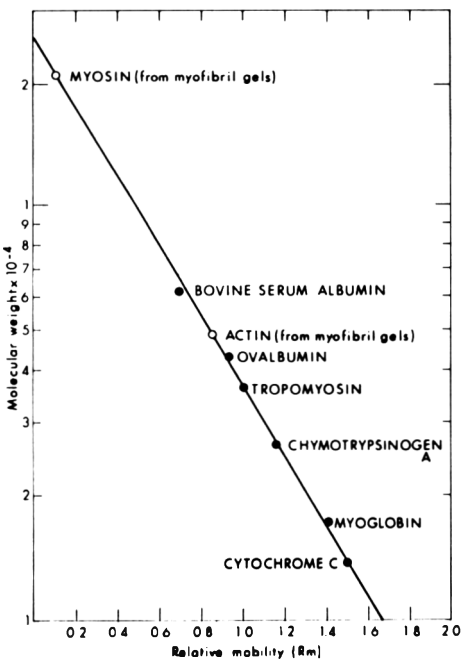

Fig. 1—Semi-log of molecular weight against distance of migration relative to the cardiac tropomyosin. A line of best fit has been drawn through the data points.

MATERIALS & METHODS

CHYMOTRYPSINOGEN A, cytochrome C, bovine serum albumin, myoglobin and ovalbumin were obtained from Schwartz/Mann Co., Orangeburg, N.Y. Cardiac tropomyosin was kindly donated by Dr. W.D. McCubbin, Dept. of Biochemistry, University of Alberta, Edmonton. Acrylamide Bis (N,N'-methylene bisacrylamide), and temed (N,N,N',N'-tetramethylenediamine) were obtained from Bio Rad Laboratories, Richmond, Calif. Sodium dodecylsulphate was obtained from Fisher Scientific Co., Ltd. and Coomassie Brilliant Blue from Schwartz/Mann Co.

Preparation of myofibrils and sarcoplasmic proteins

The myofibrils were prepared essentially according to the procedure described previously (Hay et al., 1973a). The supernatant from the first extraction, which consists of 0.25M sucrose, 1mM EDTA, 0.05M Tris pH 7.6, was used as the source of sarcoplasmic proteins.

SDS – gel electrophoresis

The method employed was similar to that described by Weber and Osborn, (1969). 8.5% acrylamide and bis-acrylamide in a 45:1 weight ratio was used in gels which were run in 8 mm (OD) × 100 mm tubes. The gels and the upper and lower reservoirs contained 50 mM sodium phosphate (pH 7.1) and 0.1% SDS. SDS-protein complexes were prepared by incubating myofibrils, sarcoplasmic proteins and protein standards (conc 3-4 mg/ml) in the presence of 1% SDS and 0.5 mM DTT at 40°C for ½ hr. Following incubation, 60% glycerol was added in the ratio of 1:1 (v/v) to increase the density of the protein solution. In the presence of potassium ion, the samples were kept at 40°C to prevent crystallization when applying the samples to the gels. 50 μg of the myofibrillar and sarcoplasmic proteins and 4 μg of each standard protein were applied to the gels. Protein concentrations were determined by the biuret procedure of Gornall et al. (1949) and Robson et al. (1968).

Electrophoresis was performed at 20–25°C at a constant current of 5 mA/tube for 5 hr. The gels were stained in a solution of Coomassie Brilliant Blue (0.5g dissolved in 227 ml of 50% methanol and 23 ml glacial acetic acid) for 2 hr and destained in a quick gel destainer (Canalco Co.) for 25 min in a solution containing 7½% (v/v) acetic acid and 5% (v/v) methanol. The molecular weights of the protein components were determined by comparison of their mobilities with proteins of known molecular weight as described by Weber and Osborn (1969) and Dunker and Rueckert (1969).

RESULTS & DISCUSSION

THE MOLECULAR weight calibration of the SDS polyacrylamide gels is depicted in Figure 1. Tropomyosin (molecular

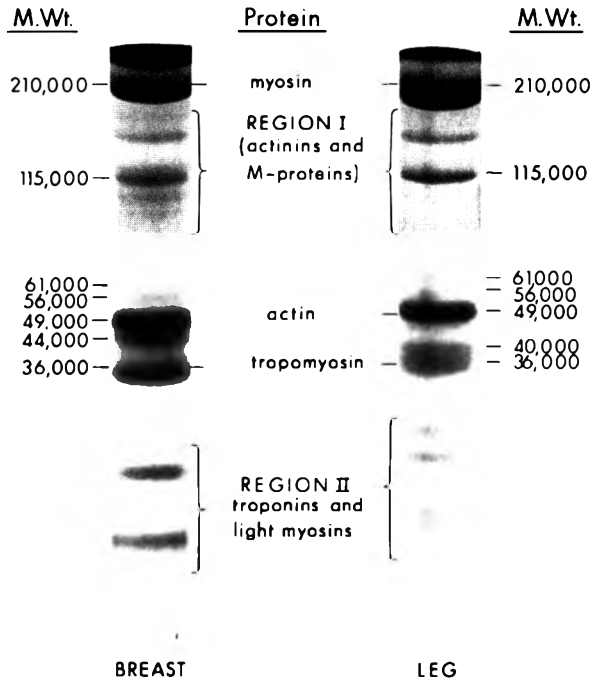

Fig. 2—Electrophoretograms of myofibrillar proteins from 0 hr breast and leg chicken muscle. The conditions for electrophoresis are described in Materials & Methods section. Tentative identification of the bands is indicated.

weight, 36,000 Daltons) was selected as the reference for relative mobility, since it is an easily identified component in the myofibrillar protein gels, and since both breast and leg tropomyosin exhibit the same mobility as the cardiac tropomyosin standard.

The SDS-gel electrophoresis patterns of the myofibrillar proteins from 0 hr chicken breast and leg muscle are shown in Figure 2. The major contractile proteins, myosin, actin and tropomyosin, together with several minor protein components are readily discerned.

The molecular weight ranges and tentative identification of the bands is also included in this figure. (Generally, identification of the minor bands proved difficult because of the lack of authentic standards. Although we record actin to have a molecular weight of 49,000 and α-actinin to have a molecular weight of 115,000, higher than the accepted 46,000 and 95–105,000, respectively, comparison with literature values of molecular weights for myofibrillar components is perhaps the most reliable means available for evaluation of these bands). The ill-defined bands which appear above the myosin bands are probably myosin aggregates. The bands within the 80,000–160,000 Dalton region (region I) probably include the proteins α-actinin (Sender, 1971; Penny, 1972), β-actinin (Maruyama, 1971) and M-protein (Masaki and Takaita, 1972).

The major protein obtained in a crude α-actinin extract prepared according to Goll et al. (1970) migrated the same distance as the 115,000 Dalton component. However, this value is higher than the molecular weight for α-actinin reported by Sender (1971) and Penny (1972) using gel electrophoresis. The assortment of bands in the 10,000–25,000 Dalton region (region II) probably correspond to the light myosin chains (Sarkar et al., 1971; Dow and Stracher, 1971; Weeds and Pope, 1971; Lowey and Risby, 1971), and troponins (Hartshorne and Pyun, 1971; Ebashi et al. 1971; Greaser and Gergely, 1971; Wilkinson et al., 1972).

With regard to the remaining bands, Sender (1971) noted two "tropomyosin" bands at 52,000 and 55,000 Daltons when his proteins were dissolved in 6M urea–1% SDS. Without urea, his tropomyosin migrated a distance corresponding to 36,000 Daltons. Two faint bands at 56,000 and 61,000 Daltons can be observed in both the leg and breast gels of Figure 2, but their intensities were unaffected by pretreatment with urea-SDS. In the leg sample, the 40,000 Dalton component just noticeable above the tropomyosin might be troponin-B (Dabrowska et al., 1973). This band is only present in the leg myofibrils. Lastly, just below the actin component in the breast sample alone, there is a band corresponding to 44,000 Daltons. The close migration of this band to actin refutes the suggestion that it is troponin-B. This band more likely represents the subunits of the M-line of protein chicken breast myofibrils reported and characterized by Morimoto and Harrington (1972).

The differences in the banding patterns between the breast and leg myofibrils are probably a reflection of the intrinsic differences between white and red muscle types. Champion et al. (1970) observed marked qualitative differences in the starch gel electrophoretograms from red and white pork muscle types, while Lowey and Risby (1971) and Sarkar et al. (1971) have been able to relate these differences specifically to the light myosin components.

The changes in the protein components of myofibrils prepared from intact carcasses at several periods of aging in the cold are shown in gel patterns presented in Figures 3 and 4 for breast and leg muscle, respectively. In the case of breast myofibrils, minor changes in banding patterns and intensities can be seen in both

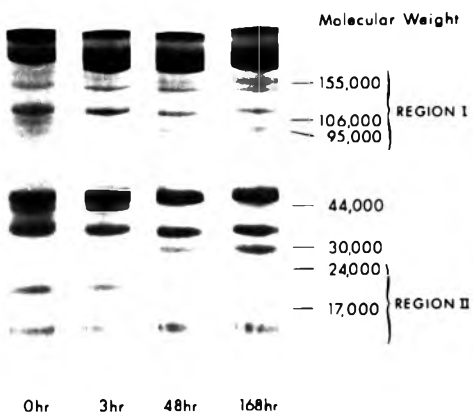

Fig. 3—SDS-gel electrophoresis of myofibrillar proteins extracted from chicken breast muscle aged 0, 3, 48 and 168 hr.

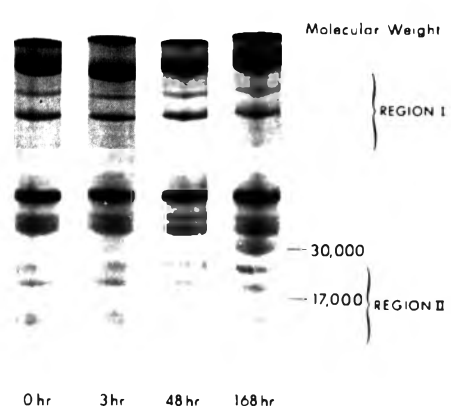

Fig. 4—SDS-gel electrophoresis of myofibrillar proteins extracted from chicken leg muscle aged 0, 3, 48 and 168 hr.

region I and, to a lesser extent, in region II. In region, I, note the development of a band at 155,000 Daltons increasing in intensity as aging proceeds. Similarly, note a faint band near 95,000 Daltons also increasing in intensity with aging. Less apparent is a band at 106,000 Daltons in the 0 hr sample, which decreases in intensity at 3 hr and 48 hr, and has vanished in the 168 hr gel. It is clear from these studies that the protein corresponding to α-actinin (115,000 Daltons) does not diminish with aging, an observation in keeping with the results of Penny (1972) who showed no change in the amount of α-actinin isolated from fresh or aged bovine muscle. In region II, a band at about 24,000 Daltons has increased slightly in intensity and a very faint band has appeared at 17,000 Daltons in the 48 and 168 hr gels.

The above noted changes in regions I and II are not nearly so marked as the clearly defined decrease and disappearance of the 44,000 Dalton component directly below the actin band in the 0 and 3 hr gels, and the appearance and increase in intensity observed for the 30,000 Dalton band in the 48 and 168 hr gels. Since the 44,000 band has disappeared totally in the 48 hr gel, while the 30,000 band increases in intensity from 48 to 168 hr, it is unlikely that the 30,000 band is resulting from degradation of the 44,000 component. Alterations in the solubility of these proteins might have contributed to these changes, but the absence of the coincident bands in the 0.25M sucrose extracts (Fig. 5) makes this explanation unlikely. The observation that the intensity of the M-line of the in situ chicken breast sarcomeres shows a marked decrease in the early stages of aging (Hay et al., 1973a) may be significant here if in fact the 44,000 Dalton component is the M-line protein described by Morimoto and Harrington (1972). The disappearance of the M-line in electron micrographs and the 44,000 Dalton component in the gels of breast myofibrils may not be entirely coincidental. This might be supported by the lack of a 44,000 Dalton component in leg muscle, and the fact that in situ leg myofibrils do not lose their M-line structure even after 168 hr aging (Hay et al., 1973a).

The appearance of the 30,000 Dalton component, which is most prominent in breast muscle after 168 hr of aging, but also appears in 168 hr leg muscle (see Fig. 4) is the most noticeable alteration in the gel patterns with aging. It does not appear to be derived from the minor protein components, since they show no changes in rates of migration or intensity relative to the appearance of the 30,000 Dalton component. As actin and tropomyosin show no apparent change in intensity, this unknown protein may be derived from myosin. If this band is derived from myosin, one might expect a heavy subunit of myosin to appear in the 170–180,000 Dalton range of corresponding gels. Unfortunately, the width of the myosin band is broad enough to encompass this region, and little definition is possible on these gels. Degradation of myosin occurring during postmortem aging has been suggested by Guenther and Turba (1969) and Wu and Sayre (1971). Stone and Perry (1972) in a study of the papain fragmentation of rabbit skeletal myosin, identified a 26,000 Dalton component by SDS-gel electrophoresis, and suggested that it represented a specific cleavage product of subfragment 1. Hayashi (1972) has followed the changes in the patterns of SDS-gel electrophoretograms during digestion of myosin and HMM with trypsin and revealed a component with a molecular weight of 27,000 which he indicated was derived from the f-subunit of myosin. These references may support the interpretation that myosin may be degraded during aging and that the 30,000 Dalton band may be one product of this degradation.

Degradation of troponin-B might also be implicated in the production of the 30,000 Dalton band, as others have shown troponin-B can be degraded to a 30,000 Dalton fragment. The relative intensities of the bands in the gels indicate that this is unlikely.

In the case of the leg myofibrils (Fig. 4) little change can be detected in the banding pattern of region I and region II, with the possible exception of the development of a smudge at about 17,000 Daltons in the 168 hr case. The only real change to be found in these gels is the appearance of a 30,000 Dalton component just below the band corresponding to tropomyosin in the 168 hr gel, which corresponds to the protein band appearing in later stages of aging in the breast muscle case. Again, mere changes in solubility will not suffice to explain the appearance of the band, since the 0.25M sucrose extracts of leg muscle (Fig. 6) show no bands coincident with 30,000 Daltons.

The differences noted between breast and leg sarcoplasmic proteins (Fig. 5 and 6) are due in part to the fact that red muscle is characteristically deficient in glycolytic enzymes. The minor changes in the 150,000 to 220,000 Dalton region in both breast and leg sarcoplasmic proteins may reflect some changes in solubility and/or denaturation of the proteins in this region. Neelin and Rose (1964) and Neelin and Ecobichon (1966) have already reported minor changes in starch gel electrophoretic patterns of sarcoplasmic proteins extracted from fresh and aged chicken muscle. No attempt has been made to identify the sarcoplasmic protein bands.

The principal findings of this study are the disappearance of the 44,000 Dalton component from breast myofibrils in the early stages of aging, and the appearance of a 30,000 Dalton component in the myofibrils of both leg and breast muscle during and after 48 hr postmortem. While some proteolysis has been shown to occur in muscle throughout postmortem aging (Khan and van den Berg, 1964; Miller et al., 1965; Davey and Gilbert, 1966; Parrish et al., 1969) it was not thought to play an important role in the changes associated with the development of tenderness in meat. Recently, Goll et al. (1971) has challenged this view and suggested that a very specific type of proteolysis may be responsible for many of the changes in postmortem myofibrils. As a result of his studies on trypsin-treated myofibrils, he suggested that fission of only a limited number of peptide bonds would be sufficient to produce the postmortem alterations usually observed in muscle. Certainly, only a few specific breaks in the peptide chain of heavy myosin would be necessary to produce the changes observed in the gel patterns pre-

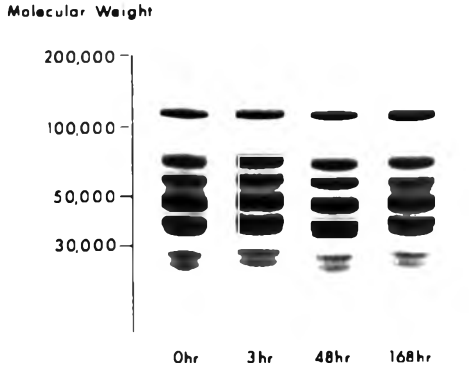

Fig. 5–SDS-gel electrophoresis of sarcoplasmic proteins extracted from chicken breast muscle aged 0, 3, 48 and 168 hr

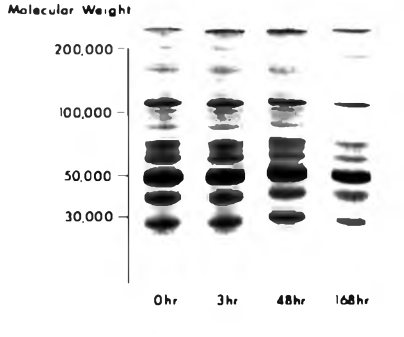

Fig. 6–SDS-gel electrophoresis of sarcoplasmic proteins extracted from chicken leg muscle aged 0, 3, 48 and 168 hr.

sented here. Catheptic enzymes native to chicken muscle (Caldwell, 1970) might well produce this type of proteolysis. It is questionable, however, if proteolysis is an important factor in postmortem changes in leg muscle, since detectable changes occur only after 48 hr aging, when most of the changes associated with tenderness development are known to have already occurred. Perhaps proteolysis in relation to tenderness may only apply to certain muscle types. Changes in the protein bands in the range indicated by region I in Figures 3 and 4 are of interest. Similar molecular weight proteins are thought to be located in the Z-line and M-line and a relationship may exist between the disappearance of these structural features in in situ myofibrils of chicken breast muscle (Hay et al., 1973a) and the changes in region I of the corresponding gel patterns apparent with aging. Added support for the existence of such a relationship is provided by the observation that leg muscle shows neither perceptible changes in Z-line or M-band, nor an alteration of the gel patterns in region I throughout postmortem aging.

In conclusion, the results of this study show clearly that specific alterations occur in the proteins of the myofibril during postmortem aging, and suggest that a specific and limited protein hydrolysis is the most likely cause of these changes.

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EFFECTS OF SODIUM CHLORIDE AND CONDENSED PHOSPHATES ON THE WATER-HOLDING CAPACITY, pH AND SWELLING OF CHICKEN MUSCLE

INTRODUCTION

THE EFFECT of sodium chloride (NaCl) and condensed phosphates on water-holding capacity, pH and meat swelling has been adequately demonstrated by several investigators (Swift and Ellis, 1956; Wierbicki et al., 1957b; Hamm, 1960; Shults and Wierbicki, 1972). Sodium salts of condensed phosphates increase the pH of chilled meats, thus increasing their water-holding properties; sequestering of heavy metal and calcium ions in meats contribute also to the water-holding capacity; and apparently some polyphosphates can split actomyosin into components with higher water-holding properties (Hamm, 1960; Wierbicki et al., 1963; Yusui et al., 1964; Shults and Wierbicki, 1972). However, the exact mechanism by which NaCl and polyphosphates, in combination, affect the meat is not clearly defined.

Shults and Wierbicki (1972) found that tetrasodium pyrophosphate was the most effective food-grade phosphate for increasing the meat pH, swelling and the water-holding capacity of beef muscle. Maximum effects on reduction of the meat shrinkage (reciprocal of water-holding capacity) were obtained by the addition of 0.5% pyrophosphate or tripolyphosphate. Higher levels of phosphate concentrations had little additional effect on the swelling or the water-holding capacity, especially in the presence of 1% NaCl. Sodium hexametaphosphate or metaphosphate had only small effects on the beef muscle.

Some research on the use of NaCl and phosphates in chicken meat has been conducted, using both light and dark meat. Froning (1965, 1966) reported that the addition of polyphosphates (Kena) to ground chicken meat in a range of 0.5–1.0% was desirable for improving the quality of chicken rolls. He reported that an addition of 6% polyphosphates to the chilling water significantly reduced cooking losses and increased moisture retention in cooked chicken rolls. Mountney and Arganosa (1962) and Schermerhorn and Stadelman (1964) reported that a concentration of 4% or more of polyphosphates added to the chilling water decreased water uptake. This decrease became very pronounced at a concentration of 12%.

The objectives of these studies were to determine the relative effects of NaCl and different condensed phosphates on swelling and pH of raw, white chicken meat and the water-holding capacity of the cooked meat, with emphasis on the water-holding capacity (reciprocal of meat shrinkage).

METHODS & MATERIALS

Meat

The raw material utilized for these studies was fresh, boneless breast of chicken (broilers). Skin and fat were removed and the meat was ground through a 3/16-in. grinding plate and thoroughly mixed. The ground meat was stored in a refrigerator (2–4°C) before use.

Additives

The food-grade, condensed phosphates studied were: Sodium tripolyphosphate (TPP), sodium hexametaphosphate (HMP), tetrasodium pyrophosphate (PP), a commercial preparation of HMP (Foodfos), Kena FP-28 (a mixture of TPP, PP and sodium acid pyrophosphate), and Curafos 22-4 and 11-2 (commercial mixtures of TPP and HMP). Kena FP-28 is a special mixture of polyphosphates recommended for use in poultry products. All phosphates studied were obtained by the courtesy of Calgon Corporation, Pittsburgh, Pa. Other commercial mixtures, Curafos 22-4 and Curafos 11-2, are not specifically recommended by the manufacturer for use in poultry.

The phosphates and NaCl were dissolved first in distilled water. Meat samples were weighed out and various solutions were added in the ratio of 10 ml solution to 100g of meat. When additions of NaCl were higher than 1.5% in the meat, the appropriate amount of salt was added directly to 100g ground meat samples along with 10 ml of water or the phosphate solution. The whole was mixed and used after standing in the refrigerator overnight. All determinations were run twice in duplicate. The data reported are averages of four readings.

Methods

Meat shrinkage (water-holding capacity). The water-holding capacity was determined by the method of Wierbicki et al. (1957a) with modifications by Shults and Wierbicki (1972).

Meat swelling (water-binding capacity). The method by Wierbicki et al. (1962) was used with modifications by Shults and Wierbicki (1972).

pH readings. The pH of the meat samples was read by immersing the electrodes directly into the meat, using a Beckman Zeromatic pH meter. Readings were taken prior to weighing the samples for the shrink determination.

Statistical analysis. The data were subjected to statistical analysis using analysis of variance (Steel and Torrie, 1960) and multiple range test (Duncan, 1955).

RESULTS & DISCUSSION

Effects of polyphosphates

The effects of the various phosphates (added at a 0.5% level) on the pH, swell-

Table 1—Effects of phosphates with and without sodium chloride on the pH, swelling and water-holding capacity of chicken muscle

	0% NaCl				1.0% NaCl			
Polyphosphates	pH	% Swelling	% Shrink	Multiple Range Test[a]	pH	% Swelling	% Shrink	Multiple Range Test[a]
Tetrasodium pyrophosphate	6.3	188	22		6.3	203	13	
Kena FP-28	6.1	185	24		6.0	191	15	
Sodium tripholyphosphate	6.1	180	25		6.2	191	16	
Curafos 22-4	6.1	183	29		6.0	185	18	
Curafos 11-2	5.9	146	29		6.0	174	18	
Sodium hexametaphosphate	5.9	151	28		5.8	171	19	
Foodfos	5.9	137	29		5.8	171	19	
Control (No phosphates)	5.7	102	31		5.7	125	22	

[a] Significance at the 5% level; applies to percent shrink only.

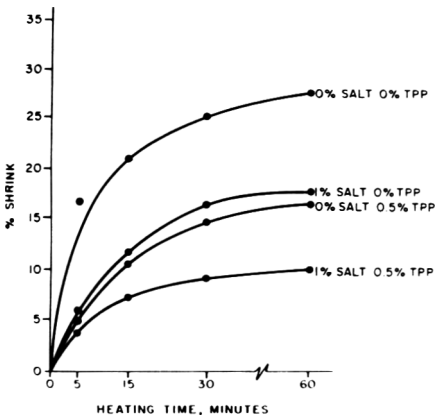

Fig. 1—Effect of heating time at 70° C on the shrinkage of chicken muscle.

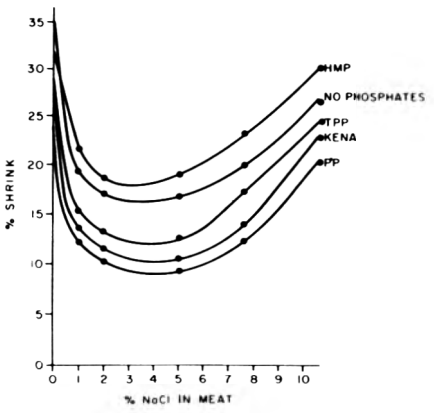

Fig. 2—Effect of NaCl on the shrinkage of chicken muscle at 70° C.

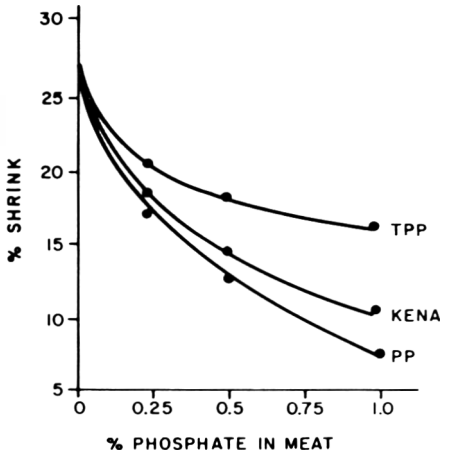

Fig. 3—Effect of polyphosphates, without NaCl, on shrinkage of chicken muscle at 70° C.

ing and water holding capacity (WHC) are shown on Table 1. These phosphates were tested with and without 1% NaCl added to the ground chicken meat. In the samples without NaCl, pyrophosphate (PP) exerted the greatest effects on the pH, swelling and WHC followed by Kena, TPP and Curafos 22-4. Analysis of the WHC data showed that PP caused significantly lower shrinkage than the other phosphate samples. No statistical differences were found between the Kena and TPP samples, but they had significantly less shrinkage than Curafos 22-4, Curafos 11-2, HMP and Foodfos samples. The addition of 0.5% PP resulted in a 9% reduction of shrinkage in the cooked meat and 86% increase in swelling of the raw meat.

In samples with 1% NaCl added, no signfificant differences were found in the shrink data for PP and Kena samples. However, PP sample was significantly lower than samples with 22-4, 11-2, HMP and Foodfos. The swelling data show that PP had the greatest effect and Kena and TPP were equally effective. The results indicate that PP is the most effective phosphate, followed by Kena and TPP. The addition of 1% NaCl and 0.5% PP resulted in a total reduction in shrinkage of 18% in the cooked meat and 101% increase of swelling in the raw meat.

Effects of heating time on meat shrinkage

Figure 1 shows relative effects of TPP, NaCl and a combination of TPP and NaCl on the water-holding capacity (reduction of meat shrinkage) of white chicken meat during heating at 70°C for 5–60 min. The data indicate a synergistic effect of TPP and NaCl on water-holding capacity of the meat. They also indicate that 30 min heating time used throughout this study is sufficient to obtain the relative effects of the additives on the water-holding capacity of chicken meat.

Effect of NaCl concentration

Figure 2 shows the effects of NaCl concentration ranging from 0–10% on the meat shrinkage at 70°C when it was used alone and in combination with 0.5% of TPP, Kena, PP and HMP. The addition of 1% NaCl dramatically decreased the meat shrinkage. Reduction in shrinkage of the meat continued up to a concentration of 3–5% NaCl, depending on the phosphate. Over 5% NaCl concentrations had an adverse effect on the meat shrinkage. The presence of the phosphates did not alter this trend. However, there was a definite synergistic effect, with the exception of HMP. The greatest relative effect in reducing the meat shrinkage was shown by PP, followed by Kena and TPP. With the exception of HMP, the effect of NaCl and other condensed phosphates on chicken meat is similar to the effect found previously for beef (Shults and Wierbicki, 1972).

Effect of phosphate concentrations on meat shrinkage

To test the effects of phosphate concentrations up to 1% on meat shrinkage, the three most promising phosphates, PP, Kena and TPP, were evaluated with and without NaCl. Figure 3 shows the results of the three phosphates on meat shrinkage when tested without NaCl. The greatest effect was shown by PP. At 0.5% concentration, PP and Kena showed only slight differences. TPP showed a lesser effect on the shrinkage; a very small additional reduction in meat shrinkage oc-

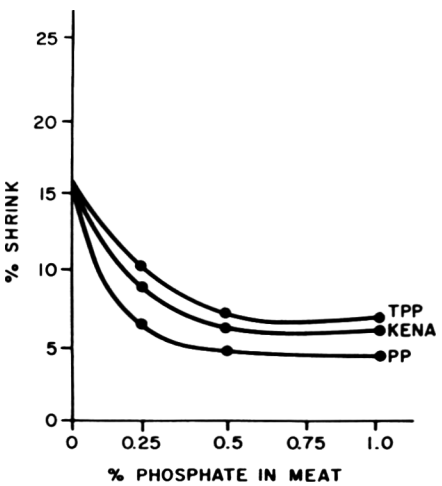

Fig. 4—Effect of polyphosphates and 1% NaCl on the shrinkage of chicken muscle at 70° C.

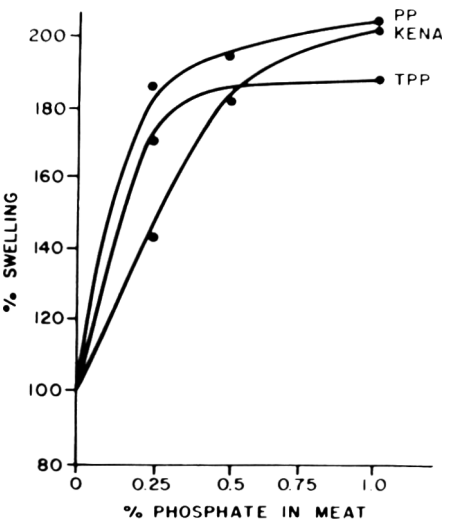

Fig. 5—Effect of polyphosphates, without NaCl, on the swelling of raw chicken muscle.

curred when the concentration of TPP was increased from 0.5–1.0%. Kena and PP continued to show a significant reduction of meat shrinkage up to a 1.0% concentration.

Figure 4 shows the results obtained with 1% NaCl added to the meat samples. The same trends were obtained as for the samples without the NaCl. However, the data indicate that when NaCl is present, further reductions in meat shrinkage did not occur when the concentration of the three phosphates was increased from 0.5 to 1.0%. PP has the greatest effect on meat shrinkage in the range of 0.25–0.5%.

Effect of polyphosphate concentrations on swelling

The three phosphates that had the greatest effects on meat shrinkage were evaluated to determine their effects on meat swelling of the raw meat. Figure 5 shows the effect of PP, Kena and TPP without NaCl on meat swelling. PP and TPP showed the greatest effect on the swelling at a concentration of 0.25%. Kena showed little effect at the 0.25% level. The three phosphates yielded similar results at 0.5%. The sample with Kena continued to show improvement up to the 1.0% level, whereas TPP and PP showed very little improvement above 0.25%. The addition of the phosphates resulted in an 80–100% increase in the meat swelling.

Figure 6 shows the effects of the three polyphosphates in combination with 1% NaCl on the meat swelling. Compared to the results of Figure 5, 1% NaCl alone increased the meat swelling from 100 to 150%. In combination with the phosphates, the swelling increased by an additional 30–50%. With PP and TPP, the greatest initial effects on swelling were obtained at 0.25%, then increasing only slightly up to 1.0% phosphate additions. Kena increased continuously the meat swelling up to the 1.0% addition.

Table 2–Effect of combinations of polyphosphates and 1% NaCl on the water-holding capacity of white chicken muscle

Detm	ml of juice per 20g muscle										
no.	AAA[a]	AAB	ABB	AAC	ACC	BBB	BBC	BCC	CCC	ABC	1% NaCl
1	2.5	3.2	3.6	2.9	2.5	3.5	2.7	2.9	2.2	2.6	4.3
2	3.0	2.7	3.4	3.0	2.4	3.9	3.0	2.9	2.4	2.1	4.6
3	2.5	2.6	2.8	2.5	2.4	3.8	3.3	2.3	1.9	2.1	4.2
4	2.4	2.8	3.2	3.0	2.8	3.4	2.5	2.4	2.1	2.4	4.5
$\bar{X}$	2.60	2.83	3.25	2.85	2.52	3.65	2.87	2.62	2.12	2.30	4.40
(% Shrink)	(13.0)	(14.2)	(16.2)	(14.2)	(12.6)	(18.2)	(14.3)	(13.1)	(10.6)	(11.5)	(22.0)
	Multiple range test (Significance at the 5% level)										(Control)
P-combinations:	CCC	ABC	ACC	AAA	BCC	AAB	AAC	BBC	ABB	BBB	1% NaCl
ml/20g:	2.12	2.30	2.52	2.60	2.62	2.83	2.85	2.87	3.25	3.65	4.40

[a] A = sodium tripolyphosphate; B = sodium hexametaphosphate; C = sodium pyrophosphate

Effect of polyphosphate concentrations on pH

The effects of the three polyphosphates (PP, TPP, and Kena), with and without 1% NaCl, on the pH of the meat are shown in Figure 7. As the data indicate, the three polyphosphates increased the pH of the chicken meat. Pyrophosphate had the greatest effect, followed by Kena and TPP. These results confirm that there is a direct relationship between pH and the shrinkage and swelling of the meat. Addition of 1% NaCl slightly decreased the pH, while greatly increasing the water-holding capacity (decreased the meat shrinkage). This confirms generally accepted hypothesis that increase in the ionic strength of the media (caused by the addition of NaCl), in addition to pH, is responsible for the water-holding capacity of meats (Hamm, 1960; Wierbicki et al., 1963).

Effect of polyphosphate combinations

Several combinations of sodium tripolyphosphate (designated as A) sodium hexametaphosphate (designated as B) and tetrasodium pyrophosphate (designated as C) were evaluated, along with 1% NaCl, for their effect on the WHC to determine a best combination for use in chicken meat. These combinations are listed in Table 2. The total amount of polyphosphates added to the sample was 0.45%. Each polyphosphate was added in 0.15% increments. Therefore, the sample listed AAA is 0.45% TPP, the sample listed

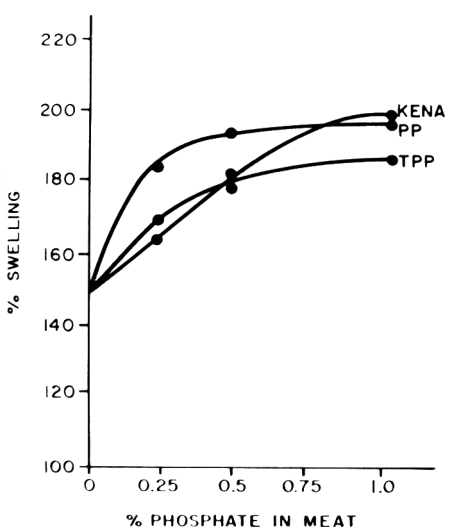

Fig. 6–Effect of polyphosphates and 1% NaCl on the swelling of raw chicken muscle.

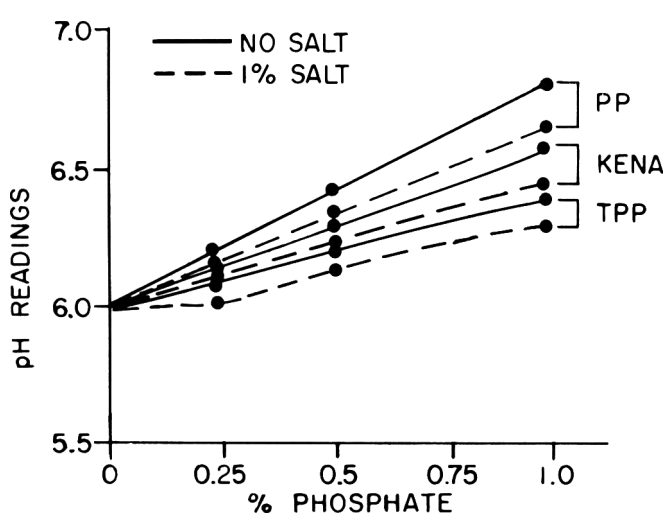

Fig. 7–Effect of polyphosphates, with 1% and without NaCl, on the pH of raw chicken muscle.

AAB is 0.3% TPP and 0.15% HMP and so forth. The shrink determinations were run four times in duplicate and the data statistically analyzed to determine differences among the combinations.

Statistical analysis showed that CCC (0.45% PP) yielded the lowest shrink values and was significantly different from all the other combinations except ABC (0.15% PP, 0.15% TPP and 0.15% HMP). However, ABC (TPP, HMP and PP), ACC (TPP and PP), AAA (TPP) and BCC (HMP and PP) were not significantly different.

CONCLUSIONS

THE OVERALL RESULTS indicate that at an addition of 0.25–0.5% sodium salts of (a) pyrophosphate; (b) a mixture of one part tripolyphosphate, one part hexametophosphate and one part pyrophosphate; (c) the commercial phosphate mixture, Kena FP-28; or (d) tripolyphosphate are the most efficient polyphosphates for the reduction of the loss of natural juices during cooking of chicken meat.

The reduction of the loss of natural juices during cooking is greatly increased when the polyphosphates are used in a combination with common salt, sodium chloride.

Addition of 0.3% polyphosphates to raw chicken meat before cooking is considered sufficient for obtaining optimum reduction of cooked-out juices from the meat during thermal treatment.

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THE ASSOCIATION OF STRUGGLE DURING EXSANGUINATION TO GLYCOLYSIS, PROTEIN SOLUBILITY AND SHEAR IN TURKEY PECTORALIS MUSCLE

INTRODUCTION

VIGOROUS MUSCULAR activity by turkeys, associated with handling and exsanguination process, is a frequent cause of lowering carcass grade through bruises and tears. Furthermore, the degree of struggling postexsanguination affects the response to electrical stimulation and rigor time course of the pectoralis (Ma et al., 1971). The practice of electrical stunning has been generally curtailed due to an increased incidence of wing and keel bone breakage. This curtailment permits more struggling. Khan and Nakamura (1970) demonstrated that accumulation of lactic acid in chicken muscle results in increased shear values.

The exact mechanism by which rapid glycolysis influences muscle shear properties is unknown. Sink et al. (1965) demonstrated that porcine muscle which experiences a brief rigor time course also exhibits short sarcomeres. Such muscle may also exhibit low protein solubility—a factor which, like short sarcomeres, may result in increased shear values. Moreover, low protein solubility may result in lower muscle yield after thermal processing. The present study was designed to study the interrelationships among stunning, struggling, postmortem muscle glycolysis, protein solubility and shear values of turkey pectoralis muscle.

EXPERIMENTAL

Experiment 1

Sixteen toms (age, 5 months), half of which were permitted to struggle freely postexsanguination while the other half were restrained in a metal cone, were dispatched and determinations made as described by Ma et al. (1971). Sarcomere length determination was conducted hourly until lengthening occured. pH was measured with probe electrode. Muscle was prepared by the method of Borchert and Briskey (1964), and adenosine triphosphate (ATP) and lactic acid were determined (enzymatically on perchloric acid extracts) at the same time intervals as sarcomere length determination.

Experiment 2

Sixteen toms (age, 5 months) were divided into equal groups of struggled and restrained and dispatched as in Experiment 1. Protein solubility samples were prepared according to Borchert and Briskey (1964) and extracted as outlined by Helander (1957). Sarcoplasmic protein was extracted with 0.03M KPO_4 buffer, pH 7.4 and total soluble protein with 1.1M KCl, 0.1 KPO_4 buffer, pH 7.4. Protein concentration of extracts was quantitated by the biuret method (serum albumin standard) and total nitrogen was determined by macro-Kjeldahl. Intact breast muscle samples, secured from turkey carcasses exposed to 4°C at 24 hr postmortem, were blast frozen, followed by thawing overnight at 4°C. Weight lost after thawing was calculated as thawing loss. For shear determination, meat was cooked in a Cryovac bag to 80°C and 2.54 cm diameter cores were sheared on a Warner-Bratzler apparatus.

Experiment 3

This was similar to Experiment 2, with two differences. Ten young birds (age, 3 months) were utilized and electrical stunning was compared to struggling. Water-holding capacity was determined according to Wierbicki et al. (1957) on meat heated at 60°C for 30 min.

Experiment 4

Twenty-four tom turkeys (age, 6 months), selected in a commercial processing plant, underwent the regular processing procedure, including chilling in a 0°C ice slush overnight and freezing. Prior to evisceration, the degree of rigor of the pectoralis muscle was determined by tactile evaluation and recorded on a subjective scale (5 = rigor, 1 = prerigor). Each frozen turkey was divided in half and thawed at 2–4°C. Approximately 700g of breast muscle was cut from each half and rolled in a covered aluminum foil pan. The samples were cooked to an internal temperature of 80°C in a 180°C oven. A 16-member taste panel evaluated tenderness based on a hedonic scale of extremely good (8) to extremely poor (1). Shear was determined by using a Warner-Bratzler shear press with a 1 cm^2 cross-sectional area strip.

Statistical analysis

Results were subjected to analysis of variance and significance of means determined by t test (Steel and Torrie, 1960) and by correlation analysis and linear regression analysis.

RESULTS & DISCUSSION

POSTEXSANGUINATION struggling elevated electrical stimulatory response threshold ($P < 0.05$) and decreased contractility duration ($P < 0.01$, Table 1). Significant ($P < 0.01$) decrease in rigor time course also resulted from struggling. These findings agree with previous research on turkey pectoralis (Ma et al., 1971). This anaerobic muscle may exhibit patterns of postmortem metabolism similar to pale, soft, exudative (PSE) porcine muscle. Further support of this suggestion is provided by findings that levels of lactate, ATP and pH are significantly different between treatments (Table 2). Sair et al. (1970) used curare to prevent muscular stimulation and magnesium to retain total phosphorylated creatine in muscle. They reported that these treatments prevented development of PSE muscle in stress-susceptible pigs postmortem. High pH and ATP concentration were noted in anesthetized rabbit muscles by Bendall and Lawrie (1962). High ATP levels in restrained turkeys reduced the rate of lactate accumulation.

In the present study, accumulation of lactic acid as a result of glycolysis occurred more rapidly in struggling than restrained turkeys. These rates are comparable to or even exceed those reported for PSE porcine muscle (Briskey, 1964) as some muscles from struggling birds completed glycolysis (pH 5.50) within 5 min postmortem. Turkey pectoralis muscle is composed almost completely of α-white (fast contracting, anaerobic) muscle fibers (Wiskus et al., 1973).

Muscle from restrained birds exhibited shorter ($P < 0.01$) sarcomeres at 1 and 2 hr postmortem than from strugglers (Ta-

Table 1—Effect of struggling on parameters of response to electrical stimulation of tom turkey pectoralis muscle (experiment 1)

	Restrained (N=8)		Struggled (N=8)	
Muscle parameters	Mean	S.D.	Mean	S.D.
Rigor completion (min)	233**	64	92	33
Threshold (volts)	4*	1	44	40
Contractility duration (sec)	207**	35	104	6

* $P < 0.05$
** $P < 0.01$

ble 2). Interpretation of these results is difficult because sarcomere shortening may have been induced by homogenization of prerigor myofibrils (Ma et al., 1971). Sarcomere lengthening was described by Takahashi et al. (1967) on chicken pectoralis.

No significant difference between muscle from restrained and struggled turkeys (experiment 2) was found for protein extractibility, thawing loss, cooking loss and shear value (Table 3). Total protein solubility (24 hr postmortem) was not different between treatments, yet it was correlated to shear value ($N = 16$, $r = -0.54$, $P < 0.05$).

Similar results were obtained for electrical stunning (experiment 3, Table 4) with the exception of thawing loss which was significantly ($P < 0.05$) less in stunned birds; however, consistent data trends suggest that stunning did have beneficial effects. An interesting statistic was calculated from these data. Decrease in myofibrillar protein solubility between 0 and 24 hr postmortem was negatively correlated to shear value ($N = 10$, $r = -0.58$, $P < 0.05$). This unexpected result was interpreted, after graphical analysis of the data, as indicating the pH decline was rapid enough in some turkeys to denature muscle proteins at death. Consequently, postmortem time would result in little further decrease in solubility. In contrast, extent of protein denaturation at death in other turkeys was not as high; hence, higher percent protein denaturation occurred postmortem.

The reason for not finding significant differences between treatments may include the difficulty of eliminating struggling, as even electrical stunning does not completely prevent it. Other factors not studied in this experiment, e.g., emotional stress and heat stress may also influence glycolysis in turkey breast muscle. The inability to identify these factors may also explain lack of clear difference of muscle properties between struggled and restrained birds. Factors associated with protein solubility and pH decline are apparently involved with postmortem muscle toughening. The almost complete lack of aerobic metabolism in pectoral muscle is an important factor.

A preliminary study using turkeys treated with nembutal, which minimizes muscular contraction during exsanguination, demonstrated that these muscles exhibit extremely long time courses of rigor (344 min) and very slow pH decline rates (pH 7.2, 7.0, 6.4, 6.1 and 6.0 at death, 1/3, 1, 2 and 3 hr postmortem). These results suggest that despite the anaerobic nature of pectoral muscle, it is not obligatory that the muscle experience rapid postmortem glycolysis. The potential exists, in the absence of contraction stimuli, for slow or moderate rates. Therefore, minimization of struggling in turkeys, ante- and postexanguination would slow down the pH decline rate and improve muscle properties. Commercially-used electric stunners are generally not able to cause total inhibition of struggling.

Shear value and panel tenderness data (experiment 4) were plotted against subjective rigor scores. Panel tenderness was correlated to shear value ($r = -0.58$, $P < 0.01$, $N = 24$). A clear tendancy was noted for an increase in shear (decrease in tenderness) as rigor time decreases. Linear regression analysis indicated that 75% of the variation in shear value was accounted for by the recorded differences in rigor extent as determined by tactile evaluation on the evisceration line.

It was not possible, from the results of the present study, to make definite conclusions concerning the mechanism of postmortem toughening of the pectoralis muscle. Protein solubility changes, as influenced by pH decline, were apparently involved in shear value increases in experiments 2 and 3. In experiment 3, a significant correlation coefficient ($r = -0.79$, P

Table 2—Effects of struggling on lactate, ATP, pH and sarcomere length as function of time post mortem of turkey pectoralis muscle (experiment 1)

Muscle parameters	Time postmortem (hr)	Restrained (N=8) Mean	Restrained (N=8) S.D.	Struggled (N=8) Mean	Struggled (N=8) S.D.
Lactate (μM/g)	0[a]	36.7	17.5	46.5	26.6
	½	48.4*	7.9	59.0	10.7
	1	59.8	8.8	64.2	10.9
	2	62.1	11.9	62.8	10.8
	3	59.0	11.1	62.8	10.9
ATP (μM/g)	0[a]	2.7*	0.3	2.2	0.6
	½	2.5*	0.4	1.9	0.5
	1	2.8	0.6	2.4	0.6
	2	2.7	0.7	2.2	0.6
	3	2.3	0.8	2.2	0.6
pH	0[a]	6.3**	0.2	5.9	0.2
	½	6.0	0.2	5.8	0.2
	1	5.9	0.2	5.8	0.2
Sarcomere length (μ)	0[a]	0.9	0.1	1.1	0.2
	1	1.0**	0.1	1.6	0.3
	2	1.1**	0.3	1.7	0.3
	3	1.5	0.4	1.8	0.1
	4	1.7	0.2	–	–
	5	1.8	0.1	–	–

[a] 5 min postmortem
* $P < 0.05$
** $P < 0.01$

Table 3—Effects of struggling on response to electrical stimulation, pH decline rate, protein solubility[a] and tenderness of tom turkey pectoralis muscle (experiment 2)

Muscle parameters	Restrained (N=8) Mean	Restrained (N=8) S.D.	Struggled (N=8) Mean	Struggled (N=8) S.D.
Threshold (volts)	4	5	29	42
Contractility duration (sec)	251*	79	150	116
pH 0 min	6.6**	0.2	6.2	0.2
pH 15 min	6.4**	0.3	6.1	0.2
pH 30 min	6.3*	0.3	6.0	0.1
Sarcoplasmic protein, 0 hr	30.1	1.5	29.8	2.1
Sarcoplasmic protein, 24 hr	27.3	5.4	27.9	2.6
Myofibrillar protein, 0 hr	51.7	7.8	52.4	4.9
Myofibrillar protein, 24 hr	40.8	2.3	41.8	7.0
Thawing loss, %	0.9	2.5	0.9	1.1
Cooking loss, %	29.3	2.3	29.2	2.3
Shear value (kg)	4.7	1.4	5.7	1.8

[a] % soluble protein of total protein
* $P < 0.05$
** $P < 0.01$

Table 4—Effects of electrical stunning and struggling on response to electrical stimulation, protein solubility[a] and tenderness of young tom turkey pectoralis muscle (experiment 3)

Muscle parameters	Elec. stunned (N = 5)		Struggled (N=5)	
	Mean	S.D.	Mean	S.D.
Rigor completion (min)	318*	92	143	73
Threshold (volts)	2*	2	24	20
Contractility duration (sec)	338*	133	140	39
Sarcoplasmic protein, 0 hrb	18.9	0.6	18.7	1.1
Sarcoplasmic protein, 24 hr	16.8	1.4	16.2	1.6
Myofibrillar protein, 0 hrb	22.5	4.3	20.1	5.5
Myofibrillar protein, 24 hr	15.6	3.6	14.2	3.3
Thawing loss, %	0.2*	0.2	0.5	0.3
Cooking loss, %	31.6	3.8	34.7	4.6
Shear value (kg)	6.7	1.2	7.4	1.9

[a] % soluble protein of total protein
[b] 5 min postmortem
* $P < 0.05$

< 0.01) was calculated between sarcomere length (24 hr) and shear. In contrast, the correlation of these two parameters was low and not significant ($P > 0.05$) in experiment 2. Howard and Judge (1968) noted that sarcomere length was related to tenderness of bovine longissimus dorsi muscle as determined on the medial but not the lateral portion of that muscle. Welbourn et al. (1968) reported that no significant correlation between sarcomere length and shear value occurred in pectoralis muscle. In their study, measurements of sarcomere length took place at 3 hr postmortem at which time some muscles may still be in a prerigor state. Hegarty and Hooper (1971) and Ma et al. (1971) reported that the use of prerigor muscle could lead to misleading results when measuring fiber diameter and sarcomere length due to contraction of samples during homogenization.

In general, the results of this study are in agreement with those reported by Khan and Nakamura (1970) and suggest that an effective method for electric stunning may be one possibility for improving turkey pectoralis muscle tenderness. Khan and Nakamura (1972) reported that high pH meat retained higher protein solubility and sulfhydryl content and experienced less protein degradation during a 40-wk storage at −10°C than low pH meat. It is possible that similar differences exist between fast- and slow-glycolysis pectoralis muscle.

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EFFECTS OF INTRAMUSCULAR COLLAGEN AND ELASTIN ON BOVINE MUSCLE TENDERNESS

INTRODUCTION

CONSIDERABLE RESEARCH has been reported concerning the factors related to beef tenderness (Wilson et al., 1954; Herring et al., 1967; Goll et al., 1963; Kauffman et al., 1964; McClain et al., 1965; Adams et al., 1960; Smith and Carpenter, 1970; Cross et al., 1972; Shimokomaki et al., 1972 and Pfeiffer et al., 1972). Presently identified relationships are only able to account for 30–40% of the observed variation in meat tenderness. Collagen has been extensively investigated, both with regard to its physical and chemical properties and to its relationship to tenderness (Hill, 1966; Goll et al., 1964a, b; Parrish et al., 1962; Kruggel et al., 1970; Kruggel and Field, 1971 and Shimokomaki et al., 1972). Age-associated changes in collagen have been reported (Hill, 1966; Herring et al., 1967; Kauffman et al., 1964; Goll et al., 1963; 1964a; Tuma et al., 1963 and Shimokomaki et al., 1972). Elastin and its role in meat tenderness has been less extensively researched; perhaps because it forms a lesser proportion of the total connective tissue than collagen and because of the inherent difficulty in studying a protein which is characteristically insoluble during heating. The objectives of this study were: (a) to determine the singular and combined effects of elastin and collagen on the tenderness of bovine muscle tissue; (b) to document age-associated changes in bovine muscle connective tissue; and (c) to determine the relative concentrations of elastin and collagen in various bovine muscles.

EXPERIMENTAL

15 HEREFORD FEMALES in three chronological age groups (Group I = 305–642 days; Group II = 1382–1396 days; and Group III = 3635–5096 days) were used in this study. Following a 7-day storage period in a 2°C cooler, the biceps femoris (BF), semimembranosus (SM), rectus femoris (RF), semitendinosus (ST) and longissimus dorsi (LD) muscles were removed from the left side of each carcass. Individual muscles were frozen at −34°C and stored in a −23°C freezer. Four steaks (2.54 cm thick) were removed from the center of each muscle. The two center steaks (5.1 cm × 7.6 cm) were used for sensory panel and shear force determinations. Steaks for sensory panel evaluation were cooked in a 175°C electric oven for 50 min to an internal temperature of 72°C. A trained 6-member panel evaluated three slices (6 mm thick) from each steak for juiciness and tenderness by use of an 8-point rating scale (8 = extremely juicy or tender and 1 = extremely dry or tough). Juiciness evaluations were separated into (a) fluid release during the first 5–10 chews (initial juiciness) and (b) fluid release during the last 5–10 chews (sustained juiciness). Tenderness determinations consisted of (a) the initial impression of tenderness or toughness during the first 5 to 10 chews, (b) the toughness or tenderness of the muscle fiber component and (c) the amount of connective tissue residue remaining at the end of mastication. Warner-Bratzler shear values were obtained on five cores (1.27 cm in diameter) from each muscle.

Proximate analysis

Muscle samples were frozen in liquid nitrogen and powdered in a high speed blender. Moisture was determined as the weight loss of duplicate 3-g samples after drying for 24 hr at 102°C. Fat content was determined as the weight loss of the dried samples after 8 hr of extraction with diethyl ether.

Hydroxyproline analysis

Frozen powdered samples (4g) were heated for 70 min at 77°C in one-fourth strength Ringer's solution and separated into supernatant and residue fractions following the procedure of Hill (1966). Each fraction was individually hydrolyzed in 6N HCl for 6 hr at 1 atm pressure and 102°C. The hydroxyproline content was determined as outlined by Woessner (1961). Total collagen content (mg/g) was computed according to three methods: (a) multiplying the combined hydroxyproline contents of the supernatant plus residue fractions by 7.25 (Goll et al., 1963); (b) subtracting the hydroxyproline content actually contributed by elastin from the hydroxyproline content of the residue and multiplying the corrected hydroxyproline content of residue and supernatant fractions by 7.52; (c) multiplying the hydroxyproline content of the residue by 7.25 and that of the supernatant by 7.52.

Elastin analysis

Elastin was isolated from each of the five muscles using the procedure outlined by Cross et al. (1973). After removal of the myofibrillar and sarcoplasmic proteins and the lipid portion of the sample, the residue was selectively hydrolyzed to remove the collagen portion of the connective tissue fraction.

Statistical analysis

Data were reduced using regression analysis and analysis of variance as outlined by Snedecor and Cochran (1967) and the mean separation technique of Duncan (1955). Since age group X muscle interactions were not significant, data for various muscles within age groups and for various age groups within muscles were combined.

RESULTS & DISCUSSION

SIMPLE CORRELATION coefficients for sensory panel ratings, shear force value and chronological age are presented in Table 1. With the exception of ratings for amount of connective tissue, sensory panel ratings were closely interrelated and probably mutually dependent. Ratings for amount of connective tissue were significantly ($P < 0.01$) related to initial tenderness in all age groups but were associated ($P < 0.01$) with muscle fiber tenderness only for the combined population. Ratings for initial tenderness are related to the tenderness effects of both the muscle fiber (actomyosin effect) and connective tissue (background effect) components. Shear force values were significantly correlated with ratings for initial tenderness (3 of 4 data groups), muscle fiber tenderness (3 of 4 data groups) and amount of connective tissue (age group II). The latter findings and those of Cover et al. (1962) and Bouton and Harris (1972) suggest that shear force values are more closely related to muscle fiber properties than to connective tissue components in cooked muscle. Actual age of the animal from which samples were obtained was significantly correlated with all five of the sensory panel ratings and with shear force value for the combined age group. Increases in chronological age over the range included in the combined age group were associated with increases in juiciness (initial and sustained) and decreases in tenderness (initial, muscle fiber, amount of connective tissue and resistance to shear).

Simple correlation coefficients between certain chemical components and sensory panel ratings for juiciness are

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Table 1—Simple correlation coefficients for sensory panel ratings, shear force value and actual chronological age

Evaluation	Age[c] group	Sensory panel rating: Sustained[a] juiciness	Initial[a] tenderness	Muscle[a] fiber tenderness	Amount[b] of connective tissue	Shear force value	Actual age (days)
Initial juiciness rating	I	0.94**	0.43*	0.45*	−.38*	−.10	0.11
	II	0.92**	0.54**	0.37	0.15	−.29	0.16
	III	0.94**	0.45*	0.60**	−.12	−.22	−.26
	Combined	0.94**	0.36**	0.38**	−.18	−.09	0.26*
Sustained juiciness rating	I		0.40*	0.38*	−.36	−.15	0.06
	II		0.54**	0.35	0.19	−.10	0.10
	III		0.51**	0.65**	−.09	−.28	−.09
	Combined		0.35**	0.33**	−.16	−.05	0.34**
Initial tenderness rating	I			0.96**	0.43*	−.56**	0.30
	II			0.71**	0.71**	−.13	0.07
	III			0.90**	0.64**	−.49**	−.14
	Combined			0.88**	0.63**	−.47**	−.24*
Muscle fiber tenderness rating	I				0.34	−.44*	0.31
	II				0.23	−.32	−.14
	III				0.34	−.56**	−.22
	Combined				0.35**	−.49**	−.24*
Amount of connective tissue rating	I					−.36	0.01
	II					0.39*	−.04
	III					−.16	−.07
	Combined					−.17	−.32*
Shear force value (kg)	I						−.13
	II						−.43*
	III						0.23
	Combined						0.35**

[a] Means based on an 8-point scale (8 = extremely tender or juicy; 1 = extremely tough or dry)
[b] Means based on an 8-point scale (8 = no connective tissue; 1 = abundant amount)
[c] Mean chronological ages for the three groups were as follows: age group I = 483.4 days; age group II = 1376.6 days; and age group III = 4087.6 days. Combined = age groups I, II and III. n = 25 for each age group and n = 75 for combined age groups.
** $P<0.01$
* $P<0.05$

Table 2—Simple correlation coefficients between certain chemical components and measures of tenderness for the combined age groups[c]

Measurement[d]	Initial juiciness[a]	Sustained juiciness[a]	Initial tenderness[a]	Muscle fiber tenderness[a]	Amount of connective tissue rating[b]	Shear force value (kg)	Actual age (days)[c]
Fat, WTB (%)	0.15	0.07	−.02	0.01	−.01	0.08	0.05
Fat, MFB (%)	0.13	0.05	0.01	0.05	0.02	0.06	0.01
Moisture (%)	−.28*	−.24*	0.17	0.16	0.23	−.10	−.32*
Collagen, WTB, unadj. (mg/g)	0.18	0.12	−.05	0.03	−.20	0.07	0.08
Collagen, MFFB, unadj. (mg/g)	0.15	0.09	−.03	0.05	−.15	0.08	0.04
Soluble collagen, unadj. (%)	−.18	−.17	0.02	−.08	0.31*	−.17	−.57**
Collagen, WTB, adj. (mg/g)	0.19	0.12	−.04	0.03	−.20	0.06	0.06
Collagen, MFFB, adj. (mg/g)	0.16	0.10	−.02	0.05	−.15	0.07	0.02
Soluble collagen, adj. (%)	−.19	−.18	0.01	0.08	0.31*	−.16	−.57**
Hydroxyproline, total (mg/g)	0.18	0.12	−.05	−.03	−.21	0.07	0.08
Elastin, WTB (mg/g)	−.03	−.01	−.10	−.03	−.10	0.13	0.19
Elastin, MFFB (mg/g)	−.06	−.03	−.08	−.03	−.08	0.12	0.14
Collagen/elastin ratio	0.18	0.11	0.13	0.11	−.03	−.05	−.08

[a] Means based on an 8-point scale (8 = extremely tender or juicy; 1 = extremely tough or dry)
[b] Means based on an 8-point scale (8 = no connective tissue; 1 = abundant amount)
[c] Mean chronological ages for the three groups were as follows: age group I = 483.4 days; age group II = 1376.6 days; and age group III = 4087.6 days. Combined = age groups I, II and III. n = 75.
[d] Abbreviations were as follows: WTB = whole tissue basis, MFB = moisture free basis, MFFB = moisture and fat free basis, unadj. = hydroxyproline times 7.25 and adj. = hydroxyproline from the residue fraction minus hydroxyproline from elastin times 7.52.
** $P < 0.01$
* $P < 0.05$

Table 3—Mean values for sensory panel ratings, shear force values, percentages of fat and moisture and connective tissue components for muscles from three chronological age groups

	Chronological age groups[a]					
	I		II		III	
Component	Mean	S.D.	Mean	S.D.	Mean	S.D.
Initial juiciness rating[b]	5.12[e]	1.16	4.88[e]	0.94	5.77[f]	1.16
Sustained juiciness rating[b]	4.83[e]	1.04	4.63[e]	0.95	5.59[f]	0.92
Initial tenderness rating[b]	5.50[e]	1.00	5.24[ef]	0.92	4.87[f]	1.22
Muscle fiber tenderness rating[b]	5.95[e]	0.95	5.79[ef]	0.81	5.44[f]	1.01
Shear force value, kg.	5.26[e]	1.50	5.95[ef]	1.28	6.56[f]	1.54
Amount of connective tissue rating[c]	5.46[e]	1.15	4.75[f]	1.28	4.29[f]	1.53
Fat, WTB (%)[d]	3.00[e]	1.10	2.55[e]	0.93	3.31[e]	2.00
Fat, MFB (%)[d]	12.73[e]	4.53	11.04[e]	4.00	13.36[f]	7.34
Moisture (%)	76.63[d]	1.12	76.91[e]	1.32	75.50[f]	1.58
Collagen, WTB, unadj. (mg/g)	7.32[e]	2.73	6.68[e]	1.91	7.58[e]	3.27
Collagen, MFFB, unadj. (mg/g)	35.97[e]	13.87	32.99[e]	10.80	36.50[e]	17.97
Soluble collagen, unadj. (%)	10.66[e]	6.31	3.45[f]	1.36	2.21[f]	0.67
Collagen, WTB, adj. (mg/g)	7.06[e]	2.80	6.42[e]	1.91	7.24[e]	3.37
Collagen, MFFB, adj. (mg/g)	34.84[e]	14.04	31.61[e]	10.76	34.88[e]	18.40
Soluble collagen, adj. (%)	11.44[e]	6.78	3.71[f]	1.39	2.43[f]	0.77
Hydroxyproline, total (μg/g)	1007.06[e]	376.44	921.70[e]	262.91	1041.72[e]	452.69
Elastin, WTB (mg/g)	4.41[e]	1.94	4.56[e]	2.82	5.26[e]	2.63
Elastin, MFFB (mg/g)	21.86[e]	9.92	22.55[e]	14.28	24.91[e]	12.42
Collagen/elastin ratio	2.12[e]	1.76	2.05[e]	1.66	1.86[e]	1.54

[a] Mean chronological ages for the three groups were as follows: age group I = 483.4 days; age group II = 1376.6 days; and age group III = 4087.6 days.
[b] Means based on an 8-point scale (8 = extremely tender or juicy; 1 = extremely tough or dry).
[c] Means based on an 8-point scale (8 = no connective tissue; 1 = abundant amount).
[d] Abbreviations were as follows: WTB = whole tissue basis, MFB = moisture free basis, MFFB = moisture and fat free basis, unadj. = hydroxyproline times 7.25 and adj. = hydroxyproline from the residue fraction minus hydroxyproline from elastin times 7.52.
[e,f] Mean values in the same horizontal row bearing different superscripts differ significantly ($P < 0.05$).

presented in Table 2. Increases in percent fat were not associated with significant changes in initial juiciness, sustained juiciness, initial tenderness or muscle fiber tenderness ratings. Percent moisture was negatively related to both initial and sustained juiciness ratings but was not significantly related to either of the tenderness scores. In the present study, none of the chemically determined connective tissue components were significantly associated with sensory panel evaluations of tenderness. That neither fat nor moisture would be related to tenderness ratings was not anticipated, since it is generally assumed that such relationships exist over wide ranges in maturity.

Ratings for amount of connective tissue were positively correlated with both unadjusted and adjusted percents of soluble collagen (Table 2). However, the relationships between measures of tenderness and chemically determined connective tissue components in the present study were lower than those reported by Herring et al. (1967) and Smith and Carpenter (1970). Chemical measures of connective tissue were more closely associated with ratings for amount of connective tissue than with ratings for muscle fiber tenderness. Adjustments of values for total collagen and percent soluble collagen resulted in slightly higher correlations with tenderness, but little advantage was observed by adjusting for the content of elastin in the residue fraction.

Mean values for sensory panel ratings, shear force values and certain chemical parameters are characterized according to differences in age in Table 3. Initial and sustained juiciness ratings, initial and muscle fiber tenderness ratings, shear force values, amount of connective tissue ratings and percentages of fat (MFB) and moisture differed significantly ($P < 0.05$) among age groups. Samples from animals in age group III were rated higher in initial and sustained juiciness but were less tender (initial and muscle fiber ratings. shear force values) than samples from animals in age group I. Samples from age group II were intermediate in tenderness to those from samples in age groups I and III. Fat content (WTB) did not differ significantly among age groups, whereas fat content (MFB) was significantly higher in samples from age group III. Moisture was significantly lower ($P < 0.05$) in age group III when compared to age groups I and II. Samples from age group III generally differed in most traits from those in age group I and occasionally from those in age group II which is attributed to the age differentials involved (1 yr vs. 4 yr vs. 10 yr).

Contents of collagen, elastin and

Table 4—Mean separation analysis for certain traits of individual bovine muscles

	Semimembranosus		Semitendinosus		Biceps femoris		Rectus femoris		Longissimus dorsi	
Component	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Initial juiciness rating[a]	4.78[d]	0.96	4.93[e]	0.98	6.26[f]	1.14	5.58[e]	1.04	4.73[d]	0.90
Sustained juiciness rating[a]	4.59[d]	0.91	4.70[d]	1.04	5.88[e]	1.01	5.31[e]	0.84	4.60[d]	0.85
Initial tenderness rating	4.54[f]	1.25	5.39[de]	0.85	5.29[de]	0.88	5.97[d]	0.76	4.82[ef]	1.06
Muscle fiber tenderness rating[a]	5.29[ef]	1.06	5.87[de]	0.57	6.21[d]	0.59	6.24[d]	0.85	5.01[f]	0.93
Shear force value, kg.	5.70[d]	1.28	5.79[d]	0.96	5.81[d]	1.62	5.37[d]	1.52	6.92[e]	1.81
Amount of connective tissue rating[b]	4.11[f]	1.31	5.15[e]	1.04	3.42[g]	0.99	6.03[d]	0.96	5.44[de]	0.95
Fat, WTB (%)[c]	2.09[d]	0.82	3.08[de]	1.31	3.05[de]	1.14	3.26[e]	1.69	3.28[e]	1.82
Fat, MFB (%)[c]	8.96[d]	3.41	13.03[de]	5.33	12.72[de]	4.57	13.86[e]	6.37	13.30[e]	6.51
Moisture (%)	76.83[e]	1.34	76.39[de]	0.74	76.08[de]	1.50	76.81[e]	1.99	75.62[d]	1.32

[a] Means based on an 8-point scale (8 = extremely tender or juicy; 1 = extremely tough or dry)
[b] Means based on an 8-point scale (8 = no connective tissue; 1 = abundant amount)
[c] Abbreviations were as follows: WTB = whole tissue basis, MFB = moisture free basis, MFFB = moisture and fat free basis, unadj. = hydroxyproline times 7.25 and adj. = hydroxyproline from the residue fraction minus hydroxyproline from elastin times 7.52.
[d,e,f,g] Means in the same horizontal row with different superscripts are significantly different ($P < 0.05$).

hydroxyproline and collagen/elastin ratios were not significantly different among the three age groups (Table 3). The latter finding is in general agreement with Cross et al. (1972) and Wilson et al. (1954). Samples from age group I (Table 3) had significantly ($P < 0.05$) higher percents of soluble collagen than samples from age groups II and III. Numerous researchers have shown that as age increases, percent soluble collagen decreases (Hill, 1966; Herring et al., 1967; Smith and Carpenter, 1970; Cross et al., 1972).

Differences in chemical and palatability traits among individual muscles are presented in Tables 4 and 5. The BF muscles were assigned the highest numerical ratings for initial and sustained juiciness, but did not differ in either fat or moisture percentages from the other muscles. The RF muscles were significantly more juicy than the LD muscles, yet the two groups of muscles did not differ in fat or moisture percentages.

The LD muscles had among the lowest values for amount of connective tissue, total collagen, hydroxyproline and elastin content; and had the highest percentage of soluble collagen (Table 5), all of which would have been expected to contribute to greater tenderness. The LD muscles were rated low in tenderness by all subjective panel scores and by the shear force values (Table 4); thus connective tissue contributed little to the observed toughness of these LD muscles. The ST muscles were intermediate in total collagen and low in soluble collagen which agrees with the sensory panel evaluations of tenderness, but were high in elastin content, which does not reflect the observed differences in tenderness (Table 4). The RF muscles were intermediate in actual percent of soluble collagen, in total collagen and in elastin content, yet the RF muscle was considered high in tenderness. It is apparent that tenderness ratings and resistance to shear depended more upon muscle fiber properties than upon connective tissue components. Cross et al. (1972) found that relationships between chemical or histological traits and tenderness differed among anatomically adjacent muscles and that attempts to identify those parameters operative in determining tenderness differences using combinations of muscles obscure the factors of greatest importance for specific muscles.

Mean values for sensory panel ratings for individual muscles from animals in each age group are presented in Table 6. Initial and sustained juiciness ratings were highest for the BF muscles in age groups I and III, but no significant difference was evident among muscles in age group II. The BF muscles had the greatest amount of subjectively determined connective tissue in age group I and among the greatest amounts in age groups II and III, but these differences were not reflected in either initial tenderness ratings or shear force values. The LD muscles were among the lowest in numerical values for initial and muscle fiber tenderness ratings in all age groups, but few of these differences

Table 5–Mean values for connective tissue components for individual bovine muscles

Component[a]	Semimembranosus		Semitendinosus		Biceps femoris		Rectus femoris		Longissimus dorsi	
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Collagen, WTB, unadj. (mg/g)	5.64^{d}	1.71	7.55^{cd}	2.35	8.69^{b}	2.36	7.99^{bc}	3.50	6.11^{cd}	2.19
Collagen, MFFB, unadj. (mg/g)	26.70^{b}	8.35	36.89^{bc}	11.50	42.24^{c}	13.49	40.64^{c}	19.57	29.31^{b}	11.27
Soluble collagen, unadj. (%)	4.31^{b}	3.07	3.81^{b}	2.84	4.68^{b}	3.70	5.49^{b}	4.25	8.91^{c}	8.90
Collagen, WTB, adj. (mg/g)	5.25^{d}	1.82	7.01^{cd}	2.42	8.50^{b}	2.35	7.81^{bc}	3.56	5.97^{cd}	2.21
Collagen, MFFB, adj. (mg/g)	24.99^{b}	8.99	34.26^{bc}	11.74	41.27^{c}	13.33	39.81^{c}	19.82	28.56^{b}	11.36
Soluble collagen, adj. (%)	4.91^{b}	3.65	4.19^{b}	3.09	4.91^{b}	3.87	5.83^{b}	4.55	9.45^{c}	9.53
Hydroxyproline, total (μg/g)	771.64^{d}	230.55	1039.89^{cd}	324.22	1197.59^{b}	325.98	1100.98^{bc}	482.84	840.71^{cd}	302.80
Elastin, WTB (mg/g)	4.94^{c}	2.24	7.15^{b}	2.65	4.51^{cd}	2.42	4.19^{cd}	1.56	2.93^{cd}	1.50
Elastin, MFFB (mg/g)	23.72^{b}	10.94	34.85^{d}	12.80	21.95^{bc}	12.37	21.13^{bc}	7.66	13.88^{c}	7.21
Collagen/elastin ratio	1.44^{b}	1.07	1.12^{b}	0.55	2.57^{b}	1.99	2.26^{b}	1.62	2.67^{b}	2.02

[a] Abbreviations were as follows: WTB = whole tissue basis, MFB = moisture free basis, MFFB = moisture and fat free basis, unadj. = hydroxyproline times 7.25 and adj. = hydroxyproline from the residue fraction minus hydroxyproline from elastin times 7.52.
[b,c,d] Means on the same horizontal row with different superscripts are significantly different ($P < 0.05$).

Table 6–Mean values for sensory panel ratings and shear force values for individual muscles from animals in each age group

Muscle	Age group I	Age group II	Age group III	Age group I	Age group II	Age group III	Age group I	Age group II	Age group III
	Initial juiciness rating[a]			Sustained juiciness rating[a]			Amount of Connective tissue rating		
Semimembranosus	4.3^{b}	4.8^{b}	5.2^{b}	4.1^{b}	4.5^{b}	5.2^{b}	4.9^{b}	4.4^{bc}	2.9^{b}
Semitendinosus	4.6^{b}	4.5^{b}	5.7^{c}	4.4^{b}	4.1^{b}	5.6^{bc}	6.0^{c}	4.1^{b}	5.4^{cd}
Biceps femoris	6.8^{c}	5.0^{b}	6.9^{c}	6.2^{c}	4.9^{b}	6.5^{c}	3.8^{d}	3.8^{b}	2.6^{b}
Rectus femoris	5.3^{b}	5.4^{b}	6.0^{bc}	4.9^{b}	5.2^{b}	5.7^{bc}	6.6^{c}	5.6^{cd}	5.9^{c}
Longissimus dorsi	4.5^{b}	4.7^{b}	4.9^{b}	4.4^{b}	4.5^{b}	4.9^{b}	5.8^{bc}	5.9^{d}	4.6^{d}
	Initial tenderness rating[a]			Muscle fiber tenderness[a]			Shear force value (kg)		
Semimembranosus	4.9^{c}	4.9^{b}	3.9^{c}	5.5^{c}	5.6^{b}	4.9^{bc}	5.2^{b}	5.7^{b}	6.3^{b}
Semitendinosus	5.8^{bc}	4.8^{b}	5.6^{b}	6.2^{cd}	5.6^{b}	5.8^{b}	5.5^{b}	5.7^{b}	6.1^{b}
Biceps femoris	5.7^{bc}	5.3^{b}	4.9^{bc}	6.3^{d}	6.3^{b}	5.9^{b}	6.0^{b}	5.4^{b}	6.0^{b}
Rectus femoris	6.4^{b}	5.7^{b}	5.8^{b}	6.7^{d}	6.0^{b}	5.9^{b}	3.9^{b}	6.2^{b}	6.0^{b}
Longissimus dorsi	4.8^{c}	5.5^{b}	4.1^{c}	5.1^{b}	5.4^{b}	4.5^{c}	5.7^{b}	6.7^{b}	8.4^{c}

[a] Means based on an 8-point scale (8 = extremely tender or juicy; 1 = extremely tough or dry)
[b,c,d] Means in the same vertical column with different superscripts are significantly different ($P < 0.05$).

Table 7—Mean values for certain chemical components for individual muscles from animals in each age group

Muscle	Age group I	II	III	Age group I	II	III	Age group I	II	III
	Fat, WTB (%)[a]			Moisture (%)			Collagen, WTB, adj. (mg/g)[a]		
Semimembranosus	1.8[c]	2.4[b]	2.1[b]	77.4[c]	77.2[bc]	75.9[b]	4.9[b]	5.8[b]	4.9[b]
Semitendinosus	3.4[b]	3.2[b]	2.6[b]	76.5[bc]	76.5[bc]	76.1[b]	8.6[c]	6.4[b]	6.0[bc]
Biceps femoris	3.6[b]	2.2[b]	3.4[b]	75.6[b]	77.6[b]	75.0[b]	8.9[c]	7.5[b]	9.0[bc]
Rectus femoris	3.1[b]	2.5[b]	4.2[b]	77.1[bc]	77.5[bc]	75.9[b]	7.3[bc]	6.5[b]	9.6[c]
Longissimus dorsi	3.1[b]	2.5[b]	4.3[b]	76.5[bc]	75.8[c]	74.6[b]	5.5[bc]	5.8[b]	6.6[c]
	Collagen, MFFB, adj. (mg/g)[a]			Soluble collagen, adj. (%)[a]			Hydroxyproline (μg/g)		
Semimembranosus	23.8[b]	28.4[b]	22.7[b]	8.9[b]	3.3[b]	2.5[b]	738.2[b]	839.4[b]	737.3[c]
Semitendinosus	42.5[c]	31.8[b]	28.5[bc]	7.9[b]	2.6[b]	2.1[b]	1234.2[c]	971.9[b]	913.6[b]
Biceps femoris	43.9[c]	38.1[b]	41.8[bc]	9.4[b]	3.4[b]	1.9[b]	1249.8[c]	1068.5[b]	1274.5[b]
Rectus femoris	36.9[bc]	32.9[b]	49.9[c]	11.0[b]	3.7[b]	2.8[b]	1033.2[bc]	924.2[b]	1345.5[b]
Longissimus dorsi	27.2[bc]	26.8[b]	31.8[bc]	19.9[c]	5.7[c]	2.8[b]	799.9[bc]	804.5[b]	937.7[b]
	Elastin, WTB (mg/g)[a]			Elastin, MFFB (mg/g)[a]			Collagen/elastin Ratio		
Semimembranosus	5.4[bc]	4.4[b]	5.1[b]	25.9[bc]	22.1[bc]	23.2[b]	0.9[b]	1.9[b]	1.4[b]
Semitendinosus	6.2[b]	7.7[b]	7.5[b]	31.3[b]	37.8[b]	35.5[b]	1.4[b]	1.1[b]	0.9[b]
Biceps femoris	3.9[cd]	4.6[b]	4.9[b]	19.4[cd]	23.5[bc]	22.9[b]	3.4[b]	1.7[b]	2.6[b]
Rectus femoris	3.8[cd]	3.9[b]	4.8[b]	19.9[cd]	19.4[c]	24.1[b]	2.3[b]	1.7[b]	2.8[b]
Longissimus dorsi	2.6[d]	2.2[b]	3.9[b]	12.8[d]	9.9[c]	18.9[b]	2.6[b]	3.8[b]	1.7[b]

[a] Abbreviations were as follows: WTB = whole tissue basis, MFFB = moisture and fat free basis, unadj. = hydroxyproline times 7.25 and adj. = hydroxyproline from the residue fraction minus hydroxyproline from elastin times 7.52.
[b,c,d] Means in the same vertical column with different superscripts are significantly different ($P < 0.05$).

were consistent enough for statistical significance. There were no significant differences in shear force values among muscles in age groups I and II while in age group III the LD muscles had significantly higher ($P < 0.05$) shear force values than the RF, BF, SM or ST muscles.

Total collagen and percent soluble collagen (Table 7) differed among muscles but the observed variability was not consistent among age groups. Collagen content did not differ among muscles in age group II, but in age group III, RF and BF muscles were considerably higher in total collagen (WTB) than the SM muscles. The LD muscles were significantly higher ($P < 0.05$) in the adjusted percent of soluble collagen than all other muscles in age groups I and II. The differences among muscles in percent soluble collagen and total collagen (WTB) were greater in the younger age groups. Since adjusted percentages of soluble collagen did not alter the muscle to muscle comparative order from that achieved using unadjusted values (Table 7), there seems to be little advantage for making an adjustment for the amount of hydroxyproline contributed by elastin to the residue fraction.

The elastin content (WTB) of the LD muscles in age group I was significantly lower ($P < 0.05$) than that for the SM and ST muscles, but it was generally observed that the ST muscles had the highest elastin content across all age groups. The ratio of collagen to elastin did not differ significantly among the muscles in any of the age groups. In view of the values reported in this study, the assumed collagen to elastin ratio of 3:1 seems rather high.

Regression equations for predicting tenderness ratings, amount of connective tissue ratings and shear force values are presented in Table 8. The simultaneous consideration of variation in all of the chemical and histological traits studied (data not presented in tabular form) accounted for only 19.5, 26.1, 26.1 and 15.7% of the variation in initial tenderness rating, muscle fiber tenderness rating, amount of connective tissue rating and shear force value, respectively. Consideration of three chemically determined connective tissue components (concentration of collagen and elastin and percent soluble collagen) accounted for 0.02, 0.02, 12.45 and 0.04%, respectively, of the variation in these same tenderness attributes (Table 8). In all of the equations, percent soluble collagen was of greatest comparative importance (among the independent variables) in accounting for variations in organoleptic or mechanical measures of tenderness, but soluble collagen content was significant ($P < 0.05$) in its effect only in the equation for predicting amount of connective tissue ratings which agrees with Smith and Carpenter (1970). These data confirm that knowledge of chemical parameters does not explain a large portion of the variation in muscle tenderness.

In conclusion, connective tissue is a

Table 8—Regression equations for predicting tenderness ratings, amount of connective tissue rating and shear force value for combined age groups and muscles

Independent variables[c]	Standard partial regression coefficients: Initial tenderness rating[a]	Muscle fiber tenderness rating[a]	Amount of connective tissue rating[b]	Shear force value
Collagen, WTB, adj. (mg/g)	0.04	0.03	−.15	0.04
Soluble collagen, adj. (%)	−.08	−.09	0.28*	−.14
Elastin, WTB (mg/g)	−.03	−.03	−.04	−.10
C.D. ($R^2 \times 100$)	0.02	0.02	12.45	0.04

[a] Means based on an 8-point scale (8 = extremely tender or juicy; 1 = extremely tough or dry)
[b] Means based on an 8-point scale (8 = no connective tissue; 1 = abundant amount)
[c] Abbreviations were as follows: WTB = whole tissue basis, MFB = moisture free basis, MFFB = moisture and fat free basis, unadj. = hydroxyproline times 7.25 and adj. = hydroxyproline from the residue fraction minus hydroxyproline from elastin times 7.52.
* $P < 0.05$

contributing factor to toughness of meat. Total concentrations of connective tissue components (collagen and elastin) were not closely related to ratings for muscle fiber tenderness or amount of connective tissue. However, percent soluble collagen was significantly related to the contribution of connective tissue to toughness, as assessed by the sensory panel. Tenderness differs among muscles from various anatomical locations because of the variation in the traits or factors responsible for tenderness (e.g., myofibrillar or connective tissue). Elastin concentration was not consistently related to variations in tenderness of bovine muscles.

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VIABILITY OF Staphylococcus aureus IN INTERMEDIATE MOISTURE MEATS

INTRODUCTION

TYPICAL intermediate moisture foods (IMF) have water activities from 0.6–0.85 and moisture contents from 20–50% on a dry solid basis, although in some cases the moisture content may be higher, depending on the method of preparation and the quantity of humectants added.

IMF exhibit hysteresis phenomena in their water sorption isotherms (Labuza, 1968). Two different isotherms are obtained depending upon the direction of the water transfer (desorption or adsorption). In order to determine the differences, if any, between samples of the same water activity but different moisture contents, the viability of *Staphylococcus aureus* in intermediate moisture meats prepared by desorption and adsorption methods was investigated.

In the present study, the bacteriostatic and bactericidal effects of glycerol, 1,2-propanediol and 1,3-butanediol on *S. aureus* were investigated in intermediate moisture meats and laboratory culture media.

MATERIALS & METHODS

Food systems

Strained chicken (Gerber brand baby food) and ½–1 in. cubes of commercial lean pork loin were used to prepare IMF systems with three different humectants: glycerol (certified A.C.S., Fisher Scientific Corp.), 1,2-propanediol (laboratory grade, Fisher Scientific Corp.) and 1,3-butanediol (high purity, Celanese Chemical Co.). The direct-mix technique (Labuza et al., 1972a) was used to prepare IM-strained chicken and the equilibrating solution technique (Labuza et al., 1972a) was used for IM-pork dices.

The strained chicken was thoroughly mixed with the desired amounts of humectant in a stainless steel chamber of an Omni-Mixer homogenizer (Sorvel, Inc.). These constituted the desorption samples. Approximately 10g were placed into 3 oz screw cap jars, weighed and stored at 25°C. To prepare the adsorption samples, one-half of the desorption samples were frozen in open 3 oz screw cap jars, freeze dried for 24 hr at 0.1 mm Hg at 77°F in a Virtis Laboratory Freeze Dryer, Model 10-MR-TR and placed into desiccators equilibrated at various water activities corresponding to that in the desorption samples with saturated salt solutions as shown in Table 1. When equilibrium (constant weight) was reached after 3–7 days, the jars were removed from the desiccators, closed, weighed and stored together with the desorption samples at 25°C. Several samples were separated for moisture content and a_w determinations and the rest were inoculated with the bacterial suspension as explained below. The initial pH of all samples was constant at 6.4.

Desorption IM-pork dices were prepared by immersing portions of about 10 cubes (½–1 in. in size and approximately 10g each) of raw pork loin into infusion solutions in 500 ml Erlenmeyer flasks. The equilibrating solutions contained 2.5% NaCl, distilled water and humectants in different proportions, whereas the meat to infusion solution weight ratio was kept constant at 1:3.

After heating the immersed samples at 96°C for 15 min and soaking for 24 hr at 4°C, the meat dices were removed, drained, gently dried with sterile cheesecloth, placed into 3 oz screw cap jars, weighed and stored at 25°C. In the first run of these experiments it was observed that a relatively low rate of diffusion of humectant(s) into the samples was obtained at 4°C; therefore, in the subsequent runs, the samples were soaked in a less concentrated solution at 25°C for 24 hr. Half of these samples were used to prepare the adsorption samples by freeze drying and rehumidification in evacuated desiccators with saturated salt solutions (Table 3).

Total moisture and water activity measurements

The moisture content of all IMF samples was determined by the vacuum oven method at 29 in. of Hg and 60°C for 24 hr.

Table 1—Parameters of IM-strained chicken IM-systems (Humectant: glycerol)

Added glycerol (% wt)	Water activity @ 25°C	Moisture content (g H_2O/g solids)	pH
Desorption samples (Direct Mix)			
22.0	0.920	1.56	6.4
32.0	0.860	1.13	6.4
50.0	0.750	0.64	6.4

Sat. salt sol	Water activity @ 25°C	Moisture content (g H_2O/g solids)	Calc glycerol conc (% wt)	pH
Adsorption samples (Freeze dried/Rehumidified)				
KNO_3	.920	1.43	23.2	6.4
KCl	.860	1.03	33.6	6.4
NaCl	.750	0.57	52.3	6.4

Water activity was determined by Hygrosensor transducers (serial No. 553266, 549757 and 550500, Hygrodynamics, Inc.) attached to an electric hygrometer indicator (Model 15-3001, Hygrodynamics, Inc.). The sensors were inserted through the lid of 600 ml glass jars containing 20–30g of test sample. The jars were tightly closed and all measurements were carried out in a constant temperature room at 25(± 1)°C. The output of the hygrometer indicator was connected to a high speed response chart recorder (Model V.O.M.7. Bausch and Lomb) and the samples were allowed to equilibrate for at least 8 hr. The error of the water activity measurement was ± 0.5%. Sensors were recalibrated against saturated salt solutions prior to use.

Bacterial inoculation of food samples and humectant solutions

Staphylococcus aureus S-6 strain was used in this study. For each experiment 40 ml of Brain Heart Infusion (BHI) broth (Difco) in a 250 ml Erlenmeyer flask was inoculated from a stock culture slant and incubated for 16–20 hr at 37°C on a rotary shaker at 200 rev/min. The cells, from the stationary phase culture, were harvested from BHI broth medium by centrifugation at 5900 × G for 10 min, washed twice and resuspended in double-distilled water at 25°C. The washed cell suspension (about 5×10^9 cells/ml) was subsequently diluted to the appropriate concentration for inoculating IMF samples and BHI broth with added humectants. Except where noted, 0.1% peptone water (Difco) was used as diluent. All the growth media, diluent tubes and humectant solutions were heat sterilized prior to use by autoclaving at 121°C for 15 min.

The bacterial cells were suspended, prior to inoculation into the IMF sample, in a water-glycerol solution at the same water activity as the food sample for 1–2 hr at 25°C. This treatment had no bactericidal effect at the concentrations used.

IM-chicken samples were inoculated to a final concentration of 4.5×10^6 cells/g. The samples were carefully mixed with the inoculum using sterilized glass rods and stored at 25°C. IM-pork cubes were inoculated by pipetting 0.1 ml of the water-glycerol suspension of cells over the surface of the meat piece to a final concentration of $4–8 \times 10^5$ cells/g. The desorption and adsorption samples of each run were inoculated in the same way and at the same time.

The viability of *S. aureus* was also investigated in BHI broth with different combinations of humectants. In these test media, the dry BHI concentration was maintained constant at 37g BHI/1000 ml of solution and the pH of all test samples was adjusted to 7.1. The initial concentration of bacteria was $4–8 \times 10^5$ cells/ml and the inoculated solutions were incubated at

25°C on a rotary shaker at 200 rpm. At different intervals, 0.1 ml samples were taken for viable count determination.

Storage conditions and viable count determinations

IMF samples were stored in screw cap jars at 25°C. At different intervals (up to 2 months) samples were removed from storage for viable count determinations.

The food homogenates of IM-strained chicken samples were prepared by blending 1g of sample with 9 ml of 0.1% peptone water in sterilized plastic vials with glass beads and shaking by hand at ambient temperature. Homogenates of IM-pork dices were prepared by blending the meat samples (½–1 in. cubes) with 0.1% peptone water (1:9 weight ratio) in a 400 ml stainless steel chamber of an Omni-Mixer homogenizer (Sorval, Inc.) for 5 min at 400 rpm. Aliquots (0.1 ml) of appropriate serial dilutions were spread on the surface of duplicate BHI agar plates. After spreading, the plates were incubated aerobically at 37°C for 48 hr and the visible colonies were counted.

RESULTS

THE PARAMETERS of the IM-strained chicken system are summarized in Table 1. The IM-strained chicken samples (desorption and adsorption) were prepared at three different water activities (0.92, 0.86 and 0.75) in the intermediate moisture range. The glycerol concentration of the adsorption samples were calculated, assuming that no glycerol was lost during the freeze-drying process. It was observed that the contribution of strained chicken solids to the final a_w was very small and relatively high concentrations of glycerol were needed to sufficiently depress the a_w.

Table 2 shows the composition of the infusion solutions used to prepare the IM-pork dices by the equilibrating solution technique. Several combinations of humectants were used and the parameters of IM-pork systems are shown in Table 3. The total humectant concentration of these samples was estimated from the a_w lowering capacity of the different humectants and the pork solids. At all $/a_w$'s investigated, the adsorption samples had less moisture content than the corresponding desorption samples at the same a_w. This hysteresis phenomenon was greater at the lower $/a_w$'s and the adsorption and desorption curves became closer as the a_w was increased (Fig. 1).

The viability patterns of *S. aureus* during storage of the IM-strained chicken system with glycerol as water-binding agent are shown in Figure 2. At 0.92 a_w, the viable counts in desorption and adsorption samples increased during the first days and then remained stationary at about 10^8 cells/g; after 20 days a slow decrease in viability was observed. The increase in the viable fraction was confirmed to be due to *S. aureus*. At 0.86 and 0.75 a_w the viable counts decreased continuously with a higher death rate being observed at the lower a_w. Water activity remained constant during the storage period in all samples tested.

Results on the viability of *S. aureus* during storage of the IM-pork dices with glycerol as humectant are shown in Figure 3. At 0.915 a_w in desorption samples and 0.920 a_w in adsorption samples, the viable population increased during the first 30 days and then decreased slowly. At 0.88 a_w, in the desorption samples, the viable counts remained stationary for 15 days and then started to increase at a lower rate than in the 0.915 a_w samples.

Table 2—Equilibrating solutions for the preparation of IM-pork dices

Composition of equilibrating solution[a] (% wt)					
Water	Glycerol	1,2-Propanediol	1,3-Butanediol	NaCl	Equilibrating Temp°C[b]
48.75	48.75	–	–	2.5	4
24.4	73.1	–	–	2.5	4
–	97.5	–	–	2.5	4
58.5	39.0	–	–	2.5	25
58.5	–	–	39.0	2.5	25
58.5	–	39.0	–	2.5	25
39.0	58.5	–	–	2.5	25
39.0	45.5	–	13.0	2.5	25
39.0	45.5	13.0	–	2.5	25

[a] The weight ratio pork/infusion solution was kept constant at 1:3. In every run the fresh pork was distributed in portions of about 10 cubes each.

[b] The samples soaked in the infusion solution were heated for 15 min at 96°C and incubated for 24 hr at the equilibrating temperature.

Table 3—Parameters of IM-pork dices

	Desorption samples			Adsorption samples			
Humectant	Water activity (@ 25°C)	Moisture content (g H_2O/g solids)	Humectant (% wt)[a]	Sat salt soln	Water activity (@ 25°C)	Moisture content (g/H_2O/g solids)	Humectant (% wt)[a]
Glycerol	0.880	0.932	26.5	KCl	.860	0.772	28.9
Glycerol	0.805	0.710	36.8	$CdCl_2$	.820	0.675	37.6
Glycerol	0.730	0.640	51.7	NaCl	.750	0.585	53.5
Glycerol	0.915	1.130	20.1	KNO_3	.920	1.110	20.3
1,3-Butanediol	0.925	1.180	18.2	KNO_3	.920	1.060	19.3
1,2-Propanediol	0.915	1.080	20.2	KNO_3	.920	1.090	20.1
Glycerol	0.865	0.871	26.8	K_2CrO_4	.880	0.860	27.0
Glycerol-butanendiol ratio 3.5:1	0.875	0.903	28.1	K_2CrO_4	.880	0.845	29.0
Glycerol propanediol ratio 3.5:1	0.865	0.858	26.9	K_2CrO_4	.880	0.852	27.0

[a] Estimated from the water activity lowering capacity of the various humectants.

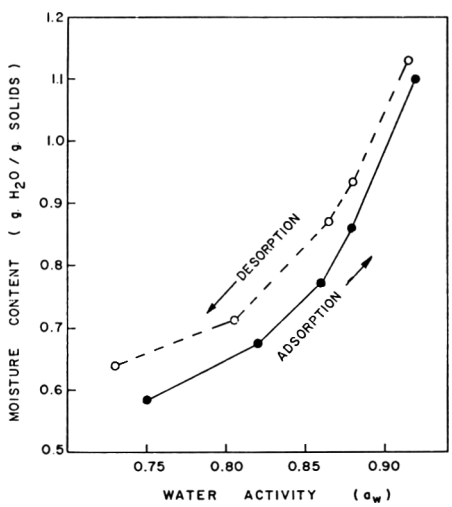

Fig. 1—Sorption isotherm of IM-pork cubes and glycerol at 25°C.

On the contrary, in the adsorption samples at the same a_w, the cells died slowly during the first 3 wk, after which, an increase in the death rate was observed. It is important that the opposite effects (growth vs. death) were observed in samples with the same a_w but with different moisture contents. The results indicate that the maximum a_w that inhibits growth of *S. aureus* in adsorption systems is higher than 0.88, whereas a_w lower than 0.88 is needed to inhibit growth in the desorption system of IM-pork dices with glycerol as the water-binding agent. Below 0.88 a_w, viable counts decreased during storage. Lower a_w resulted in higher death rates. In addition, at the same a_w, death rates in adsorption samples were higher than in desorption samples. Regardless of the preparation method (desorption or adsorption), at moisture contents of 0.932g H_2O/g solids or above the viable counts increased at higher rates with increasing moisture contents, conversely at moisture contents of 0.871 H_2O/g solids or below, the viable counts decreased at higher rates with decreasing moisture contents.

The effects of the different humectants used to prepare the IM-pork systems on the viability of *S. aureus* are shown in Figures 4 and 5. These results indicate that: (a) at high a_w (0.915–0.925), 1,2-propanediol and 1,3-butanediol have a bactericidal effect on *S. aurueus* cells which cannot be explained by the water-binding capacity of the humectants. At the same a_w, the bactericidal effect was absent in samples prepared with glycerol.

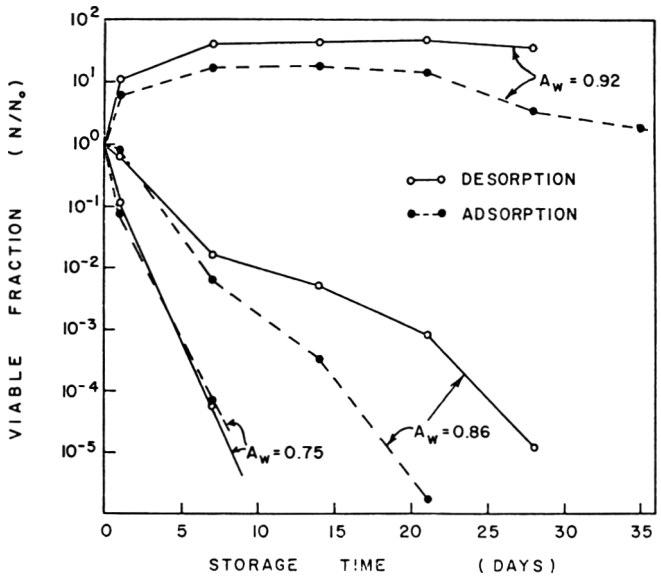

Fig. 2—Viability of Staphylococcus aureus in IM-strained chicken at 25° C prepared with glycerol.

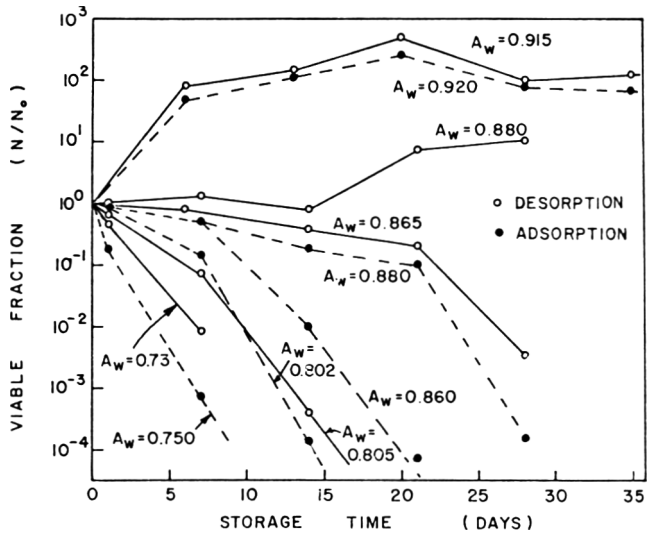

Fig. 3—Viability of Staphylococcus aureus in IM-pork cubes at 25° C prepared with glycerol.

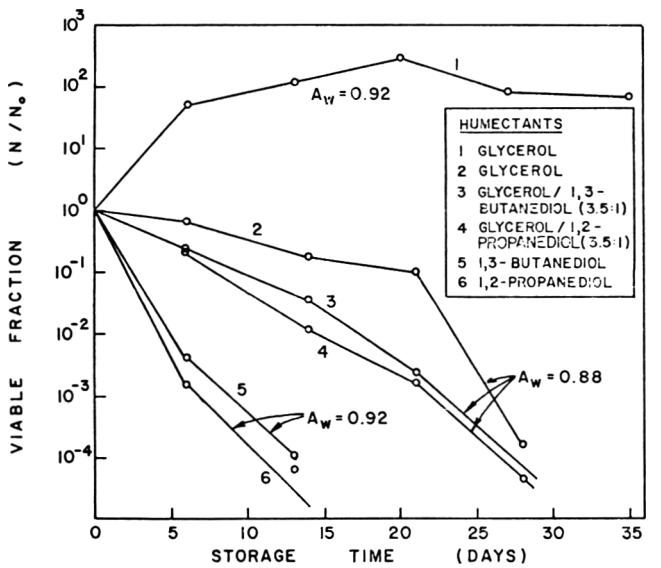

Fig. 4—Effect of humectants on the viability of Staphylococcus aureus in IM-pork cubes prepared by adsorption.

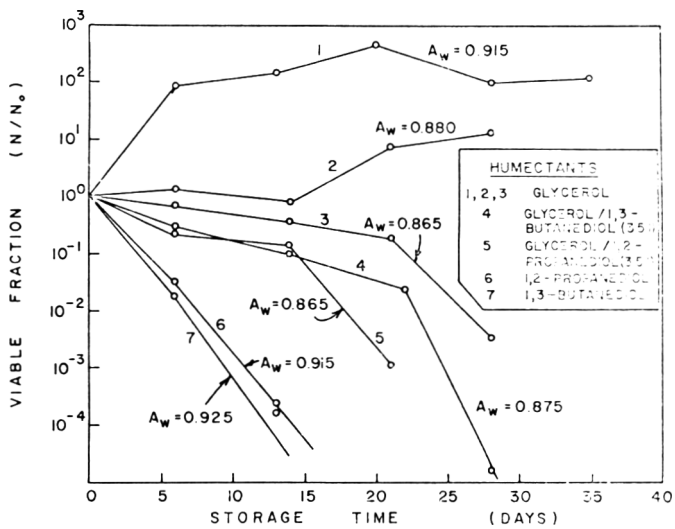

Fig. 5—Effect of humectants on the viability of Staphylococcus aureus in IM-pork cubes prepared by desorption.

(b) Regardless of the humectant used to lower the a_w, in samples prepared by adsorption, the death rate of *S. aureus* is higher than in desorption samples at the same a_w.

Results on the viability of *S. aureus* in BHI broth with different humectant concentrations are shown in Figures 6, 7 and 8. For each humectant, a bacteriostatic concentration was found where the viable population remained approximately constant for at least 20 hr. These concentrations were 40, 18 and 15% weight for glycerol, 1,2-propanediol and 1,3-butanediol respectively. These concentrations corresponded to a_w of 0.865 for glycerol, 0.95 for 1,2-propanediol and 0.97 for 1,3-butanediol. Below the bacteriostatic concentrations, the viable counts increased, although longer lag periods, lower growth rates and lower maximum cell concentrations were observed with increasing humectant concentrations.

Above the bacteriostatic concentrations, the results are somewhat different for the various water-binding agents. In the case of 1,2-propanediol and 1,3-butanediol, the viable counts decreased at increasing rates as the humectant concentration was increased above the bacteriostatic level. On the other hand, in the case of glycerol, the viable population was not as strongly affected as with 1,2-propanediol and 1,3-butanediol at concentrations higher than 40%.

DISCUSSION

SCOTT (1953) reported that regardless of the medium used, the minimum a_w required for growth of *S. aureus* was 0.86 under aerobic conditions. Other experiments (Scott, 1957; Christian, 1963) supported the fact that the biological response to a particular a_w is, at least for some organisms, largely independent of the type of solutes and the total moisture content of the substrate. The results obtained in this investigation showed that the a_w of the medium is not the only determining factor regulating the biological response of *S. aureus* but also the moisture content plays an important role. IM-pork dices at 0.88 a_w prepared by desorption and adsorption methods showed different biological responses.

Labuza et al. (1972b) studied the viability of several microorganisms in intermediate moisture strained pork prepared by adsorption and desorption methods. They reported that *S. aureus* cells were able to grow in desorption samples at 0.92 a_w, but not at 0.86 a_w, in close agreement with the present results. However, in the adsorption samples the viable population decreased at all a_w investigated (0.67–0.92 a_w). These results disagree with the experiments reported here, in which growth was observed in the adsorption samples at 0.92 a_w.

Glycerol inhibited growth of *S aureus* at 0.88 a_w in adsorption samples and at 0.865 a_w in desorption samples. These results are in good agreement with the values reported in the literature for the minimal a_w in various media (Scott, 1953; Marshall et al., 1971; Troller, 1971). At 0.92 a_w, in the samples prepared with 1,2-propanediol or 1,3-butanediol, the cells died rapidly. These results indicated that these two humectants have a bactericidal effect on *S. aureus* cells that cannot be solely explained in terms of their water-binding characteristics.

There is no reason to expect that solute with different properties such as ionic strength, cell permeability and cell utilization would have exactly the same effects at the same level of a_w. A simple approach to this complex problem (Kushner, 1971) is to consider that a high humectant concentration produces two effects: lowering the a_w and, superimposed on this, the specific action of the humectant molecules. Other experiments (Baird-Parker and Freame, 1967; Calhoun and Frazier, 1966; Marshall et al., 1971) have shown that microbial response to a particular water activity depends on the solute(s) used to lower the water activity of the growth medium.

In Brain Heart Infusion medium, the minimum concentrations of humectants found to be inhibitory for the growth of *S. aureus* were: 40, 18 and 15% weight for glycerol, 1,2-propanediol and 1,3-butanediol, respectively. It should be noted that these bacteriostatic concentrations represented quite different levels of water activity: 0.865 for glycerol, 0.95 for 1,2-propanediol and 0.97 for 1,3-butanediol. These results again show that besides the water-binding properties of 1,2-propanediol and 1,3-butanediol, there is a bactericidal effect which depends on the chemical structure of the humectant molecules.

Even though in these experiments *S. aureus* was the only organism investigated, several published results indicate that *S. aureus* is one of the most resistant microorganisms to the bactericidal effects of various polyhydric alcohols (Robertson et al., 1948; Olitzky and Mattly, 1967). Robertson et al. (1948) reported that the lowest concentrations found inhibitory for *S. aureus* were 15% for 1,3-butanediol and 20% for 1,2-propanediol. There values are in close agreement

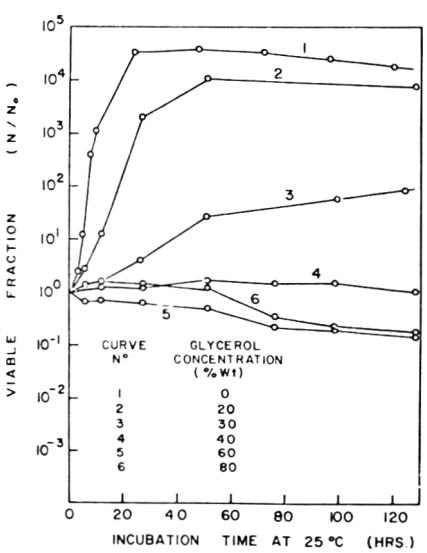

Fig. 6—Effect of glycerol on Staphylococcus aureus in brain heart infusion medium.

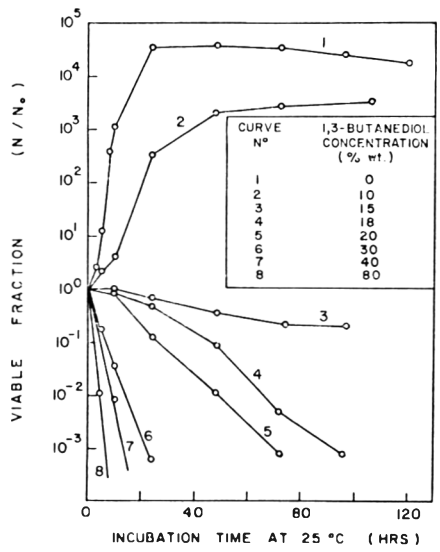

Fig. 7—Effect of 1,2-propanediol on Staphylococcus aureus in brain heart infusion medium.

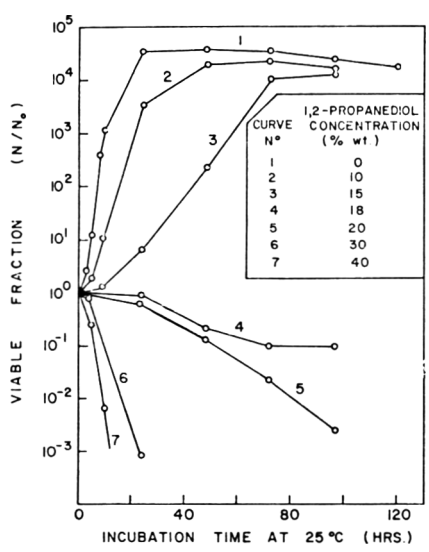

Fig. 8—Effect of 1,3-butanediol on Staphylococcus aureus in brain heart infusion medium.

with the results reported in this investigation.

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INTERACTION OF FORMALDEHYDE WITH FISH MUSCLE IN VITRO

INTRODUCTION

DURING FROZEN STORAGE of fish muscle there is a progressive decrease in (a) extractable whole myofibrils (Childs, 1973b) and (b) protein extractable in high ionic strength salt solutions (Dyer, 1951). Much work has been performed in an attempt to explain this latter phenomena and a great deal of effort has been expended in observations on the interactions of breakdown products such as free fatty acid with fish muscle (see review by Connell, 1968). In some fish species, reduction of trimethylamine oxide yields formaldehyde (Harada and Yamada, 1971), which has been shown to cause a marked decrease in the amount of protein soluble in 0.50μ saline solutions (Castell, 1971). It thus appears plausible that formaldehyde may play some role in in situ protein, and myofibril, insolubilization in fish muscle. This study was undertaken to determine the quantitative and qualitative effects of formaldehyde on salt soluble proteins and whole myofibrils in vitro, and to obtain possible insights to its in situ effects.

EXPERIMENTAL

PACIFIC TRUE COD (*Gadus macrocephalus*) was obtained one day post-catch, filleted, blocked and frozen in 1 × 3.75 × 21 in stainless steel trays in a −40°C blast freezer. 15 hr later, the blocks were sawed into 1× 3.75 × 1.6 in portions, packed in evacuated, moisture-vapor proof film and held at −40°C until analysis at 0, 3 and 6 months.

Extraction of proteins and whole myofibrils

Prior to extraction, all connective tissue in the portion of white muscle to be analyzed was carefully dissected and discarded. Following this, the high ionic strength soluble proteins and whole myofibrils were extracted and quantified as previously described (Childs, 1973a,b).

Reaction with formaldehyde (FA)

Salt-soluble proteins. All procedures were performed at 4°C. FA was added to the extraction buffer (5% NaCl, 0.02M $NaHCO_3$) in the form of reagent grade formalin (37–38% FA). 5g of fish muscle and 145 ml of extraction buffer + FA were placed in a Virtis homogenizing flask equipped with a baffle plate to prevent foaming. The sample was homogenized for 3 min at medium speed and the homogenate then centrifuged for 20 min at 1000 × G. The supernatant was decanted and its protein content determined in the same manner as in the control.

Whole myofibrils. All procedures were performed at 4°C. FA was added to the initial extraction buffer (0.025M KCl, 0.039M boric acid, 0.005M disodium EDTA, pH 7.1) in the form of reagent grade formalin (37–38% FA). 8g of fish and 40 ml of extraction buffer + FA were placed in a small blender jar and mildly homogenized at low speed for 1 min. This homogenate was centrifuged at 600 × G for 15 min. The pellet was resuspended in extraction buffer without formaldehyde. No additional exposure to formaldehyde was employed and the remainder of the extraction and quantification procedure were performed as previously described (Childs, 1973a).

Electrophoresis of myofibrillar proteins

Myofibrillar proteins were separated by sodium dodecyl sulfate (SDS)-acrylamide gel electrophoresis as previously described (Childs, 1973a). The high ionic strength salt soluble proteins were prepared for electrophoresis by diluting with an equal volume of buffer (0.01M phosphate at pH 7.0, 8M urea, 1.0% β-mercaptoethanol and 1.0% SDS) and then electrophoresed in the same manner as whole myofibrils. Because 90–95% of fish muscle proteins are salt soluble (Ravesi and Anderson, 1969), sarcoplasmic proteins were surely present in the salt soluble protein isolate, but in such small amounts that they did not stain deeply with Amido Schwartz.

RESULTS

Studies with protein soluble at 0.50μ

There was a marked decrease in the amount of extractable protein in fresh and frozen samples following exposure to FA (Fig. 1). The rate of decrease in extractable proteins was less at higher concentrations of formaldehyde. The significant differences in the amount of extractable protein between fresh and

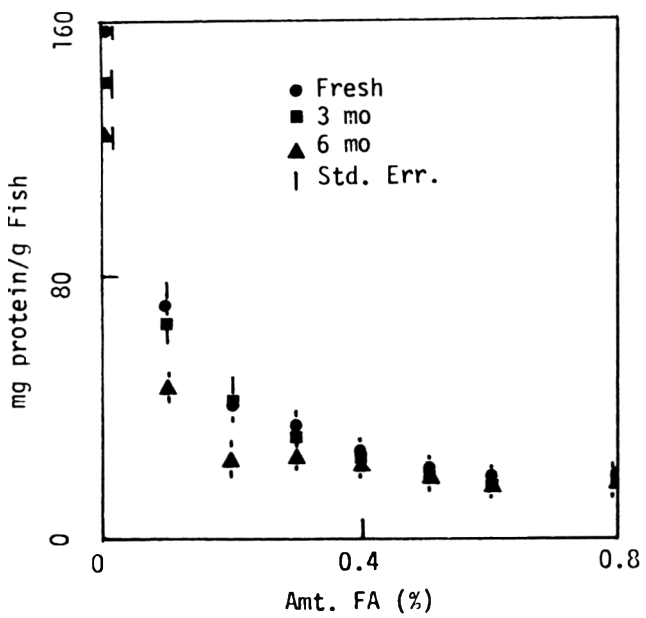

Fig. 1—Effect of formaldehyde on the extractability of salt-soluble proteins of true cod. Each point is the mean of four replicates and the bar represents the standard error of the mean. The FA concentrations are added FA. None of the sample contained > 0.0015% endogenous FA. Points with the same abcissa value having different exponents are significantly different ($P < 0.05$).

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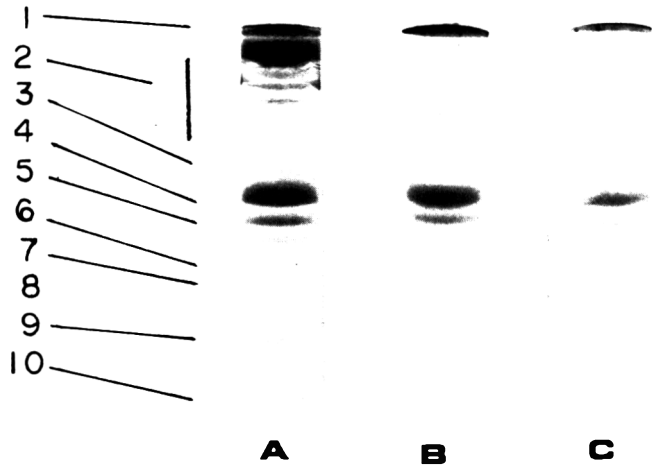

Fig. 2—SDS-acrylamide gel electropherograms of salt-soluble proteins extracted in the presence of FA. The identity of the protein species is based on studies of Wilkinson et al. (1972) with rabbit myofibrils, and subsequent adaptation to fish muscle by Childs (1973a). The bands are: (1) heavy chains of myosin; (2) actinins (?); (3) actin; (4) 37,400 mol wt component; (5) tropomyosin; (6) light chain of myosin; (7) inhibitory factor; (8) Ca inhibitory factor which may not occur in fish muscle; (9) light chain of myosin; (10) light chain of myosin; (A) Control; (B) 0.04% FA; (C) 0.40% FA.

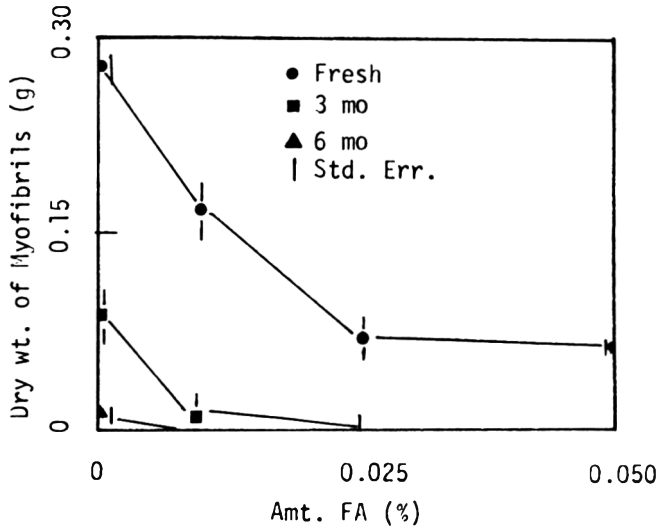

Fig. 3—Dry weight of extractable myofibrils as a function of exposure of fish muscle to formaldehyde. Each point is the mean of four replicates and the bar represents the standard error of the mean. The FA concentrations are added FA. None of the samples contained > 0.0015% endogenous FA.

frozen stored samples observed at lower concentrations of FA did not continue at higher FA concentrations.

SDS-acrylamide gel electrophoresis of the extracted proteins allowed delineation of the relative reactivity of protein fractions to FA in this system (Fig. 2). A given protein was judged to be insolubilized when it was absent from the electropherogram. Using this criteria, the most reactive molecular species were tropomyosin and heavy chains of myosin. These were completely eliminated from the extractable fraction by a 0.04% formaldehyde solution. Proteins which were less reactive included what may be the actinins and the inhibitory factor. The least reactive proteins in these experiments (i.e., the only ones still present on the electropherogram after exposure to 0.40% FA) were actin and the 37,400 mol wt component.

Whole myofibril studies

The effect of FA on extractability of whole myofibrils is objectively quantified in Figure 3. The whole myofibrils lost their extractability at much lower FA concentrations than did the myofibrillar proteins. Approximately 75% of the whole myofibrils were insolubilized following exposure to only 0.05% FA (Fig. 3), while 0.2% FA was required to insolubilize a similar fraction of salt soluble proteins (Fig. 1).

SDS-acrylamide gel electrophoresis of whole myofibrils indicated that the extracted myofibrils had complete protein complements (Fig. 4).

DISCUSSION

PREVIOUS STUDIES in this laboratory have indicated that the whole myofibril loses its solubility before all of its component proteins (Childs, 1973b). In the study reported here, it has been shown that a protein denaturant can cause a similar phenomena. If one juxtaposes the loss in whole myofibril extractability (Fig. 4) with the SDS-acrylamide gel determination of myofibrillar protein species (Fig. 2), it would appear that the decrease in myofibril extractability may parallel the

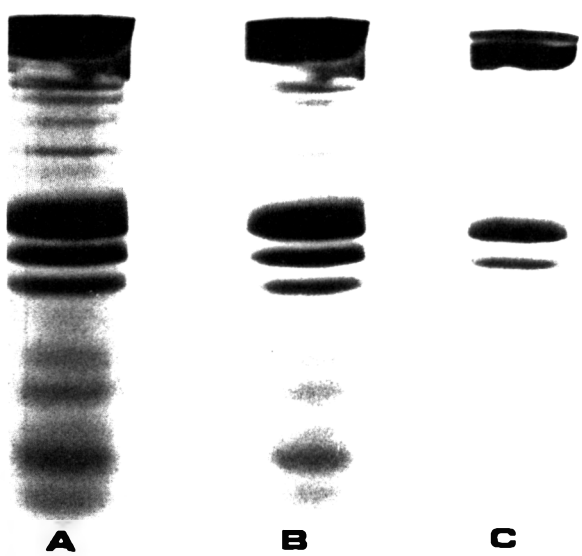

Fig. 4—SDS-acrylamide gel electropherograms of whole myofibrils extracted in the presence of FA. For identity of protein bands see caption to Fig. 2. (A) Control; (B) 0.025% FA; (C) 0.05% FA.

loss of myosin and tropomyosin solubility. This would suggest that if these proteins lose their extractability, then the entire myofibril becomes insolubilized. This suggestion is given additional strength by the observation that the small amount of myofibrils which could be extracted after exposure to FA contained myosin and tropomyosin (Fig. 4). Unfortunately, current methodology does not easily allow a final proof that the solubility of the whole myofibril may be a function of the solubility of one or two constituent proteins. It would be necessary to delineate the nature of the insolubilized proteins and whole myofibrils as well as the soluble ones, and further to observe them simultaneously. However, solubilization of these proteins with guanidine hydrochloride in the presence of sodium borohydride (Buttkus, 1969) may make such a scrutiny possible.

It is most difficult from this data, and other data in the literature, to evaluate the role of FA in loss of extractability of whole myofibrils and myofibrillar proteins in situ. When the data of Babbitt et al. (1972) concerning muscle concentration of FA were converted to equivalent FA concentrations in the extraction buffer, it was found that a 75% decrease in extractability of protein was noted at 0.003% FA. In this study, at least 0.2% FA was required to obtain a similar loss in extractability. This suggests that either (a) FA has little effect on protein extractability in situ, or (b) FA is a more efficient denaturant in a frozen fish matrix than in a buffer-fish extraction matrix. This latter suggestion is given credence by the work of Buttkus (1967).

In addition, it could be that the actual in situ site of insolubilization is the whole myofibril, and decreases in extractable salt soluble protein are a reflection of increasing numbers of myofibrils which cannot be dissociated into component protein by 0.50μ salt. Scrutiny of this hypothesis will require direct experimentation with the insolubilized pellet.

A number of other factors have been suggested as causal agents in insolubilization of fish myofibrillar proteins including (a) changes in the concentration of tissue solutes; (b) dehydration; (c) fatty acids (reviewed by Connell, 1968); and (d) oxidation of disulfide groups and hydrogen bonding (Buttkus, 1969). Investigations concerning the effects of each of these factors on the whole myofibril and its constituent proteins are underway in this laboratory.

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EFFECT OF TEMPERATURE ON LIPID EXTRACTION AND FUNCTIONAL PROPERTIES OF FISH PROTEIN CONCENTRATE (FPC)

INTRODUCTION

FISH PROTEIN concentrate (FPC) has been defined as a product resulting from water and oil removal from fish, thus increasing the concentration of the protein and other nutrient material (Stillings and Knobl, 1971). Several processes have been developed for producing FPC (Finch and Liston, 1970; Library of Congress, 1970) and can be classified as chemical (solvent extraction), biological (enzymatic digestion), or physical (mechanical pressing). Of these, the solvent extraction process is the closest to commercial adaptation.

Solvent (alcohol) extracted FPC is recognized internationally as a source of high-quality animal protein. Past research has centered mainly on the nutritional and engineering aspects of the product and process, respectively. Interest in functional properties of FPC is more recent and, for this reason, not as yet well documented.

Functional properties of a food ingredient are those properties that are capable of imparting specific desirable characteristics to a processed food (Johnson, 1970). Some of these are: solubility, wettability, emulsifying ability and water dispersible with no settling. At the present time FPC made by isopropyl alcohol (IPA) extraction of whole fish at 70–80°C (Fishery Leaflet 584) is considered to have relatively poor functional properties. It is practically odorless, tasteless and has a light grey to tan color. Furthermore, it has very limited water solubility, wettability and dispersibility characteristics. It does, however, absorb more fat than it does water.

The proteins involved in binding (whether water holding or emulsion binding) have been found to be due mainly to the myofibrillar proteins: myosin, tropomyosin, actin and actomyosin (Donnelly et al., 1966). These proteins are extremely susceptible to damage even in frozen storage. Between 40 and 60°C there is a substantial decrease in the solubility of the myofibrillar protein and above 60°C there is almost complete insolubility (Hamm, 1966). Coinciding with loss of solubility is a decrease in water holding capacity. In beef muscle, these effects begin at about 35°C and are most serious between 40 and 50°C.

Therefore, the purpose of this research was to determine the effect of extraction temperature on lipid extraction and functional properties for the purpose of defining conditions and to extract the lipids and water from whole fish with IPA with minimum damage to the integrity of the fish proteins.

MATERIALS & METHODS

Fish

Red hake (*Urophycis chuss*), a lean fish of about 3–5% fat content, was used because it has been approved by the FDA for FPC production (Federal Register, 1967). The experimental material consisted of several batches caught during the spring of 1970. The areas of capture were Pt. Judith, R.I. and Pt. Pleasant, N.J. Approximately 300 lb of fish from each batch were brought to the laboratory of the National Marine Fisheries Service, College Park, Md. and stored at −20°C until used.

Processing

Isopropyl alcohol:azeotrope 91% v/v was used in accordance with a five-stage countercurrent procedure as outlined by Brown and Miller (1969) and modified by chilling overnight the liquid filtrate (miscella-2) to 3°C prior to contact with the raw fish. This chilling process causes lipids to precipitate from solution. These lipids are predominantly triglycerides and, if not removed, tend to redeposit onto the solids in the first stage (Dambergs, 1969). The miscella-2, after removal of the lipids, was then reheated to the desired temperature of extraction, prior to contact with the raw fish.

Random samples of frozen whole fish were removed from frozen storage and ground in a Hobart grinder (one pass through a 1/4-in. end plate followed by a second pass through a 1/8-in. end plate). An 800-g sample of ground fish was removed for solvent extraction. The solvent to lipid free-moisture free solid ratio was held constant in each experiment at 10:1 w/w. Each experiment was limited to five stages of extraction. Separation of solids from liquid was by filtration using a Buchner funnel, #1 Whatman filter paper, and a vacuum filter flask.

The sample of fish was placed in a reaction vessel equipped with baffles to ensure turbulent agitation (McPhee and Brown, 1971). To the flask was added the correct amount of solvent and the mixture was stirred at the desired temperature of 20, 40 or 50°C for 10–15 min. The slurry was then filtered. The miscellas (liquid filtrates), other than the first, were retained for subsequent countercurrent extractions. The first-stage miscella was discarded. Fresh solvent was used for the final stage of extraction. Seven to 10 replications were made at each temperature.

After the final stage of extraction, the wet solids were dried under vacuum at a temperature of 45–55°C for 30 min. The residual IPA of the solids was about 1% by weight. The dry solids were then milled in a Wiley mill through a 40-mesh screen.

The Food & Drug Administration (FDA) specifications call for a maximum residue of 250 ppm of IPA in FPC (Federal Register, 1967). However, in order to meet this level, steam stripping is required. This method cannot be used if functionality is to be preserved as denaturation of the proteins ensues (Dubrow, 1971). As a consequence, all analyses were performed on the dry, nondesolventized solids.

Chemical evaluation

Lipids. Residual lipid content was determined in accordance with the method of Smith et al. (1964). A characterization of the lipids was performed using column and thin-layer chromatography. Silicic acid (Mallinckrodt AR grade 100 mesh) was washed with methanol, and dried overnight at 105°C. Enough chloroform was then added to the dried material to make a slurry which was then introduced into a glass column equipped with a fritted disc filter, glass wool and Teflon stopcock. The dimensions of the column were 2.0 × 20.0 cm.

Approximately 100 mg of lipid were placed onto the column and eluted at a rate of 3 ml/min with the following systems: chloroform, chloroform-methanol (70:30 v/v) and finally methanol. The eluates were evaporated under nitrogen in a 50°C water bath and the residue weighed. The residue was then prepared for thin-layer chromatography (TLC).

Gelman SA ITLC sheets were activated by dry heat at 120°C for 3 hr (Haer, 1969). For separation of neutral lipids (chloroform eluate), a solvent mixture consisting of hexane-ethyl ether-acetic acid (95-5-1 v/v) was used as the moving phase. The ITLC sheet was dried and then placed in iodine vapors for detection of unsaturated lipid spots. Standard lipid mixtures were also spotted, as references.

Other components. Crude protein, volatiles, and ash content of the raw fish and FPC samples were determined in accordance with methods described in AOAC (1965). Since only

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small insignificant differences in protein and ash content were found, these data are not included in the results.

Functional properties

Salt soluble protein. Because tests of protein solubilty of fish, and particularly FPC, have not been thoroughly standardized (Ravesi and Anderson, 1969), an empirical method for determining soluble protein in FPC was established experimentally (Dubrow, 1971) by Biuret reaction.

The procedure was as follows: 2g of FPC were added to 50 ml of cold 5% NaCl (in 0.02M $NaHCO_3$, pH 7.5) in a 125-ml flat bottom extraction flask. The material was magnetically stirred for 3 hr at room temperature. This method produced very little foam and negligible heat. After extraction, the dispersion was filtered through #1 Whatman filter paper. From the filtrate, 2.0 ml were removed and added to 6.0 ml of Biuret reagent (Snow, 1950a, b). The reaction mixture was stirred and allowed to stand for 30 min for color development. The percent transmission of light was determined spectrophotometrically in a Bausch and Lomb Spectronic 20 at 540 mμ and compared to a standard protein. Each sample was prepared in duplicate.

Emulsifying capacity. 2g of FPC were blended (Waring Blendor, Model #1083) in a pint-size blendor jar with 20 ml of 5% NaCl (in 0.02M $NaHCO_3$) for 3 min at low speed. 20 ml of corn oil were then added to the blendor and the entire mixture blended for 1.5 min at low speed. The mix was then poured into three graduated test tubes in 10-ml portions. The test tubes were placed in a water bath (at about 98°C) for 30 min after which they were cooled in an ice water bath. Since FPC is more lipophylic than hydrophylic, measurements were taken of the volume of water separated. If oil separated at the same time, measurements were also taken of this phase. Emulsion stability was calculated as the percentage of water (total) that separated from the system.

Color measurement-reflectance. Only two peaks of absorption are normally found in the visible range for FPC's made by alcohol extraction: a major peak at 410 mμ and a minor peak at 535 mμ. Reflectance at 410 mμ (Soret band) has been used as an indicator of lightness of FPC by Canadian researchers (Power, 1964). To determine the effect of process variables, FPC's were analyzed for percent reflectance by the following method: approximately 5.0g were placed in a 35 × 10 mm tissue culture dish (Falcon Plastics) and tapped to remove air pockets. Samples of the compressed protein concentrate were measured for percent reflectance of light between the wavelengths of 390 mμ and 700 mμ by means of a Beckman Model DB recording spectrophotometer fitted with a diffuse reflectance attachment. A magnesium oxide block was used as a standard. Conversion to tri-stimulus color characteristics, in terms of C.I.E. (Commission International de L'Eclairge) coordinates, was performed in accordance with pre-selected wavelength technique (Beckman Application Data Sheet, DK-78-MI).

Suspended solids. A 2-g sample was mechanically agitated by a magnetic stirrer with 50 ml of distilled water for 3 hr. The slurry or suspension was then poured into a 100-ml graduated cylinder and allowed to stand for 90 min or longer. This allowed particles to settle and equilibrium to be established. A 5-ml aliquot of the supernatant was removed and placed into a tared aluminum weighing dish and dried overnight at 103°C. The percent suspended solids was then determined from the dry weight. All samples were run in duplicate.

Wettability. This evaluation was subjective and based upon the extent of wetting when particles were applied to the surface of a water medium. If particles wetted (this is clearly visible) within 30 sec, the sample was ranked as "excellent." If, after this period, slight agitation (magnetic stirring) was required to wet the particles, the sample was ranked "good." However, if particles required from 5–10 min to wet, they were "fair" and any longer period merited only a "poor" rating.

pH. pH was determined on a 4% slurry, from the test for suspended solids, prior to the settling period. A Fisher "Acumet" pH Meter, Model #210 was used for this determination.

Table 1—Composition characteristics of residual lipid determined by separation of extract by silicic acid column chromatography

Sample	Lipid composition Chromatography solvent: Chloroform	Chloroform:methanol	Methanol
	Weight %		
FPC (20°C extraction)	68.6	27.4	4.0
FPC(50°C extraction)	52.1	34.4	13.5
M-2 ppt[a]	65.0	32.8	1.5
Nonextracted whole hake	65.6	24.0	10.5

[a] Composition of lipid precipitate from Miscella-2 at 20°C extraction.

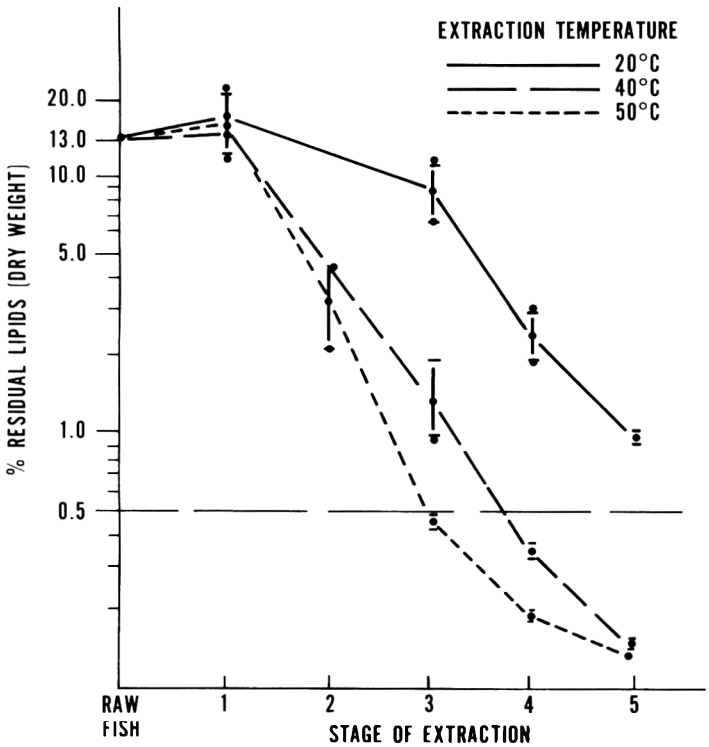

Fig. 1—Effect of temperature on extraction of lipids from whole red hake with isopropyl alcohol (five-stage countercurrent)

RESULTS & DISCUSSION

Lipid extraction.

As illustrated in Figure 1, a countercurrent alcohol extraction at 20°C failed to extract sufficient lipid, after five stages, to meet FDA regulations which state that the lipid content of FPC must be $< 0.5\%$. These FPC's had a residual lipid content of $1.00 \pm 0.24\%$. A similar extraction involving a first-stage crosscurrent extraction followed by four countercurrent stages showed that the lipids can be reduced to about 0.4% (Dubrow, 1971). This system, however, requires greater solvent usage.

Increasing the extraction temperature to 40°C reduced the lipids to less than

0.5% in four stages of extraction. The FPC produced at this temperature had a residual lipid content of 0.35 ± 0.10%. The addition of a fifth stage at this temperature reduced the residual lipid further to 0.15 ± 0.06%. IPA extraction at 50°C resulted in the residual lipid content being slightly less than 0.5% in three stages of extraction. However, for quality control purposes it would be preferable to control the residual lipid to well below this level. Thus, four stages at 50°C extraction, yielded a lipid content of 0.20 ± 0.06%.

It was of interest to note that in all treatments, the first stage solids contained a higher lipid percentage than did the starting raw material. Although the miscella-2 (M-2) was cooled and the precipitated heavy lipids removed, there still was an apparent redeposition of lipids from the miscella onto the raw fish solids.

Table 1 shows the solubility characteristics of the lipids in (1) FPC's extracted at 20 and 50°C; (2) M-2 precipitate from the 20°C extraction; and (3) raw hake. The data on the FPC residual lipids from 50°C extractions are similar to those reported by Medwadowski et al. (1967) for an FPC produced by extraction at 70–80°C.

It was shown that the lipid composition of the M-2 precipitate was essentially the same as the residual lipid in the FPC from the same extraction. The relative percentage difference in chloroform soluble lipids between FPC extracted at 50°C and that extracted at 20°C could be the result of triglyceride breakdown. However, the combined extracts of chloroform and chloroform-methanol had a relative percentage about equal to that of the raw fish. The combined extracts from FPC extracted at 20°C amounted to about 96%, which possibly indicates that at low temperatures of extraction more methanol solubles or phospholipids are extractable with IPA. Thin-layer chromatography of the chloroform eluted lipids showed a predominance of triglycerides while chloroform-methanol eluates were found to contain mono- and diglycerides, with a trace of triglycerides and fatty acids. The fatty acid composition of whole raw hake lipids and of the M-2 lipid precipitate indicated only a slight difference in composition (Table 2).

Table 2—Fatty acid composition of lipids from whole red hake precipitated lipid from an N-2 miscella. Extraction temperature was 20°C.

Fatty acid	Whole red hake	M-2 ppt lipid
	----% of total----	
14:0	1.15	0.88
16:0	15.25	12.65
16:1	4.49	3.69
17:0	0.58	0.40
18:0	5.00	5.15
18:1ω9	29.15	35.28
18:2ω6	1.11	0.97
18:4ω3	0.72	0.64
20:4ω6 + 20:3ω3	3.28	2.70
20:4ω3	0.66	0.51
20:5ω3	11.00	15.00
22:4ω6	0.64	0.87
22:5ω6	0.83	0.57
22:5ω3	2.65	3.03
22:6ω3	12.60	11.31

Functional properties

The effect of extraction temperature on the soluble protein, suspended solids, emulsifying capacity, wettability and pH of isopropyl alcohol extracted whole fish solids is shown in Table 3. Both the salt soluble protein and suspendable solids decreased with an increase in extraction temperature. These two parameters were found to be directly correlated, r = 0.81. The increase in extraction temperature from 20 to 40°C, resulted in a decrease of about 30% in protein solubility and suspended solids. An increase in temperature to 50°C resulted in a further decrease to about 70% of the soluble protein found in the FPC extracted at 20°C.

The emulsifying capacity of the FPC's also decreased with an increase in extraction temperature. Only those FPC's processed at 20°C formed emulsions, which were stable to heat. The FPC's from the other temperature treatments showed a release of both water and oil.

One of the variables affecting emulsifying capacity is the concentration of soluble protein (Carpenter and Saffle, 1965). Since the data have shown a decrease in soluble protein with an increase in extraction, it would be expected that there would be a consequent loss in the FPC's emulsifying and binding power (since there would be less soluble protein per unit weight). It has been reported that the soluble protein of comminuted poultry meat, extracted with IPA, similarly failed to produce a stable emulsion when extracted at 40 to 50°C (Toledo, 1970). However, when the poultry meat was extracted at 30°C, a stable emulsion was obtained. Heat denaturation of poultry meat also resulted in unsatisfactory binding (Vadehra and Baker, 1970).

The pH of the FPC's produced at the various extraction temperatures showed an increase in alkalinity with an increase in temperature. The pH of the raw fish, prior to extraction, was between 6.7–6.8 and after extraction at 20 and 50°C, the pH's of the FPC's were found to be 7.37 ± 0.12 and 7.60 ± 0.07, respectively.

Studies of rabbit muscle proteins, after various time-temperature treatments, also showed that the pH of the muscle tissue increased with heating (Paul et al., 1966). This increase was attributed to a loss of free acidic groups by the formation of new stable cross-linkages. These authors further found a decrease in myobribrillar protein solubility with an increase in temperature from 52.4% at 40° (after 10 min of heating) to 21.6% at 50°C (10 min of heating).

The data obtained in this study appear to corroborate the findings of other investigators with respect to the effect of heat upon the pH of fish muscle and the resultant FPC.

None of the samples of FPC's were wettable. For the most part, they remained on the surface of water and required external energy to wet them. The samples were rated "fair" to "poor."

The effect of extraction temperature did not appear to significantly alter the wetting characteristics of the FPC's judged to be "poor" from a 50°C extraction were also "poor" from a 20°C extraction.

The poor wetting properties of the FPC can be overcome, however, by the addition of a surfactant. Applying a surface active agent (2% Tween 80) to the solids, prior to drying, produced excellent results (Rasekh and Stillings, unpublished data).

The chemistry of color in FPC is primarily the chemistry of the myoglobin and hemoglobin (Henderson and Dubrow,

Table 3—Effect of temperature of extraction on the functional properties of FPC's produced by extraction of whole hake with isopropyl alcohol

Functional property	Extraction temperature		
	20°C	40°C	50°C
Soluble protein	26.95% ± 1.90[a]	18.88% ± 4.01[a]	8.30% ± 1.27[a]
Suspended solids	26.22% ± 3.92	16.02% ± 2.15	9.12% ± 0.86
Emulsifying capacity[b]	100	70[c]	62.5
Wettability	Fair to poor	Fair to poor	Fair to poor
pH	7.37 ± 0.12	–	7.60 ± 0.07

[a] Standard deviation
[b] Percent oil-water bound after heat treatment
[c] Separation of water and oil phase

1972). Table 4 shows the C.I.E. coordinates and Y (lightness) values obtained from the reflectance measurements. These data show that the FPC's processed at 20° were slightly darker than the FPC's processed at 40°C, whereas the FPC's processed at 50°C were slightly darker than the FPC's processed at 20°C. These differences may be due to the extent of heme oxidation prior to solvent extraction. In general, there were two peaks of absorption, one major one at 410 mμ (Soret band), and a minor peak at 535 mμ (ferrihemochromes). Also observed was a trace absorption at 627 mμ of the FPC's processed at 20°C, which was not as noticable in the other treatments.

Table 4—Calculated C.I.E. coordinates for FPC's produced from whole red hake by solvent extraction with isopropyl alcohol at 20, 40 or 50°C

Temperature of extraction	C.I.E. coordinates		
	x	y	Y
IPAcc			
20°C	0.3488	0.3510	.3828
40°C	0.3422	0.3523	.4047
50°C	0.3471	0.3564	.3774

CONCLUSIONS

THE FOLLOWING conclusions of the effect of processing temperature on lipid extraction and functional properties from whole red hake (*Urophycis chuss*) are presented: IPA performed more efficiently in removing lipid with an increase in extraction temperature. The major component of the residual lipids was triglycerides and the major polar lipid was phosphatydyl choline (lecithin). All FPC's showed a decrease in protein solubility and suspended solids and an increase in pH with increased temperature of extraction. FPC's processed from whole hake at 20°C had better functional properties than those processed at either 40 or 50°C. FPC's formed stable emulsions when extracted at 20°C. All failed to exhibit this property when extraction temperature was 40°C or higher. Wettability of FPC was not improved regardless of extraction temperature.

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IN VITRO DIGESTIBILITY OF PROTEIN IN YOGURT AT VARIOUS STAGES OF PROCESSING

INTRODUCTION

YOGURT, a cultured product made from whole or partially skimmed milk, is acidified and coagulated by two bacteria, namely, *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. The basic process for the commercial preparation of yogurt involves the formation of a yogurt mix, pasteurization, homogenization, partial, cooling, addition of the yogurt culture, incubation to form the acidified coagulum, and refrigeration of the final product. Humphreys and Plunkett (1969) have written an excellent review on the manufacture of yogurt.

Although yogurt has become a popular dairy product in recent years, it will probably retain its special nutritional image which dates back to Metchnikoff who attributed longevity of Bulgarians to their consumption of 'yoghurt' (Foster et al., 1957). Literature devoted solely to the therapeutic value of yogurt is quite similar to the clinical studies of acidophilus milk, being primarily concerned with gastrointestinal treatment. In a study on the effect of yogurt containing prune whip on constipation, Ferrer and Boyd (1955) found that of 194 elderly patients suffering from constipation and other chronic geriatric illnesses, 187 patients required no laxatives during the period of administration of the yogurt. However, the validity of this work must be questioned due to the probable influence of the prunes. Yogurt has also been found to be effective in the treatment of diarrhea caused by a virus or antibiotic treatment (Niv et al., 1963). Raffle (1956) observed that yogurt was effective in recolonizing the bowels of infants treated with antibiotics, and Shapiro (1960) also found that adults with antibiotic-induced symptoms responded favorably to yogurt therapy.

In addition to the beneficial effects of yogurt cited above, many workers have mentioned that yogurt is more easily digested than milk. However, little research has been devoted specifically to substantiate these claims or to assess the processing variables which may influence digestibility characteristics. Davis and Latto (1957) in their advocation of cultured milks, particularly acidophilus milk, have commented that such fermented products can often be taken by people who cannot digest ordinary milk. They stated that the curd formed by ingestion of cultured milks is much finer than that formed by milk. Hermann (Steyn, 1969) also remarked that yogurt is more easily digested, although as far as is known, the fermented milk has essentially the same food value as the original milk from which it is made. Damrau (1954) reported that yogurt is more easily digested than ordinary milk–one container being digested in 1 hr as compared to 3 hr for the same amount of milk. Smetinoff (1971) reported that over 90% of yogurt is digested within 1 hr, as compared to only 30% of milk.

Only one short study (Anonymous, undated) was available which represented a comparison of the digestibility of yogurt and homogenized milk. Results of this study suggested that the proteins in yogurt are digested more rapidly than those of homogenized milk. However, the experiments were not designed so as to have the same ratio of protein and gastric juice in both products. The finer curd particles observed in the treated yogurt led the workers to speculate that the more rapid rate of digestion was due to increased surface area.

Doan and Dizikes (1942) compared the digestion characteristics of various types of milk with human milk. They devised a satisfactory in vitro method for determining gastric digestibility and curd characteristics. The size of the curds present at various stages of digestion was determined by a screening method and the amount of each curd size was estimated by nitrogen determination. Buttermilk (pH 4.5 or lower), evaporated milk and superheated soft curd milk exhibited excellent digestibility characteristics. Lactic acid milk (pH 5.4 to 5.7), boiled milk and homogenized milk were found to have digestion characteristics much improved over ordinary milk. Milk treated with locust bean gum (0.1%) was found to be affected adversely by such treatment. This work indicated that low pH, homogenization and heat treatment have a positive influence on digestibility.

In the manufacture of yogurt, milk is exposed to heat treatment, homogenized and acidified so it is reasonable to suspect that it is readily or easily digested. Some yogurts contain stabilizers such as guar gum, carrageenan, or gelatin which may have a negative effect on digestibility. Other factors in the manufacture of yogurt may also affect the digestibility. Accordingly, the purpose of this study has been to determine the effect of various steps in the processing of yogurt upon its digestibility characteristics. The index of digestibility was based upon a quantitative evaluation of one o more of the following: (1) total nitrogen; (2) nonprotein nitrogen; and (3) free amino acids, of samples treated in an in vitro digestion system.

EXPERIMENTAL

Sampling

Samples (#1, #2, #3, #4 and #5) were obtained at various stages in the processing scheme for yogurt in a large commercial plant (Fig. 1). This product is incubated in the retail container and is prepared without the addition of preservatives or stabilizers. Flavored samples were also supplied by the above manufacturer, the fruit yogurts being prepared by adding the preserves to the container prior to filling with the yogurt mix.

Another brand, processed by a different procedure (Swiss style, i.e., vat incubated), containing sorbic acid as a preservative and gelatin as a stabilizer was purchased from a local store for one of the studies. Lemon-flavored yogurt was purchased for this study as a plain yogurt was unavailable.

Digestion method

A modification of the method of Doan and Dizikes (1942) was chosen as a satisfactory basic digestion procedure. Digestibility was measured by the ease of degradation of curd particles in a simulated gastric digestive system. This method involved a peptic digestion procedure conducted in a digestion apparatus which provided a mild squeezing action, simulating peristalsis. The product of digestion was separated into various curd fractions based upon particle size and the total nitrogen content of these fractions was determined.

Digestion apparatus. The apparatus used in this study had a motor-driven unit attached to a thermostatically controlled water bath (Fig. 2). Mounted across the top of one-half of the bath was a wooden board which supported the AC-

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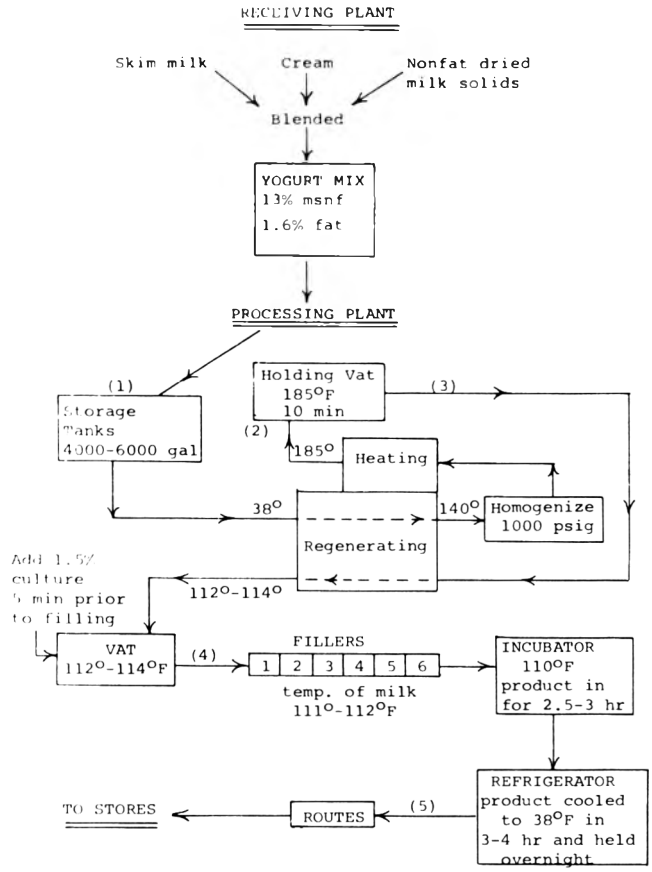

Fig. 1—Schematic of yogurt processing steps and sampling stations.

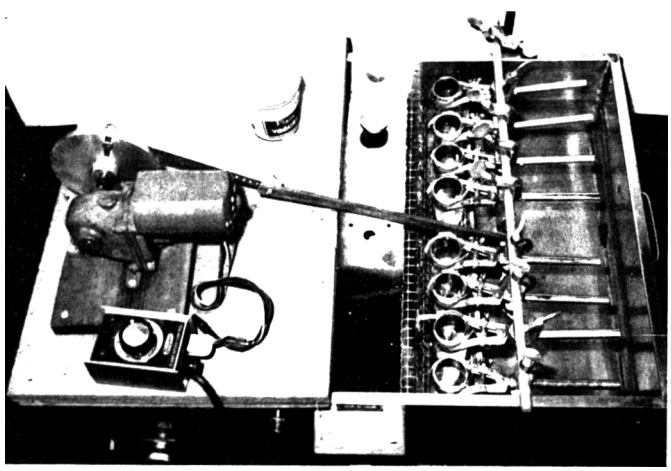

Fig. 2—Digestion apparatus.

DC gearhead reduction motor and speed control. This motor unit was attached to an eccentric which activated a perforated metal leaf in a mild squeezing action against the stationary center plate within the bath. The rate of simulated peristalsis action was maintained at 11 strokes per minute. In the closed position there was a 0.5-in. clearance between the leaf and plate, while in the open position the space was 3.5 in.

Plastic Whirl-Pak pouches (NASCO, Fort Atkinson, Wis.) were used as digestion tubes. Eight 6.5-in. by 3-in. tubes were attached to metal rings secured with extension clamps. Each clamp was held by a dual clamp holder attached to a metal bar placed across the upper edge of the water bath so that the tubes were suspended between the squeezing plates.

Peptic digestion. The digestion mixture used in this study consisted of 0.5 ml of normal hydrochloric acid, 2.0 ml of a 2.1% solution of New Zealand veal rennet extract and 5.0 ml of a 1% solution of swine pepsin (1:10,000).

Four digestion tubes were used for each sample analyzed, 7.5 ml of the artificial digestion mixture being added to each tube prior to placing in the water bath at 37.2°C to temper for 5 min. Then 50 ml of the sample, previously tempered to 37.2°C, were added to each tube by means of either a 50-ml pipette or a 50-ml graduated cylinder, depending upon the viscosity of the samples. Firm bodied yogurt samples were weighed out to approximately 50g in disposable aluminum weighing dishes. The samples were allowed to stand for 15 min, after which 10 ml of the 1% solution of pepsin were added to each tube. One tube was then removed as the control, representing zero time of digestion. The mechanism for simulating peristalsis was set in motion and continued for the 3-hr period of digestion. After each half hour of digestion, 0.75 ml of normal hydrochloric acid was added to each tube to progressively lower the pH. At the end of 1, 2 and 3 hr, the second, third and fourth tubes from each sample group were removed for analysis.

Each tube removed from the apparatus was emptied into a beaker containing 25 ml of 40% formaldehyde and approximately 75 ml of water. The tube was rinsed with distilled water and emptied into the beaker, the final volume being approximately 250 ml. Samples in formaldehyde solution were allowed to stand for at least 24 hr in order to harden the curd particles.

Curd particle size determination. The curd particles of different sizes were separated by a screening method. A piece of moistened filter paper (Whatman #2) was placed in a Buchner funnel attached to a suction flask and aspirator, actually a Buchner water booster. A telescoping screen unit consisting of 4-, 10-, 20- and 40-mesh individual, 5-in. screens was set on top of the filter paper. The formaldehyde treated samples to be fractionated were then poured through the 4-mesh screen and washed thoroughly with distilled water until all particles capable of passing through the 4-mesh had been rinsed onto the next screen. The 4-mesh screen was removed and the 10-mesh, 20-mesh and 40-mesh screens, as well as the filter paper, were treated similarly.

Nitrogen determination

The curd particles from each screen plus the filter paper, and an aliquot of the filtrate were transferred to separate Kjeldahl flasks and analyzed for nitrogen. In this study, when the amount of curd particles from an individual screen or the filter paper was greater than 5–7g, that fraction was divided between two Kjeldahl flasks and a total value was calculated for the fraction, or a representative sample of the fraction was analyzed. Nitrogen determinations were made according to the improved Kjeldahl method for milk prescribed by AOAC (1965). However, caprylic alcohol was used instead of paraffin to reduce frothing during the digestion.

Total free amino acid determination

Analyses were also made to determine changes in free amino acids using a modified ninhydrin colorimetric procedure (Rosen, 1957).

Analyses

Study no. 1: [A preliminary study to determine differences in digestibility between (a) the raw mix and samples taken during various stages of processing, and (b) various fruit-flavored yogurts as compared to plain yogurt.] Samples from the various stages of processing, as well as raspberry, apricot, strawberry and boysenberry fruit-flavored yogurts, and blueberry and raspberry Swiss-style yogurts were digested by the previously described method. All fruit samples were stirred 50 times by hand in order to distribute the fruit preserves. A Beckman pH meter (Model H2) was used to determine the pH of the refrigerated and tempered samples, and of the digested samples after 0, 1, 2 and 3 hr of digestion. Curd particle size and Kjeldahl nitrogen determinations were made on all samples.

Study no. 2: [A study to determine the time required for the raw mix to reach the same stage of digestion as the finished product.] The finished yogurt sample was analyzed after the usual 0-, 1-, 2- and 3-hr digestion periods, while the raw mix sample was examined after 0-, 3-, 6- and 9-hr intervals. Digestibility was determined by the prescribed method of curd fractionation and nitrogen determinations.

Study no. 3: [A study to determine the changes in free amino acid and nonprotein nitrogen fractions of yogurt during various stages of manufacture.] Samples were subjected to the digestion method, removed from the apparatus at 0-, 1-, 2- and 3-hr intervals, and the contents of each digestion tube were emptied into separate centrifuge bottles. Samples were centrifuged in an International centrifuge for 10 min at 2500 rpm and filtered (Whatman #42). To completely precipitate the proteins, 5g of trichloroacetic acid TCA (12%) and 5g of filtrate were weighed into plastic centrifuge tubes, centrifuged in a Superspeed Angle centrifuge (Servall) for 10 min at about 3000 rpm, and filtered (Whatman #42). The Kjeldahl method was applied to aliquots of the final filtrate in order to determine nonprotein nitrogen. A 1-ml aliquot of the filtrate was diluted to 100 ml used for the determination of free amino acids by the modified ninhydrin method.

Study no. 4: [A study of the digestibility characteristics of nonstabilized yogurt versus yogurt stabilized with gelatin.] Differences in the total nitrogen, nonprotein nitrogen, and free amino acids of the finished products were examined after in vitro digestion.

RESULTS & DISCUSSION

Study no. 1.

The results of pH readings before and after hourly intervals of digestion are shown in Table 1. It was quite evident that the finished product, acidified by the lactic acid culture, had a pH considerably lower than the processed milks. The pH of the samples was slightly lower after tempering, still lower with the addition of the enzyme mixture, and progressively decreased with the addition of hydrochloric acid at 0.5-hr intervals throughout the digestion process.

The distribution of protein in the yogurt samples, before and during digestion, is shown in Table 2. Incomplete results were obtained in a few instances due to difficulties in the Kjeldahl procedure. However, comparison of the 2-hr data demonstrates the distributive trend of this study. There were only small differences noted in the protein distribution among the preheating, homogenization and holding stages. In comparing the raw mix with the final product, however, there was a definite decrease in the amount of large curd particles (4 mesh), and a significant increase in the precipitate and filtrate portions. Thus, on the basis of this procedure, the finished yogurt product was found to be far more digestible than the mixture from which it was made.

These data concur with the observations of Doan and Dizikes (1942) which indicated that buttermilk, heated milk, and homogenized milk were more easily digested than untreated milk.

There was little variation in the pH values (Table 3) for the various fruit yogurts. The protein distribution data (Table 4) demonstrated that there were no significant differences in the digestibility

Table 1—pH values for samples from various stages in the processing of yogurt, before and during digestion

Stage of digestion	Sample[a] 1	2	3	4	5
	pH				
After refrigeration	6.77	6.74	6.75	6.58	4.14
After tempering	6.44	6.35	6.47	6.38	4.01
Control[b]	5.31	5.19	5.50	5.36	3.89
After 1-hr digestion	4.78	4.77	4.79	4.77	3.82
After 2-hr digestion	4.12	4.23	4.07	4.15	3.59
After 3-hr digestion	3.58	3.61	3.59	3.64	3.29

[a] 1—Raw mixture prior to heating; 2—After homogenization, regeneration, and heating to 85°C; 3— After 10 min holding at 85°C; 4—After cooling 44.4-45.5°C and adding culture; 5—Finished product

[b] Sample representing zero time of digestion, i.e., removed from digestion apparatus after 15 min tempering period and the addition of pepsin, but prior to the start of simulated peristalsis

Table 2—Distribution of protein (%) in yogurt samples from various stages in processing, before and during digestion

SAMPLE	4 mesh	10 mesh	20 mesh	40 mesh	ppt	filtr
	%					
[1] Control	incomplete results					
1 hr	incomplete results					
2 hr	56.624	–[a]	–	–	4.640	38.736
3 hr	49.514	0.678	0.619	0.324	3.772	45.093
[2] Control	incomplete results					
1 hr	60.157	1.114	0.393	0.426	0.754	37.156
2 hr	51.617	1.109	0.571	0.539	3.551	42.613
3 hr	incomplete results					
[3] Control	1.683	5.892	24.208	11.052	6.002	51.163
1 hr	incomplete results					
2 hr	51.506	2.551	0.676	0.738	1.659	42.870
3 hr	36.373	2.505	0.977	0.902	1.378	57.866
[4] Control	incomplete results					
1 hr	incomplete results					
2 hr	29.246	4.474	1.574	1.436	4.115	59.155
3 hr	31.206	6.113	1.341	0.831	1.930	58.579
[5] Control	–	–	–	–	57.983	42.017
1 hr	–	–	1.134	1.572	49.031	48.263
2 hr	–	–	–	–	41.962	58.038
3 hr	–	–	–	–	36.667	63.333

[a] Dash (–) means no curd particles on the mesh.

of the various flavored yogurts. These values compared favorably with the plain yogurt (Sample #5, Table 2). Although some protein was observed in the screenings, this was undoubtedly due to the protein found on or in the particles of fruit.

Study no. 2.

Protein distribution data show that after 6 hr of digestion the raw mixture had achieved approximately the same stage of digestion as the finished product after 3 hr of digestion (Table 5). The percent protein in the filtrate fraction of the raw mix after 6 hr was 71.87%, and the finished product after 3 hr contained 71.73%. Thus, the data indicate that the raw mixture required approximately twice the time to reach the same stage of digestion as the finished yogurt. However, it should be recognized that even after 9 hr of digestion, 15% of the protein in the raw mixture was still in the form of curd particles. Therefore, caution should be exerted in interpreting the data since a loss of enzymatic activity may have occurred after several hours of incubation.

The results of this study indicate that the proteins of yogurt are digested twice as rapidly as the basic yogurt mix. This is in agreement with the conclusions drawn in an earlier yogurt digestion study in which the rates of gastric digestion of yogurt and homogenized milk were compared (Anonymous, undated). It should be noted, however, that the total solids contents of homogenized milk and yogurt differ which probalby influenced the results of the earlier study. The total solids contents of both the basic yogurt mix and the final yogurt product were held constant in the present study, thus eliminating this factor as a variable.

Study no. 3.

Analyses which were made to determine quantitatively the nonprotein nitrogen and free amino acid fractions of the yogurt samples (Table 6) indicate that the free amino acids present in the finished yogurt are more than twice the amount present in the raw mixture. After 0 hr (control) and 1 hr of digestion, the raw mix had free amino acid contents of 2.6 and 3.9 μM/ml respectively, while the finished product showed corresponding values of 7.3 and 7.6. The free amino acid content of the raw mixture increased at a more rapid rate than in the finished yogurt during digestion. When the results of each sample are plotted as a bar graph against the free amino acid contents, extrapolation of the raw mixture (Sample #1) to 6 hr indicates the probable attainment of the same level of free amino acids as in the finished product after 3-hr digestion. Thus, based upon the free amino acid content, the raw mix required twice the time to reach the same stage of digestion as the finished product. It was also evident that the percent nonprotein nitrogen increased througout digestion. Nonprotein nitrogen values showed a nonsignificant, but positive correlation with free amino acid values, the correlation coefficient being 0.689.

The results of this study indicate that the quantity of free amino acids increases during yogurt manufacture, from the yogurt milk to the final product. Rasic et al.

Table 3–pH values for fruit flavored yogurts, before and during digestion

Stage of digestion	Sample[a]					
	Rasp	Apr	Straw	Boysen	Ss Rasp	Ss Blue
	pH					
After refrigeration	4.34	4.22	4.21	4.03	4.39	4.36
After tempering	4.16	4.03	4.10	3.96	4.23	4.20
Control	4.11	4.01	3.79	3.77	4.20	4.13
After 1-hr digestion	3.87	3.88	3.73	3.69	4.03	3.96
After 2-hr digestion	3.55	3.60	3.41	3.40	3.67	3.60
After 3-hr digestion	3.25	3.29	3.13	3.05	3.33	3.19

[a] Raspberry, apricot, strawberry, boysenberry, Swiss-style raspberry and Swiss-style blueberry yogurts

Table 4–Distribution of protein (%) in various flavored yogurts, before and during digestion

	4 mesh	10 mesh	20 mesh	40 mesh	ppt	filtrate
	%					
Raspberry						
Control	–[a]	2.179	1.533	1.937	57.744	36.607
1 hr	–	2.661	1.096	2.778	46.561	46.905
2 hr	–	2.418	1.188	–	37.907	58.487
3 hr	–	1.811	1.523	1.481	32.178	63.007
Apricot						
Control	–	1.018	0.952	2.693	44.266	51.071
1 hr	–	1.179	1.142	2.983	41.102	53.595
2 hr	–	–	1.068	1.913	34.386	62.633
3 hr	–	–	1.105	2.485	32.493	63.918
Strawberry						
Control	–	1.078	1.110	1.273	45.971	50.568
1 hr	1.463	1.428	1.219	1.463	38.247	56.180
2 hr	1.697	2.910	1.455	1.172	35.157	57.609
3 hr	–	1.395	1.006	0.844	22.811	73.944
Boysenberry						
Control	–	–	1.366	1.400	47.190	50.044
1 hr	–	–	0.742	0.766	28.794	69.698
2 hr	–	–	1.168	1.515	36.997	60.320
3 hr	–	–	1.151	1.637	30.445	66.767
Swiss style raspberry						
Control	–	–	1.123	0.682	59.180	39.015
1 hr	–	1.096	0.709	0.773	38.343	59.080
2 hr	–	0.859	0.892	0.693	30.872	66.684
3 hr	–	0.984	0.923	0.677	27.132	70.284
Swiss style blueberry						
Control	–	0.424	1.043	0.912	43.983	53.639
1 hr	–	0.828	1.017	0.866	40.628	56.664
2 hr	–	0.849	0.926	0.810	35.640	61.776
3 hr	–	0.693	0.948	0.693	26.723	70.944
Overall ranges						
Control	–	0.0–2.2	1.0–1.5	0.7–2.7	44.0–59.2	36.6–53.6
1 hr	0.0–1.5	0.0–2.7	0.7–1.2	0.8–3.0	28.8–46.6	46.9–69.7
2 hr	0.0–1.7	0.0–2.9	0.0–1.5	0.0–1.9	30.9–37.9	57.6–66.7
3 hr	–	0.0–1.8	0.9–1.5	0.7–2.5	22.8–32.5	63.0–73.9

[a] Dash (–) means no curd particles on the mesh.

Table 5—Distribution of protein (%) in raw mixture and finished yogurt, before and during digestion

	4 mesh	10 mesh	20 mesh	40 mesh	ppt	filtr
			%			
Raw mixture						
Control	67.376	–[a]	–	–	0.323	32.301
3 hr	52.989	–	–	–	6.839	40.172
6 hr	26.387	–	–	–	1.742	71.871
9 hr	15.408	1.318	0.745	0.630	2.006	79.893
Finished product						
Control	–	–	–	–	59.580	40.420
1 hr	–	–	0.508	0.393	46.143	52.956
2 hr	–	–	–	–	32.143	67.460
3 hr	–	–	–	–	28.268	71.732

[a] Dash (–) means no curd particles on the mesh

Table 6—Nonprotein nitrogen (NPN) and free amino acid (FAA) contents of TCA filtrates obtained from yogurts during various stages of manufacture, before and during digestion

	Control		1 hr		2 hr		3 hr	
Sample	NPN (%)	FAA (μM/ml)	NPN (%)	FAA (μM/ml)	NPN (%)	FAA (μM/ml)	NPN (%)	FAA (μM/ml)
1	0.161	2.6	0.195	3.9	0.252	5.6	0.240	6.2
2	0.156	2.7	0.221	3.7	0.255	5.6	0.277	5.4
3	0.161	3.2	0.212	3.7	0.260	4.8	0.311	4.8
4	0.184	5.6	0.238	6.7	0.288	7.3	0.308	8.1
5	0.249	7.3	*	7.6	0.345	8.2	0.365	9.2

* No datum

Table 7—Distribution of protein (%) in nonstabilized yogurt and yogurt stabilized with gelatin, before and during digestion

	4 mesh	10 mesh	20 mesh	40 mesh	ppt	filtr
			%			
Nonstabilized yogurt						
Control	–[a]	–	–	–	59.580	40.420
1 hr	–	–	0.508	0.393	46.143	52.956
2 hr	–	–	–	–	32.540	67.460
3 hr	–	–	–	–	28.268	71.732
Stabilized yogurt						
Control	–	–	–	–	57.673	42.327
1 hr	–	–	–	–	52.262	47.738
2 hr	–	–	–	–	40.299	59.701
3 hr	–	–	–	–	39.319	60.681

[a] Dash (–) means no curd particles on the mesh

Table 8—Nonprotein nitrogen (NPN) and free amino acid (FAA) contents of TCA filtrates obtained from nonstabilized yogurt and yogurt stabilized with gelatin, before and during digestion

	Control		1 hr		2 hr		3 hr	
	NPN (%)	FAA (μM/ml)	NPN (%)	FAA (μM/ml)	NPN (%)	FAA (μM/ml)	NPN (%)	FAA (μM/ml)
Nonstabilized yogurt	0.246	6.4	0.263	6.1	0.303	8.1	0.325	8.7
Stabilized yogurt	0.317	6.1	0.356	7.6	0.372	8.3	0.452	9.2

(1971) found that a significant increase in the biological values of proteins from different kinds of yogurt occurred during yogurt manufacture. The biological values of the proteins were estimated from a pepsin, pancreatin digest index, which was based on the release of eight essential amino acids. Admittedly, the methods of Rasic et al. (1971) were more sophisticated than the total amino acid determinations used in this study, but it may be stated that an increase in free amino acids occurred during yogurt manufacture.

Miller and Kandler (1964) found that yogurt manufactured with *L. bulgaricus* and *S. thermophilus* had about 300–500 mg free amino acids (FAA) per liter, while strongly acidified yogurt had considerably more free amino acids. In comparing the data obtained in this study to those of Miller and Kandler, it is evident that the raw mix after 0 hr and 1 hr of digestion yielded values which were within this range, i.e., 341 and 510 mg FAA/liter. The finished product, however, after the same digestion intervals had free amino acid contents which were considerably higher, i.e., 956 and 995 mg FAA/liter. Higher values may have been due to the different methods used to determine the free amino acid contents.

Study no. 4.

In this experiment the protein distribution data (Table 7) show that although both yogurts were highly digestible, the nonstabilized product had a definitely greater quantity of protein available in the filtrate throughout the digestion period, whereas the stabilized product had a larger quantity of protein in a precipitated form. Although the stabilized yogurt showed higher nonprotein nitrogen and free amino acid values than the nonstabilized yogurt (Table 8), these values did not differ significantly. Although Doan and Dizikes (1942) found that the digestibility of milk treated with locust bean gum was affected adversely by such treatment, there was little or no difference observed in the digestibility of the yogurts, based upon the criteria of this study.

CONCLUSIONS

THE PURPOSE of this study was to determine the effect of various steps in the processing of yogurt upon its digestibility characteristics; the digestibility index being the distribution of total protein and protein fractions after in vitro digestion. The results have led to the following conclusions: (a) yogurt is more digestible in vitro than the raw mixture from which it is made; (b) there is no significant difference in the digestibilities of various fruit-flavored yogurts, their characteristics being very similar to that of plain yogurt;

(c) the raw mixture requires twice the amount of time to attain the same stage of digestion as the finished yogurt; (d) the finished yogurt contains twice the amount of free amino acids as the raw mixture; and (e) there is little or no difference in the digestibilities of nonstabilized and stabilized yogurts.

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MOLECULAR SPECIES OF PHOSPHORYLASE IN POSTHARVEST POTATO TUBERS

INTRODUCTION

STORAGE of potato tubers at 4°C generally causes degradation of starch and accumulation of free sugars (sucrose, glucose and fructose). The tendency of tubers to accumulate reducing sugars may be determined by variety (Akeley et al., 1965), cultural practices (Hart and Smith, 1966), physiological stage of development (Yamaguchi et al., 1966) and environmental parameters (Yamaguchi et al., 1964). Because of the economic importance of this phenomenon, much attention has been given to understanding the metabolic control of carbohydrate metabolism in potato tubers.

Phosphorylase (α-1,4-glucan: orthophosphate glucosyltransferase, E.C. 2.4.1.1) is believed to play a focal role in the metabolism of starch (Whelan, 1961; Nordin and Kirkwood, 1965; Henderson, 1968). Hyde and Morrison (1964) reported that phosphorylase activity was generally greater in tubers stored at 4°C than those conditioned for 4 wk at 21°C. For example, extracts from Kennebec tubers exhibited a 25% reduction in total phosphorylase activity after conditioning.

Several laboratories have reported that plant phosphorylases exist in isozyme species (Siepmann and Stegemann, 1967; Gerbrandy and Verleur, 1971; Tsai and Nelson, 1969; DeFekete, 1969). Gerbrandy and Verleur (1971) observed nine electrophoretically distinguishable bands with phosphorylase activity in extracts from immature potato tubers and one isozyme in extracts from mature potatoes. During periods of starch breakdown in growing tubers and cotyledons of *Vicia faba* and *Phaseolus vulgaris* the fast moving bands possessed a relatively high activity. Tissues undergoing starch synthesis showed more activity in the slower moving zones. Recently, Lee (1972) presented evidence that phosphorylase, from sweet corn, exists in multiple forms composed of three monomer species.

We have examined the electrophoretic patterns of phosphorylase in low temperature stored and conditioned potato tubers. The two varieties examined differed widely in their propensity to accumulate reducing sugars during storage at 5°C.

MATERIALS & METHODS

Materials

Mature potato tubers (*Solanum, tuberosum* L. var. Monona and var. Kennebec) were stored at 5°C for 1–4 months after harvest. Potatoes were conditoned at 20°C for up to 4 wk. Kennebec tubers accumulate substantial amounts of reducing sugars during low temperature storage, whereas the Monona variety does not undergo appreciable loss of starch (Deppen, 1968; Haard, 1971).

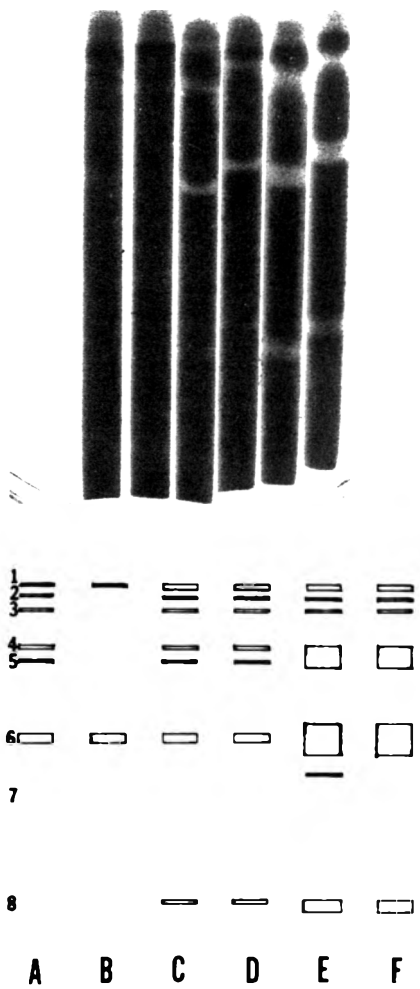

Fig. 1—Photographic and diagrammatic representation of polyacrylamide gels of extracts of cold stored tubers assayed for starch hydrolysis: (A) Kennebec, 1-hr assay at 30°C; (B) Monona, 1-hr assay at 30°C; (C) Kennebec, 5-hr assay; (D) Monona, 5-hr assay; (E) Kennebec, 18-hr assay; (F) Monona, 18-hr assay. Electrophoresis was at 3 ma/tube for 2.5 hr. Sample volume applied to the gel was 50 µl.

Preparation of tissue extracts

Tubers were peeled, thinly sliced and frozen in liquid nitrogen. The frozen tissue was powdered in an Omnimix blender for 1 min. A 25-g sample of powder was mixed with 50 ml of an isolation medium containing sodium citrate (0.1M, pH 6.0); sucrose (0.4M); $Na_2S_2O_5$ (0.0020M); Na_2SO_4 (0.0035M); and EDTA (0.002M). The homogenate was centrifuged for 60 min at 60,000 × G and 5°C. The resulting supernatant fraction was directly assayed for phosphorylase or applied to gels for electrophoresis.

Gel electrophoresis

Polyacrylamide gel electrophoresis was performed according to Maurer (1968) using a 7% acrylamide gel and Tris-glycine buffer, pH 8.3. Gels assayed for the synthetic reaction contained 0.1% oyster glycogen (Calbiochem) as a primer; and, those which were assayed for the starch degradation reaction, contained 0.2% amylopectin (Calbiochem). Electrophoresis was performed for 2.5 hr at 3 ma per tube and at a temperature of 5°C. Phosphorylase activity was demonstrated in the gels by one of two procedures. For the synthetic reaction (gels containing glycogen primer) the electrophoresed gels were incubated in 0.1M sodium citrate (pH 4.3) and 0.025M glucose-1-phosphate (Sigma Chemical Co.) for 1–18 hr at 30°C. For the degradative reaction (gels containing amylopectin) the gels were incubated in 0.1M sodium citrate and 0.1M sodium phosphate (pH 6.0) for 1–18 hr. After incubation, gels were stained with a solution of 0.01M I_2 and 0.014M KI. Starch synthesis was indicated by a dark blue disc on the gel and the degradative reaction was evidenced by clear zones in a blue background.

Phosphorylase assay

Extracts were assayed for phosphorylase activity by the procedure of Lee (1966), using sodium maleate buffer (0.10M, pH 6.3).

RESULTS & DISCUSSION

EXTRACTS prepared from cold stored Monona and Kennebec tubers showed very similar distribution of activity on polyacrylamide gels (Fig. 1 and 2). Gels assayed for the degradative reaction contained one major zone (Zone 6) which was of identical Rf for both Monona and

Kennebec (Fig. 1). A 1-hr incubation time was sufficient for Kennebec to develop four additional zones of low activity while Monona showed only one minor species after this assay time. Following 18 hr incubation both gels revealed an active species with high mobility (Zone 8) and in addition, Kennebec showed a faint second zone of activity (Zone 7). The most conspicuous difference between extracts of two varieties was the greater apparent activity of zones with an Rf less than the major zone (Zone 6).

Gels assayed for the synthetic reaction showed similar activity for the two potato varieties. Both samples showed one predominent species (Zone 5) which usually appeared as a doublet during the initial minutes after iodine stain was applied. Although the Rf of this zone was different from that of the major zone observed with degradative gels they were shown to be the same species. This was accomplished by incubation of the degration gel in the synthesis assay medium. A 1-hr incubation was sufficient to reveal three additional minor zones in Kennebec extracts; these minor zones appeared in Monona gels after longer incubation times (Fig. 2). Zone 8 observed with the degradative assay, did not appear on synthetic gels after 48 hr incubation.

The gel patterns of phosphorylase from conditioned Monona and Kennebec were identical to those observed in cold stored tubers. There was no obvious change in phosphorylase associated with the conditioning of either potato variety. Extracts from germinating tubers showed patterns of activity which differed markedly from the dormant tubers. These samples were distinguishable by a more prominent activity in zones of high electrophoretic mobility. These results were consistent with the data communicated for germinating Binjtl potato tubers (Gerbrandy and Verleur, 1968).

The phosphorylase activity of extracts ranged from 1.0–1.2 (ΔA_{700} nm/min-ml) and was the same for the two varieties. This was consistent with the similarity in total activity evidenced on gels.

The data reported here indicate that the low temperature induction of starch degradation in potato tubers is not related to the appearance of additional total phosphorylase or to the formation of unique isozyme or molecular species as appears to occur during tuber germination. The greater apparent activity of minor phosphorylase species in extracts of Kennebec may be a meaningful observation. Further studies in our laboratory will be directed toward the isolation of these molecular species and understanding the metabolic control of phosphorylase catalyzed starch degradation in vitro.

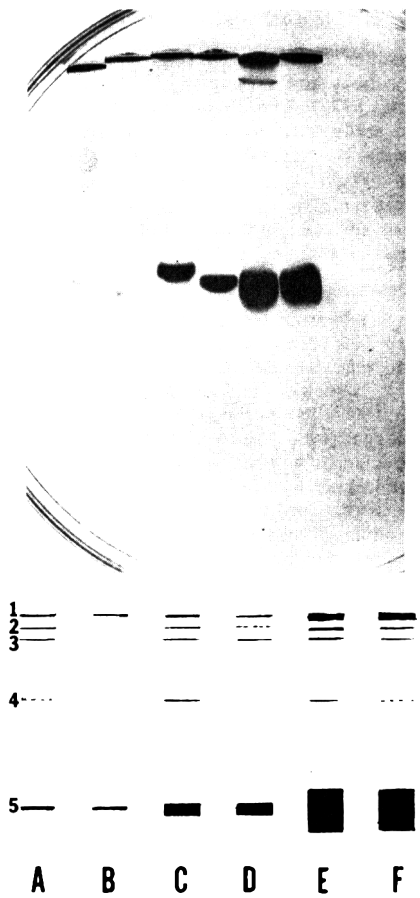

Fig. 2–Photographic and diagrammatic representation of polyacrylamide gels of extracts of cold stored tubers assayed for starch synthesis (See legend to Fig. 1).

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CORN ODOR CLASSIFICATION FROM LOW-RESOLUTION GAS-CHROMATOGRAPHIC PROFILES OF HEAD SPACE VOLATILES

INTRODUCTION

CLASSIFICATION of grain odor, at present, depends on human judgement usually as exercised by trained grain inspectors. Analytical criteria do not yet exist. A procedure based on objective data should, therefore, give results which agree with the inspectors' judgements. As in any sensory evaluation, perfect agreement cannot be expected. Conventionally, an agreement between two panels at a 95 percent ($p < 0.05$) confidence level is considered satisfactory. If a sensory panel classified 20 corn samples, by a two-choice test, as "good" or "bad" and agreed with inspectors' judgements for 15 samples, the probability is less than 0.05 that results of the two-choice test occurred by chance. Similar criteria might be applied to the classifications derived from gas chromatographic (GC) data.

An earlier work (Dravnieks et al., 1973), showed that GC patterns of headspace volatiles of corn could be correlated, through use of computerized stepwise discriminant analysis, to the odor quality of corn. These patterns were obtained by collection of the volatiles on Apiezon L-coated Teflon powder supports, reconcentration by a cryogenic process, and a 1-hr gas-chromatographic analysis with a programmed temperature rise.

Although this demonstrated the feasibility of an objective corn odor classification, the process of volatiles collection and analysis was clearly too long and too cumbersome for routine use. The next logical step was to determine whether accelerated procedures of volatiles analyses could still supply enough compositional information to distinguish the corn samples with normal odors from those with odor defects.

EXPERIMENTAL

GRAIN HEADSPACE contains many volatile components of low concentration (Hougen et al., 1971; Dravnieks et al., 1973), making preconcentration necessary before analysis. Organic nonpolar polymers with large surface areas and considerable thermal stability have come into use as ambient-temperature adsorbents to collect organic vapors from air in the presence of water vapor (Dravnieks et al., 1971; Dravnieks and O'Donnell, 1971; cf. Krumperman, 1972). The pre-concentration process in our study was based on use of such polymers. Special consideration was given to the design of the collection and analysis systems to permit a short sampling/analysis cycle, assure stability of flow and temperature parameters and reduce the occurrence of artifacts.

Adsorbent

A polyaromatic nonpolar polymer, Chromosorb 105 (Johns-Manville, 1970), 60/80 mesh, with reported surface area of 600–700 m^2/g, was selected as an ambient-temperature adsorbent. It was purified by heating in a zero-grade nitrogen stream at 250°C for 24 hr. The vapor adsorber consisted of thin-wall (0.01 in.) stainless steel tubing, 3/8 in. o.d. and 4 in. long, containing approximately 1g of the polymer secured by Pyrex glass wool plugs. Desorption was effected by heating the adsorber and passing electrical current through its thin wall, to 180°C, with an initial overshoot to 200° C. The relatively large diameter of the adsorber is needed to permit rapid headspace sampling; at the same time, because of the limited thermal conductivity of the polymer, it lengthens the desorption by delaying the establishment of the final desorption temperature throughout the polymer. The selected cross-section is a compromise.

Gas-chromatographic column

The column tubing was 1/8-in. o.d. thin-wall Teflon inner-coated stainless steel, 6 ft long. The packing was 60/80 mesh Supelcoport, (an acid-washed, silanized ceramic type support, Supelco Co., Bellefonte, Pa.) coated with 10 wt % SP-1000 stationary phase. This phase is similar, in its polarity, to Carbowax 20M, but is thermally more stable and produces adequately shaped peaks with most organic compounds, including free fatty acids thought to be of possible importance in vapors from spoiled grain.

Other features

In the path of the sample vapors valving was avoided, to reduce the possibilities of vapor trapping and leakages at the valve connections. All tubing was joined by silverbrazing wherever possible. Temperature or pressure cycling in the column also was avoided, to eliminate the sources of instability in performance as much as possible, and to facilitate rapid recycling to the analytical phase of the process.

Practical considerations

It was desired to reduce the evaluation time to 10–15 min, so that the entire system could recycle to its starting condition, ready to take a headspace sample from another charge of grain, within that time. This precluded maintaining scrupulous cleanliness in the sample container or in the tubing connecting this vessel to the sampling port. It was hoped that each sample would sufficiently overwhelm the residual traces of volatiles from the previous sample to permit a classification. Consequently, cf. the following description, the corn sample vessel was merely wiped with a fresh paper napkin to remove the previous sample's dust, the glass wool plug holding the corn was replaced with a fresh plug, and the new sample was placed into the vessel.

Description of apparatus and samples

Figure 1 shows the principal parts of the apparatus. Approximately 1 liter of corn is placed in a 3 in. i.d. glass vessel, A, provided with a flanged Teflon ring seal; a glass wool plug, B, holds the grain. Nitrogen is supplied to the vessel through a tee, C, with excess nitrogen exiting through flowmeter, D. The connecting tubes are Teflon. Initially, before connecting the bottom Teflon tubing to the device's sampling port, E, 100–200 ml of nitrogen are used to nominally replace the previous sample's vapors in this tubing, by the new sample's vapors. The sampling port, E, consists of a commercial Teflon compression plug (Conax fitting, Conax Corp., Buffalo, N.Y.) A 1/8 in. o.d. stainless steel tubing stub, inserted into the sampling vessel's Teflon tubing, is used to connect this tubing to the device. When the sampling is completed, this stub is withdrawn and the compression fitting is sealed by a stainless steel plug.

The adsorber, F, connects via solenoid valve, G, to the peristaltic sampling pump, H. Volume of the gas sample is measured with wet-test meter, I. A blower, J, assures maintaining ambient temperature in F during sampling. A compound manometer, K, monitors pressure at the end of the adsorber: negative with respect to the atmospheric during the sampling, positive during the analysis.

The vertical position of the adsorber is an important feature; it assists in maintaining more equal temperature around the periphery of the adsorber, and assures that the thermocouple, L, which is used to monitor the skin temperature of the adsorber, indicates the highest temperature. The vertical position also prevents undesirable cross- or against-the-carrier-gas-flow internal convection currents such as occur in other positions in the adsorber during its heating cycle.

Carrier gas is supplied through flowmeters and a constant flow control valve, R, at 20

ml/min. During sampling, the solenoid valve, T, is open, U is closed; the carrier gas enters the system between the GC column, V, and an 8 in. long 0.006 in. i.d. stainless steel capillary, Q. The purpose of this connection is to supply the column with carrier gas despite a reduced pressure in the adsorber, F. During sampling, a small amount of carrier gas flows through the capillary, Q, towards F, and thus protects the column from an undesirable intrusion of the sample vapors.

Sampling duration is 90 sec; volume taken typically 1.42 liters (0.05 cu ft). Then G is closed, H is stopped, and port E closed with a rod. 2 min are allotted for an equilibration of the pressures before the analysis begins. The port E and all tubing connecting it to F and Q are constantly heated by heating tape, while the capillary Q and the column V are in an aluminum block thermostatted at 68°C.

The sample injection and analysis occur as follows. Simultaneously, the blower, J, is stopped, T is closed, U is open, and the heating of the adsorber is initiated by feeding 100A current, from a TV filament transformer, M, through leads m. The electrical resistance of leads (stainless steel tubing leads are used) is dimensioned so that they heat up similarly to F; therefore, the upper lead m does not generate a cold spot in the sample elution path on the adsorber skin. An optical contact pyrometer, P, operated from the thermocouple, L, and a relay, O, begins to regulate the adsorber's temperature as soon as a pre-set temperature limit is reached. A Variac N supplies the current to M.

The column effluent is monitored by a hydrogen flame ionization detector, W. An electrometer, X, feeding its output through an electronic integrator, Y, produces a gas-chromatographic record on the chart of the recorder, Z.

Data reduction

The conventional gas-chromatographic data, such as supplied by an electronic integrator with a digital printout, consist of enumeration of the peak retention times and the areas under each of the peaks. The retention times of the same peak in analyses of different samples shift if the peak represents several gas-chromatographically unresolved components occurring in different ratios. Shifts also can be caused by variations in the operational parameters: flow rate, temperature, rate of temperature rise in the adsorber, moisture content of the sample (water vapor present in substantial amounts can temporarily change the polarity of the stationary phase), and definition of the starting point. It was shown earlier (Dravnieks et al., 1973) that reduction of such data to sets of uniform variables suited for a later computerized statistical treatment of many samples requires assumptions such as whether a peak in one sample may be considered to correspond to a certain peak in the other samples; it also was shown that the statistical pattern discrimination is relatively tolerant of the method by which gas-chromatograms are subdivided into variables.

With a view to eventual automatic data and sample classification, the simplest possible mathematical logic was selected to reduce the gas chromatograms to sets of variables. Digital printer ZZ printed out the integrated electrometer current, equivalent to the area under the recorder-drawn trace, separately for each 10-sec interval of the GC analysis. An external timing circuit, ZZZ, directed by a synchronously driven cam, served to obtain such mode of operation from the integrator-printer combination.

The first integration was initiated when the adsorber reached 180°C; the chromatogram was continued for 7.5 min, and thus resulted in a set of 45 uniform variables. Each variable approximately represented the total organics in the volatiles collected for the respective 10-sec period. In essence, this expressed the complex GC profile in terms of vertical slices of uniform width, but different lengths; the process might be likened to the use of a contour gage in reproducing irregular shapes in carpentry.

Samples

52 corn samples were analyzed. US grain inspectors classified the odor of 20 of these as "good." The rest of the samples had "musty" or "sour" odor to various extents; four of the musty samples carried also an "insect odor" note.

RESULTS & DISCUSSION

TYPICAL BLANK and three gas-chromatographic profiles are shown in Figure 2. The "good corn" chromatogram is supplied with time marks for the start of the adsorber heating, the start of integration, and the end of the adsorber heating, coincident with the end of the integration. The Hydrocarbon (Kovats) Index scale marks also are indicated. The two analyses of the same musty corn sample demonstrate the degree of the instrumental reproducibility. The electronic attenuation of the signal from the integrator to the recorder was 1:32. The zero level for the integration was set equivalent to the electrometer current observed just prior to the start of the adsorber heating. This

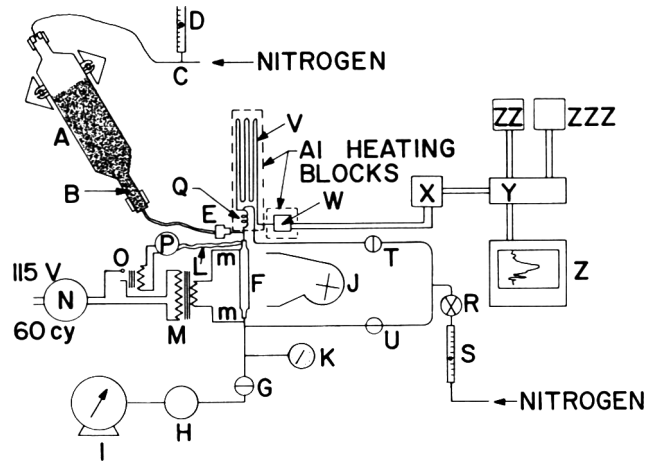

Fig. 1—Corn volatiles sampling and analysis apparatus.

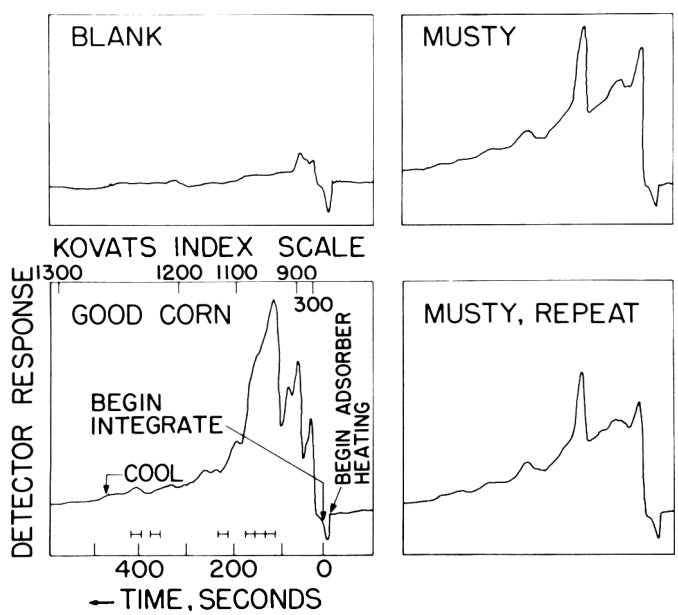

Fig. 2—Examples of blank and corn volatiles GC profiles.

blank current was 2 to 8 picoamperes: lower after longer conditioning, higher when analyses followed each other in short sequence. The total organics in the gas chromatogram of the "good corn" shown, per calibration with alkanes, was of the order 10^{-5} g.

The overall time requirement for each analysis was 12 min: 1.5 min for sampling, 1–2 for changes to the analysis mode, 7.5 min for the analysis proper, and the remainder for cooling the adsorber and connection of the next sample. The sample in the container A was changed during the analysis of the preceding sample.

Selection of GC variables and transforms for pattern classification

The digital printer provided 45 variables per sample. Of these, the two first and the three last 10-sec intervals seemed to be relatively feature-free and were omitted from consideration. The remaining 40 were paired, reducing the chromatogram to 20 consecutive 20-sec intervals; this simplification was taken to obtain the number of variables for experimenting with various transforms more suited within the capacity of the computer programs used.

An earlier study (Dravnieks et al., 1973) indicated that the following transforms of the "peak size" variables might have merit, and were considered in the pattern discrimination: logarithms of the peak areas [after the usual addition of one unit to area, to obtain log (area) = 0 when the peak is absent], areas expressed as percent of the sum of the areas of all peaks, and some peak area ratios. Additional transforms considered were squares of the areas and of the percent areas; the rationale of this parabolic transform is that e.g., the "goodness" of corn odor may require an optimum concentration of a compound, while lower and higher concentrations produce odor defects.

Furthermore, it was observed that musty corn samples tend to exhibit lower concentrations of total organics, sour corn tends to have higher values, and the good odor samples tend to have intermediate values. Consequently, codes 0 and 1 were used to designate if the total organics are within or outside the intermediate range (between 10^{-5} and 2.4×10^{-5} g).

A peak tended to occur, on the relatively heavy background, at 400 sec. To emphasize its possible influence, a derived experimental variable was introduced: the difference between the area corresponding to the 20-sec interval at 400 sec and the area of the preceeding 20-sec interval.

As a result of all these considerations, the information on the samples now was contained in 104 variables—original and those obtained by various transforms. This number exceeded the capacity of the UCLA Health Sciences Facilities Program BMDO7M Stepwise Discriminant Analysis program used in the previous study (Dravnieks et al., 1973). Hence the BMDO2R Stepwise Regression Analysis Program was used to pre-screen the variables, using a group of randomly selected (13 good, 21 bad) samples. All those variables which after seven regression steps exhibited F-ratio values (these denote the levels of the statistical significance) of unity or higher, were taken as the candidate variables for the step-wise discriminant analysis. There were 21 such variables.

Stepwise discriminant analysis

The study of the corn odor discrimination process was envisioned to consist of three stages: first, a "nose" such as described under Experimental, is used to obtain GC profiles of many samples; second, a "teacher" in form of a high-performance computer (UUC 1108), takes a group of such profiles and teaches a mathematical model to produce an equation, into which certain selected features of the profiles can be inserted to calculate the relative probabilities of each sample to be either "good" or "bad"; third, the model is then applied to additional samples to check to what extent it has learned to discriminate unrelated corn sample odors. If such process shows a promise, a relatively simple digital circuit could be designed to supply the "nose" with a primitive "brain" which would process the information generated by the "nose" in the exact form prescribed by the "teacher" and indicate "good" or "bad." In the present study, such a circuit was not actually designed, but its usefulness was mathematically probed.

Randomly selected 13 good and 21 bad corn samples were used to develop the discriminant function, using BMDO7M Stepwise Discriminant Analysis. The computer program was terminated by choice at the seventh step. 13 "good" samples were all classified "good;" out of 21 "bad" samples, 19 were classified "bad", two were termed "good." The results are plotted in Figure 3. The F-ratio (7 and 26 degress of freedom) attained was 7.60, indicating that such success of discrimination could have occurred by chance with a probability $p < 0.001$.

The GC profile ranges utilized in this discrimination are indicated by segments in the "good" corn profile in Figure 2.

The most significant discriminating variable was the square of the percent area of the interval between 160th and 180th sec, followed by the ratio in the areas of this interval to the area of 120th to 140th sec interval, then by the difference between the area of the 240–400 sec and 160–180 sec intervals, and by the percent area of the 220–240 sec interval. Other variables had less significance.

When this function was applied to an additional group of 18 corn samples (7 good and 11 bad), 13 were classified correctly. If two panels would agree on 13, the agreement would be considered statistically marginally significant ($p = 0.05$). All good samples were classified correctly, with erroneous classifications of "good" for five bad samples. It appears that not all GC profile features that would lead to a "bad" sample were accounted for sufficiently in the initially developed discriminant equation. The misclassification of two bad samples as "good" in Figure 3 indicates a similar effect. It could be reasonably speculated that there are more ways to be "bad" than "good" as far as the GC profiles are concerned.

This first attempt at the statistical analysis of the profiles obtained indicates that simple volatile analyses, even after gross simplifications on the raw data.

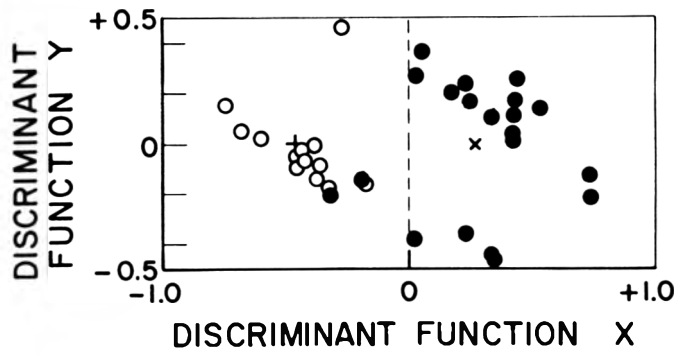

Fig. 3—Statistical discriminant analysis of corn volatiles GC profiles. (Each circle represents one sample: ○ good samples; ● off-odor samples; + denotes the center for the good samples; X denotes the center of the off-odor samples.)

supply sufficient information for corn odor classification. A simple circuitry, such as in pocket-size electronic calculators, able to perform a few elementary arithmetic operations, should suffice to convert the data into the classification readouts.

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Use of a company or product name does not imply approval or recommendation of the product by the U.S. Dept. of Agriculture to the exclusion of others which may also be suitable.

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CHEMICAL AND SENSORY PROPERTIES OF PIMENTO LEAF OIL

INTRODUCTION

KEMP (1925) analyzed the volatile components of pimento leaf (*Pimento dioica* L.) oil and reported the presence of eugenol (95.5% in free form or 96.6% total), caryophyllene, an unsaturated acid (?) $C_{13}H_{14}O_4$, an unsaturated dibasic acid (?) $C_{10}H_{14}O_4$, and an aldehyde or ketone (?). Several other workers (Anon., 1914; Anon., 1919; Campbell, 1922; Chiris, 1924; Finnemore, 1926; Gildemeister and Hoffman, 1961; Guenther, 1950) have reported eugenol concentrations in pimento leaf oil ranging from 32 to 97%. Nabney and Robinson (1972) recently identified 34 volatile compounds from a steam-distilled sample of pimento berry oil. The purpose of the present study was to identify major volatile constituents in the leaf oil and to conduct a preliminary screening of the sensory properties of the volatiles.

EXPERIMENTAL

Chemical studies

Apparatus. Preparatory gas-liquid chromatography was done on a Varian Aerograph Model 202 equipped with thermal conductivity detectors. The helium flow rate was 40 ml/min. Two 10-ft × 0.250-in OD glass columns were used: one packed with 3% Carbowax 20M on 60/80 mesh Chromosorb G, and the other with 1% SF 96(50) on 70/80 mesh Chromosorb G. The latter column was used for final separations of fractions of the oil.

Two open-tubular capillary columns, 152-m × 0.76-mm ID stainless steel, were also used in the study. One was coated with Carbowax 20M, the other with SF 96(50). Each column was housed in a Varian Aerograph Model 1200 gas chromatograph equipped with a flame-ionization detector. Flow rates for both columns were 6 ml/min nitrogen, with 30 ml/min hydrogen, and 200 ml/min air at the detector.

Infrared spectra were obtained from thin films of sample between sodium chloride plates or from solutions in a 0.1-mm salt microcavity cell, with carbon disulfide [chromatoquality, Matheson, Coleman and Bell] or tetrachloroethylene [Mallinckrodt reagent grade] as solvent. Spectra were scanned on a Perkin-Elmer Model 257 Infrared Spectrophotometer equipped with a 5× beam condenser.

Mass spectra were obtained with a Finnigan Model 1015 gas chromatograph-mass spectrometer system equipped with a System/150 computer control and data handling system. The column used was the open tubular capillary column coated with Carbowax 20M.

Materials and procedure. A sample of commercial pimento leaf oil was obtained from the research laboratories of McCormick and Company, Baltimore. Injections of 60 μliter of oil were made onto the preparatory Carbowax 20M column, which was programmed from 50–225°C at a rate of 2°C/min. Major fractions were collected and sealed in thin-walled glass capillary tubes for further analysis. The large amount of eugenol present in the oil produced a very broad peak which obscured part of the chromatogram. In some experiments, most of the eugenol was removed by dissolving the oil

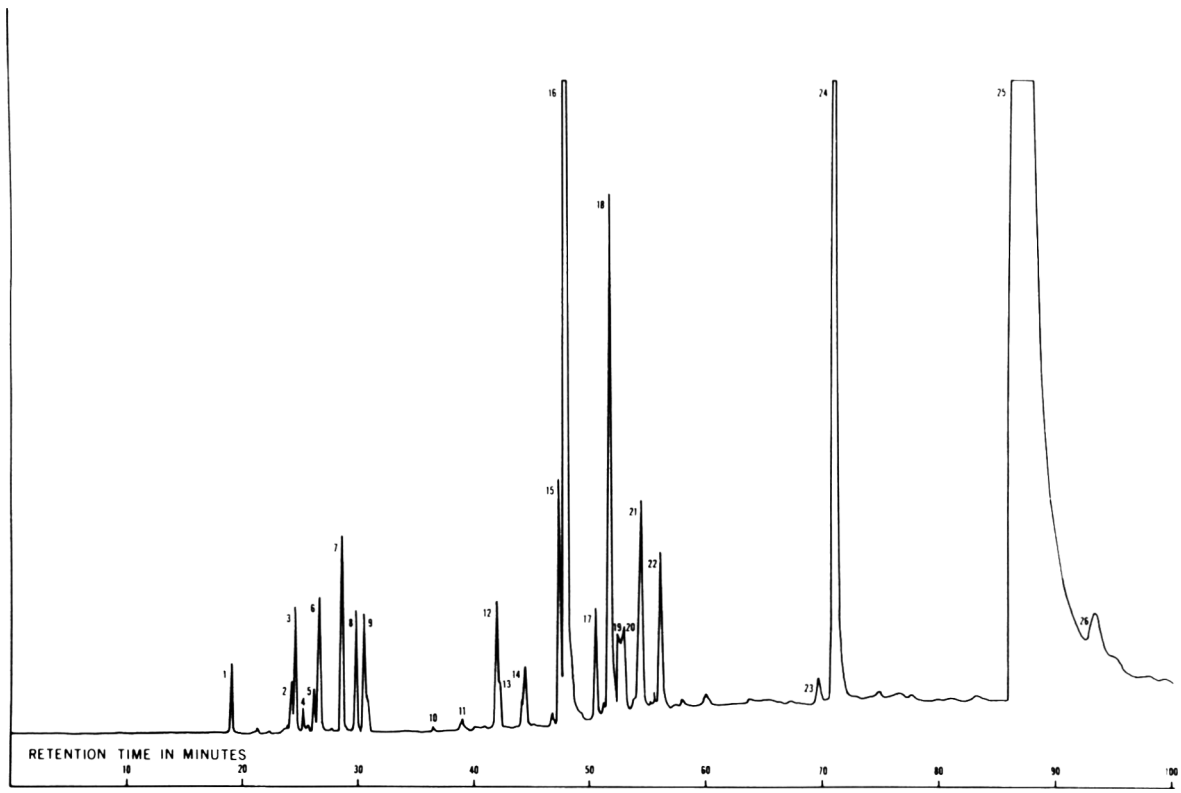

Fig. 1—Typical gas chromatogram of pimento leaf oil on Carbowax 20M column.

in an equal volume of pentane, shaking the solution with excess 2M KOH and saving the organic phase. In oil thus treated, virtually all of the eugenol was removed from the organic phase, and several new peaks were noted in the area of the chromatogram previously masked by the eugenol peak. These new peaks were also collected for further study.

Further separations of components of the oil were made on the preparatory SF 96(50) column, isothermally at 75°C (peaks 1–9), 125°C (peaks 12–22), and 175°C (peaks 24 and 25). Purified components were recollected and subjected to analysis by infrared spectrophotometry. Spectra of several commercially available compounds were compared with those from pimento leaf oil as a means of identification. Reference spectra from the literature were also surveyed as aids in the identification process.

In the mass spectral analysis a sample of the extracted pimento leaf oil was injected onto the Carbowax 20M capillary column, which was programmed from 50–170°C at a rate of 2°C/min. Effluent from the gas chromatograph was split, with approximately 1% going into the mass spectrometer and the remainder to a flame-ionization detector. Ion current from the mass spectrometer was integrated and calibrated at each mass number in the scan, and recorded on magnetic tape. These data were later processed by computer to produce plots of relative abundance vs. m/e for selected scans. Prior to plotting, a background spectrum was subtracted from each sample spectrum, and the peaks obtained were normalized with respect to the base peak. The spectra obtained were compared with spectra previously reported in the literature.

As a further confirmation of identities, the Kovats Indices of many of the peaks were determined. Samples of the extracted oil, and commercial samples of tentatively identified compounds, when available, were injected along with bracketing normal hydrocarbon standards on the capillary Carbowax 20M and SF 96(50) columns, using isothermal conditions. Retention times of all compounds were recorded by an Infotronics CRS 104 digital integrator. Output from the integrator was recorded on a T-33 standard teletype, utilizing paper tape and time-share mode to the campus computer center. The Kovats Indices were calculated from the retention times by a Burroughs B5500 computer.

Table 1—Final list of descriptive terms used for odors of compounds in pimento leaf oil and extract

Sweet	Yeasty
Floral	Spicy
Fruity	Clove-like
Citrus	Minty
Wood-like	Nutty
Pine-like	Sweaty
Celery-like	Salty
Grassy-green	Medicinal
Straw or hay	Kids' white paste
Tea-like	Burnt
Meat-like	Earthy
Buttery	Sulfury
Vegetable oil	Sharp
Mineral oil	Penetrating
Soapy	Acrid
Waxy	Cooling-menthol

Sensory studies

Triangle test of heated and unheated samples. *Sample, apparatus and operating conditions.* A triangle sensory test was used to determine whether noticeable thermal degradation of the oil occurred during passage through the gas chromatograph.

"Heated" samples were prepared by injecting 10 μliter of pimento leaf oil onto the preparatory SF 96(50) column, which was operated isothermally at 225°C. This column was used because it is more thermally stable at this temperature than is the Carbowax 20M column. The 225°C temperature was selected because it was the temperature limit used in the chemical and sensory studies; also, the retention times of all components were low, thus minimizing loss of lower-boiling components during the trapping procedure. The total effluent was collected in U-shaped glass capillary tubes immersed in a dry ice-ethanol slurry. The tubes were sealed by flame after collection and stored in a freezer until used.

Panel. Three panel members, two female and one male, were selected from faculty members and students with previous experience in sensory studies. In addition, two female nonexperienced faculty members or students were included on each panel.

Procedure. On each test day, two sets of samples were prepared. A sample tube containing material previously collected from a chromatograph was crushed with a mortar and pestle and transferred to a porcelain cup which had a numbered aluminum lid. A nonchromatographed sample was prepared by injecting a 10 μliter portion of pimento leaf oil into a glass

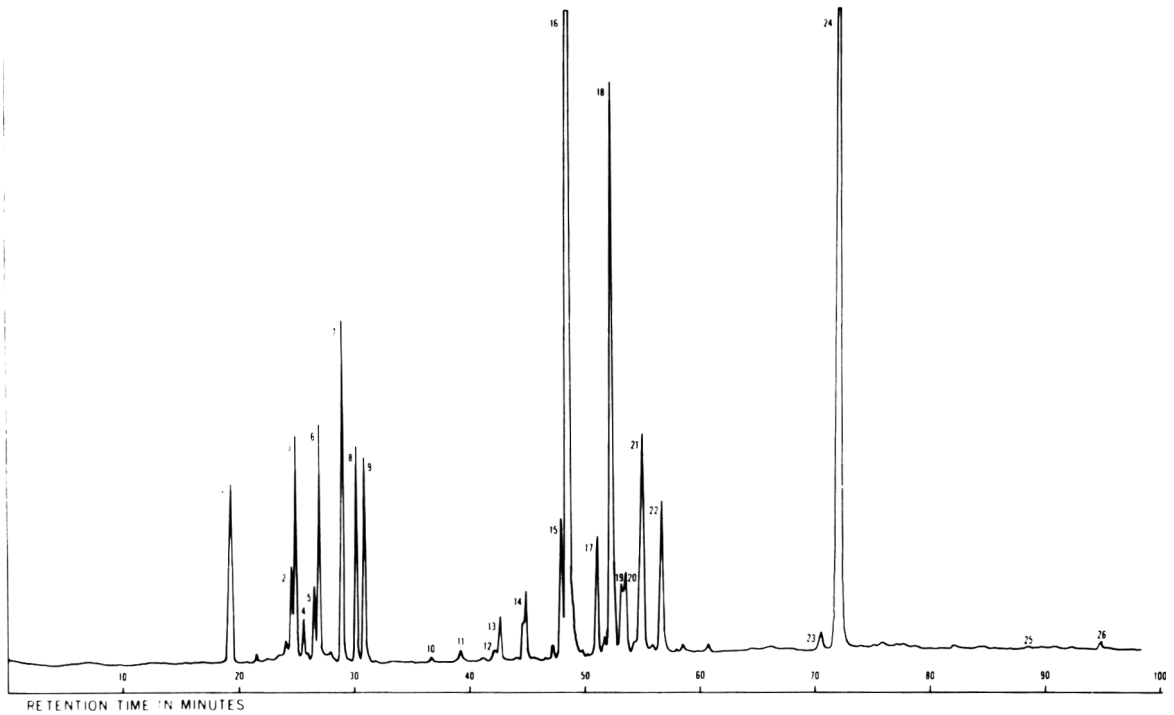

Fig. 2—Typical gas chromatogram of pimento leaf oil alkali extract on Carbowax 20M column.

capillary tube and crushing it as above. Each judge sniffed the triangle test samples and noted the number of the sample which he considered different from the other two.

Descriptive characterization of GC effluent. *Sample, apparatus and operating conditions.* Both pimento leaf oil and the low-eugenol extract were used. For effluent sniffing, 15 μliter of the oil or 10 μliter of the extract, were injected onto the preparatory Carbowax 20M column, which was programmed from 50–225°C at 6°C/min. To lessen olfactory fatigue of panel members, the samples were evaluated from three segments of the chromatograph. These segments, each evaluated on separate days, were (1) pimento leaf oil peaks eluting through peak 22, requiring approximately 35 min; (2) pimento leaf oil peaks eluting after peak 22, requiring approximately 17 min; and (3) extract peaks eluting after peak 22, also requiring approximately 17 min. The segment one portion of the alkali extract was not sniffed, but segments two and three were evaluated to determine odor differences from the removal of eugenol.

To facilitate sniffing, a 70-ml glass cup was attached to the exit port of the gas chromatograph as described by Potter and Daye (1970). Laboratory air was bubbled through two Drechsel, tall-form, gas wash bottles which were half filled with distilled water and then introduced into the bottom of the sniffing cup. This was done to minimize the drying effect of the carrier gas on the nasal membranes of the panelists.

Panel. The three experienced panel members who participated in the preliminary triangle test also served on this panel, along with two female students who had experience with sensory procedures.

Procedures. A preliminary set of samples was sniffed by each judge to become familiar with the procedure and to provide terms which could be used as the basis for a terminology common to all the judges. The judges sniffed the effluent at their own discretion and described each odor perceived by any term(s) that occurred to them, including terms denoting odor qualities and examples of materials with similar odors.

A rough grouping of terms was made on the basis of apparent similarities to facilitate discussion by panel members. Several reference materials were available for sniffing, but no attempt was made to provide a reference for every term. Some terms in the original group were eliminated because they were only used once or twice to describe odors, or were somewhat redundant with other terms. The 32 terms finally agreed upon for further use in the study are indicated in Table 1.

Each complete three-segment evaluation was repeated twice. During each run, the judge had the final list of descriptive terms available for reference and was asked to use those terms as often as possible to describe the odors perceived. He was, however, allowed to use another term if he felt it was more descriptive of the odor. Also, the judges were seated facing away from the recorder during these runs to remove any bias from the judge seeing where peaks occurred on the chromatogram. The presence or absence of a time bias was not determined.

RESULTS & DISCUSSION

TYPICAL CHROMATOGRAMS of pimento leaf oil and of the alkali-extracted oil on the Carbowax 20M capillary column are shown in Figures 1 and 2, respectively. The compounds identified are listed in Table 2, along with the methods of identification.

In the triangle test there were eight correct judgements out of a total of 20, which was not statistically significant (Amerine et al., 1965). This indicated that differences between chromatographed and nonchromatographed samples were minor and would not seriously affect further sensory studies. Also, this was a good indication that the materials studied chromatographically were indeed responsible for the odor of pimento leaf oil, and were not merely chemical artifacts.

Results of the descriptive characterization study are given in Table 3. Terms used three or more times to describe the odor at one point on the chromatogram are listed, along with the retention distance and compound name, for those compounds identified in the chemical study.

The terms spicy or clove-like, which were often used to describe the overall oil odor, occurred at only four different points on the chromatogram, three of which were very small peaks. The fourth was eugenol, the major compound present in the oil, and which thus had a great influence on the odor of the oil.

The two terms used most often to describe different points on the chromatogram were grassy-green and floral. Mineral oil, nutty, earthy, wood-like and fruity were other terms used relatively more often than spicy or clove-like.

No significant odors were unmasked by the removal of eugenol, although several small new peaks did become visible on the chromatogram. Descriptions of odors in this area of the chromatogram were in poor agreement, probably since odor intensities of the eluting compounds were very low.

The difference in sensitivity between the chromatographic detector and the human nose was well illustrated in several instances. There were many responses given by panelists to areas where only

Table 2—Compounds identified in pimento leaf oil

		Infrared spectrophotometry		Mass spectrometry	Kovats index	
Peak[a]	Compound	Comparison with reference compound	Comparison with reference spectra	Comparison with reference spectra	Comparison with reference compound	Comparison with literature value
1	α-Pinene	X	X[b]	X[h,i,j]	X	X[m]
2	Sabinene			X[i,j]		X[m]
3	α-Phellandrene	X	X[b]	X[i,j]		
5	Limonene			X[h,i,j,k]	X	X[m]
6	1,8-Cineol	X	X[c]		X	X[m]
7	cis-β-Ocimene			X[i,l]		X[m]
7	γ-Terpinene			X[h,i,j]		X[m]
7	trans-β-Ocimene			X[i]		X[m]
8	p-Cymene	X	X[b]	X[i]	X	X[m]
9	Terpinolene		X[b]	X[h,j]		X[m]
11	α-Copaene			X[m,n,o]		X[m]
13	Linalool	X	X[d]	X[p,q]	X	X[m]
14	α-Gurjunene		X[e]	X[o]		X[g]
15	1-Terpinen-4-ol		X[d]			X[m]
16	β-Caryophyllene		X[e]	X[n]		X[m,s]
17	Alloaromadendrene		X[f]	X[m]		X[m]
18	α-Humulene		X[e]	X[n,o]		X[m]
21	α-Selinene		X[e]	X[n]		
21	α-Muurolene			X[m,o]		X[m]
22	δ-Cadinene		X[e]	X[n]		X[m]
24	Eugenol methyl ether		X[g]		X	
25	Eugenol	X	X[g]	X[r]	X	
26	Isoeugenol			X[r]		

[a] Peak numbers refer to numbering in Figure 1.
[b] Mitzner et al. (1965)
[c] Mitzner and Mancini (1969)
[d] Mitzner et al. (1968)
[e] Wenninger et al. (1967)
[f] Wenninger and Yates (1970)
[g] "Sadtler Standard Spectra" (1961)
[h] McLafferty (1963)
[i] Ryhage and von Sydow (1963)
[j] Thomas and Willhalm (1964)
[k] Bunau et al. (1969)
[l] Budzikiewicz et al. (1964)
[m] Maarse (1971)
[n] Moshonas and Lund (1970)
[o] von Sydow et al. (1970)
[p] von Sydow (1963)
[q] Willhalm et al. (1964)
[r] Stenhagen et al. (1969)
[s] Sakai et al. (1967)

Table 3—Results of the descriptive characterization of the effluent from the gas chromatograph of pimento leaf oil

Constituent	Retention distance[a]	Responses[b]
TP	2.6	Sulfury (7)
TP	4.2–7.7	Yeasty (7)
TP	10.1	Buttery (7)
α-Pinene	12.8	Pine-like (5)
TP	13.9	Meat-like (3), Fruity (3), Sweet (3)
TP	14.2	Sweet (3)
TP	15.5	Grassy-green (5)
TP	16.1	Acrid (3)
TP	17.3	Fruity (8)
α-Phellandrene	19.8	Mineral oil (3), Kids' white paste (3)
TP	20.6	Floral (3), Nutty (3)
Limonene	21.4	Mineral oil (3), then Grassy-green (3)
1,8-Cineol	22.2	Cooling-menthol (6)
TP	22.9	Meat-like (5)
cis-β-Ocimene,δ-terpinene, trans-β-Ocimene	23.6	Citrus (3), Penetrating (3) Grassy-green (3)
p-Cymene	24.4	Meat-like (3)
Terpinolene	25.0	Mineral oil (5), Grassy-green (3)
TP	25.6	Mineral oil (3), then Nutty (3)
TP	27.1	Nutty (3)
TP	29.2	Grassy-green (4)
TP	30.4	Mineral oil (4)
TP	31.7	Earthy (7)
TP	32.0	Nutty (5)
TP	32.5	Wood-like (3)
α-Copaene	33.4	Burnt (3)
TP	34.6	Green (3)
TP	35.6	Floral (8)
TP	36.8	Vegetable oil (6)
α-Gurjunene	37.3	Floral (4)
TP	38.6	Grassy-green (5)
1-Terpinene-4-ol	39.0	Earthy (6), then Grassy-green (4)
β-Caryophyllene	40.4–41.7	Soapy (4), Cheesy (4), Nutty (4), Sweaty (3)
TP	41.9	Almonds (4)
Alloaromadendrene	42.3	Licorice (3), then Kids' white paste (5)
α-Humulene	44.2	Sweaty (6) (back side of peak)
δ-Cadinene	46.6	Minty (5), Sweet (5), Waxy (3)
TP	47.9	Floral (5), Grassy-green (3)
TP	48.1	Green (3)
TP	49.4	Fruity (3)

[a] Retention distances are listed in inches of chart paper; 1 in. = 3 min. TP = trace peak or no detectable peak.
[b] The number of responses out of a total possible of 10 is given in parenthesis.

trace peaks, or no detectable peaks, were eluting. This was especially true in the low-retention-distance sections of the chromatogram, where several trace peaks were described identically at least seven out of ten possible times. The odors involved were sulfury, yeasty, buttery, fruity and earthy. Only a few of the larger peaks on the chromatogram were described by a single term in more than 50% of the judgements, the major exception being the eugenol peak. The larger peaks were often described with changing terms by a judge; the character of the odor might change with concentration, or under the chromatographic conditions used in this study what appeared as one peak on the chromatogram might actually have been two or more compounds which had different odors. Further clarification of the contribution of odorant volatiles awaits identification of trace components that were unidentified in this study.

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THE NATURE OF CAROTENOID ESTERIFICATION IN TANGERINES

INTRODUCTION

ALTHOUGH FRUIT xanthophylls are known to be esterified (Goodwin, 1965) information on the nature of their esterification is lacking. Curl and Bailey (1956) reported the presence of xanthophyll esters in Valencia oranges, though they did not determine the nature of esterification. Philip et al. (1971) isolated capsanthin dilaurate from paprika and extensive esterification of xanthophylls is believed to occur during leaf senescence (Chichester and Nakayama, 1965). The present investigation was undertaken to study the nature of esterification of xanthophylls in tangerines.

MATERIALS & METHODS

Extraction

Two varieties of tangerines, viz., Kinnow mandarins (a hybrid of Kinnow mandarin with sour oranges was used in this study) and Dancy tangerines, were obtained in March, 1973 from the University of Arizona Citrus Experiment Station located at Tempe, Ariz. The fruits were hand peeled and the peel and pulp were worked up separately.

The peels (5 kg) were blended with 50% methanol (5 liters) in a Waring Blendor and filtered. The pulp (5 kg) was mixed with an equal volume of 50% methanol and filtered. The cakes were extracted with acetone (5 liters). The acetone extracts were evaporated to dryness (all evaporations were carried out in a rotary evaporator under vacuum below 40°C). The residue was dissolved in ethyl ether and the ether layer washed with distilled water. The ether extract was dried over anhydrous sodium sulfate and then evaporated to dryness. The yields of acetone extractives were as follows: Kinnow mandarin peels, 55g/5 kg; Kinnow mandarin pulp, 34g/5 kg; Dancy tangerine peels, 48g/5 kg; Dancy tangerine pulp, 30g/5 kg.

Carotenoid separation

The unsaponified carotenoids were subjected to a preliminary purification on a column of neutral alumina (activity I). The carotenoids were developed with petroleum ether. Two separated zones were observed on the column. The first zone was eluted with petroleum ether containing 2% ether. This zone was mostly essential oils and carotenes and therefore was discarded. The second zone containing carotenoid esters was eluted with petroleum ether containing 5–10% ether. The eluate was evaporated and the residue was subjected to TLC separation on silica gel G using acetone-petroleum ether (5 + 95) for development. The major ester bands were isolated in larger quantities by preparative TLC and were repurified on silica gel G using the same solvent system for development.

Carotenoid identification

After determining the R_f value, visible spectrum and infrared spectrum, the ester was saponified with 10% KOH in methanol (2 hr at room temperature). The saponified carotenoid was extracted with ether. The ether extract was washed free of alkali, dried over anhydrous sodium sulfate and evaporated. The saponified carotenoid was purified by TLC on silica gel G using acetone, benzene and petroleum ether (20:10:70) for development. The purified carotenoid was identified by R_f value, visible (Perkin-Elmer 202 UV-Visible spectrophotometer), infrared (Perkin-Elmer 337 IR spectrophotometer), NMR spectra (Varian T-60 NMR spectrometer) and chemical tests. The aqueous phase remaining after saponification was neutralized with HCl (1 + 2) and the fatty acid was extracted with ether. The ether was evaporated and the fatty acid was methylated by the method of Metcalfe et al. (1966). The methyl ester was separated on GLC (12% DEGS) and identified by comparison of its retention time with authentic compound.

Reduction

The pigment (2–5 mg) was dissolved in ethanol (10 ml) and an excess of sodium borohydride (1g) was added. When the reduction was complete (30 min), the mixture was transferred to a separatory funnel containing ether (50 ml). The mixture, after shaking, was washed with distilled water, the ether layer dried over anhydrous sodium sulfate and evaporated. The reduced pigment was purified on silica gel G using acetone, benzene and petroleum ether (20:10:70) for development.

Preparation of laurates

The natural pigment (2–5 mg) was dissolved in an excess of pyridine (10 ml) and the solution was transferred to a separatory funnel. Lauroyl chloride (10 drops) was added dropwise and the mixture was shaken vigorously for 5 min. Petroleum ether (100 ml) was added and the mixture, after shaking, was washed with dilute HCl (1 + 2), followed by distilled water. After removing the solvent, the laurate was purified by thin-layer chromatography on silica gel G using acetone and petroleum ether (5 + 95) for development.

RESULTS

ON PARTITION between 95% methanol and petroleum ether, the unsaponified carotenoids from the pulp and peel of Kinnow mandarins and Dancy tangerines were epiphasic. The unsaponified carotenoids separate well on silica gel G thin layer plates with a solvent system con-

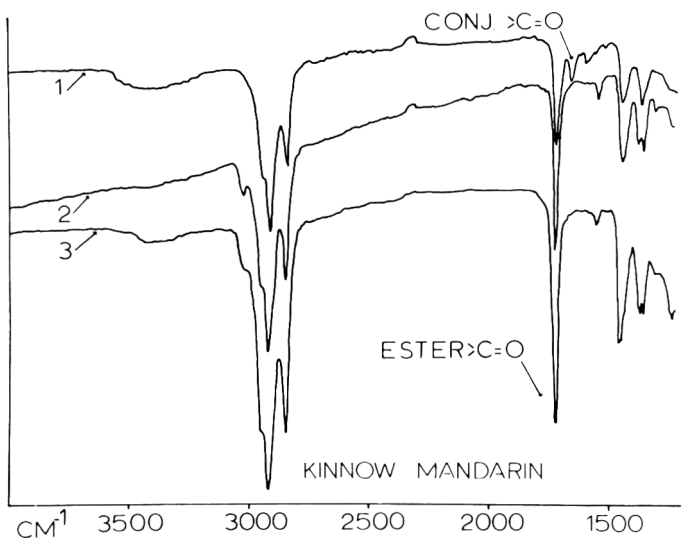

Fig. 1—The infrared spectra from 1200–4000 cm^{-1} (KBr disc) of β-citraurin laurate (1), cryptoxanthin laurate (2) and an unidentified laurate (3) isolated from Kinnow mandarin peels.

taining only 5% acetone in petroleum ether, whereas the polarity of the solvent (acetone, benzene and petroleum ether in the ratio of 20:10:70) had to be increased for the separation of saponified carotenoids (Table 1). These evidences indicated that the hydroxyl groups are not free in the tangerine xanthophylls.

Figure 1 shows the infrared spectra (1200–4000 cm^{-1}) of esters of β-citraurin, cryptoxanthin and an unidentified xanthophyll isolated from Kinnow mandarin peels, and Figure 2 shows the infrared spectra (1200–4000 cm^{-1}) of esters of cryptoxanthin, β-citraurin and reticulataxanthin isolated from Dancy tangerine peels. The strong absorption bands at 1725 cm^{-1} unequivocally prove the presence of ester carbonyls. The weak absorption bands due to conjugated carbonyls occur at lower frequencies at 1660 cm^{-1}. The absence of -OH vibrations also indicates that the hydroxyl groups are bound.

Thin-layer separations of the major pulp and peel carotenoids were similar except for relative proportions, and the peel contained higher amounts of total carotenoids than the pulp.

The spectral properties of a number of carotenoid laurates isolated from Kinnow mandarin and Dancy tangerine peels and related compounds are described below. The R_f values of these compounds are given in Table 1. The laurate component of the esters was identified by methylation, GLC separation and comparison of retention time with authentic compound.

Cryptoxanthin laurate

Cryptoxanthin laurate (λ_{max} in hexane 429, 455 and 483 nm) was present in both Kinnow mandarins and Dancy tangerines. Cryptoxanthin was identified by λ_{max} in hexane (428), 454 and 482 nm; infrared bands (KBr disc) at 33-3600 (-OH), 2950, 2925, 1550, 1450, 1375, 1350, 1300, 1155, 1105, 1025, 990 and 950 cm^{-1}; NMR (CCl_4) signals at 8.03 (in-chain methyls), 8.30 (cyclohexenic methyl), 8.93 (*gem* dimethyl) and 8.95 (*gem* demethyl) τ.Cryptoxanthin isolated from corn and synthesized cryptoxanthin laurate gave identical R_f values with those isolated from tangerines.

Table 1–R_f values (silica gel G) of carotenoid esters and derivatives from Kinnow mandarin and Dancy tangerine peels

	R_f Values	
Carotenoid	Acetone-pet. ether (5 + 95)	Acetone-benzene-pet. ether (20 + 10 + 70)
Cryptoxanthin	0.09	0.51
Cryptoxanthin laurate	0.91	1.00
β-Citraurin	0.00	0.37
Reduced β-citraurin	0.00	0.21
β-Citraurin laurate	0.22	1.00
Reticulataxanthin	0.00	0.30
Reduced reticulataxanthin	0.00	0.20
Reticulataxanthin laurate	0.21	1.00
Unidentified laurate	0.85	1.00

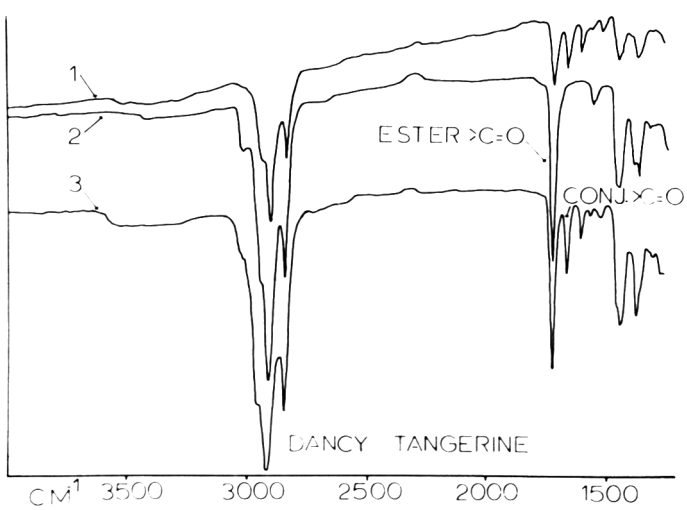

Fig. 2–The infrared spectra from 1200–4000 cm^{-1} (KBr disc) of β-citraurin laurate (1), cryptoxanthin laurate (2) and reticulataxanthin laurate (3) isolated from Dancy tangerine peel.

β-Citraurin laurate

β citraurin laurate (λ_{max} in hexane 435, 455 and 484 nm) was present both in Kinnow mandarins and Dancy tangerines. β-citraurin was identified by λ_{max} in hexane 438, 458 and 486 nm; infrared bands (KBr disc) at 33-3600 (-OH), 2950, 2925, 2860, 1660 (conj. > C=0), 1600, 1555, 1510, 1440, 1400, 1350, 1300, 1260, 1170, 1145, 1030, 990 and 955 cm^{-1}; NMR (CCl_4) signals at 0.6 (-CHO), 8.00, 8.13 (vinyl methyl α to > C=0), 8.23 and 8.93 τ. The hypsochromic shift of the reduced β-citraurin (λ_{max} in hexane 400, 423, and 447 nm) corresponded to one conjugated carbonyl. The synthesized β-citraurin laurate gave an R_f value identical with that isolated from Kinnow mandarin and Dancy tangerine.

Reticulataxanthin laurate

Reticulataxanthin laurate (λ_{max} in hexane 467 and 493 nm) was found only in Dancy tangerines. Reticulataxanthin (Yokoyama et al., 1965) was identified by λ_{max} in hexane 467 and 495 nm; infrared bands (KBr disc) at 33-3600 (-OH), 2950, 2925, 2860, 1660 (conj. C=0), 1600, 1510, 1440, 1350, 1240, 1170, 1030, and 955 cm^{-1}; NMR (CCl_4) signals at 7.80 (end of chain methyl α to C=0), 8.03, 8.24, and 8.93 τ. The hypsochromic shift of the reduced reticulataxanthin (λ_{max} in hexane 425, 445 and 473 nm) corresponded to one conjugated carbonyl group. The synthesized reticulataxanthin laurate gave an R_f value identical with that isolated from Dancy tangerine. Reticulataxanthin oxidizes rapidly to β-citraurin.

Unidentified laurate

This compound (λ_{max} in hexane 420, 445 and 475 nm) was present only in Kinnow mandarins. The visible spectrum (λ_{max} 421, 446, 475 nm) and infrared spectrum (infrared bands at 2950, 2925, 2860, 1550, 1450, 1360, 1350, 1240, 1155, 1100, 990 and 950 cm^{-1}) of the hydrolyzed pigment indicated a structure similar to α-cryptoxanthin. The carotenoid could not be isolated in sufficient quantities for proper identification.

Minor carotenoid esters

Several minor esters were observed on thin-layer plates. They were not in sufficient quantities for proper identification. The esters from Kinnow mandarin and Dancy tangerine peels were combined

separately. The combined esters on hydrolysis and methylation gave methyl laurate as well as methyl myristate on the basis of identification by comparison of retention time with authentic compounds on gas liquid chromatography.

DISCUSSION

THE EPIPHASIC NATURE of unsaponified carotenoids of Kinnow mandarin and Dancy tangerine indicates that most, if not all, the xanthophylls in these fruits are esterified. Although esterification does not change the visible absorption properties significantly, esterification increases the solubility of carotenoids in lipids with which they are associated. The role of carotenoids in producing off-flavors in foods is little understood, although carotenes are important flavor precursors in black tea (Sanderson et al., 1971). Carotenoids impart a pleasing yellow-to-orange color to citrus juices. The significance of carotenoid esters in stability of color and flavor during processing and storage of citrus products should be evaluated carefully, particularly to determine whether maintenance of the ester configuration provides advantages over the unesterified configuration.

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FLAVONOL GLYCOSIDES OF SOUR CHERRIES

INTRODUCTION

THE ANTHOCYANIN pigments of sour cherries are well known (Harborne and Hall, 1964; Dekazos, 1970; Shrikhande and Francis, 1973), but very little work has been done to identify the yellow flavonoid pigments. Shaller and Von Elbe (1970) confirmed the presence of kaempferol-3-rutinosides and indicated the possible presence of kaempferol-3-glycosides in the fruits of Montmorency cherries. Olden and Nybom (1968), working with three varieties of cherry leaves, separated their polyphenols and predicted the presence of the rutinosides and the 3-glucosides of quercetin and kaempferol. Their prediction was mainly based on comparison of the R_f values with literature values, without identifying the compounds involved. The presence of quercetin-3-glucoside was indicated in the leaves of *prunus cerasus* by Geissman (1956).

Considerable interest lies in the identification of flavonol pigments, due to their potential as chemical markers in plant taxonomy, their role along with anthocyanins in providing total coloration to fruit and fruit products and perhaps their role as bioflavonoids for retarding the ascorbic acid oxidation in fruit juices (Harper et al., 1969).

The present study deals with the identification of flavonol pigments in the fruits of sour cherries (*prunus cerasus* L., var. Montmorency).

MATERIALS & METHODS

Extraction

Sour cherries (*prunus cerasus* L., var. Montmorency), obtained from a local grower near Amherst, were frozen until required. About 2 kg of these frozen cherries were macerated with about 2 liter of 70% methanol and stored overnight at 4°C. The macerate was filtered and the residue was extracted again as outlined above, stored at 4°C and filtered. Both filtrates were combined, and vacuum evaporated to a final volume of 250 ml. This aqueous contract was shaken twice with an equal volume of petroleum ether to remove carotenoids, chlorophylls and waxy materials. The extract was then shaken with three equal portions of ethyl acetate. The aqueous extract containing anthocyanins was discarded. Both the ether and ethyl acetate extracts were combined and evaporated to dryness under vacuum at 30°C. The yellow residue was dissolved in 10 ml of methanol. This methanol solution was streaked evenly on six sheets of Whatman No. 3 filter paper and developed in 15% HoAC.

Chromatographic methods

Whatman No. 3 filter paper was used for all separations and purifications and Whatman No. 1 for R_f data in descending manner in both cases. The following solvents were used for chromatography:

BAW–1-butanol:glacial acetic acid:water (4:1:5)

B_2AW–1-butanol:glacial acetic acid:water (6:1:2)

BBPW–1-butanol:benzene:pyridine:water (5:1:3:3)

Forestal–glacial acetic acid:12N HCl:water (15:3:82)

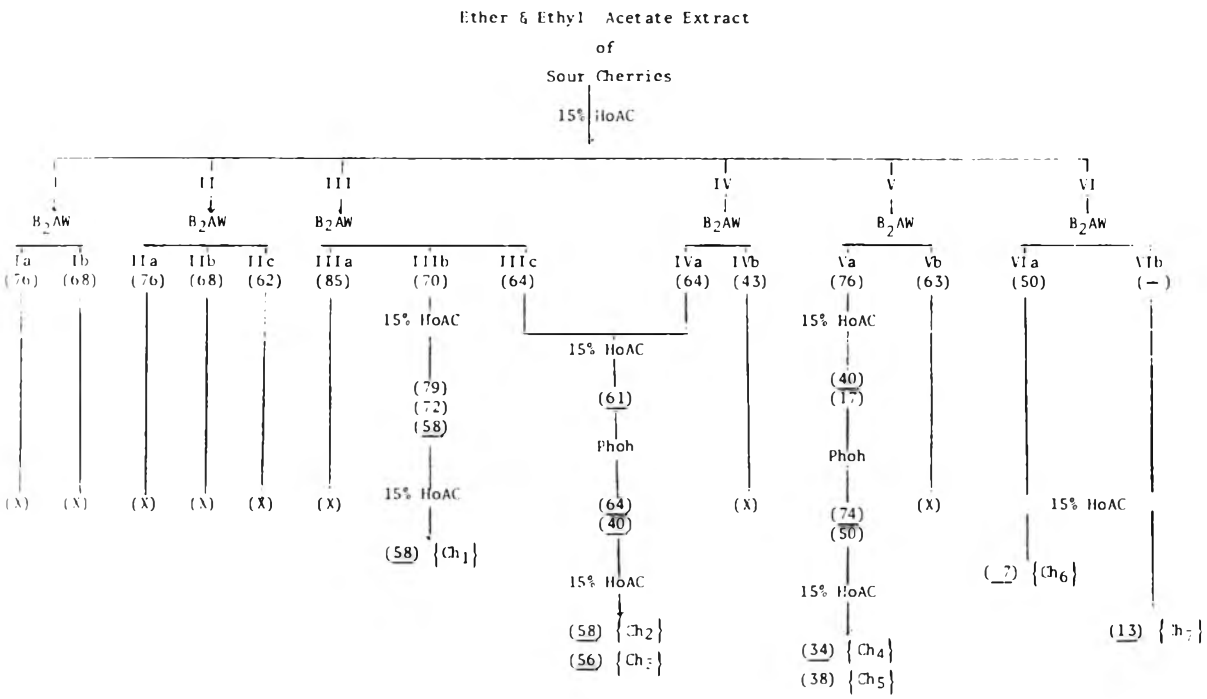

Fig. 1–Separation scheme for flavonol glycosides and polyphenols from sour cherries. (X) indicates bands were discarded; (–) indicates flavonol glycosides. The values in parenthesis are R_f values × 100.

15% HoAC—glacial acetic acid:water (15:85)
Phoh—water saturated with phenol
Water—Distilled water

Aglycones

The aglycones were obtained by heating purified pigment in methanol with 2N HCl in a boiling water bath for 1 hr. The aglycones were extracted with three successive 1-ml portions of ether and evaporated to dryness at room temperature. The aglycones were dissolved in a minimum amount of methanol and spotted on No. 1 paper with authentic quercetin and kaempferol. The chromatograms were developed overnight in BAW, Phoh and Forestal solvents.

Sugars

The solutions remaining after the extraction of aglycones were treated with di-n-octylamine to remove the mineral acid, washed with chloroform to remove residual amine and evaporated to dryness at room temperature. The sugar crystals were dissolved in water and spotted on No. 1 paper along with authentic glucose, galactose, rhamnose, xylose and glucuronic acid. The chromatograms were developed in BBPW and Phoh solvents for 30 hr, essentially by the method of Partridge (1949).

Spectral data

Spectra for each flavonol were determined using a Perkin Elmer 450 recording spectrophotometer by the method outlined by Mabry et al. (1970). Each purified pigment band on paper was cut into small pieces and shaken for 10 min with 50 ml spectral grade methanol. An equal portion of blank paper was similarly eluted. Both elutes were filtered using Whatman No. 3 paper, evaporated to dryness and dissolved in 10 ml of methanol. The spectral shifts with sodium methoxide, sodium acetate, boric acid and aluminum chloride were recorded for each pigment.

Authentic pigments

Purified quercetin, quercetin-3-galactoside and quercetin-3-rhamnoside were available from previous work on cranberries (Lees and Francis, 1971). Commercial preparations of kaempferol and quercetin-3-rutinoside were obtained from K. & K. Laboratories, New York.

Chromatographic separations

The methanol extract of flavonoids was streaked on No. 3 filter paper and developed descendingly in 15% HoAC. The chromatograms when viewed under UV light indicated the presence of several bands ranging in color from bright blue, whitish blue, blue green to dull brown. Only the brown bands (five in number) and a bright yellow band near the origin were cut and eluted with methanol, concentrated, reapplied on No. 3 paper and developed in B_2AW. Each band separated into a number of bands (Fig. 1). At this stage, the papers were fumed with ammonia and viewed under UV light, to locate the yellow or yellow brown bands characteristic for flavone and flavonol pigments (Mabry et al., 1970). The separated bands having deep blue, whitish blue and greenish blue colors were discarded (Shaller and Von Elbe, 1970). Bands IIIb, IIIc, IVa, Va, VIa and VIb were retained, eluted, concentrated and rerun in 15% HoAC. Band IIIb again separated into three bands and since one fraction had a dull brown color, it was retained. Pigments IIIc and IVa were combined due to their similar R_f values. At this stage, pigments were considered to be pure enough for determination. However, during the development for R_f determination in Phoh, both pigments IVa and Va again separated into two discrete spots of R_f (0.64, 0.40) and (0.74, 0.50) respectively. After seeing the above separations, both pigments were streaked on No. 3 paper and developed in phenol overnight. The separated bands were eluted, concentrated and run three times in succession in 15% HoAC in order to remove traces of phenol.

In the above separations, the seven flavonoid compounds obtained, were numbered from Ch_1 to Ch_7. The R_f values in four solvent systems, color reactions with or without ammonia in UV light are recorded in Table 1.

RESULTS & DISCUSSION

Identification

The R_f data (Table 1), spectral data (Table 2), aglycone and sugar analysis (Table 3) are consistent with the following identification: pigment Ch_2 was kaempferol-3-rhamnosylglucoside (K-3-RG); Ch_3 was quercetin-3-hamnosylglucoside (Q-3-RG); Ch_4 was quercetin-3-glucoside (Q-3-G). Pigment Ch_4 had an R_f value very close to authentic Q-3-galactoside. The similar behavior of glucoside and galactosides is well known (Harborne, 1967).

Pigment Ch_6 had an R_f value very similar to that of quercetin-7-glucoside and quercetin-4′-glucoside (Harborne, 1962). Preliminary attempts to hydrolyze CH_6 with 2N HCl failed to give any aglycone. The resistance to acid hydrolysis of Q-7-glucoside and Q-4′-glucosides is well established (Horhammer et al., 1956 and Harborne, 65); Harborne (1965) considered this resistance due to their insolubility in aqueous media. However, if excess ethanol was present during hydrolysis, the aglycone could be obtained within 1 hr. Accordingly, the method for hydrolysis of pigment Ch_6 was modified by adding excess methanol and refluxing the mixture for 2 hr. After this modification, both quercetin and glucose were obtained. The pigment Ch_6 failed to give any spectral shift with boric acid (Table 2), indicating that either the 3′ or 4′ hydroxyl in the pigment was glycosidated. Glycosidated pigments in the 3′ position are very rare. Usually, 4′-glycosides or methylation produces a hypsochromic shift in the spectra of flavonols (Jurd, 1962). This pigment has an absorption maximum of 361 nm consistent with the above finding. This differs from 7-substituted flavonols where spectral differences in either band I or band II are similar in the corresponding aglycone (quercetin: 372, 255; Q-7-glucoside: 372, 255). With the above assumptions, pigment Ch_6 must be quercetin-4′-glucoside. This pigment was also isolated from gorse flowers by Harborne (1962). The pigment Ch_6 has a rather low shift of 2 nm with sodium

Table 1—R_f values[a] of cherry flavonols and their color reactions in UV light

Cherry flavonols[b]	Solvents				Color[c]		Suggested structure[b]
	BAW	H_2O	15%HoAC	Phoh	UV	UV +NH_3	
Ch_1	70	25	60	65	DBr	DBr	K-3-R-4′-GA
Ch_2	64	26	58	64	DBr	YG	K-3-RG
Ch_3	58	23	56	40	DBr	Y	Q-3-RG
Ch_4	74	9	38	50	DBr	Y	Q-3-G
Ch_5	–	17	34	74	DBr	YG	K-3-G
Ch_6	50	00	7	38	BY	BY	Q-4′-G
Ch_7	–	3	13	–	BY	BY	K-4′-G
Authentic							
Q-3-RG	58	26	54	41	DBr	Y	–
Q-3-R	81	19	51	52	DBr	Y	–
Q-3-GA	73	8	35	54	DBr	Y	–
From Harborne (1962)							
Q-7-G	32	00	10	40	–	–	–
Q-4′-G	48	1	12	35	–	–	–

[a] R_f value (X 100)
[b] G = glucose; GA = galactose; R = rhamnose; Q = quercetin; K = kaempferol.
[c] DBr = dull brown; YG = yellow green; BY = bright yellow; Y = yellow

Table 2—Spectral properties of cherry flavonols

Cherry flavonols	Reagent					
	Methanol	NaAC	NaAC & boric acid	$AlCl_3$	$AlCl_3$ & HCl	Sodium methoxide
	Absorption maxima (nm)					
Ch_1	340,255	–	–	–	–	–
Ch_2	354,278	387,286	354,278	400,278	400,278	410,278
Ch_3	355,255	375,263	375,257	405,257	405,257	408,261
Ch_4	359,254	379,264	379,254	400,259	400,254	410,254
Ch_6	361,255	341,257	361,255	410,255	410,255	410,255

Table 3–Products of acid hydrolysis of cherry flavonols

Cherry flavonols	Aglycones, R_f value			Sugars, R_f value R_g of glucose = 1	
	Forestal	BAW	Phoh	BBPW	Phoh
Ch_1	0.35	0.89	0.59	1.78	1.71
				0.79[a]	1.04[a]
Ch_2	0.35	0.88	0.59	0.97	0.95
				1.78	1.69
Ch_3	0.22	0.77	0.22	0.97	0.94
				1.78	1.71
Ch_4	0.21	0.77	0.22	0.98	0.94
Ch_6	0.23	0.75	0.24	0.95	0.98
Authentic aglycones					
Quercetin	0.23	0.75	0.25		
Kaempferol	0.38	0.90	0.58		
Authentic sugars					
Glucose				1.00	1.00
Galactose				0.89	1.11
Xylose				1.43	1.25
Rhamnose				1.78	1.71

[a] Sugar tentatively identified; needs further confirmation

Table 4–Products of acid hydrolysis of cherry flavonols

Pigment	Aglycone	Sugars
Ch_1	kaempferol	rhamnose,galactose
Ch_2	kaempferol	rhamnose, glucose
Ch_3	quercetin	rhamnose, glucose
Ch_4	quercetin	glucose
Ch_6	quercetin	glucose

acetate in band II and a 20 nm hypsochromic shift in band I.

The pigment Ch_1 did not change color after fuming with ammonia. It gave faint spots of rhamnose and galactose as the sugars and kaempferol as the aglycone. It had a UV maximum of 340 nm. Usually, substitution of the 3-hydroxyl does not produce such large hypsochromic shifts in the flavonol (K, 367 nm); however, substitution of both 3 and 4′ could produce such shifts (Mabry et al., 1970). We tentatively identify this pigment as K-3-rhamnoside-4′-galactoside. Unfortunately, due to the very minute quantity of this pigment, it was not possible to make more conclusive identification.

Pigments Ch_5 and Ch_7 were also present in very minute quantities. On the basis of their R_f values alone, Ch_5 may be kaempferol-3-glucoside and Ch_7 may be kaempferol-4′-glucoside.

The resolving power of phenol in separating Q-3-RG and K-3-RG is noteworthy. Ryan (1971) also noticed the same phenomenon with the quercetin and kaempferol glucosides of strawberries. It is interesting to note that both Q and K-rutinoside and Q and K-glucoside have very close R_f values in 15% HoAC and H_2O and have a greater spread of R_f in phenol (Harborne, 1967). The inclusion of phenol may be considered as a potential solvent for the separation of flavonol pigments similar in structure. However, removing phenol from the pigment requires three to four runs in 15% HoAC, otherwise phenols themselves may interfere with the spectra.

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ISOLATION AND IDENTIFICATION OF SOME FLUORESCENT PHENOLIC COMPOUNDS IN CRANBERRIES

INTRODUCTION

FLUORESCENT PHENOLS play a role as intermediates in biosynthetic pathways (Hanson and Zucker, 1963; Harborne, 1964a; Levy and Zucker, 1960; McCalla and Neish, 1959) and have a number of physiological functions such as inhibitors of spore germination and growth of some parasitic microorganisms (McCalla and Neish, 1959). They are involved in enzymatic browning (Walker, 1962; Tate et al., 1964), and might be degradation products of more complex phenolic compounds in tissue.

The identification of such compounds has been the subject of many studies and the majority of those found have been hydroxycinnamic acid derivatives which were methylated or combined with organic substances. These compounds have been reported in tea leaves, the leaves and fruit of apples (Cartwright and Roberts, 1954; Cartwright et al., 1955), canned apricots (El-Sayed and Luh, 1965), pear puree (Sioud and Luh, 1966), cling peaches (Luh et al., 1967), Montmorency cherries (Schaller and Von Elbe, 1970), tomato fruit wall tissue (Walker, 1962), and in many plants (Pridham and Saltmarch, 1963; Harborne, 1960). Harborne and Corner (1961) identified the glucoside and other sugar esters of hydroxycinnamic acid derivatives from potato tissue and also reported sugar-benzoic acid derivatives in a number of plants (Harborne, 1964b). Steck (1967) also identified some of these compounds from a number of plants.

This report concerns the isolation and identification of fluorescent phenolic compounds in cranberries where they may play an important role in the development of color and astringent flavor.

A total of 11 fluorescent fractions were isolated, two of which later proved to be identical. This compound, identified as the 4-glucoside of caffeic acid, was recovered from both the ethylacetate and aqueous extracts. Tentative identification of six of the compounds are as follows: glucose derivatives of ferulic and gentisic acids; glucose esters of hydroxycinnamic and cinnamic acids; a glucose derivative of cinnamic acid; and a derivative of arabinose, glucose and ferulic acid. Three compounds remain unidentified.

MATERIALS & METHODS

Authentic compounds

Sugars, hydroxycinnamic acid derivatives and benzoic acid derivatives were obtained from K & K Laboratories Inc., 121 Express St., Plainview, NY 11803.

Preparation and extraction

Early Black variety cranberries, *Vaccinnium macrocarpum*, AiT, were obtained from a local commercial source and stored at −29°C until used. 2 kg of frozen cranberries were thawed and macerated with 95% ethanol in a Waring Blendor for 5 min. The crude mash was removed by filtering under vacuum through Whatman No. 1 filter paper using celite as a filter aid. The filtrate was evaporated until all ethanol was removed and the residue extracted three times with 400 ml of ethylacetate in a separatory funnel. The ethylacetate extract was rinsed with a small amount of water in a separatory funnel to remove free sugars and pigments and then passed through sodium sulfate. Both the ethylacetate and aqueous extracts were evaporated using a rotary evaporator under vacuum at 30°C. In order to collect an adequate amount of sample, this procedure was repeated three times.

Solvent systems

The following solvent systems were used for chromatography.

- BAW – 1-butanol:acetic acid:water (4:1:2)
- BEW – 1-butanol:ethanol:water (4:1:2:2)
- B_zAW – benzene:acetic acid:water (125:72:3)
- BPW – 1-butanol:pyridine:water (14:3:3)
- BEAA – 1-butanol:ethanol:ammonia-ammonium carbonate buffer (40:11:19. 1.5N with respect to both ammonia and ammonium carbonate)
- 2% HOAc – 2% acetic acid

Separation and isolation

The ethyl acetate fraction was applied to Whatman No. 3 papers (46 × 57 cm), and developed in 2% acetic acid. The bands were located by examination under ultraviolet light with or without fuming ammonia. The bands

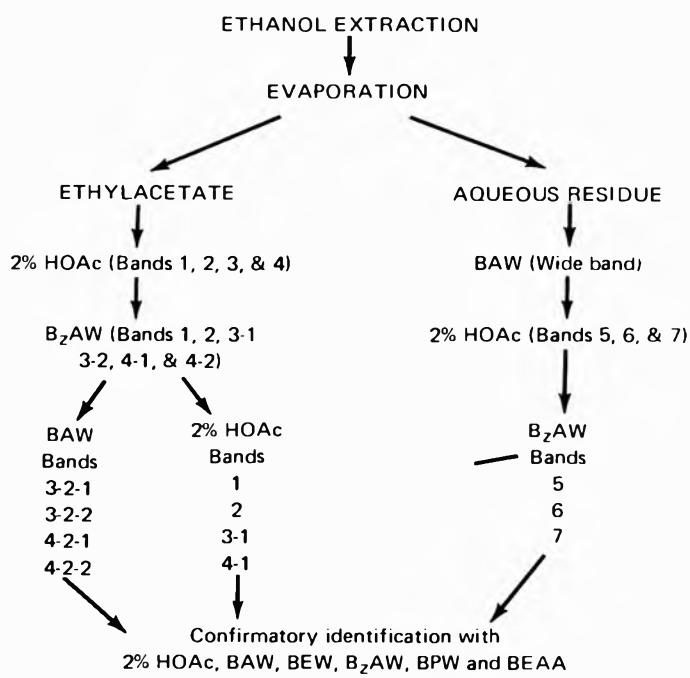

Fig. 1—Separation and isolation of phenolic compounds.

were eluted with methanol and further purified according to the scheme in Figure 1. A total of 11 bands were isolated from both the ethyl acetate and aqueous extracts.

UV spectra

UV spectral curves were measured with a Perkin-Elmer 450 Spectrophotometer using 1-cm cuvettes. All spectra were standardized with a blank methanol solution obtained by eluting the solvent from another portion of the chromatogram. Identification was further confirmed by adding two drops of 3% aluminum chloride and then three drops of 1% aqueous sodium hydroxide to cause a shift in peaks.

Chromatogenic reagents

All isolated samples were spotted on chromatographic papers and sprayed with the following reagents:

(a) **1% Ethanolic ferric chloride.** The chromatogram was sprayed with a freshly prepared reagent. The production of intense green, brown or blue color is due to the formation of a complex iron salt. Compounds with two adjacent hydroxyl groups show a green color (Swain, 1953; Harborne, 1964a).

(b) **Ferric chloride-potassium ferricyanide.** This reagent consists of equal volumes of 0.5% ferric chloride and 0.5% potassium ferricyanide. The chromatograms were sprayed with freshly prepared reagent and rinsed with 2% hydrochloric acid and then with distilled water. Phenolic compounds produce blue spots (Luh et al., 1967).

(c) **DPNA (diazotized p-nitroaniline).** This reagent was prepared by mixing three reagents, 0.5% p-nitroaniline in 2N HCl, 5% $NaNO_2$ and 20% sodium acetate (1:10:30) in an ice bath (Luh et al., 1967). Characteristic brown or yellow colors are produced by phenolic acids (Swain, 1953). Phenolic compounds with methoxy substituents only, do not couple with this reagent (Dunlap and Wender, 1960).

(d) **Hoepfner reagent.** This reagent consists of 0.5% acetic acid and 0.5% sodium nitrite in equal proportions. Different colors indicate a different number of hydroxy-substituted groups and ester linkages of cinnamic acid derivatives. A spray with 2% NaOH also shows different color reactions (Schaller and Von Elbe, 1970).

IR spectra

Absorption spectra were measured by Perkin-Elmer 337 Grating Infrared Spectrophotometer using the ultra micromethod with a condenser.

Hydrolysis

Each isolated sample was dissolved with 2 ml of water and 2 ml of acetone in a 25 ml round flask and 4 ml of 2N HCl was added. The flask was refluxed on a steam bath for 55 min. The condenser was then removed and the acetone allowed to evaporate. Hydrolysates were cooled and extracted three times with anhydrous ether. The ether extract was evaporated in a desiccator. The aqueous residue was evaporated with a hair dryer. Ether extracts were developed with authentic compounds using six solvent systems. The aqueous residue was developed along with quinic acid in BAW and 2% acetic acid. The developed papers were sprayed with sodium metaperiodate and sodium nitroprusside and piperazine (Cartwright and Roberts, 1955). Quinic acid showed up as an orange-yellow spot.

If the hydrolysates were sugars, the aqueous residue was extracted with 10% di-n-octylmethylamine in chloroform to remove acids (Lederer and Lederer, 1957). The aqueous neutral sugar solutions were then developed with authentic sugars on BBPW for 1 day and phenol solution for 2 days. Developed papers were rinsed with aniline hydrogen phthalate reagent and heated at 105°C for 5 min after drying. Sugars were detected with UV light.

Methylation and hydrolysis

The sample was dissolved in 10 ml of acetone, 0.2 ml of dimethylsulphate and 0.5g of potassium carbonate were added and the mixture refluxed on a steam bath for 1 hr. The condenser was then disconnected and the acetone allowed to evaporate. The methylated product was dissolved in 10 ml of acetone and filtered through Whatman No. 1 paper. The filtrate was concentrated and hydrolysed as previously described. The final product was extracted with anhydrous ether and developed along with authentic compounds on BAW, B_2AW, 2% acetic acid and BEAA systems.

RESULTS & DISCUSSION

11 COMPOUNDS were isolated by paper chromatography. Eight of these compounds, referred to as: 1, 2, 3-1, 4-1, 3-2-1, 3-2-2, 4-2-1 and 4-2-2, were isolated from ethylacetate extracts. Three compounds referred to as: 5, 6 and 7 were isolated from an aqueous extract of the residue.

The Rf values (Table 1) of 11 isolated compounds were determined but they were not the same as the Rf values for any of the authentic compounds listed in Table 1. The fluorescent responses (Table 1) under UV light could not be used for identification due to many different compounds having a similar fluorescent color. The maxima and minima from UV spectra are shown in Table 2. These are characteristic of hydroxycinnamic acid derivatives. Spectral shifts caused by $AlCl_3$ and NaOH give an indication of the aromatic substituents. Unfortunately no spectrum was identical with that of the authentic compounds. Chromatogenic reagents (Table 3) showed that the isolated compounds were phenolic compounds having substituents such as hydroxyl, carboxylic, methoxy and glucosidic groups. However no compound showed an 0-dihydroxyl group on the aromatic ring. The ether-soluble products of hydrolysis (Table 4) for compounds 3-1 and 5 were identical and were identified as caffeic acid. The hydrolysis product from compound 6 was identified as ferulic acid and that from compound 7 was identified as gentisic acid (2.5-dihydroxy benzoic acid). The concentration of some hydrolysates was so low that Rf values were not obtained. Quinic acid was not found in the aqueous hydrolysates. Methylation and hydrolysis (Table 5) was carried out with com-

Table 1—Rf values and color under UV light for isolated compounds from cranberries and for authentic compounds

Cranberry compd	Color[a]		Solvent systems						
	UV	UV + NH_3	BAW	B_2AW	BPW	BEAA	BEW	2% HOAc	
1	W	W	0.74	0.16	0.59	0.05	0.75	0.27	
2	PB	BrB	0.81	0.54	0.76	0.35	0.85	0.30	
3-1	B	GY	0.52	0.03	0.08	0.04	0.26	0.53	0.70
3-2-1	BG	GY	0.50	0.10	0.54	0.20	0.54	0.51	0.62
3-2-2	N	B	0.60	0.08	0.43	0.02	–	0.54	0.70
4-1	B	G	0.51	0.05	0.46	0.18	0.55	0.50	0.63
4-2-1	B	G	0.58	0.11	0.60	0.31	0.65	0.61	0.70
4-2-2	N	B	0.65	0.07	0.55	0.02	0.19	0.56	0.68
5	B	BY	0.53	0.02	0.06	0.02	0.26	0.52	0.70
6	B	G	0.58	0.10	0.58	0.31	0.65	0.63	0.70
7	B	B	0.52	0.03	0.07	0.03	–	0.70	
Authentic compd									
chlorogenic	B	GY	0.54	0.03	0.12	0.04	0.28	0.54	0.72
caffeic	BW	BW	0.78	0.23	0.51	0.07	0.75	0.22	
ferulic	P	P	0.81	0.72	0.58	0.12	0.83	0.28	
p-coumaric	N	P	0.86	0.60	0.70	0.18	0.89	0.37	
gentisic	BrB	BrB	0.85	0.31	0.26	0.17	0.66	0.53	
m-coumaric	P	P	0.88	0.60	0.68	0.23	0.90	0.41	
o-coumaric	W	P	0.90	0.66	0.80	0.23	0.91	0.44	
2,3-dihydroxy cinnamic	WG	WB	0.90	0.90	0.72	0.05	0.92	0.40	
3,5-dihydroxy cinnamic	B	P	0.90	0.89	0.66	0.27	0.90	0.18	
3,4-dihydroxy cinnamic	P	P	0.87	0.86	0.57	0.18	0.82	0.29	

[a] W = white; P = purple; Br = bright; B = blue; G = green; GY = green-yellow; N = none

pounds 2 and 3-1. The latter produced ferulic acid which indicated that glucose was substituted at position 4 of caffeic acid. The water soluble sugars in the aqueous extracts of the hydrolysates were identified by chromatography on paper in BBPW and phenol along with authentic sugars. Glucose was identified in all eleven compounds isolated from cranberries. Arabinose was detected in No. 6 as well as glucose.

Compound 1

Compound 1 might be a phenolic compound with one hydroxy group or carboxy group due to the positive color reactions obtained with ferric chloride-potassium ferricyanide and DPNA. It produced glucose on acid hydrolysis without changing the Rf value of the compound. No explanation is given of this observation at this time.

Compound 2

Compound 2 might be a phenolic compound with one hydroxy group attached to the aromatic ring ortho to an oxygen-linked group attached to the same ring. This is indicated by the positive color reactions obtained with ferric chloride-potassium ferricyanide and DPNA, and the UV spectral shifts caused by $AlCl_3$ and NaOH. The Rf values of the compounds produced with methylation and methylation-hydrolysis are the same indicating that no carboxylic group was present. The glucose produced after hydrolysis without a change in Rf value is again ambiguous as noted previously for compound 1.

Compound 3-1

Compound 3-1 produced Rf values in six solvent systems which were below that of chlorogenic acid indicating that this compound might be a glucoside linkage of caffeic acid as reported by Steck (1967). The product of hydrolysis was identical to caffeic acid in six systems. Chromatogenic reagents showed this compound to have a hydroxy or carboxy group but not 0-di-hydroxy substituents in the aromatic ring. IR spectra (Fig. 2) also showed that this compound had an aromatic ring with a hydroxy or carboxyl group and two adjacent hydrogens. This is indicated by the OH stretching at about 3400 cm^{-1}, CH_3 asymmetry and symmetry stretch at 2925 cm^{-1} and 2850 cm^{-1}, aromatic skeletal vibration at 1600 cm^{-1}, 1580 cm^{-1}, 1500 cm^{-1} and 1450 cm^{-1}, OH bending at 1380 cm^{-1} and 1250 cm^{-1}, and the carbonyl group or carboxylic acid stretching at 1700 cm^{-1}. The spectrum at 800 cm^{-1} indicates two adjacent hydrogens in the aromatic ring. The product of acid hydrolysis from the aqueous extract was glucose. In the UV spectra, the hypsochromic shift of 2 nm (Pridham, 1963) from caffeic acid (Fig. 3) and a large bathochromic shift from

Table 2—UV absorption maxima and minima and spectral shifts with $AlCl_3$ and NaOH of seven compounds isolated from cranberries and authentic compounds

Cranberry compd	absorption maxima[a] λ min	λ max	$AlCl_3$	Shift NaOH
2	247	308	+	322
3-1	263	322	+	365
3-2-1	260	309	–	
3-2-2	268	308	–	363
4-2-1	260	308	–	365
4-2-2	257	308	–	365
6	204	314	–	
Authentic compd				
chlorogenic acid	264	328	+	371
caffeic acid	262	324	+	348
ferulic acid	257	314	+	345

[a] diffuse +; no shift –

Table 3—Color responses of spray reagents with compounds isolated from cranberries and with authentic compounds

Cranberry compd	Color responses of spray reagents[a] $K_3Fe(CN)_6$	1% $FeCl_3$	DpNA	Hoepfner	Hoepfner base
1	B	Br	Br	–	–
2	B	YBr	T	–	–
3-1	B	GBr	T	Y	O
3-2-1	B	YBr	–	SIY	–
3-2-2	B	YBr	Br	SIY	–
4-1	B	YBr	T	SIY	–
4-2-1	B	Br	T	Y	–
4-2-2	B	Br	T	SIY	–
5	B	GBr	T	Y	–
6	B	–	–	–	–
7	B	YBr	–	Y	Y
Authentic compd					
chlorogenic	B	GGr	Y	YO	RO
caffeic	B	dG	Br	R	O
ferulic	B	Br	T	YBr	OY

[a] B = blue; Br = brown; Y = yellow; G = green; Gr = grey; O = orange; R = red; T = tan; d = dark; SI = slightly

Table 4—Rf values of ether-soluble compounds in the hydrolysates

Compounds	Solvent systems BAW	B_zAW	BPW	BEAA	BEW	2% HOAc	Identity
3-1	0.78	0.22	0.51	0.08	0.74	0.22	caffeic
3-2-1	0.78	0.21	–	0.08	0.73	0.15	
3-2-2	0.88	0.19	0.42	0.16	–	0.29	
4-1	0.79	0.70	–	0.07	–	0.26	
4-2-1	0.84	0.81	0.82	–	–	0.23	
4-2-2	0.88	–	0.61	0.19	–	0.32	
5	0.77	0.21	0.50	0.07	–	0.24	caffeic
6	0.80	0.71	0.56	0.13	0.81	0.26	ferulic
7	0.85	0.31	0.26	0.17	0.66	0.51	gentisic
chlorogenic	0.78	0.22	0.51	0.08	0.74	0.24	caffeic

Table 5–Rf values of the ether-soluble products of methylation and hydrolysis

Compounds	Solvent systems				Identification
	BAW	B_2AW	2% HOAc	BEAA	
2	0.84	0.83	0.38	0.78	Unidentified
3-1	0.83	–	0.28	0.14	Ferulic acid

Table 6–Tentative identification of fluorescent phenolic compounds isolated from cranberries

Compound	Tentative identity
1	Phenolic compound with one hydroxy or carboxy group
2	Phenolic compound with two hydroxy groups
3-1	4-glucoside of caffeic acid
3-2-1	Glucose derivative of cinnamic acid
3-2-2	Glucose derivative of a phenolic compound with a hydroxyl and an ethylene group
4-1	Glucose derivative of ferulic acid
4-2-1	Glucose ester of cinnamic acid
4-2-2	Glucose ester of hydroxycinnamic acid
5	Identical with 3-1 above
6	Feruloylglucose arabinoside or feruloylarabinoside
7	Glucose derivative of gentisic acid

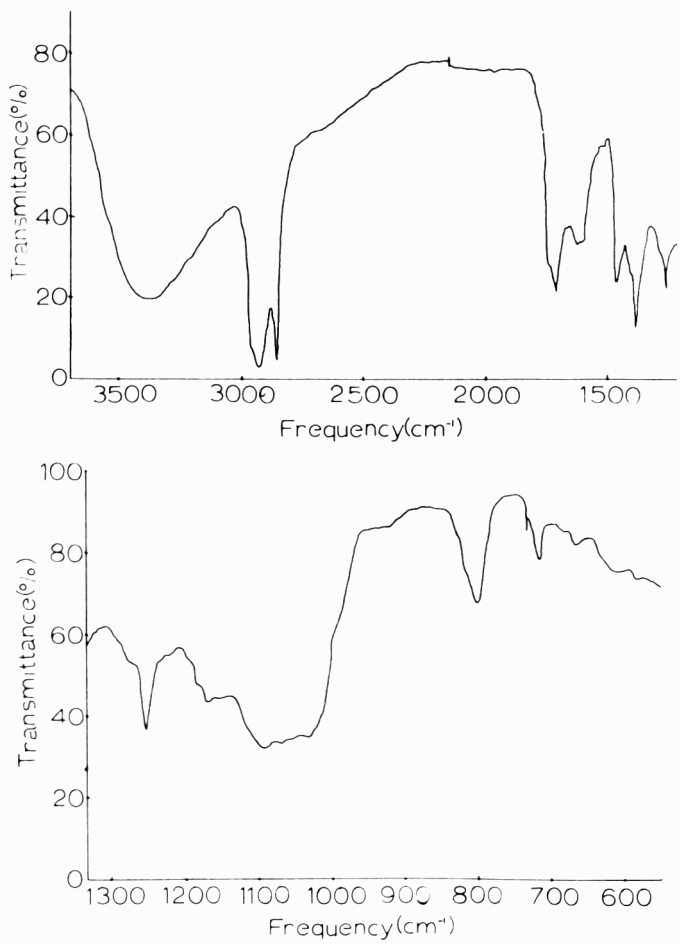

Fig. 2–IR spectra of compound 3-1 obtained for original ethylacetate extract from cranberries.

322 nm to 365 nm are characteristic of 4-glucoside-caffeic acid (Harborne and Corner, 1961). If this compound were the 3-glucoside of caffeic acid, the hypsochromic shift would be more than 10 nm from the UV maxima of 324 nm (Pridham, 1963). In addition, it produced ferulic acid on methylation-hydrolysis. Therefore the evidence indicates that this compound is the 4-glucoside of caffeic acid.

Compound 3-2-1

The color reactions of chromatogenic reagents indicated that this might be a phenolic compound and the Rf values obtained with a 2% HOAc showed that this compound might have an ethylene group. The UV spectrum showed a maximum absorption at 309 nm without shifts when aluminum chloride and sodium hydroxide were added. This indicated that it might be a cinnamic acid derivative linked with substituents other than a hydroxyl group. Production of glucose on acid hydrolysis along with the other evidence suggests a cinnamic acid-glucose derivative.

Compound 3-2-2

The color reactions obtained indicate that this compound might be a phenolic compound with a hydroxyl or carboxylic group. Rf values in 2% HOAc showed that this compound may have an ethylene group. In addition maximum absorption at 308 nm along with a bathochromic shift caused by NaOH indicate that it might be a cinnamic acid derivative with a hydroxyl group attached to the aromatic ring. The product of acid hydrolysis was glucose. IR spectra showed aromatic and hydroxyl characteristics and no fluorescence was produced by UV light alone. This suggests that the compound might be hydroxycinnamic acid linked with glucose. However, there was no hydroxycinnamic acid produced under acid hydrolysis. Therefore, the compound is probably a phenolic compound containing a hydroxyl group and an ethylene group.

Compound 4-1

The color reactions obtained with chromatogenic reagents and IR spectra showed that hydroxyl or carboxylic groups are attached to the phenolic portion. On acid hydrolysis, glucose was produced. The ether soluble product of acid hydrolysis might be ferulic acid. The Rf values of this compound were identical with those of ferulic acid in three solvent systems but lower in BEAA. It is likely that this compound is a glucose derivative of ferulic acid.

Compound 4-2-1

The color reactions of chromatogenic reagents indicated that this compound has carboxylic or hydroxyl groups attached to the phenolic portion. UV spectra showed a large bathochromic shift

with NaOH indicating the presence of a hydroxyl group. Chromatography with 2% HOAc showed the presence of an ethylene group in this compound. It produced glucose on hydrolysis. However, it was not possible to identify the aglycone from the Rf values of this compound and those of its hydrolysates. It may be a glucose derivative of cinnamic acid. The linkage might be a glucose ester as suggested by the Rf values of 0.60 on BPW (Steck, 1967).

Compound 4-2-2

The color reactions of the chromatogenic reagents and the large bathochromic shift shown by UV absorption with sodium hydroxide indicate that this compound has a hydroxyl group attached to a phenolic portion. Chromatography with 2% HOAc showed an ethylene group. Glucose was produced by hydrolysis. However, the Rf values of this compound and those of its hydrolysate did not give an indication of its identity. This compound may be a glucose derivative of hydroxycinnamic acid with the glucose in an ester linkage as suggested by the Rf value of 0.55 in BPW (Steck, 1967) and no fluorescence under UV light alone.

Compound 5

The color reactions of the chromatogenic reagents, Rf values on six systems and products of hydrolysis of this compound are the same as compound 3-1. Therefore, this compound was identified as the 4-glucoside of caffeic acid.

Compound 6

This compound showed a blue color with ferric chloride-potassium ferricyanide. Also there was no shift in the UV spectra with $AlCl_3$ and NaOH. This indicated that this compound did not have either a carboxyl or hydroxyl group attached to the phenolic portion. On hydrolysis, it produced glucose and arabinose. The hydrolysate of the ether extract was identified as ferulic acid. An ethylene group was also shown with 2% HOAc. Therefore, this compound may be either feruloylglucose arabinoside or feruloylarabinose glucoside in view of the Rf value in BPW (Steck, 1967) and no shift in the UV spectra.

Compound 7

This compound showed a blue color with ferric chloride-potassium ferricyanide reagent. Glucose was the product of hydrolysis and the hydrolysate in the ether extract was identified as gentisic acid. The absence of an ethylene group was shown with 2% HOAc. Therefore, this compound was identified as gentisic acid linked with glucose at the hydroxyl or carboxyl group.

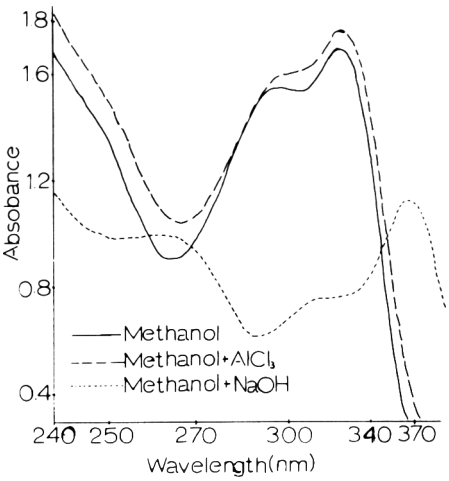

Fig. 3–UV spectra of compound 3-1 showing absorption shifts due to the addition of $AlCl_3$ and NaOH.

The tentative identifications are listed in Table 6. It appeared that compound 3-1 occurred in the highest concentration, compound 2, the next highest, and all others were very low in concentration.

None of the compounds has been previously reported in cranberries. The isolation and identification was complicated by the very low levels of these compounds in cranberries and the unavoidable losses in paper chromatography. The amounts isolated were too low for NMR studies to determine the position of the substituents. It is not unexpected that compounds such as these would be found in plant tissue in view of the association of cinnamic and benzoic acid derivatives in lignin synthesis and other metabolic reactions (Pridham and Saltmarsh, 1963; Harborne, 1964a; Ibrahim and Towers, 1960).

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EFFECT OF METALLIC IONS ON COLOR AND PIGMENT CONTENT OF CRANBERRY JUICE COCKTAIL

INTRODUCTION

APPARENT INCREASES in contamination of food, air and water supplies have occurred. However, the effects of some of the contaminants, introduced before and after treatment and processing, are not nearly as well established. Related work on anthocyanin (ACY) pigment stability has led to the present study of the effect of metallic contaminants on the stability of these pigments, and on the color of cranberry cocktail. To the drink manufacturer/bottler, processing water is a prime vehicle for metallic contamination.

Levels of various metals in processing water will vary with geographic location, as well as the immediate source, i.e., well, spring, or open surface water. Of the metals included in this study, McKee and Wolf (1963) found copper to occur naturally up to 0.05 ppm and its presence in greater concentrations indicates a form of pollution. As this metal may lead to some undesirable color reactions in food products, forming tannates and sulfates, Britain has established a limit of 2.0 ppm in ready-to-drink beverages, while the U.S. Public Health Service (1962) recommends a limit of 1.0 ppm in drinking water. Similarly, iron may form chelates with organic matter which subsequently precipitates; a dark violet color is produced with tea. Cohen et al., (1960) report the taste threshold in spring water to be 1.8 mg/liter, and the PHS recommended a maximun for drinking water of 0.3 ppm. McKee and Wolf (1963) state that tin, while not generally present in natural water, may occur in trace amounts when stored in tin containers. There is no PHS recommended maximum for this metal. While aluminum precipitates as the hydroxide, indicating levels in water would be very low, this metal was included in this study with the other, heavier, metals because of the well known aluminum chloride shift in the spectra of ACY pigments with orthohydroxyl groups in the B ring (Geismann, et al., 1953; Asen and Seligman, 1957). Similar metal-ACY complexes affecting color have been reported by Somaatmadja et al., (1964) for copper, Jurd and Asen (1966) for iron, and Salt and Thomas (1957) for tin. In addition, heavy metals, particularly copper and iron, have been reported by Sondheimer and Kertesz (1953), MacKinney et al. (1955), Nebesky et al. (1949) and others to act as catalysts in the oxidation and loss of ascorbic acid, which is of considerable interest as cranberry beverages are fortified with this vitamin. Also, Dekker and Dickenson (1940) and Silverblatt et al. (1943) have proposed a mechanism whereby aerobic loss of ascorbic acid leads to the formation of hydrogen peroxide. Sondheimer and Kertesz (1952) and Timberlake (1960a, b) recognized this formation, which would in turn react with the ACY pigments to produce breakdown products leading to formation of a brown resinous precipitate in fruit juices. Markakis et al. (1957) were able to recover 85% of labelled ACY from such a precipitate.

Other factors found to affect ACY stability include oxygen content (Starr and Francis, 1968), sugars (Mechter, 1953), temperature (Kertesz and Sondheimer, 1948) and light (Nebesky et al., 1949; Esselen et al., 1946; and Van Buren et al., 1968). Finally, pH exerts a large effect on ACY stability, primarily as determined by Jurd and co-workers (Jurd, 1963; Jurd and Geissman, 1963; and Jurd and Asen, 1966). ACY are found to exhibit greatest stability at low pHs and suffer color changes to green and blue near and over neutrality.

MATERIALS & METHODS

Test pack preparation

Stock solutions of cupric chloride, ferric chloride, stannic chloride and aluminum chloride in concentrations 100,000 times the U.S. Public Health Service level, were freshly prepared. Appropriate dilutions were prepared and aliquots delivered into pint bottles with an automatic pipette. Variables included copper, aluminum, iron, tin and a combination of the latter two, at three concentrations (5, 25 and 50 ppm for copper; 1, 5 and 10 ppm for the others). The iron/tin combinations used were at levels of 1/1, 1/5 and 1/10 ppm. All dilutions were prepared such that the same volume was delivered into all bottles. Cranberry juice cocktail was prepared at pHs 2.2 by addition of HCl and 2.7, each with and without the current level of commercial fortification of ascorbic acid (30 mg/6 fl oz). These lots were then pasteurized at 195°F, in a plate heat exchanger, and filled into the bottles to overflowing. The underside of the caps was swept with nitrogen and the bottles capped, inverted, cooled and subjected to room temperature (75° ± 3°F) storage for the study period. Samples were withdrawn from storage at the designated intervals and analyzed as described.

Total pigment content

Pigment content was determined by the pH Differential Method of Fuleki and Francis (1968) which utilized optical density measurements at 515 mm of two sample aliquots buffered at pH 1.0 and pH 4.5. The differential, divided by the average of the extinction coefficients for the four major ACY pigments of the cranberry (77.5), yields total ACY content in mg/100 ml of cocktail.

Spectral data

Cocktail samples were run on a G.E. Recording Spectrophotometer equipped with a custom-made brass water-jacketed constant temperature cell. The Davidson and Hemmendinger integrator provided X, Y and Z values which were converted to the chromaticity coefficients x and y.

Table 1—Analysis of Variance for the effect of the individual metals, and storage time, on the total anthocyanin content of samples of cranberry cocktail.

	F Values[a]							
	AN		AY		CN		CY	
	Time	Metal	Time	Metal	Time	Metal	Time	Metal
Cu	1060**	6.8 **	271**	2.61	498**	6.00**	532**	5.33*
Al	3312**	16.3 **	1050**	.79	266 **	5.63**	539**	0
Fe	1048**	1.20	169**	2.05	805 **	5.06*	1142**	1.60
Sn	3092**	4.46*	864**	.66	254 **	1.00	427**	2.00
F/S	856**	9.09**	817**	4.02*	61.4**	.16	453**	5.0 *

[a] The F values for significance at the 5%(*) and 1%(**) level for time were 3.26 and 5.41, respectively, and for metals, 3.49 and 5.95. The AN, AY, CN and CY series are described in Figures 1 and 2.

ΔE values

X, Y and Z values were entered on data processing cards by pH/AA series, and ΔE values were calculated by the Friele, MacAdam, Chickering (FMC) program on a CDC 3600 Research Computer.

Determination of base levels of metals

Naturally occurring levels of copper, aluminum, iron and tin in distilled water used in cocktail manufacture and in the finished cocktail, were determined by the methods of Banick and Smith (1957), Motojima and Ishiwatari (1965), Banick and Smith (1957) and Newman and Jones, (1966), respectively.

RESULTS & DISCUSSION

PRIOR TO the initiation of the storage study outlined above, a series of initial trials were conducted to permit selection of the lowest level of each metal that would yield a discernible change relative to the control after a 24-hr period. This level was then used as the maximum concentration for use in the storage study. As a basis for these initial trials, multiples of 1 to 1000× of the maximum allowable or recommended levels of each metal by the U.S. Public Health Service (U.S.P.H.S. Drinking Water Standards, 1962) were used. From inspection of the resultant spectral curves and E values (Starr, 1971), the final levels were obtained.

The storage study was designated to determine whether or not the four metals and one bi-metal combination (iron/tin) all behave in a similar manner regarding pigment content, whether or not the respective metals all act in the same manner at the different concentrations, and how they react relative to each other and the control with increasing storage time.

The four series used were AN, AY, CN and CY where A and C refer to pH 2.2 and pH 2.7, respectively, while Y and N refer to the fortified (ascorbic acid) and the unfortified groups, respectively. This fortification (Y) provided 177μg/AA/ml (30 mg/6 oz), while the AA content of the unfortified group (N) was 21.2 μg/ml (3.6 mg/6 oz).

An analysis of variance (Table 1) was computed to determine the level of significance of the effects of the various metals on ACY content. The effect of time is significant to at least the 1% level in all cases, while the effect of the separate metals varies. Nevertheless, it appears that the effect of the separate metals is generally greater in the absence of added AA (N group) than in its presence (Y group).

As the analysis of variance was calculated using averages of total ACY present after exposure to the three concentration levels used, it was also considered desirable to examine the effects of the individual concentrations of a given metal on total ACY, both relative to the control and to the other concentrations used. Figures 1 and 2 are graphical displays of the results of the Multiple Range Test which tests for significant differences between total ACY content for the means of the separate concentrations, using data from each sampling period. Starred boxes denote a significant difference (95% level) between the two treatments tested. Asterisks outside each block indicate the 95%+ level of significance for the metal taken as a whole, for that series, as determined from the analysis of variance. A larger number of such comparisons are significant in the unfortified (N) series (25 vs. 10), which must occur as this test is merely a more penetrating examination of the trends described by the analysis of variance.

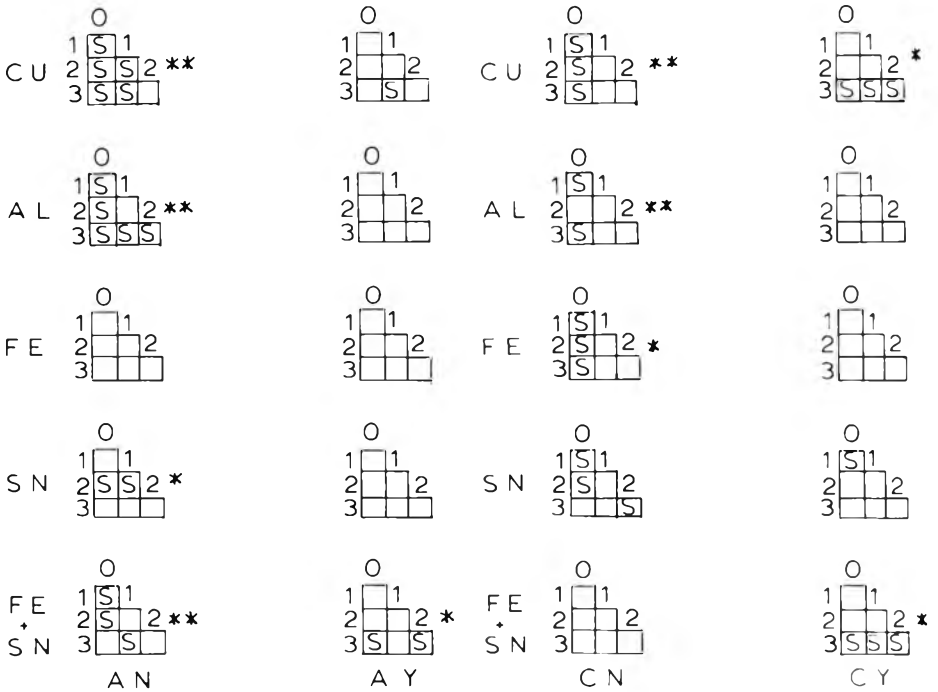

Fig. 1—Comparisons of means of pigment contents for three levels of metals. Copper was added for the 1, 2, 3 experiments at 5, 25 and 50 ppm. The other metals were added at 1, 5 and 10 ppm. The AN series refers to samples at pH 2.2 with no added ascorbic acid. The AY series refers to samples at pH 2.2 with 30 mg/178 ml added ascorbic acid. The S indicates significance at the 95% level. The asterisks indicate significance at the 95 and 99% level for the block as a whole.

Fig. 2—(See headings for Fig. 1) The CN and CY designations, respectively refer to samples at pH 2.7 without added ascorbic acid and with 30 mg/178 ml added ascorbic acid.

The fact that not all metals affected pigment content in each of the four major series indicated that an interaction has occurred between the variables. Accordingly, a factorial analysis of variance was carried out to determine the extent and significance of these interactions. Table 2 gives the levels of significance of the interactions possible, as broken down by main groups. The higher F values for interactions comprising a given factor, e.g., time or metals or levels, indicate a

Table 2—Factorial analysis of variance of total pigment content.

	F Values[a]			
	AN	AY	CN	CY
Metals	2.54	6.132**	13.2 **	24.4 **
Levels	2.11	0.943	9.94	2.12
Time	1385 **	1597 **	1673 **	3438 **
ML	2.11	3.208*	1.89	5.53 *
MT	0.437	3.491*	1.50	3.40 *
LT	0.521	0.585	0.75	0.20

[a] A single and double asterisk indicates significance at the 95 and 99% level. The AN, AY, CN and CY series are described in Figures 1 and 2.

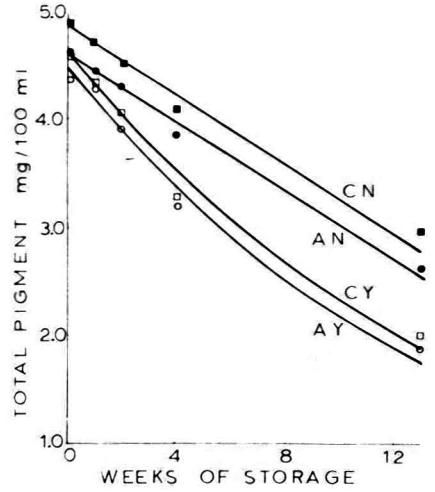

Fig. 3—Pigment content of cranberry cocktail after storage. Designations CN, AN, CY and AY are defined in Fig. 1 and 2.

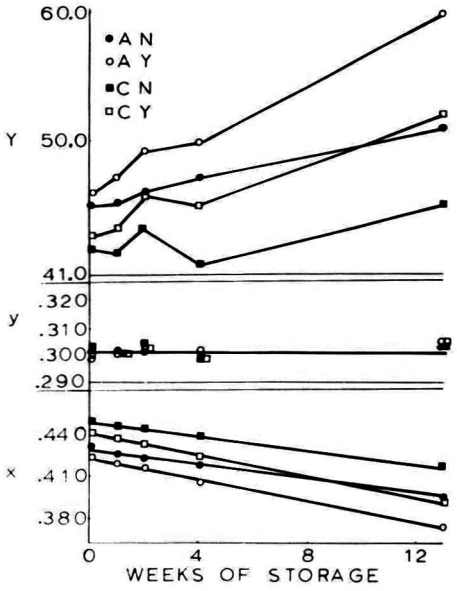

Fig. 4—Color of cranberry cocktail after storage in CIE Yxy units. Designations are described in Fig. 1 and 2.

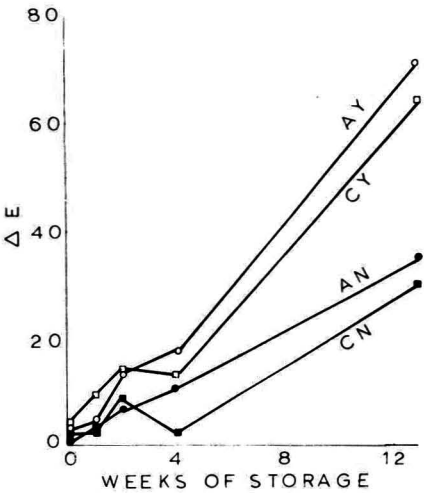

Fig. 5—Color differences between the storage valves and color at zero time of storage for cranberry cocktail. Designations are described in Fig. 1 and 2.

lower likelihood that the factor is significantly affecting pigment content; thus taken alone more randomness is associated with its presence, and no clear-cut pattern or strong general effect emerges. In the AN series, for example, the values for the interaction of (ML) and (MT) are both low, indicating a lack of randomness, and therfore, the presence of a strong effect of the common element, metals (M). Conversely, the values associated with the interaction of (ML) and (MT) in both the AY and CY series are high and significant at the 95% level; this indicates a high degree of interaction or randomness associated with metals (M) and therefore a low level of significance of the effect of metals on total ACY content. This may also be seen from Table 1.

Ascorbic acid and pH

The presence of AA in cranberry cocktail has been shown to exert a degradative effect on total ACY (Starr and Francis, 1968). However, this vitamin is also a metal chelating agent and an antioxidant; consequently its net effect could not be accurately predicted. Raw data of total pigment content (Starr, 1971), readily reveals larger losses in those series to which AA had been added—the Y group. Overall averages of these data are presented in Figure 3. At both pHs, those samples containing the added vitamin suffered greater ACY losses over the course of the study. At the same time it is apparent that the effect of pH, while consistently causing greater pigment retention at the lower level, is not nearly as dramatic as the effect of AA. In both series where AA had been added, the series with the lower pH (C group) demonstrated higher average ACY contents than the higher pH series (A group).

The above statistical tests elucidate the details of the effect of metals on total ACY content under prescribed conditions; they do not, however, give the direction of change from the control, only the relative validity of such changes. Table 3 includes the net difference between total ACY of samples and total ACY of the control. It is apparent that, with the exception of the AN series, metals in general tended to exert a small protective effect on pigment content. This effect is reversed in the AN series.

In Figure 4, the average color of the samples is plotted against storage time. The CIE Y value follows the same general trend as shown for pigment content in Figure 3. The series CN with the highest pigment content also shows the lowest Y value. The y value shows little change whereas the x (redness) shows a decrease with time. The color values are also plotted in Figure 5 as ΔE data by the Friele-MacAdam-Chickering formula representing the difference between the XYZ values of the controls at zero time and the actual storage values. The date parallel those in Figure 3 indicating that the color changes follow the anthocyanin degradation rather closely. Samples with stannic chloride added did not show a better color as suggested by Sistrunk and Cash (1970) for strawberry puree. However, the conditions were different in almost every way. Even though the general color changes followed the anthocyanin degradation pattern, examination of the samples by a visual panel indicated that some samples had an undesirable hue due to a shift to the blue/purple. This reaction may have involved other components of the mixture such as, flavonols, leucoanthocyanins and tannins. Detailed data on each treatment may be found, if desired, in the thesis by Starr (1971).

It is difficult to speculate on why the changes in pigment content occurred. The greater losses with addition of ascorbic acid, of course, are expected. The reduced losses due to ascorbic acid at the lower pH values are obviously desirable and may be due to the fact that the lower pH is further away from the pH maximums for this series of reactions. The protective efforts of the metals are less easy to explain. Aluminum (Table 3) showed more protective effect at pH 2.7 than at 2.2. Aluminum is known to chelate with cyanidin but not peonidin, but both pH values are well below the pH maximum for the chelation reaction. Cyanidin galactoside and arabinoside constitute approximately half the red pigment in cranberry juice, so even if there were a small degree of chelation with aluminum, it conceivably could account for the small beneficial effect shown in Table 3. The lesser effect shown at pH 2.2 would support the above hypothesis. It is possible that the same explanation applies to the

Table 3—Average pigment content[a] of cocktail with added metal ions minus pigment content of control

	Weeks of Storage (75° ± 3° F)				
	0	1	2	4	13
Treatment	Pigment Content Difference (mg/100 ml) Treatment Minus Control				
AN					
Cu	−.21	−.08	−.07	0	−.10
Al	−.20	−.11	−.07	−.01	−.10
Fe	−.21	+.07	−.05	+.06	−.02
Sn	−.23	−.11	−.13	0	−.09
F/S	−.15	+.01	−.06	+.04	−.12
AY					
Cu	−.38	−.09	+.04	−.02	+.13
Al	−.25	−.01	+.19	+.03	+.04
Fe	−.06	+.36	+.11	−.05	+.08
Sn	−.07	+.06	+.04	−.10	+.18
F/S	+.04	+.22	+.11	−.08	+.16
CN					
Cu	+.01	+.15	+.10	+.23	+.29
Al	+.02	+.14	+.06	+.18	+.44
Fe	−.03	+.14	3.12	+.13	+.19
Sn	−.10	0	+.02	+.10	+.15
F/S	−.17	+.09	+.02	+.07	+.09
CY					
Cu	−.28	−.10	−.08	−.05	+.07
Al	−.21	−.06	+.17	+.06	0
Fe	−.05	+.11	+.17	+.12	+.01
Sn	−.09	+.08	+.19	+.20	+.11
F/S	−.13	+.15	+.23	+.29	+.05

[a] The data for pigment content of samples with three levels of metal content were averaged

other metals, but there is little data available at these low pH values. In any event, the protective effect of the metals is very small.

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ULTRAVIOLET ABSORPTION METHOD FOR EVALUATING CITRUS ESSENCES

INTRODUCTION

CITRUS ESSENCES (aqueous aromatic solutions stripped from orange juice) are widely produced by several different processes, but there are no reliable objective methods for comparing their relative strengths. The processor needs to determine essence concentration, as an indication of relative value and the user needs to know whether a product meets his specifications and requirements. A physical or chemical measurement correlating with strength of essences would prove helpful in both respects.

The two most commonly used measures of essence strength are both unduly influenced by alcohol content. One is chemical oxygen demand (COD) (McNary et al., 1957) which measures total oxidizable organics and the other specific gravity by hydrometer which is almost solely a measure of alcohol content. Usually, more than 90% of the organic content of essences and aroma solutions (essence like solutions derived from peel, Veldhuis et al., 1972) consists mostly of methanol and ethanol with some acetaldehyde. Since these alcohols have questionable flavor influence, the usefulness of these methods as indexes of essence value is very limited.

Several methods have been suggested for measuring chemical components which contribute to essence quality. Aldehydes, esters, ketones and alcohols with four or more carbon atoms have been considered. Ismail and Wolford (1970) and Petrus et al. (1970) have developed methods for determining aldehydes in aqueous solutions using N-hydroxy benzenesulfonamide. Peleg and Mannheim (1970) reported a method for determining carbonyl concentration of aqueous solutions, using dinitrophenylhydrazine. Neither of these has proven satisfactory for measuring essence strength because results have been variable and inconsistent.

Several methods have been proposed for measuring the concentration of "less-volatile organics," here defined as the total organics excluding ethanol, methanol and acetaldehyde but including those components with greatest effects on quality. Extraction of essence and aroma solutions with methylene chloride (CH_2Cl_2) (Wolford et al., 1962) provided a non-aqueous solution of such organics for analysis by GLC or by direct gravimetric determination after evaporation of CH_2Cl_2 (Veldhuis et al., 1972). Disadvantages of these methods were the complicated and sometimes incomplete extraction procedure, the time-consuming and sometimes inexact integration of GLC peak areas and, for the gravimetric method, loss of volatile flavoring ingredients during evaporation of CH_2Cl_2. A GLC procedure for measuring these "less-volatile organics" in an aqueous solution was devised by Moshonas and Lund (1971). In a Porapak precolumn, water, methanol, acetaldehyde and ethanol were separated from the other organics, which were then analyzed in a separate GLC column. The total peak area was used as a measure of these "less-volatile organics." Another measure of this fraction is the degree of unsaturation, as determined by bromate titration (variation of the method of Scott and Veldhuis, 1966). Moshonas and Shaw (1973) showed that unsaturation in essence solutions correlated with the "less-volatile organics" fractions.

Since most of the previous analytical procedures for estimating the "less volatile organics" fraction are complicated or unreliable, a study was undertaken to evaluate UV absorption for this purpose. UV spectrophotometric examination of citrus oils has been reported as a measure of purity (Sale, 1953), but this approach has not been applied to characterize organic content of aqueous solutions. Dilute solutions of pure methanol and ethanol exhibit little UV absorption, so UV measurements would not be appreciably affected by their presence. A study was made of juice essences and peel aroma solutions comparing UV absorbance with other measures of the "less-volatile organics" fraction.

MATERIAL & METHODS

Aroma and essence solutions

Both orange aroma solutions and essence were studied. The aroma solutions were produced in the pilot plant (Veldhuis et al., 1972), either from commercial peel-oil centrifuge effluent or from fresh peel slurry. The feed material was stripped of oil by steam and the vapor fractionated. The aqueous portion of the distillate from the fractionation column was the aroma solution. Eleven essence samples were obtained from commercial sources (Redd Laboratories, Inc., Safety Harbor, Fla.), all produced in similar equipment from fresh orange juice. Several essences from other commercial sources were obtained for comparison.

The pot residues (bottoms) from the aroma solution fractionation column were also studied.

UV measurements

The UV absorption spectra were determined with a Cary Model 14 Recording Spectrophotometer. The instrument recorded the UV absorption spectra from 400 to 200 nm. The recorder chart speed was set at 2 in./min, and the spectrophotometer scanning speed at 1 nm/sec. (Each division on the chart represented 10 nm.) The absorbance range of the instrument was set to automatically switch from 0–1 to 1–2, and back, as the signal approached the upper and lower limits of the respective ranges. This allowed a wider range of organic concentrations to be tested without dilution. However, samples were diluted as required to record absorbancies in the more accurate 0–1 range.

The sample cells provided a light transmission path length of 1 cm and held approximately 2 ml test solution. Before use the cells were rinsed once with distilled water and three times with the sample.

A number of characteristic values of the UV absorption spectrum were surveyed to determine which were most meaningful and reproducible. These included: Absorbance (A) at maximum peak; absorbance (A) at selected wavelengths (200, 210 and 280 nm); the "double tangent" i.e., height at a point between tangents drawn to each side of the peak, (Sale, 1953); and the "valley" between the absorbance peak and the rising end absorption.

Analytical methods

Bromate titrations were carried out by the method of Scott and Veldhuis (1966), as modified by Moshonas and Shaw (1973) to omit the distillation step. Chemical oxygen demand (COD) determinations were carried out by a procedure similar to that given by McNary et al. (1957).

Odor tests

The odor judging panel consisted of 12 members selected from about 20 trained panelists. Each judge was presented with three samples of different UV absorbance and was asked

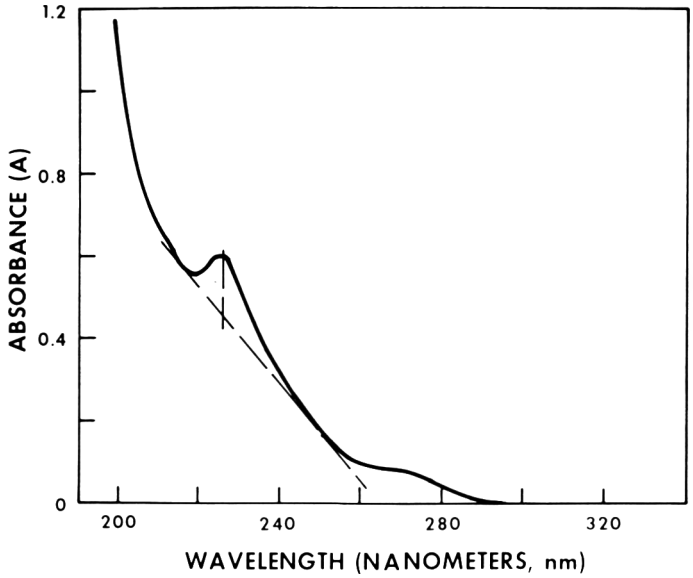

Fig. 1—Typical orange essence UV absorption spectrum.

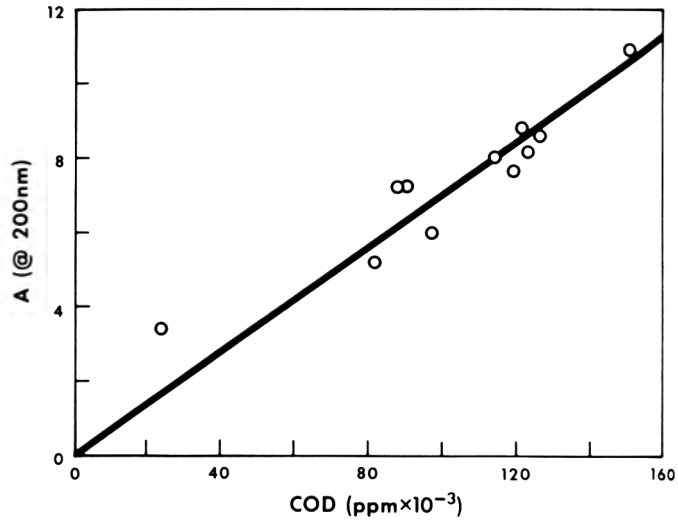

Fig. 3—Absorbance of similar type orange essences vs. COD.

to rate them on the basis of strength. A "correct" answer matched odor strength of all three samples in the same order as peak absorbance values, while an "incorrect" answer was any variation from this. Significance at the 99% level was achieved in this odor test with six correct answers and at the 99.9% level with eight correct answers (Roessler et al., 1948).

RESULTS & DISCUSSION

A TYPICAL UV absorption trace for essences and aroma solutions (Fig. 1) showed absorbance beginning to appear about 280–300 nm then increasing to a peak at 210–230 nm. The absorbance then declined slightly with decreasing wavelength before increasing again and exceeding the previous peak value. The end absorbance continued to increase rapidly to the limit of the trace, 200 nm. UV absorption spectra for aroma solutions and essences were essentially the same, with a peak around 220–230 nm and a strong end absorption. The absorbance could not be caused by acetaldehyde, methanol, or ethanol which do not absorb in this range.

It was assumed that absorbance followed Beer's law; i.e., absorbance at any wavelength is directly proportional to concentration. Dilution tests with representative aroma solutions and essences indicated that the assumption was valid. Adherence to Beer's law would be important in any useful quality control method where it would not be convenient to determine calibration curves, since most aroma solutions and essences had absorbances too high to measure without dilution.

The bottoms from the aroma fractionation column had been completely stripped of methanol, ethanol and acetaldehyde, but contained some organics. Thus, they provided samples where COD might be an accurate measure of "less-volatile organics." The linear relationship between A at the absorbance peak and COD (Fig. 2) indicates that COD would be a valid measure of "less-volatile organics" if acetaldehyde, methanol and ethanol were not present.

Although an object of the program was to determine if UV absorption could act as an index of the "less-volatile organics" in aqueous aroma solutions, there was no definitive method for measuring this fraction for comparison. Therefore, the UV data were correlated with COD or bromate titration, two other methods which have been used to approximate the organic content of aroma solutions and essences.

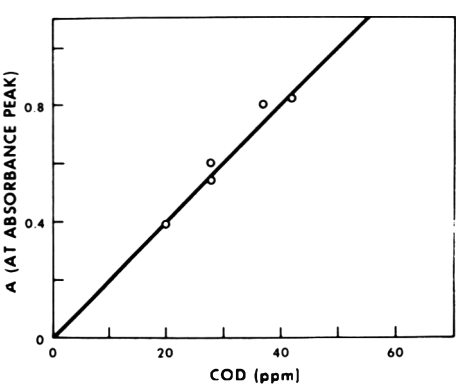

Fig. 2—UV absorbance of bottoms from aroma distillation column vs. COD.

The results of analyses of the 11 commercial essence samples comparing COD with UV measurements are shown in Figure 3. COD correlated well with A at 200 nm (correlation coefficient, r = 0.933) when the essences were from similar feed and equipment. However, as expected, there was no significant correlation between these factors for essences from differing sources, or for aroma solutions of different types. Essences or aroma solutions produced from different feeds could have large variations in the concentrations of ethanol and methanol, both absolute amounts and relative to each other. Concentrations of lower alcohols depend primarily on the storage history of the raw feed material. Long storage and elevated temperature promote fermentation and breakdown of pectin to methanol. Ethanol and methanol are not important to flavor or to measurements of UV absorption, but they usually account for 90–99% of measured COD.

The results of analyses of several aroma solutions, comparing bromate titration with UV measurements, are shown in Table 1 and Figure 4. Several different measures of UV absorption were compared with bromate titrable organics to determine which showed the best relationships. As shown in Table 1, good correlations were observed when absorbance (A) values were obtained at peak height, "double tangent," or at 200 or 210 nm. Correlation coefficients at 200 or 210 nm were higher than those for peak height or "double tangent," and all other things considered, measurements at one of the two low wavelengths would be preferred. The slope of the A vs. bromate organics line was greatest at 200 nm, indicating

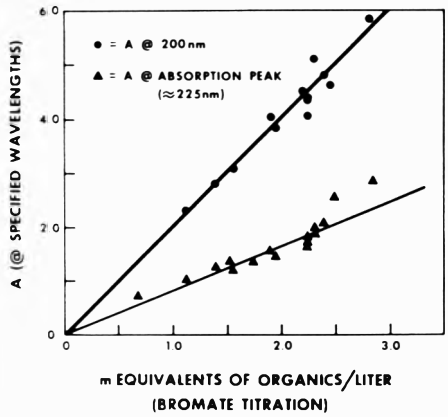

Fig. 4–A at 200 nm or absorption peak as related to bromate titrable aroma solution components.

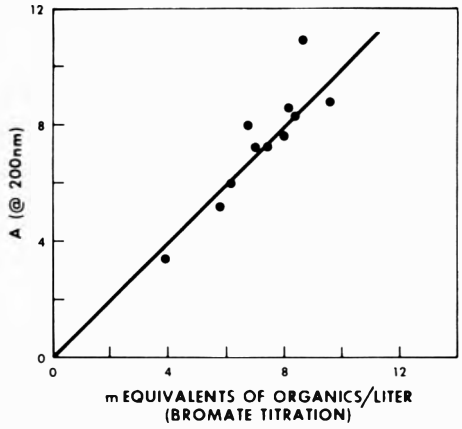

Fig. 5–Absorbance of commercial orange essences vs. bromate titrable organics at 200 nm.

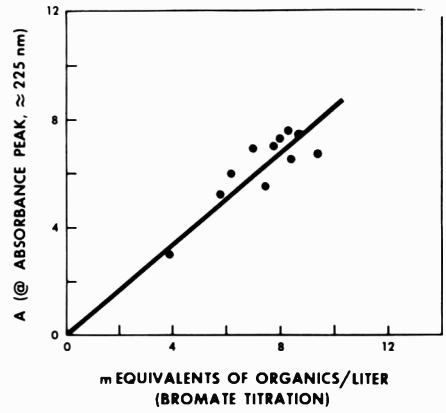

Fig. 6–Absorbance of commercial orange essences vs. bromate titrable organics at absorbance peak.

this measurement would have highest sensitivity. Any difficulties in determining absorption at 200 nm, due to the steep slope of the end absorbance, would be minimized by setting the spectrophotometer to read only at 200 nm. However, many spectrophotometers available in citrus plant control laboratories do not have the capabilities of the Carey instrument used in the study. Most inexpensive instruments are somewhat inaccurate in the 200–220 nm range, if they can measure in the range at all. In the absence of a sensitive spectrophotometer, a measurement above 220 nm, such as peak height, would be appropriate.

The correlation of UV absorbance (A) with bromate titrable organics for orange essences was similar to the results for aroma solutions. The results for 200 nm and peak height are given in Figures 5 and 6, respectively. At 200 nm wavelength, the correlation coefficient was 0.873.

Several sets of aroma solutions were presented to an odor panel for a determination of relative strengths. Table 2 shows the results of the odor tests. Samples were diluted with distilled water where noted.

Test 1 was designed to determine if the panel could detect concentration differences in one aroma solution. Test 2 compared three aroma solutions which had large differences in UV absorbance. Test 3 compared three aroma solutions which had slight differences in UV absorbance and showed that the UV measurement is a very sensitive index of odor strength. The results of all the odor strength tests were significant at the 99.9% level.

Several compounds in orange essences and aroma solutions are known to absorb in the 200–250 nm range in nonaqueous solvents. Three most common constituents of aroma solutions and essences are linalool, d-limonene and α-terpineol. By weight, they may comprise as much as 75% of the flavor organics (Lund et al., 1972). These three compounds in aqueous solution were found to produce end absorption beginning about 240 nm and increasing to high values at 200 nm. The peak in the absorbance curve of Figure 1 was most likely caused by citral (neral plus geranial) and trans-2-hexenal, which are also known constituents of essence and aroma solutions. Aqueous solutions of these compounds absorbed strongly at peak wavelength of 220–230 nm with near zero absorbance at 200 nm and above 280 nm. All such compounds which may contribute to the essence and aroma solution UV absorbance spectra have not been identified.

A compound which could prejudice absorbance values is benzoic acid, which absorbs in the UV, producing a strong peak. Peel slurry feeds for aroma solution production sometimes had sodium benzoate added for stability, as do certain commercial citrus products. These feeds, and the aroma solutions distilled from them, absorbed similarly but much stronger than solutions with no benzoate added. Thus, this UV method could not be used in cases where benzoate was used.

In summary, UV absorption would be a useful tool for industrial control work, or possibly for on-line control. Monitoring the absorbance peak or the absorption at a particular wavelength would show whether a process was in steady state or the organic content of the stream was changing. UV measurements are simple, quick, nondestructive and require no chemical reagents to provide an index of "organics" strength.

Table 1–Correlation coefficients between bromate titration and UV absorbance, A, of orange aroma solutions

Absorbance measure	Slope of A vs. bromate number	Correlation coefficient
Peak height	0.869	0.906
Double tangent	0.704	0.925
200 nm	1.99	0.960
210 nm	0.99	0.957

Table 2–Odor tests of aroma solution strengths

Test no.	Sample code	% of original concentration	UV absorbance A at peak	No. of correct selections by 12-member panel
1	A	100	2.55	11*
	A	50	1.28	
	A	25	0.65	
2	A	50	1.28	9*
	B	50	0.93	
	C	50	0.68	
3	E	50	1.01	8*
	B	50	0.93	
	D	50	0.87	

* Significant at the 99.9% level

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COMPARISON OF COLOR SCALES FOR DARK COLORED BEVERAGES

INTRODUCTION

THE COLOR SCALES currently employed on a number of colorimeters were developed for reflectance measurements. This is true even if the instrument is equipped to make transmission measurements. Since transmission data may be acquired with these instruments, the applicability of these so-called "reflectance scales" should be investigated. This research involved examination of several reflectance or surface color scales and transmission color scales to determine their usefulness for transmission colorimetry.

The main problem involved in making transmission measurements on samples is the difficulty of photocells to adjust to low luminosity situations in a manner similar to the human eye. With lightly colored, clear liquids, there is no problem. However, with darker colored liquids, the photocells do not adequately measure the visual chroma or hue of the sample. Thus the measurement may be more of a lightness or darkness measure than a color judgement. Little work has been done on the application of transmission scales to dark transparent food products. However, there have been investigations using reflectance scales in research involved with wine (Joslyn and Little, 1967; Robinson et al., 1966).

Several investigators have worked with translucent liquids and food stuffs (Little, 1964; Huang et al., 1970a, b; Gullett et al., 1972). However, their results are not applicable to transmission measurements of transparent liquids due to the highly light-scattering nature of translucent materials.

EXPERIMENTAL

SERIAL DILUTIONS of known concentrations of F.D.&C. Red #1, F.D.&C. Red #2, F.D.&C. Yellow #6, F.D.&C. Blue #1, F.D.&C. Green #1 and Cyanidin-3-glucoside, obtained from blackberries, were made for determination of the areas of confusion for various color scales. Blackberries were extracted overnight in methanol, containing 1% HCl. The methanol was evaporated under reduced pressure and the solution was filtered. Cyanidin-3-glucoside comprises approximately 95% of the total red pigment in blackberries. The appearance of only one band on a paper chromatograph confirmed that no more purification was necessary.

Tristimulus readings were taken on the samples using the General Electric Recording Spectrophotometer (GERS) (now called the Diano Hardy Spectrophotometer, Winchester, Mass.) with a tristimulus integrator (Davidson and Hemmendinger, Inc., Easton, Pa.) and the Hunterlab D25 Color Difference Meter with a spherical head attachment (D25) (Hunter Associates Lab., Fairfax, Va.). These readings were then converted by means of a computer program into the corresponding values for the twelve other color scales used in this analysis. All measurements were made using 3 mm glass cells, which were optically matched during preliminary investigations.

The samples covered almost the entire luminous transmittance range, from a C.I.E. Y ranging from 2.5–83 or a Hunter L from 15–91 at color differences ranging from one to four color difference units using the Hunter-Scofield equation (Scofield, 1943) or from two to eight color difference units by the Friele-MacAdam-Chickering (F.M.C.) (Chickering, 1967) method. The samples were easily ranked correctly by a visual panel made up of five people having good color discrimination as determined by the Farnsworth-Munsell 100 Hue Test and the ISCC Color Aptitude Test.

In order to determine if commercial measurements might be made near the area of confusion of any color scale, various dark colored commercial juices were purchased from a local supermarket. These were measured in two different cell thicknesses, 3 mm and 10 mm. The juices used were: Welch's Grape Juice and Grape Drink, Za-Rex Fruit Punch Syrup and Raspberry Syrup, Ocean Spray Cranberry Juice Cocktail and Sunsweet Prune Juice.

RESULTS

THE TERMINOLOGY used in this paper is as follows:

Area of confusion of inversion area– The region where changes in the pigment concentration of the samples fail to correlate with changes in the scale readings. This is illustrated graphically in Figures 1 and 2.

Color scale– A scale consisting of three parameters which completely defines or locates a color in a particular color solid.

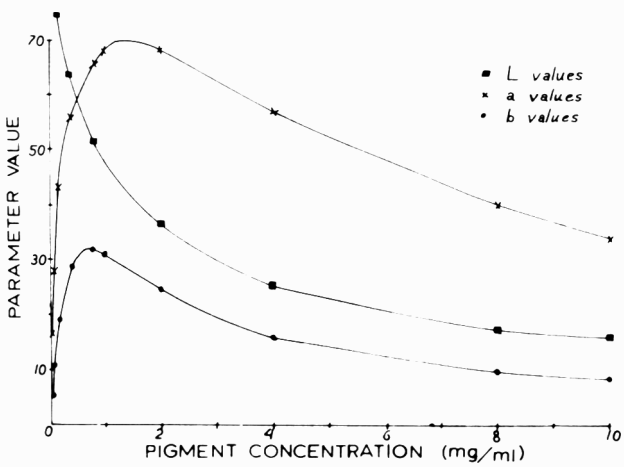

Fig. 1–L, a, b values vs. pigment concentration. (Cn-3-G)

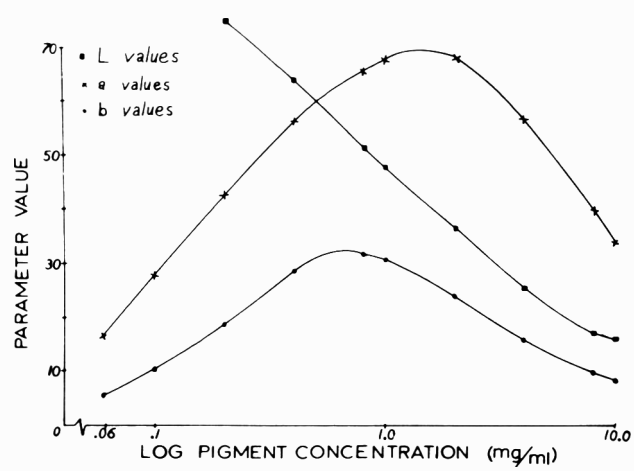

Fig. 2–L, a, b values vs. the log of pigment concentration. (Cn-3-G)

A color scale may be transformed mathematically to another system which utilizes a different color solid.

Luminous transmittance parameter—The one parameter of a color scale which defines lightness or darkness of a sample. The most commonly used luminous transmittance parameters in this investigation are C.I.E. Y and Hunter L.

Chromaticity parameters—The two parameters of a color scale which define hue and chroma at a constant luminous transmittance.

Compound functions—Those color quality parameters whose conversion formulas possess a luminous transmittance parameter in both the numerator and the denominator of the conversion equations.

Simple functions—These color quality parameters whose conversion formulas do not have a variable quantity in the denominator of their conversion equations. These are the equations for the derivation of C.I.E. X and Z (1931) and Colormaster R and B.

Root functions—Those color quality parameters whose conversion formulas are simple but have the variable terms taken to some root. Examples of these are the Glasser et al. (1952, 1958) and Adams (1942) Scales.

Color scale analysis

Converting the data from both the GERS and the D25 into the 11 remaining scales showed that with most scales, there was some area of confusion dependent upon the value of the luminous transmittance parameter. This means that the chromaticity parameters increased with pigment concentration to a certain level of luminous transmittance. Below this level, the same parameters began to decrease. The area of confusion, or inversion area, refers to this change in the chromaticity values when a certain level of luminous transmittance is reached.

Since chromaticity parameters are analogous to hue and chroma, it would be desirable, if as the colorant concentration increased, such values would increase linearly, no matter what the luminous transmittance. This does not hold, basically due to lower energy levels being transmitted through darker samples. The photocells then collect a weaker signal, which is translated into a smaller chromaticity parameter reading and also due to the shape of the color solid which is nonlinear in hue with a linear value progression. The luminous transmittance values where areas of confusion occur for different color scales are shown in Table 1. The luminous transmittance parameter used was Hunter L for all scales, including X, Y, Z, in order to simplify interpretation. The results from the two different sets of data agree quite closely.

Figures 1 and 2 show graphs which illustrate the terms "area of confusion" or "inversion area." These are graphs of the Hunter L, a, b values for cyanidin-3-glucoside (Cn-3-G). The horizontal axis is the approximate pigment concentration and the vertical axis is the value for the respective parameters. Figure 1 is the plot on rectangular coordinate paper and Figure 2 is the same data plotted on semilog graph paper. In these graphs it may be seen that luminous transmittance decreases proportionally with increased pigment concentration, but both chromaticity parameters show an inversion in certain luminous transmittance areas.

Some scale parameters did not show any inversion. These included all of the luminous transmittance parameters and those chromaticity parameters whose conversion formulas were simple functions, as defined previously. Those parameters which showed areas of confusion were the compound or root functions. Further inspection of the results showed that nearly all of the compound and root functions had areas of confusion at almost the identical range of luminous transmittance (Table 1). The only parameters which had inversions at some other luminous transmittance were the two scales specifically proposed for transmission work, the Hunter L, a', b' and the Citrus scales. Also the R_d, a_{Rd}, b_{Rd} scale, which was designed for reflectance measurements, showed an inversion area at a different luminous transmittance level. However, its inversion area was at the highest luminous transmittance level of any of the scales tested. All of the other scales are considered reflectance scales. Although each gives different numerical tristimulus values for the samples, the overall results with regard to areas of confusion were approximately the same.

Table 1—Luminosity levels at which areas of confusion occur for the chromaticity parameters of various color scales calculated from GERS X, Y, Z and D25 L, a, b values. (Pigment used was cyanidin-3-glucoside)

Color scales	References	Chromaticity parameter	Luminosity value (in terms of L) where area of confusion occurs	
			GERS data	D25 data
L, a, b	(Scofield, 1943)	a	36.8–42.9	45.9
		b	51.9–55.8	48.3
L, α, β	(Hunter, 1942)	α	36.8	34.4–37.0
		β	46.8–48.8	44.7
L', α', β'	(Hunter, 1942)	α'	36.8	34.4–36.5
		β'	46.8–48.8	44.7
L, a, b	(Glasser et al., 1958)	a_c	36.8–42.9	40.3
		b_c	42.9	40.8
L_g, a_g, b_g	(Glasser and Tory, 1952)	a_g	36.8	34.4–40.3
		b_g	42.9	40.8
L_a, a_a, b_a	(Adams, 1942)	a_a	35.4–42.9	40.3–40.8
		b_a	40.3–42.9	40.3
U*, V*, W*	(Wyszecki, 1963)	U*	40.3–42.9	44.0–44.7
		V*	48.8–53.2	48.3
%Y, x, y	(CIE, 1931)	x	25.5–29.8	29.9–34.4
		y	53.2–57.1	48.5–53.4
R_d, a_d, b_d	(Hunter, 1948)	a_d	63.0	62.8
		b_d	59.0–61.2	53.4–61.7
L, a', b'	(Hunter, 1969)	a'	19.0–21.9	19.0–21.9
		b'	35.4	29.9–37.0
Citrus Scales	(Hunter, 1967)	CR	19.0–21.9	19.0–21.0
		CY	35.4	29.9–34.4

Commercial juices

The identification of the existance of areas of confusion led to the conclusion that transmission measurements on commercial fruit juices might lead to erroneous results if they produced luminous transmittance levels equivalent to those around the areas of confusion found with model systems. For this reason, samples of various dark colored juices were purchased from a local supermarket. Using the same conditions as were employed with the model systems, tristimulus readings were made on undiluted samples of each juice. Measurements were also made in 10 mm cells because this size is easier to work with and would more likely be used in quality control testing than would the thinner 3 mm cells. Table 2 shows the raw data from the GERS and D25 for all the juices. Grape juice is probably the darkest colored commercial drink which could still be classified as transparent. Since some of the data on food coloring and pigment dilutions included solutions

darker than such commercial juices, it is believed that the results obtained would be applicable to commercial situations.

The 10 mm cells have more than three times the sample thickness of 3 mm cells, so the light beam must pass through 3 times the amount of pigment before reaching the photocells. Thus, from this logic, the chromaticity parameters ("a" and "b") for the juice in the thicker cell should be greater than those for the thinner cell, if they represent what the eye is seeing. However, in every case, except for Cranberry Juice Cocktail, the "a" and "b" values for the juice in the thicker cell were smaller than those in the thinner cell. However, it is well known that as less light is transmitted, the values for X, Y and Z all approach zero, the differences between X, Y and Z become smaller, and as a result the coordinates "a" and "b" must also decrease.

In the samples used in this study the visual panel ranked the more concentrated as being "redder" than the less concentrated, thus indicating the inadequacy of several of the scales in properly assessing colorant concentration. That is, the scales did not correlate with visual assessment under the same viewing conditions. Measurements of Cranberry Juice Cocktail correlated with colorant concentration only because in both cell thicknesses the L values were above those in an area of confusion. All other juices had at least one reading below the area of confusion.

The significance of the inversion in most of the chromaticity parameters of the scales is obvious from the fact that, except for Cranberry Juice Cocktail, the L values for the juices in 10 mm cells are at or below the areas of confusion for all scales except the Hunter L, a', b' scale.

Table 2—Tristimulus readings for some commercially available juices showing that the luminosity values fall within the area of confusion of most color scales tested

Juice	Cell thickness	X, Y, Z data			Hunter L, a, b data		
		X	Y	Z	L	a	b
Welch's Grape Juice	3 mm	19.89	9.64	3.03	29.34	57.08	13.99
	10 mm	5.85	2.67	0.81	14.50	31.40	8.80
Welch's Grape Drink	3 mm	43.01	27.57	31.76	51.13	56.22	0.22
	10 mm	14.01	6.92	3.36	24.85	48.60	10.30
Za-Rex Fruit Punch Syrup	3 mm	22.02	10.44	1.33	29.80	61.86	17.70
	10 mm	5.84	2.67	0.85	15.20	39.80	10.20
Za-Rex Raspberry Syrup	3 mm	30.97	15.42	3.61	37.18	70.90	19.07
	10 mm	15.57	7.04	0.78	24.30	54.40	15.10
Ocean Spray Cranberry Juice Cocktail	3 mm	72.78	62.78	62.86	78.65	25.77	7.38
	10 mm	44.98	29.11	16.43	53.37	50.60	17.80
Sunsweet Prune Juice	3 mm	34.03	28.92	3.43	52.70	17.91	30.11
	10 mm	6.54	3.79	0.78	17.65	26.10	10.63

Mathematical analysis of color scales

Thus far the area of confusion or inversion has been treated as an empirical observation. However, by looking at the conversion formulas themselves, scale inversion may be explained mathematically. The explanation of scale inversion could also be done by using chromaticity diagrams, but we feel the mathematical procedure is easier to understand and more direct. The conversion formulas for the Hunter L, a, b scale from X, Y, Z are:

$$L = 10Y^{1/2}$$

$$a = \frac{175(1.02X - Y)}{Y^{1/2}}$$

$$b = \frac{70(Y - 0.847Z)}{Y^{1/2}}$$

The factors 175 and 70 were derived experimentally to increase the fit of the spacing of Hunter coordinates to Munsell coordinates. On the other hand, 1.02 X and 0.847 Z are factors required to place Illuminant C at the origin of the Hunter a, b space. The X, Y, Z scale is a simple function, having no areas of confusion and all parameters increase as measurements go from the darkest to the lightest solutions. Figure 3 is a rectangular coordinate plot of the X, Y, Z raw data for cyanidin-3-glucoside dilution. It is a plot of parameter readings versus pigment concentration, and will be referred to throughout the following discussion.

Because Z is constant for a good portion of the dilution curve, it is easier to begin the mathematical discussion with the parameter involving Z. The reason for Z being constant can be seen in Figures 5 and 6 which are representative spectra of the systems tested. It may be seen that until the overall solution becomes light enough, there is not sufficient magnification of the X' peak to be measured by the photocell. X' is approximately one-sixth the size of the X peak and is located in the Z region of the spectrum. With a red material, X' should be a very high percentage of the total Z reading. When the X,

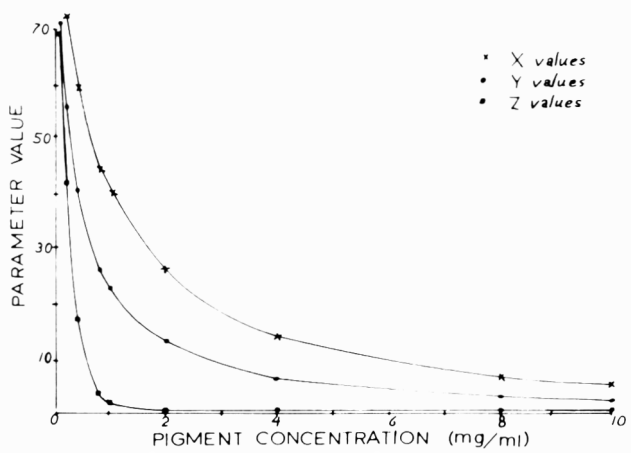

Fig. 3—X, Y, Z values vs. Cn-3-G concentration.

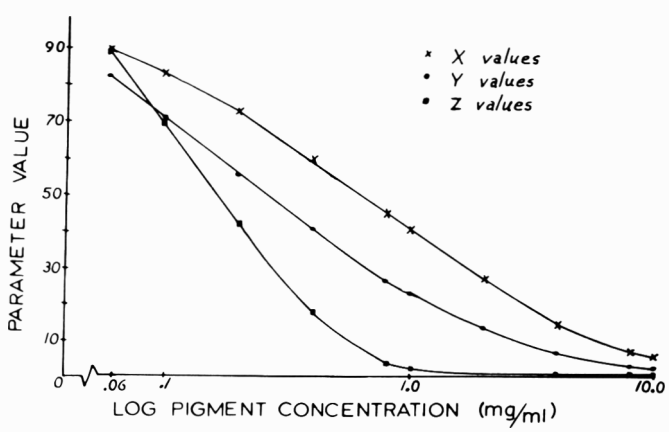

Fig. 4—X, Y, Z values vs. the log of Cn-3-G concentration.

Y, Z values are converted to the "b" parameter of the L, a, b scale, Z is only involved as a term which is subtracted from Y. If Z is constant, while Y is increasing, the numerator of the formula will increase and the overall function will increase. However, as can be seen from Figure 3, once Z starts to rise, it does so at a much faster rate than does Y. This will cause the numerator to decrease, while the denominator increases, thus causing the function to decrease. The area of confusion is the transition period where the numerator is increasing only slightly, so that the increase in the denominator causes the overall function to remain approximately constant and then decrease.

With "a" the situation is similar. Here, the numerator is 1.02 X—Y, and while neither of the variables is constant, the relative rates of increase do vary. In the lowest luminous transmittance range, X increases faster than Y, then in the middle and upper regions, Y increases faster than X. So, here again, the numerator first increases, then decreases, with the denominator increasing constantly.

The other compound scales show that the chromaticity parameters for the two Judd-Hunter scales, Citrus Scales, R_d, a_{Rd}, b_{Rd} and Hunter's transmission scale all have numerators similar to the L, a, b system in that variables with different rates of increase are subtracted from each other, while the denominator increases constantly. The two other compound function scales, Wyszecki (1963) U*, V* and the chromaticity coordinates have conversion formulas which differ in form from the others. These have just one variable in the numerator, X, Y or Z, and all three in the denominator added together. The inversion of these parameters is dependent upon the denominator. Here again, it is the relative rates of increase that control the inversion. Chromaticity coordinate "x," whose conversion formula is: X/(X + Y + Z), will serve as the example. At lowest luminous transmittance, X increases the fastest; then later Y and Z start increasing faster than X, so the overall denominator increases faster than the numerator. Thus the parameter first increases then decreases. The analogy holds for the other parameters of this type.

Root functions, such as the Glasser and Adams scales have variables taken to some root and subtracted from each other. It appears that relative rates of increase control scale inversion here also, as the formulas, without taking the equation to the root, are similar to the form of the compound function numerators. Also, the areas of confusion for these scales are in the same luminous transmittance region as those for most of the compound functions.

Simple scales, such as the C.I.E. X, Y, Z scale, do not have any areas of confusion, nor are they linear with pigment concentration. The rectangular coordinate graph of X, Y, Z readings versus pigment concentration (Fig. 3) resembled the shape of a semilog plot. Therefore, a plot of the reading versus the log of the pigment concentration was made, as shown in Figure 4. The plots of X and Y, the only ones of major concern in a red system, closely approximate a straight line for one log cycle, but veer off slightly in opposite directions on either side of the straight line portion. The linear portion of X corresponds to luminous transmittance levels of Y = 13 to 55 or Hunter L of 37 to 75. This does not include the luminosity levels of the dark colored juices. The linearity of the line, as described by linear regression analysis, showed a coefficient of determination equal to 0.964 for the linear portion and 0.931 for the entire range. The coefficients for Y over its linear region were 0.986 and 0.875 for the linear portion and the entire range respectively. However, because the plots are not linear from the initial concentration upward, this scale would not be useful for the prediction of pigment concentrations.

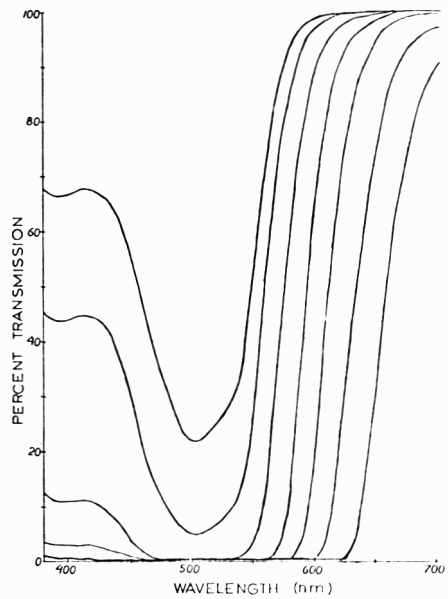

Fig. 5—Spectral curves for Cn-3-G solutions. Curves represent 0.10, 0.17, 0.69, 1.73, 3.45, 6.90 and 17.3 mg pigment per ml.

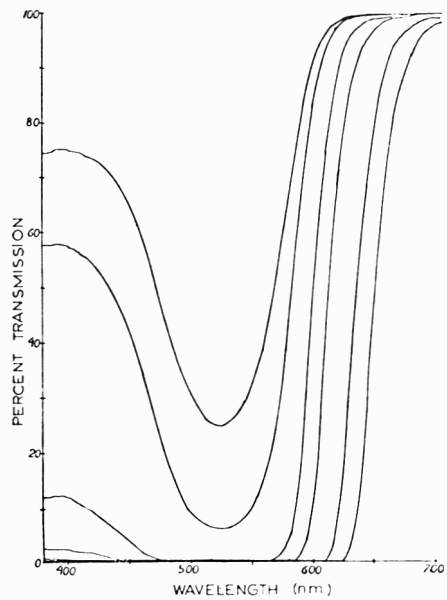

Fig. 6—Spectral curves for solutions of F.D.&C. Red #1. The curves represent 0.05, 0.10, 0.30, 0.70, 3.0 and 10.0 mg pigment per ml.

Hue and chroma functions were tested to see if chromaticity parameter inversion had any affect on them. For scales based on the Judd-Hunter color solid, hue is represented by $\tan^{-1}$ a/b and saturation by $\sqrt{a^2 + b^2}$. For chroma which is analogous to saturation, the inversion occurs precisely at the inversion point of the scale parameter of the predominating color. This means that for either red, green, blue, or yellow liquids, either "a" or "b" will be a large number, and the other much smaller; so when squared, the smaller factor contributes a relatively insignificant amount to the overall function. For a color which has almost equal contributions from both "a" and "b", such as orange or turquoise, the inversion in chroma would occur somewhere between the inversion points of the individual parameters. With hue, on the other hand, the point where inversion occurs is difficult to predict, as the "peak" hue value depends on the relative rates of increase or decrease of both "a" and "b."

Inversion in hue and chroma functions is not desirable. An inversion in the hue function would imply that the samples had a two-stage hue shift; first changing in one direction, then going back toward the hue of the darker samples. Although, on dilution, some of the colorant systems did have a visible hue shift, the shift was in one direction only. Examples of the observed hue shifts are Yellow #6 going from orange to yellow and Green #3 going from more bluish to more greenish. For the types of colorant series used in this research, the darkest samples were also the ones with the highest chroma as judged by the visual panel. Therefore, having an inversion in the chroma function does not agree with the actual system. From the above logic, one can see that errors in hue and chroma functions closely parallel errors in the chromaticity parameters and are not useful for commercial applications.

CONCLUSIONS

OF THE SCALES currently in use, only the C.I.E. X, Y, Z, the Colormaster G, R, B and all luminous transmittance scales correctly rank a pigment dilution series. Basically, this is due to the fact that all chromaticity scale values in these color scales are tied into the luminous transmittance function by a linear multiplication factor. In all of the color scales tested, none of the luminous transmittance functions had areas of confusion. Only chromaticity parameters showed inversions. Thus it appears that in systems where the addition of a pigment both darkens a sample and increases the chroma, the luminous transmittance of the sample is the key to ranking samples correctly by instrumental measurements.

None of the scales tested correlated sufficiently well with pigment concentration to be used as a quality control standard for dark-colored, transparent liquids. However, most scales are acceptible for light colored liquids.

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DEVELOPMENT OF NEW TRANSMISSION COLOR SCALES FOR DARK COLORED BEVERAGES

INTRODUCTION

COLOR is an essential property for consumer acceptance of a food product. Sales of some products, such as red wines or juices, are based for a large part on their color. At present many of the color judgments made on these products are done by human judges. One reason for this is that humans are able to see the total color impact of the product, whereas instruments just measure individual components of color. Also, as demonstrated by Eagerman et al. (1973), the color scales currently in use do not accurately relate to the pigment concentration of samples.

It is observed that the compound functions having inversion areas at the lowest luminous transmittance were the two scales developed by R.S. Hunter (1967a, b; 1969) for use with liquids. They are the L, a′, b′ scales and the Citrus Colorimeter scales. These differ mathematically from the Hunter L, a, b basically by the denominator of the conversion equation. The transmission scales have Y in the denominator as opposed to $Y^{1/2}$ for the reflectance scales. The logic used in changing the denominator was to expand the chromaticity parameters at lower luminous transmittance relative to their values at higher luminous transmittance. Figure 1 shows a graphical representation of the expansion at lower luminous transmittance levels of the L, a′, b′, labelled "a′," scale relative to the L, a, b scale at the same luminous transmittance. For the range of L values, it is shown what "a′" would be relative to constant "a" of 1 or −1. The two-dimensional graph for "b" and "b′" would be indentical. The reason why a scale was proposed which "expands" at lower luminous transmittance is that although the eye is able to adapt to lower luminous transmittance and give color judgments, photocells cannot. This expansion is presumed to be a mathematical adaption mechanism for the photocells. The proposed scale (Fig. 1) partially accomplished the desired effect. The area of confusion was lowered, but grape juice, fruit punch syrup anu prune juice, in 10 nm cells, all produced readings around the area of confusion for these scales (Eagerman et al., 1973). It appears that more "expansion" of the chromaticity parameters is necessary.

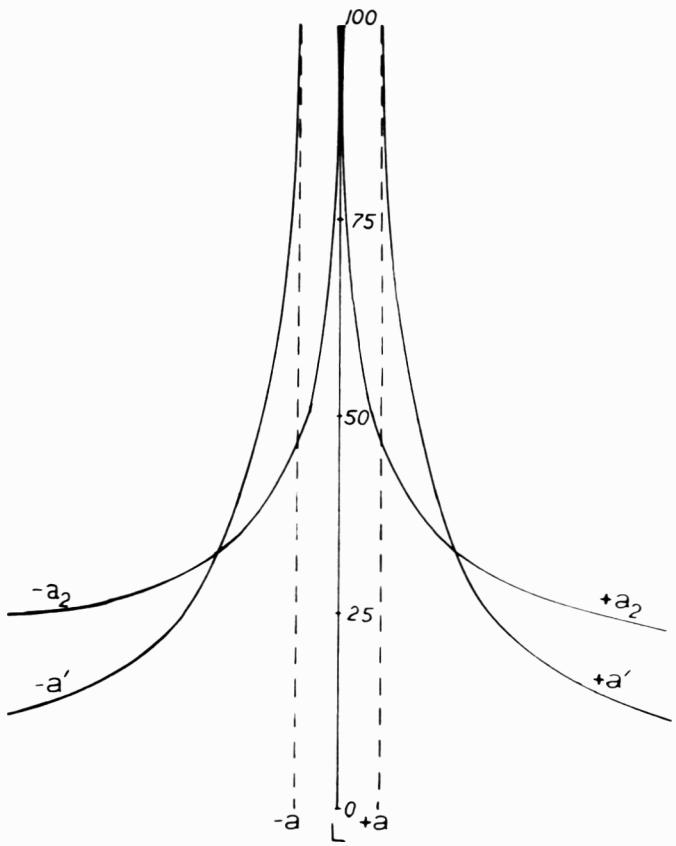

Fig. 1—A representation of L, a′, b′ and L, a_2, b_2 color scale expansion

The reasoning employed for the development of the L, a′, b′ scale provided the basis for the development of new scales specifically designed for transmission colorimetry. The systems proposed are transformations of the data obtained from different colored model systems which are reasonably successful for predicting the pigment concentration of a single colorant system from the tristimulus values obtained by colorimetric evaluation.

The object of this study was to develop a color scale suitable for use in on-line measurements of liquids, so as to adjust the color of the liquid to within present limits. Preliminary studies have been done on the feasibility of such a system for Cranberry Juice Cocktail production, using a concentrated pigment source to add color to the juice as needed (Francis and Clydesdale, 1970; Chiriboga and Francis, 1970). In one study by Staples and Francis (1968), wide-range spectrophotometry was used, rather than tristimulus values.

EXPERIMENTAL

INSTRUMENTS, mode of presentation and preparation of samples has been described previously by Eagerman et al., 1973.

The determinations of linearity with pigment or colorant concentration were done with a stock solution of 10 mg colorant per milliliter diluted at intervals of at least 0.1 mg/ml. The

intervals were different for different systems, because dilutions were initially made at different times for varying purposes. This was done with the following F.D.&C. food colorants: Red #1, Red #2, Green #3, Yellow #6 and Blue #1. A 100-fold dilution was also done using cyanidin-3-glucoside pigment, but from a starting concentration of 17.25 mg/ml. The range of luminous transmittance tested was from a C.I.E. Y of 2.5–88 which corresponds to a Hunter L of 15–91.

All computer work was done on a CDC 3600 computer (Control Data Corp.). The programs for finding the desired formulas and the subroutine for plotting the curves produced by various formulas were developed as a part of this study (Eagerman, 1972). A simple linear regression analysis program was used to determine the linearity of the plots produced by the above programs. The linear regression program used was a stored program obtained from the statistical files of the University of Massachusetts time-sharing computer system.

DISCUSSION

AS MENTIONED in the introduction, greater expansion of the parameters at lower luminous transmittance than that given by Y in the denominator was necessary. Employing this logic Y^2 was tried as the denominator, while leaving the numerator unchanged. Figure 1 shows the expansion caused by a function with Y^2 in the denominator (called "a_2"). Using Y^2 as the denominator eliminated the inversion of the parameters and created scales which were close to being linear with pigment concentration when plotted on rectangular coordinate paper. Figure 2 is a plot of the data from four different colored systems, cyanidin-3-glucoside, F.D.&C. Red #1, Red #2 and Yellow#6. Each is a plot of: $a_2 = 170(1.02X - Y)/Y^2$. However, each colored system produced lines of different slopes, even though all lines were reasonably linear.

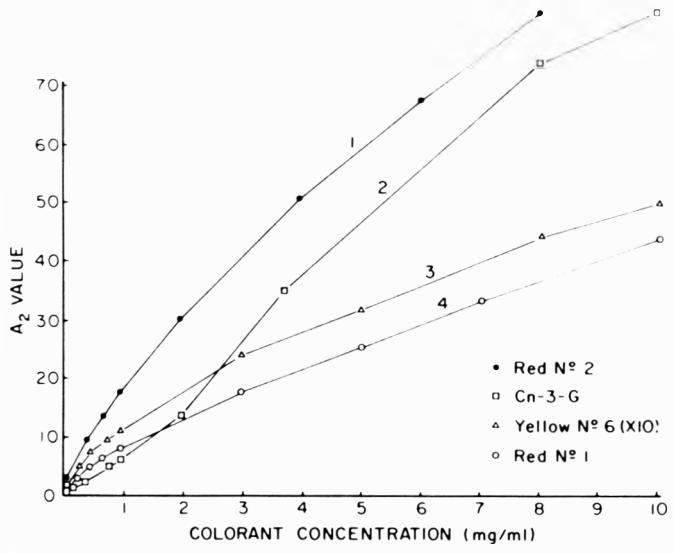

Fig. 2–Plot of "a_2" for each of the colorant systems. The coefficients of determination for the curves 1, 2, 3 and 4 are 0.9865, 0.9937, 0.9724 and 0.9901, respectively

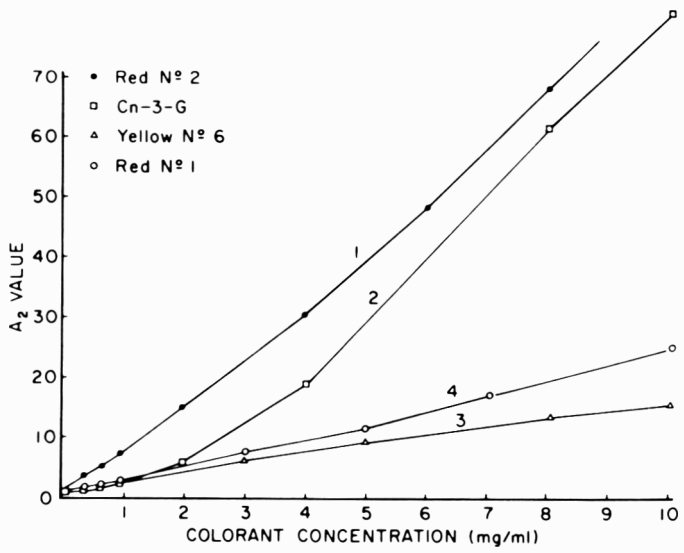

Fig. 3–Comparison of colors at the best formula for Red #1. The coefficients of determination for curves 1, 2, 3 and 4 are 0.9884, 0.9758, 0.9881 and 0.9994, respectively.

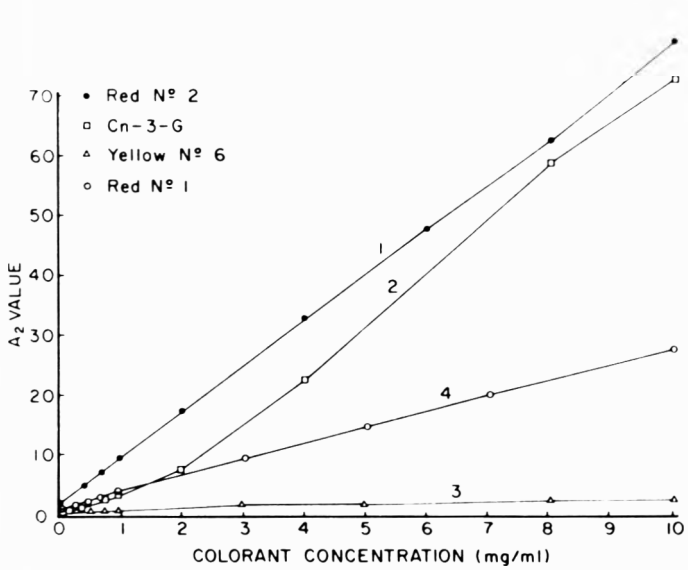

Fig. 4–Comparison of colors at the best formula for Red #2. The coefficients of determination for curves 1, 2, 3 and 4 are 0.9999, 0.9865, 0.9851 and 0.9990, respectively.

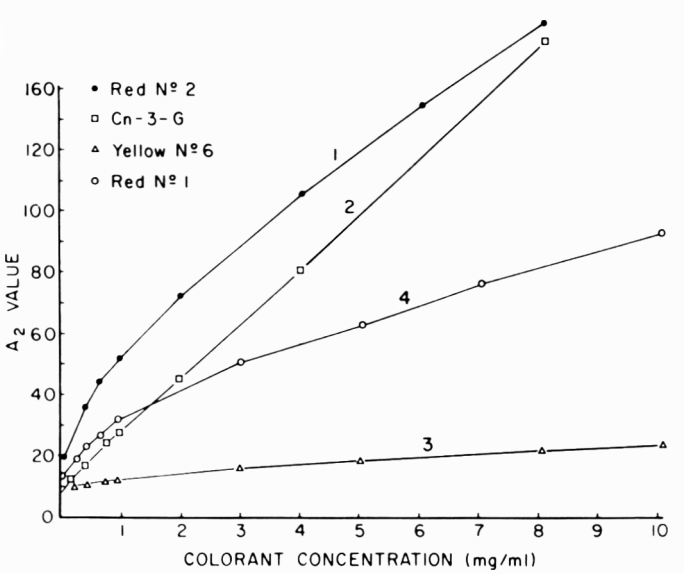

Fig. 5–Comparison of colors at the best formula for cyanidin-3-glucoside. The coefficients of determination for curves 1, 2, 3 and 4 are 0.9814, 0.9996, 0.9626 and 0.9351, respectively.

Observing that despite the differing slopes, all the curves had similar shapes, a graphical solution was attempted. Using a computer (CDC 3600) to do both the calculations and the graph plotting, various exponents and additive constants were used in several attempts to find formulas which most closely approximated linear relationships between colorant concentration and parameter values. The formulas developed will be called "a*" and "b*" to denote the relationship to the Hunter Color solid.

It was hoped that one formula for "a*" and one for "b*" would linearize all of the colored systems, but that did not happen. Because of differing slopes, the multiplying factors "straightened" each curve to a different extent. As a result, different formulas had to be found for each colorant system.

As may be seen in Table 1 and Figures 3, 4, 5 and 6, there are many possible combinations of exponents and additive constants which give plots very close to linear, and coefficients of determination better than 0.9990. The four graphs of Figures 3, 4, 5 and 6 plot the best formula for each colorant system and show the curves for the rest of the colors using that formula. The coefficient of determination, r^2, for each of the curves is included.

Changing the numerator to 100X instead of the Hunter style numerator, 170(1.02X − Y), was done to determine if there was any loss of accuracy by making this approximation. The coefficients of determination show that there is none. Also tried as an approximation for the numerator was 1000, but here the coefficient of determination dropped to approximately 0.92.

One reason why "100X" may be used as a simplification in the numerator and not "1000" is that the only portion of the curve in which the numerator is important to the overall function is in the lowest luminous transmittance region. With red samples, "X" has the largest value and is the most rapidly increasing in this area. In the lighter regions, even though the "X" numerator gives a much larger value than the Hunter style numerator, the denominator is so large that the numerator means very little. The additive term in the denominator is necessary to linearize the darkest portion, and has very little effect (less than 1%) above a "Y" of 20 or "L" of 45. Blue #1 and Green #3 are not being considered in this discussion, because their spectra are quite different, whereas the three reds and the yellow are similar.

The above process to relate color scales to pigment content bears some relationship to the use of broad band filters in absorptimetry prior to the present popularity of grating monochromators. The important difference is that the present equations utilize most of the visible spectrum instead of a small portion. It remains to be seen whether this approach will provide a more sensitive mechanism for pigment systems, which extend over a broader portion of the spectrum, than that employed with an absorptimeter. The distortion of the conventional color scales, which would occur when the coefficients are changed, will obviously affect

Table 1—Formulas and degree of linearity for color quality parameters which are linear to colorant concentration

Colorant or pigment	Parameter (a* or b*)	Formula	Slope	Coefficient of detm
F.D.&C. Red #1	a*	$\frac{170(1.02X - Y)}{Y^{2.33} - 2.5}$	2.50	0.9995
	a*	$\frac{170(1.02X - Y)}{Y^{2.35} - 2.5}$	2.43	0.9994
	a*	$\frac{100X}{Y^{2.35} - 2.5}$	2.36	0.9994
	a*	$\frac{170(1.02X - Y)}{Y^{2.35} - 3.0}$	2.45	0.9993
F.D.&C. Red #2	a*	$\frac{100X}{Y^{2.30} - 0.5}$	7.61	0.9999
	a*	$\frac{170(1.02X - Y)}{Y^{2.30} - 0.5}$	7.57	0.9998
	a*	$\frac{170(1.02X - Y)}{Y^{2.25} - 0.5}$	7.94	0.9996
	a*	$\frac{170(1.02X - Y)}{Y^{2.15} - 1.0}$	9.32	0.9993
Cyanidin-3-glucoside	a*	$\frac{100X}{Y^{1.60} - 1.5}$	18.53	0.9996
	a*	$\frac{170(1.02X - Y)}{Y^{1.60} - 2.0}$	22.25	0.9992
	a*	$\frac{170(1.02X - Y)}{Y^{1.65} - 2.0}$	20.55	0.9990
F.D.&C. Yellow #6	a*	$\frac{170(1.02X - Y)}{Y^{3.10}}$	0.0127	0.9992
F.D.&C. Green #3	b*	$\frac{120(Y - 0.847Z)}{Y^{1.65}}$	−6.47	0.9996
F.D.&C. Blue #1	b*	$\frac{120(Y - 0.84Z)}{Y^{1.90}}$	−147.6	0.9994

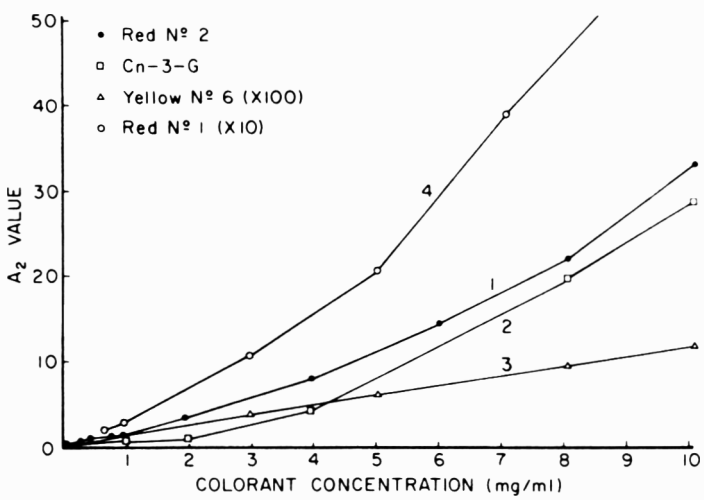

Fig. 6—Comparison of colors at the best formula for Yellow #6. The coefficients of determination of curves 1, 2, 3 and 4 are 0.9762, 0.9508, 0.9992 and 0.9749, respectively.

the relationship to visual appearance. However, if this distortion is too large, the colorimeter could be programmed internally to read out any desired scale, and, with a switch, converted back to normal operation. This principle is employed in the latest model of the Tomato Colorimeter.

CONCLUSIONS

POSSIBLY in a system where the luminous transmittance or chroma could be varied independently of each other, the procedure formulated in this investigation would not work. An example of this type of system might be one in which several different varieties of berries, each having different pigments were mixed into the same solution.

With some of the colorants used, there was a drastic hue shift over the range of luminous transmittance tested. This is what necessitated the evaluation of the readings into primarily a lightness-darkness measurement. The formulas are not strictly lightness-darkness measures, because "$1/Y^{exp}$" gives much lower correlations than using a Hunter style numerator or "X" in the numerator.

Since different formulas were necessary for each color employed, it would not be feasible to develop a colorimeter which would give accurate readings related to pigment concentration for all systems. An acceptable compromise might be to make a colorimeter which gives "a_2" and "b_2" readings, using Y^2 in the denominator. These are fair approximations for many systems.

If only one product was being worked with exclusively, a perfectly linear equation could probably be developed. As previously mentioned, a different denominator is necessary for each color, and even for the same color, different hues require different denominators. The equations for Red #1, Red #2 and the anthocyanin pigment show this to be true. This means for any other system, an exponential factor must be determined. Using the computer programs developed throughout this study such formulas would not be difficult to obtain. The most important aspect of this research is that it showed the feasibility of predicting the pigment concentrations of transparent liquids from tristimulus values.

If the new scales distort the conventional tristimulus readouts too much, the same colorimeter could easily be programmed to read the new scales as well as the conventional ones.

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FACTORS INFLUENCING COLOR DEGRADATION IN CONCORD GRAPE JUICE

INTRODUCTION

COLOR is the major attribute associated with quality in Concord grape juice. Loss of the characteristic bright, purple color results in an inferior product. Color in Concord grapes is composed of an intricate mixture of anthocyanins which are water-soluble glucosides (Ingalsbe et al., 1963). Anthocyanins are very unstable in fruit juices because of the influence of pH, metal complexes, enzymes and other chemical constituents present both in grapes and under conditions of processing and storage (Sastry and Fischer, 1952; Asen et al., 1969; Peng and Markakis, 1963).

It has been reported that there are 14 anthocyanin pigments in Concord grapes (Ingalsbe et al., 1963). Robinson et al. (1966) suggested that hydroxylation of pigment molecules affected anthocyanin stability in wines. Peonidin and malvidin were the most stable and delphinidin the least stable of the pigments.

Loss of natural color of pigments in grape juices has been shown to be accelerated by processing and storage temperature (Ponting et al., 1960; Sastry and Fischer, 1952) and oxygen (Tressler and Pederson, 1936). The pH had no marked effect on pigments at 0.5 and 0.75 pH units above and below the original pH of fruit juices (Nebesky et al., 1949), although pH markedly affected absorption spectra of metal complexes over a wider pH range (Asen et al., 1969; Jurd and Asen, 1966). Sugars, especially fructose increased the rate of pigment degradation (Tinsley and Bockian, 1960; Daravingas and Cain, 1965). Ascorbic acid (AA) increased the rate of pigment loss in the presence of O_2 (Beattie, et al., 1943; Starr and Francis, 1968). Enzymes catalyzed the breakdown of anthocyanins in fruit juices that had been heat processed (Peng and Markakis, 1963; Grommeck and Markakis, 1964; Wagenknecht et al., 1960).

Under conditions of mechanical harvest of grapes a delay of 12 hr after harvest resulted in a significant loss of color (34%) when measured by color difference meter (Morris et al., 1972). A concentration of 80 ppm SO_2 was sufficient to retard color changes in bulk bins with plastic liners.

The objective of this research was to examine some of the factors influencing pigment degradation in Concord grape juice. Factors investigated were pH, AA, temperature, buffer concentration, enzyme activity, amino acids, phenols and holding time. The purpose was to assess the major changes in pigment content and AA as influenced by these variables in order to improve the stability in grape juices and drinks.

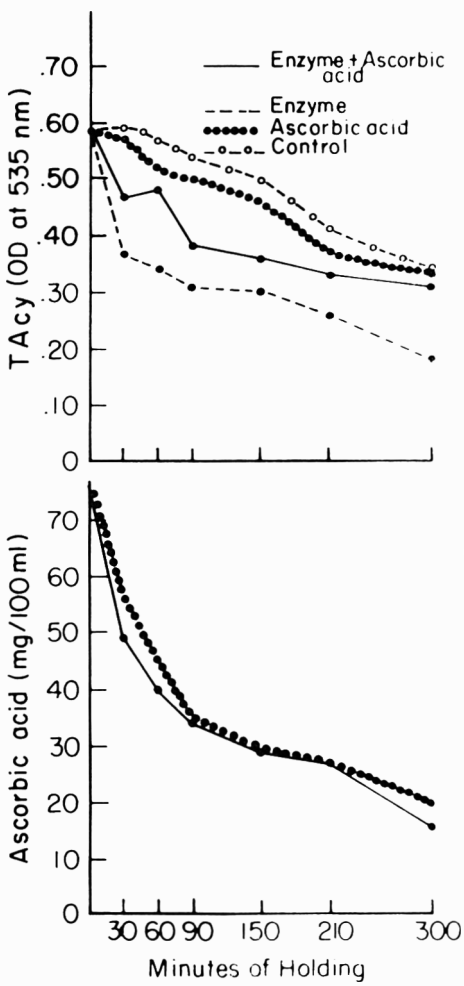

Fig. 1—Interaction of enzyme and ascorbic acid treatments and holding time at 43°C on T Acy and ascorbic acid content of Concord grape juice.

MATERIALS & METHODS

PASTEURIZED Concord grape juice was obtained in a bulk lot of 40 oz jars from Welch Foods, Inc., Springdale, Ark. and stored at 1.7°C for a source of juice. Experimental conditions were maintained in a constant temperature water bath. Each sample consisted of 100 ml, 1 part juice to 2 parts of 0.025M tartaric acid buffer, in 250 ml Erlenmeyer flasks. The pH was adjusted to 3.4 for the experiments except where pH was a variable. Air was bubbled through the samples through tygon tubes from a manifold attached to a small air pump at a flow rate of 15 ml/min. The enzyme polyphenoloxidase (PPO) was prepared from fresh Concord grapes by the method of Reyes and Luh (1960) and was added to samples at a rate equal to 10% of the activity of the grapes; that is, PPO from 10g grapes added to 90 ml of diluted juice. Temperature of water bath was main-

Table 1—Effect of pH, enzyme and holding time on T Acy and AA of Concord grape juice[a]

Main effect	T Acy OD at 535 nm	AA Mg/100 ml[c]
pH[b]		
3.0	0.403	4.49
3.4	0.380	4.46
3.8	0.347	4.39
F Value	314.14**	0.059
LSD @ 5%	0.009	NS
Enzyme		
No enzyme	0.393	5.41
Enzyme	0.357	3.47
F Value	132.14**	245.24**
Holding time at 43°C		
0 hr	0.513	16.80
1 hr	0.373	3.90
3 hr	0.350	0.58
5 hr	0.313	0.35
F Value	251.11**	1,346.65**
LSD @ 5%	0.012	0.45

[a] Six replications of the experiment
[b] pH adjustment by NaOH or tartaric acid
[c] 25 mg AA added per 100 ml

[1] Present address: Seabrook Farms Co., Inc., Seabrook, NJ 08302

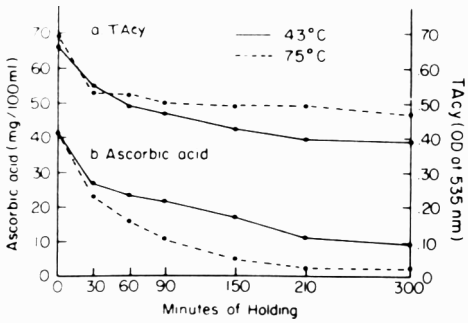

Fig. 2–Interaction of holding temperature and time of T Acy and ascorbic acid content of Concord grape juice.

Table 2–Effect of phenolic substrate on activity of PPO from Concord grapes[a]

Substrate	Conc at pH 6.0 (M)	% Relative activity
Catechol	0.01	100
Cinnamic acid	0.01	0
Coumaric acid	0.01	4
Ferulic acid	0.01	0.8
Caffeic acid	0.01	48
Tyrosine	0.01	3
Phenol	0.01	0
Hydroquinone	0.01	0
D-Catechin	0.003	58

[a] Activity in 0.1M citrate 0.2M phosphate buffer at pH 6.0

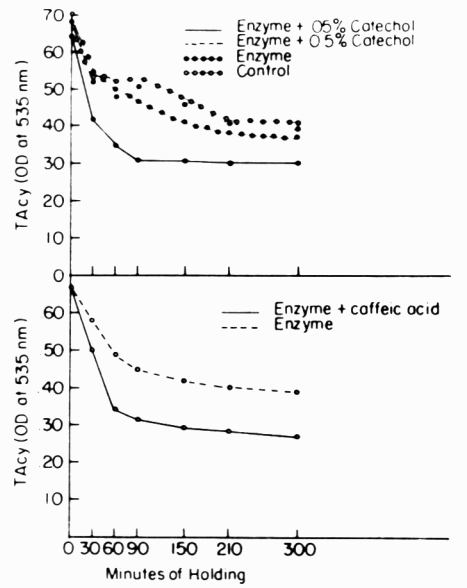

Fig. 3–Interaction of phenolic treatments and holding time at 43°C on extracted T Acy of Concord grape juice.

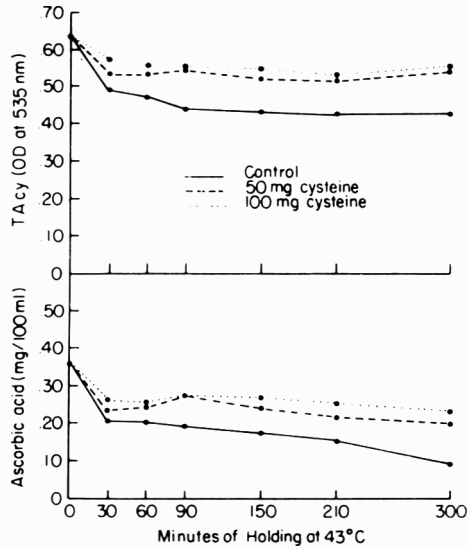

Fig. 4–Interaction of amino acids and holding time at 43°C on extracted TAcy and ascorbic acid of Concord grape juice.

Table 3–Effect of amino acids and temperature on T Acy and AA of Concord grape juice during holding[a]

	43°C		75°C	
Treatment	T Acy OD @ 535 nm	AA mg/100 ml	T Acy OD @ 535 nm	AA mg/100 ml
Control	0.480	21.0	0.538	12.5
50 mg glycine/100 ml	0.485	21.0	0.523	11.3
50 mg cysteine/100 ml	0.554	25.0	0.540	16.1
F Value	6.48**	40.74**	3.67*	18.48**
LSD @ 5% level	0.01	0.7	0.01	1.7

[a] Six replications of this experiment; 25 mg AA added per sample

tained at 43°C for enzyme studies and 75°C for the effect of high temperature. Additives were dissolved in buffer just prior to adding to samples at zero time. Substrate specificity studies were conducted with the purified grape PPO as described by Reyes and Luh (1960) except the PPO from 100g of grapes was suspended in 100 ml of 0.01N acetate buffer at pH 6.0 and diluted 1:3 with acetate buffer before using.

Flasks were sampled at 30 min intervals for 90 min, then hourly intervals thereafter. Volume changes were checked before sampling and distilled water added to replace losses. 3-ml aliquots were removed from the flasks at each sampling period and placed in small vials to cool to room temperature. 1-ml aliquots were analyzed for reduced AA by the colorimetric method of Morell (1941) except that samples were diluted with 3% metaphosphoric acid. Total anthocyanins (T Acy) were obtained by diluting 1 ml aliquots with 19 ml of extracting solvent which was composed of 95% ETOH-1.5N HCl in a ratio of 85:15 (Fuleki and Francis, 1968). Diluted samples were allowed to stand 1 hr before reading OD at 535 nm, the absorption maximum of the extract at pH 1.0.

RESULTS & DISCUSSION

MANY OF THE changes in T Acy in grape juice appear to be autodegradative and conditioned by many constituents although the variables enumerated in this study account for a large percentage of the pigment breakdown.

The greatest loss in ascorbic acid (AA) occurred during the first 90 min of holding at 43°C (Fig. 1, Table 1). The graph for AA was not drawn for samples where it was not added because of the low concentration. Changes in T Acy were more gradual during holding except in flasks that contained enzyme. AA inhibited the activity of the enzyme until most of the AA was degraded. AA actually enhanced the visual color of the juice initially because of the effect on browning reactions although browning per se was not measured by the procedure for T Acy. AA increased the reducing atmosphere of the system, thus holding the oxidation of pigments to a minimum during the first 90 min. There was little difference in the loss of AA either with or without enzyme at the 5 hr time period.

There was an interaction between holding time and temperature on T Acy and AA content of Concord grape juice (Fig. 2). The T Acy were degraded at 43°C at about the same rate as AA was oxidized. However, at 75°C, AA decreased much more rapidly; whereas, T Acy appeared to stabilize after the 30 min holding period. Beattie et al. (1943) indicated that AA was destroyed more rapidly at high temperatures. AA was added in the present research to study its influence on other variables.

Catechol (0.05%) increased the activity of the enzyme in the destruction of anthocyanins but a concentration of 0.5% inhibited the action of the enzyme as demonstrated by the decrease in T Acy (Fig. 3). Earlier studies by Peng and Markakis (1963) have shown similar results with mushroom phenolase. Caffeic acid was one of the selective substrates of the enzyme from Concord grapes as indicated by the more rapid loss of T Acy when it was added (Fig. 3). At 75°C, 0.05% Catechol had a significant protective effect on AA although caffeic acid did not affect AA (data not shown).

PPO from Concord grapes exhibited

more activity with Catechol than any of the other substrates tested in Citrate-phosphate buffer (Table 2). The enzyme characterization is the subject of a later paper.

The amino acid cysteine inhibited T Acy degradation at 43°C although at 75°C cysteine did not affect T Acy (Table 3). Glycine did not influence either T Acy or AA at 43°C but at 75°C there was a significant decrease in T Acy. Cysteine protected AA from oxidation at both temperatures. By increasing the cysteine concentration to 100mg/100ml, there was a further improvement in T Acy and protection of AA (Fig. 4). Cysteine probably supplies the additional H+ ions for the reducing atmosphere as well as blocks the action of the enzyme. In concurrent studies it has been shown that either 40 ppm cysteine or 30 ppm SO_2 was sufficient to inhibit the activity of the purified PPO when using catechol as a substrate (data not shown). Sistrunk (1972) has demonstrated the inhibition of T Acy degradation by SO_2 in Concord grape juice.

T Acy changes were more rapid at pH 3.8 than at either 3.4 or 3.0 (Table 1). Part of the change in T Acy at pH 3.8 as compared to 3.0 could have resulted from action of enzyme since enzyme activity was greater at pH 3.8 AA was not significantly influenced by pH even though there was slightly less at the higher pH. AA was degraded more rapidly in the presence of enzyme. Again, as in other experiments most of the AA was oxidized during the first hour of holding.

Concentration of buffer had a significant effect on T Acy and AA (Table 4). In samples with no added tartaric acid the strength of the buffer system was 1/3 of the original juice, approximately 0.25% acidity. Loss of T Acy was greater in this weak buffer system than in more concentrated buffer. The higher concentration (1.5%) caused more precipitation of pigment. Other studies in our laboratory have shown that higher buffer concentration resulted in more detartration and pigment loss in grape juice. Apparently AA was less stable in samples with high buffer concentration.

Table 4—Effect of buffer concentration and time on T Acy and AA of Concord grape juice[a]

Main effect	T Acy OD at 535 nm	AA Mg/100 ml[c]
Buffer[b]		
0	0.659	9.41
0.75% tartaric acid	0.671	9.98
1.5% tartaric acid	0.636	8.31
F Value	23.82**	3.61*
LSD @ 5%	0.012	1.28
Holding time @ 43°C		
0 hr	0.720	27.6
1 hr	0.677	12.2
3 hr	0.621	1.6
5 hr	0.526	1.0
F Value	281.50**	231.75**
LSD @ 5%	0.016	1.9

[a] Four replications of experiment
[b] All samples adjusted to pH 3.4 with NaOH
[c] 25 mg AA added per 100 ml

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Escherichia coli ON PECANS: SURVIVAL UNDER VARIOUS STORAGE CONDITIONS AND DISINFECTION WITH PROPYLENE OXIDE

INTRODUCTION

THE PRESENCE of pathogenic microorganisms in food commodities and their survival and growth under various storage conditions are of extreme interest to the food industry and to regulatory agencies concerned with consumer protection. Potential public health problems associated with microbial contamination of tree nuts are particularly important since nuts often receive minimal processing at the plant and may receive no further processing before incorporation into products such as bakery and confectionery items.

The presence and identification of fungi on nonprocessed (Chipley and Heaton, 1971; Hanlin, 1971; Wehner and Rabie, 1970) and processed pecans (Lillard et al., 1970) have been reported. Demonstrated presence and numbers of coliforms, in particular *Escherichia coli*, on pecans is inconsistent. Ostrolenk and Welch (1941) concluded that nutmeats within the unbroken shell did not contain coliforms but that *E. coli* could, if introduced artificially, remain viable for 68 days. Hyndman (1963) suggested the use of the presence of *E. coli* as a sanitation index for commercially produced pecan nutmeats. Wehner and Rabie (1970) failed to identify any of the 5×10^5 bacteria per g present on nonprocessed pecans as members of the coliform group while Chipley and Heaton (1971) reported 20% of the commercially shelled samples tested contained *E. coli*.

It is not uncommon for less progressive or smaller pecan growers to graze cattle, sheep, goats or swine in their orchards, thus possibly increasing the coliform contamination of nuts. A survey of pecans harvested from orchards which had been grazed with cattle revealed that 45% of the samples were positive for *E. coli* (Marcus and Amling, 1971). Therefore, potential public health problems associated with drug resistance-factor transfer between *E. coli* strains from animal and human origin (Babcock et al., 1973; Sandine et al., 1972) are of considerable importance in the pecan industry.

Chlorine and/or propylene oxide (PO) are employed by the pecan industry to disinfect inshell pecans, halves and pieces. Hanlin (1972) examined the efficiency of commercial PO treatments under controlled laboratory conditions and concluded that maximum allowable dosages were 80% effective in killing surface microflora and 64% effective in killing internal microflora of pecans. Low numbers of naturally occurring coliforms were virtually eliminated.

Realizing the general lack of information available with respect to survival and destruction of *E. coli* on pecans, an investigation was designed to determine the effect of: (1) commercially practiced storage and marketing conditions on the viability of *E. coli* on pecan halves and inshell pecans and (2) various combinations of PO concentration, exposure time, temperature and relative humidity on the destruction of *E. coli* on pecans.

EXPERIMENTAL

Materials

Standard mammouth pecan halves and Stuart variety inshell pecans were stored at 20°C until utilized as outlined below.

Inoculation

E. coli K-12 was cultured in nutrient broth (Difco) at 37°C for 18 hr. The culture was then diluted 100-fold in sterile distilled water at 20°C, resulting in 10^7 cells per ml. Pecan halves were immersed in the *E. coli* suspension for 7–9 min and inshell pecans were immersed 20 min before they were removed, drained briefly and dried in a forced-air oven at 27°C to desired moisture levels.

Moisture determination

5-g portions of ground pecans were dried at 70°C in a vacuum oven for 24 hr and the moisture content was determined by difference.

Storage conditions

All pecan samples were sealed under atmospheric conditions in glass containers. Inoculated pecan halves which had been determined to contain 3.47, 4.54 and 6.18% moisture were packed in 50-g quantities to simplify *E. coli* enumeration procedures which were performed after 1, 2, 4, 8, 16 and 24 wk storage at −7, 0, 14, 21 and 30°C. Inshell pecans (nutmeats contained 2.72% moisture) were stored in bulk quantities at 0°C for 6 and 12 wk. Pecan halves used for PO disinfection studies contained 3.91% moisture and were stored in bulk at 0°C prior to examination.

Propylene oxide disinfection

Desired relative humidities (RHs) under static conditions in sealed desiccators were obtained by the saturated salt solution method described by Rockland (1960). Saturated magnesium chloride, cupric chloride and potassium sulfate solutions resulted in relative humidities of 32 ± 1%, 68 ± 1%, or 97 ± 1%, respectively, depending upon the temperature. A zero to 2% RH was obtained with silica gel. Duplicate 50-g samples of contaminated pecan halves were deposited in mesh baskets and placed on supports above the salt solutions in the equilibrated desiccators. Sufficient liquid PO was deposited in glass petri dishes previously placed on the supports to yield upon evaporation 40, 400, or 800 ppm gaseous phase PO. Tests involving 800 ppm PO required that the pressure be released from the container during evaporation. Therefore it should be noted that these tests may have involved something less than 800 ppm in the gaseous phase of the chamber. Nuts were exposed to all combinations of the following variables: 0, 32, 68 and 97% atmospheric RH; 0, 40, 400 and 800 ppm gaseous phase PO; 20, 30 and 37°C exposure temperatures; and 4- and 16-hr exposure times.

E. coli enumeration

Procedures described by the AOAC (1970) for detection of *E. coli* on tree nutmeat halves were followed, with two exceptions. A 5-tube Most Probable Number (MPN) series instead of a 3-tube series was employed and the enumeration of *E. coli* in the inner tissue portion (comprised mostly of middle septum and packing material) of inshell pecans required the use of 15g material plus 60 ml diluent instead of the suggested 50g plus 50 ml in the case of nutmeat halves. Adjustment in MPN counts for inner tissue was made accordingly.

RESULTS & DISCUSSION

Survival of E. coli on pecans

Survival and growth of pathogenic bacteria in processed foods have been studied extensively at water activities (a_w) in the intermediate and high moisture range. Less information is available relating the effects of a_w subminimal for growth of these organisms on their survival. In a recent study reported by Christian and Stewart, (1972), *Staphylococcus aureus* and *Salmonella newport* were observed to retain viability longer in an a_w range of 0–0.22 than at a_w approaching 0.53. Differences in pH of the cake mix, skim milk, onion soup and gelatin-based dessert used in the study influenced the survival of both organisms at a_w levels ranging from 0.22–0.53. Realizing *E. coli* may be present on pecans as they are delivered to processing plants and that sanitation procedures may not eliminate

the organism, an examination of the effect of various equilibrium relative humidities (ERHs) during storage on survival was made.

Good quality control practices require that sufficient moisture be removed from inshell pecans to result in a 3.5–4.0% moisture level in the nutmeat followed by storage near freezing or below until shelling. Shelled nut quality will also be protected under such conditions; however, the packaged product is generally displayed at room temperature. Table 1 shows the effect of storage temperature and moisture on the viability of *E. coli* on pecan halves stored for up to 24 wk. Although the initial MPN was different at the three moisture levels, the combined storage temperature-moisture effect can be noted during storage period. *Escherichia coli* survived in greater numbers and for a longer time on nuts containing 3.47% moisture, the lowest moisture level examined, irrespective of storage temperature. The 6.18% moisture level proved to be most lethal to *E. coli* while 4.54% moisture was intermediate. The zero MPN registered for 6.18% moisture pecans stored at 21 and 30°C should be regarded with some caution since a massive overgrowth of fungi inhibited *E. coli* detection. Storage at −7, 0 and 14°C resulted in a protective effect for *E. coli* over storage at 21 or 30°C. This was evident at all three moisture levels but was more pronounced as the moisture level was decreased.

As the a_w of the immediate environment surrounding a bacterium is increased, the metabolic activity within the organism also increases, even though cell division may not occur. Thus, at a_w not quite sufficient to support growth, the organism undoubtedly kills itself through rapid expenditure of internal energy. This phenomenon is probably more pronounced at the 21 and 30°C temperatures studied in this experiment. It is unfortunate that moisture and temperature conditions optimum for the preservation of organoleptic quality of pecans also serve to preserve *E. coli*.

In an extensive study to determine the equilibrium moisture content of pecan halves, Woodroof and Heaton (1961) reported that pecan halves at 3.5% moisture would equilibrate at 58% RH. The 4.54 and 6.18% moistures examined in this study would correspond to ERHs of approximately 68 and 80%, respectively. The protective effect of lower a_w on the viability of *S. aureus* and *S. newport* as noted by Christian and Stewart (1972) and discussed above is in agreement with data presented here involving the degree destruction of *E. coli* on pecans at various ERHs.

Survival of *E. coli* on the shell, inner tissue and nutmeat portions of Stuart variety inshell pecans is reported in Table 2. Nutmeats of pecans examined in this experiment contained 2.72% moisture (ERH approximately 46%) and storage was at 0°C. The 20-min immersion in *E. coli* inoculum failed to produce highly contaminated nutmeats, probably due to nut morphology. There is reduced surface contact between the inner tissue and nutmeats in inshell pecans during drying, thus in effect isolating the nutmeats from potential contamination. Cross contamination is, however, likely to occur during the shelling and grading of pecans. It was noted that the 20-min immersion resulted in only a 2% increase in nut weight, whereas 8- and 64-hr dips were later demonstrated to result in 10 and 25% increases in weight, respectively. Such periods of water/nut contact in the orchard are not inconceivable and could result in nuts with much higher levels of contamination. The interesting points to be made in this study are that *E. coli* remained in relatively high numbers on the shell but were reduced markedly on the inner tissue portion during storage. As pointed out by Heaton and Woodroof (1970), moisture is not equally distributed in the nutmeat, inner shell (tissue) and outer shell portions of pecans. An equilibrated inshell pecan having 3.6% moisture in the nutmeat may have 16.8% in the inner tissue and 11.2% in the outer shell. This higher tissue moisture level (and assumed correspondingly higher a_w) may account, in part, for rapid decline in viable *E. coli* in the inner tissue.

Disinfecting power of propylene oxide

As a control, inoculated pecans were exposed to the various temperature-time-RH conditions in the absence of PO. 16 hr of exposure at 30°C and, in particular, at 37°C resulted in considerable reduction in viability. The loss in viability should be kept in mind when comparing the lethalities of 40, 400 and 800 ppm PO.

Lethal effects of PO on *E. coli* present on pecan halves are shown in Table 3. In general each concentration of PO under the same RH conditions was more effective in reducing *E. coli* population as the temperature and time of exposure were increased. Lethality was also greater at set temperature-RH conditions when PO concentration was increased. The effects of RH on the effectiveness of PO as a disinfectant under the same temperature-time conditions are not as clear cut. At 20°C, PO at a specific concentration appears to be more destructive at 33 and 68% RH, while at 30 and 37°C RHs of 32, 67, or 97% result in little difference in survivors when considering the lethal effects of temperature and RH alone. Specific PO levels were less destructive at 0% RH, re-

Table 1—Most Probable Number (MPN) of Escherichia coli per g pecan halves

Moisture (%)	Storage time (wk)	Storage temperature (°C)				
		−7	0	14	21	30
3.47	0	490,000	490,000	490,000	490,000	490,000
	1	–[a]	–	–	130,000	210,000
	2	240,000	79,000	240,000	240,000	24,000
	4	240,000	130,000	240,000	92,000	13,000
	8	17,000	130,000	130,000	–	–
	24	24,000	24,000	24,000	–	–
4.54	0	240,000	240,000	240,000	240,000	240,000
	1	–	–	–	79,000	17,000
	2	3,300	79,000	79,000	49,000	45
	4	–	–	–	4,900	3
	8	24	24,000	92,000	790	0
	16	7	16,000	920	17	0
	24	51	3,300	330	1	0
6.18	0	70,000	70,000	70,000	70,000	70,000
	1	–	–	–	55,000	55,000
	2	1,100	23,000	23,000	790	0
	4	–	–	–	0	0
	8	49	2,400	45	0	0
	16	11	1,600	0	0	0
	24	33	130	0	0	0

[a] No test performed

Table 2—Most Probable Number (MPN) of Escherichia coli per g pecan

Storage time (wk)	Portion examined		
	Shell	Inner tissue	Nutmeat
0	44,500	1400	17
6	10,500	12	17
12	7,200	0	6

Table 3–Most Probable Number (MPN) of Escherichia coli per g pecan halves. Inoculated pecans initially contained 150,000 cells per g; moisture was 3.91%

Temp (°C)	% Relative humidity	0 ppm PO Hr exposure 4	0 ppm PO Hr exposure 16	40 ppm PO Hr exposure 4	40 ppm PO Hr exposure 16	400 ppm PO Hr exposure 4	400 ppm PO Hr exposure 16	800 ppm PO Hr exposure 4	800 ppm PO Hr exposure 16
20	0	150,000	150,000	130,000	13,000	31,000	31,000	31,000	8,300
	33	150,000	73,000	125,000	32,000	7,100	7,600	7,100	150
	68	150,000	150,000	86,000	17,000	6,000	5,100	4,800	490
	97	150,000	150,000	150,000	31,000	22,000	22,000	11,000	170
30	0	38,000	17,200	33,000	2,700	32,000	3,200	5,200	2,900
	32	150,000	16,000	91,500	840	630	95	71	0
	67	90,000	53,000	60,000	33,000	560	900	14	0
	97	61,000	39,000	79,000	16,000	470	0	9	0
37	0	71,000	1,900	17,600	1,550	1,680	2	0	0
	32	41,000	4,700	7,600	0	0	0	0	0
	67	80,000	8,100	12,000	500	2,400	0	0	0
	96	20,000	4,700	5,500	2	0	0	0	0

gardless of the temperature. Bruch and Koesterer (1961) found PO to be more active at 25% than at 85% RH, however, these results were questioned by Himmelfarb et al. (1962) who exposed desiccated samples to various systems and then admitted water vapor. In the latter case desiccation may have effected tolerance to PO during the rehydration process. Tawaratani and Shibasaki (1972) reported that the moisture content of fungus spores was more effective than the RH of exposure atmosphere against the fungicidal action of PO.

All of the above reports, as well as the data presented here, seem to suggest that PO lethality is extremely dependent upon the physical state of the organism with respect to moisture content and the RH of the atmosphere under which fumigation occurs. With this in mind, an investigation of hydration/dehydration phenomena of pecan halves initially at 3.91% moisture was made. Results are shown in Figure 1. According to Woodroof and Heaton (1961) pecan nutmeats containing 3.91% moisture would equilibrate, if given sufficient time, at approximately 63% RH at temperatures ranging from 0 – 30°C. It is not surprising, as indicated in Figure 1, that pecans lost moisture when exposed to 0 and 32% RH for 16 hr and gained when exposed to 67 and 97% RH. Rates of moisture loss or gain were generally more rapid at 37 than 20°C. These data do not support the thesis that dehydration or hydration processes alone account for differences in *E. coli* tolerance to PO. However 32, 68 and 97% RH does enhance effectiveness over 0% RH.

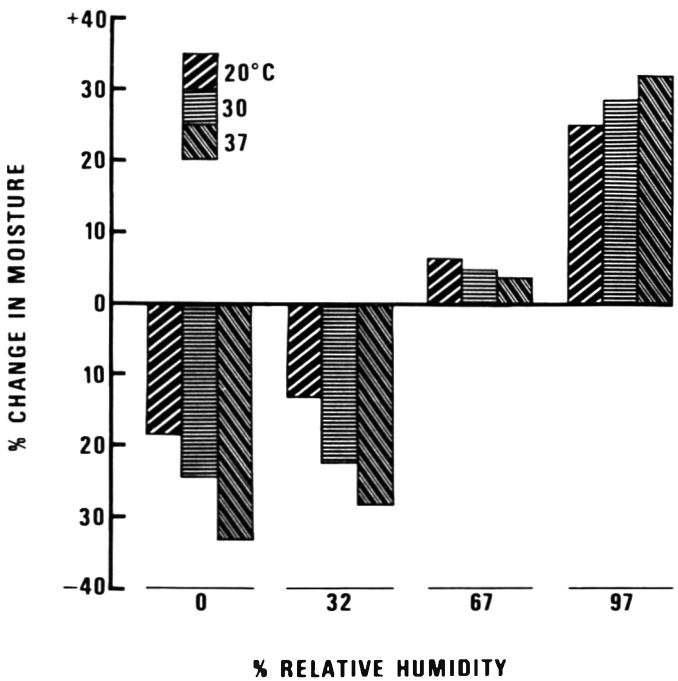

Fig. 1–Changes in the moisture content of pecan halves after exposure to 0, 32, 67 and 97% RH at 20, 30 and 37°C for 16 hr. Initial moisture was 3.91% (ERH = 63%).

There has been concern over the accumulation of toxic chlorohydrins, propylene glycol and PO residues during fumigation of foodstuffs. The possibility of such residues is enhanced by the presence of moisture (Wesley et al., 1965; Tawaratani and Shibasaki, 1972). Residue limits on nutmeats are set at 300 ppm of PO by FDA regulation 121.1076 (Federal Register, 1972). Work is planned in this laboratory to analyze PO-treated pecans for the presence of absorbed PO and propylene glycol. There is some reluctancy in advising PO fumigation levels at this time; however, PO concentrations up to 400 ppm may be required to disinfect heavily contaminated nuts.

Based on the data presented in this study, it seems advisable to fumigate pecans at RHs at or below their ERH but not at 0% RH. Disinfection was best when exposure time was extended from 4 to 16 hr; a temperature of 30°C was judged as more desirable in light of the deleterious effects treatment at 37°C might have on organoleptic qualities of the nut during subsequent storage. Such treatment conditions will increase the lethality of PO to *E. coli* and should minimize the possibility of toxic residue accumulation.

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NATURAL INHIBITORS OF NITROSATION REACTIONS: THE CONCEPT OF AVAILABLE NITRITE

INTRODUCTION

CHEMICAL FORMATION of N-nitroso compounds from nitrite and their parent amines has been well established (Mirvish, 1970, 1972; Boyland, 1971; Boyland et al., 1971; Fan and Tannenbaum, 1973). It has also been speculated that N-nitroso compounds might form from the precursors present in food systems (Lijinsky and Epstein, 1970; Wolff and Wasserman, 1972). Many reports have been published concerning the presence of nitrosamines, particularly dimethylnitrosamine (DMN), in food products (Sen et al., 1969; Sen et al., 1970; Howard et al., 1970; Telling et al., 1971; Fazio et al., 1971; Sen, 1972; Fiddler et al., 1972). There is no doubt that nitrosamines can be formed in food products if the concentrations of nitrite or nitrosatable amine are high enough and the conditions are appropriate. However, the results of various studies do not allow us to predict possible formation of significant amounts of nitrosamines in food products. This difficulty could be due to several variables which are found in the preparation and storage of food products, e.g., the concentration of nitrite and amine in the food products, the presence and concentration of other ingredients which are able to catalyze or inhibit the nitrosation, the actual processing conditions, and the subsequent time and temperature of storage. In most reports not all the variables were clearly defined or controlled.

The results from a model system may serve as a reference to predict the formation of N-nitroso compounds in foods. However, direct application of results from model systems to food systems sometimes may be misleading. Since nitrite is active in redox reactions, many chemical components in foods may react with nitrite and thus reduce the nitrite available for nitrosation. Fiddler et al. (1973) studied the effect of nitrite concentration on DMN formation in frankfurters. Only 3 μg DMN/kg frankfurters was reported when 750 mg/kg of $NaNO_2$ was added after 2 hr of processing time, whereas estimates from the results of model systems predict concentrations of 20–30 μg/kg (Fan and Tannenbaum, 1973).

Mirvish et al., 1972, and Fiddler et al., 1973, have demonstrated that ascorbic acid, sodium ascorbate, or sodium isoascorbate can block the nitrosation reaction by reacting with nitrite (Dahn et al., 1960); in addition, it can be added to comminuted meat to enhance color formation (Watts and Lehmann, 1952). In this report, we present the effect of ascorbic acid on the formation of nitrosomorpholine (NMor) in a model system. We have also studied the formation of NMor in milk, as an example of a nitrosation reaction in a simple food.

EXPERIMENTAL

Kinetics of nitrosation of morpholine with sodium nitrite in the presence of ascorbic acid

Ascorbic acid was dissolved in about 23 ml of citrate-phosphate buffer solution at the reaction pH. 1 ml of morpholine solution which was prepared by dissolving morpholine in the buffer solution was added to the ascorbic acid solution. Since morpholine is a basic compound, perchloric acid was required to adjust the pH to the reaction pH. The ascorbic acid-morpholine solution was then brought up to 25 ml in a volumetric flask. Sodium nitrite was dissolved in buffer solution, and the final volume was brought up to 25 ml. The reaction was initiated by mixing the sodium nitrite solution and the ascorbic acid-morpholine solution. The reaction rate was followed in a manner similar to that previously published (Fan and Tannenbaum, 1973). At predetermined time intervals, varying from 15 to 30 min for a total reaction time up to 2 hr, 5-ml samples were withdrawn and added to excess amounts of ammonium sulfamate crystals to quench the reaction. Usually, perchloric acid was added to lower the pH below 3.0 in order to facilitate the reaction between nitrite and sulfamate. After 15 min, 2 drops of 50% NaOH were added to bring the pH up to about 5 for extraction of nitrosomorpholine. 5 ml of dichloromethane (spectranalyzed grade) was added, and the sample was shaken on a Vortex mixer for 60 sec. After 5 min, the mixing was repeated and the sample was allowed to settle for approximately 20 min. The lower phase (dichloromethane phase) was read in a spectrophotometer at 260 nm against a blank containing all reactants except nitrite or morpholine.

Kinetics of nitrosation of morpholine with sodium nitrite in a reconstituted dry milk system

Commercial nonfat dry milk was dissolved in distilled water; the pH was 6.8. Sulfuric acid

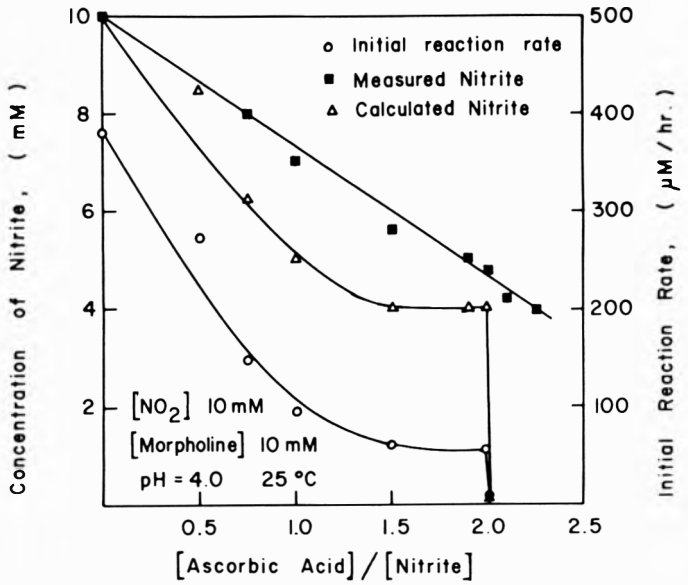

Fig. 1—Effect of the concentration ratio of ascorbic acid to nitrite on the nitrosation rate of morpholine and the residual concentration of nitrite in reaction systems 30 min after initiation.

Table 1—The effect of pH on the measurable concentration of nitrite in the nitrosation systems of morpholine and nitrite in the presence and absence of ascorbic acid[a]

Time	With ascorbic acid				Without ascorbic acid			
(min)	pH 5[b]	pH 4	pH 3	pH 2	pH 5[b]	pH 4	pH 3	pH 2
0	10				10			
15	9.4				9.6			
15+[c]		8.8	6.6	5.8		9.6	9.6	9.2
30		6.6	4.6	4.0		9.2	8.0	5.0

[a] The initial concentrations of nitrite, morpholine and ascorbic acid were 10 mM each.
[b] Initial pH of all reaction systems was 5; 15 min after the initiation of the reaction, the pH's were adjusted to 2, 3 and 4.
[c] 15+ indicates the moment immediately following the adjustment of pH.

was added if the reaction was to be conducted at lower pH (perchloric acid was not suitable for pH adjustment here, since it would precipitate proteins of milk). Solutions of morpholine and sodium nitrite at reaction pH were separately diluted by milk to the desired concentration. 25 ml each of morpholine and sodium nitrite in milk were mixed and incubated in a water bath. At predetermined intervals, 10 ml of sample was withdrawn. Ammonium sulfamate was added to the sample to quench the reaction. Perchloric acid was added to lower the pH and to precipitate the protein. The final pH was about 2.5. Samples were centrifuged at 7,000 rpm for 20 min, and the pH of the supernatant was brought up to 7 with 50% NaOH. Precipitation reoccurred when the pH was higher than 6.5. The samples were again centrifuged at 7,000 rpm for 20 min. The UV photoanalysis procedure of Fan and Tannenbaum (1971) was used to determine the concentration of nitrosomorpholine.

Recovery was complete throughout the procedure when repeated with samples spiked with known concentrations of nitrosomorpholine.

Measurement of concentration of nitrite

Samples were diluted with pH 7 buffer to appropriate concentrations, and nitrite was measured by the Griess reaction (Fan and Tannenbaum, 1971). Ascorbic acid does not interfere with this procedure for nitrite analysis over the concentration range of ascorbate used in these experiments.

Estimation of initial reaction rate

Rates were calculated by the Method of Initial Rates as described by Fan and Tannenbaum (1973). Typical reactant concentrations were 10 mM; approximately 5 points were taken for each rate measurement.

RESULTS & DISCUSSION

Ascorbic acid

Different concentration ratios of ascorbic acid to nitrite were used to study the effect of ascorbic acid on the formation of nitrosomorpholine at pH 4. The results are shown in Figure 1. Ascorbic acid has been demonstrated to act as a blocking agent for the formation of N-nitroso compounds (Mirvish, 1972). This is confirmed by the results in Figure 1. When the concentration ratio of ascorbic acid to nitrite was larger than 2, the formation of nitrosomorpholine from morpholine and nitrite was completely inhibited. When the concentration ratio was smaller than 2, the formation of nitrosomorpholine was partially inhibited, and the rate of nitrosomorpholine formation decreased with an increase in the concentration ratio of ascorbic acid to nitrite. Since Mirvish (1972) postulated that the inhibition effect was due to the reaction between nitrite and ascorbic acid, nitrite concentrations were measured 30 min after the reaction was initiated. The measured nitrite concentrations are also shown in Figure 1. There is a linear decrease of nitrite concentration as the ratio of ascorbic acid to nitrite is increased. As a comparison, the nitrite concentrations were calculated from the initial rate of nitrosation, assuming the reaction to be second order with respect to the concentration of nitrite (Fan and Tannenbaum, 1973). These calculations also appear in Figure 1. The measured nitrite concentrations were invariably higher than the calculated nitrite concentrations.

The reaction of morpholine with sodium nitrite was studied at an initial pH of 5 in the absence of ascorbic acid, and with a concentration ratio of ascorbic acid to nitrite of 1. 15 min after the initiation of the reaction, the pH of the reaction systems was decreased to 4, 3, or 2 by the addition of sulfuric acid. The concentration of nitrite was measured at zero time, 15 min, the moment immediately following the adjustment of pH, and 30 min into the reaction. The results are shown in Table 1. In the absence of ascorbic acid, the concentrations of nitrite remained unchanged before and after the adjustment of pH, while in the presence of ascorbic acid, substantial changes of concentrations of nitrite were observed immediately following the adjustment of the pH. There were also nitrite losses at 30 min at pH 2 and 3, which were independent of ascorbic acid and caused by volatilization of nitrogen oxides.

It is possible that nitrite may react with ascorbic acid and become unavailable for participation in other nitrosation reactions. Upon the addition of color reagents (sulfanilic acid and naphthylethylenediamine) for the measurement of nitrite, nitrite bound to ascorbic acid is released and thus measurable. Therefore, measured nitrite concentration (by the Griess reaction) is higher than the nitrite concentration calculated from the nitrosation rate of morpholine. The binding between nitrite and ascorbic acid is pH sensitive. In the presence of ascorbic acid, lowering the pH resulted in immediate loss of some measurable nitrite. The chemistry of this process is unclear.

Milk

To investigate the formation of N-nitroso compounds in more complex food systems, the nitrosation of morpholine by nitrite was studied in reconstituted dehydrated milk. This is considered the simplest food system for the study of kinetics of nitrosamine formation, since the precipitation of protein by the addition of perchloric acid conveniently prepares the sample for analysis by the colorimetric method. The results of nitrosomorpholine formation in milk system appear in Table 2. The rate of nitrosation in milk was retarded compared with the reaction in buffer solution at pH 6 and 25°C. The measurement of residual nitrite in the reaction systems by the color reagent showed that the nitrite concentration remained constant for at least 24 hr in both the buffer system and in the milk system. The results indicated again that nitrite which is measurable colorimetrically may not necessarily be active as a nitrosating agent.

Originally, it was suspected that nitrite might be bound to protein and not be available for nitrosation. Ultrafiltration was therefore employed to separate protein from the reaction solution. The measurement of nitrite in the ultrafiltrate indicated that all the nitrite was recovered in the filtrate and was not bound to protein. In fact, above pH 6, nitrite

Table 2—Nitrosomorpholine formation from morpholine and nitrite[a] at pH 6 and 25° C in a reconstituted dehydrated milk system and in the presence of casein

Conc of dry milk (%)[b]	Conc of casein (%)[b]	Nitrosation rate (μm/hr)
0[c]	0[c]	48
5	0	12
10	0	24
0	5	47

[a] Concentrations of both nitrite and morpholine are 15 mM each.
[b] Dry milk and casein solution were prepared in distilled water.
[c] The reaction was carried out in pH 6 buffer.

could be quantitatively recovered from milk even after days of storage. Nitrosation of morpholine in the presence of casein, which is the major component of milk protein, gave the same reaction rate as the reaction conducted in buffer solution. This also indicates that nitrite is not bound to the major milk protein. Further experiments are needed to explain why nitrosation is retarded in a milk system. The mystery is further compounded by the observation that nitrosation in a 10% milk system is faster than in a 5% milk system (Table 2).

The complex chemistry of both the ascorbic acid and the milk systems requires further investigation. However, both sets of results indicate the possibility that nitrite may be bound in several complex forms in foods, not all of which are available for nitrosation of amines, but some of which are measured as nitrite by the Griess reaction.

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IMMOBILIZATION OF MICROBIAL LACTASES BY COVALENT ATTACHMENT TO POROUS GLASS

INTRODUCTION

MEDICAL RESEARCH (Kretchmer, 1971, 1972) shows that low levels of intestinal lactase (β-galactosidase, EC 3.2.1.23), or a low tolerance for lactose, is common to many humans after early childhood, thus restricting the acceptability of many dairy products. However, new low-lactose foods can be produced by hydrolysis of the lactose of milk and milk products to monosugars with lactases (Dahlqvist et al., 1973; Eskamani et al., 1973; Kosikowski and Wierzbicki, 1971, 1973; Kosikowski et al., 1972; Wendorff et al., 1971; Wierzbicki, 1971; Wierzbicki and Kosikowski, 1973; Woychik and Wondolowski, 1972; 1973). Enzymatic hydrolysis of oligosaccharides in soy milk prior to consumption is also desirable to overcome similar symptoms produced by this alternate economical high-protein beverage (Sugimoto and Van Buren, 1970).

The renewed search for safe and inexpensive lactases suitable for modification of dairy products is exemplified by a comparative study of 23 crude microbial lactase preparations (Wierzbicki, 1971).

Enzymes immobilized to water insoluble carriers offer important potential advantages of reusability, minimization of product contamination, and shorter processing times (Wingard, 1972; Zaborsky, 1973). Lactases from two microorganisms have been immobilized: those of *Escherichia coli* (Broun et al., 1973; Bunting and Laidler, 1972; Eskamani et al., 1973; Kay and Crook, 1967; Robinson et al., 1971; Sharp et al., 1969; Stasiw et al., 1972) and those of *Aspergillus niger* (Woychik and Wondolowski, 1972, 1973). Five microbial lactases isolated in our laboratories and three types of commercial preparations were covalently coupled to diazotized porous glass and compared for stability and initial hydrolytic activity towards lactose in skim milk and acid whey.

MATERIALS & METHODS

Enzyme and substrate

Lactase preparations from various lactic acid bacteria, yeasts and molds were produced in the Cornell Laboratories (Wierzbicki, 1971). In addition, lactases from *A. niger* were provided by Baxter Laboratories, Inc., Chicago, Ill.; from *Saccharomyces lactis* by Enzyme Development Corp., New York, N.Y. and from *Asperigillus saitoi* by Seishiu Pharmaceutical Company, Ltd., Noda, Chiba, Japan. The latter sample is an acid protease preparation (Molsin) with lactase activity (Sugimoto and Van Buren, 1970; Wierzbicki, 1971).

Lactose in fresh skim milk or cottage cheese whey containing about 4% lactose, served as a natural substrate in all experiments.

Glass

Porous glass particles donated by Corning Glass Works, Corning, N.Y. were either of particle diam 120/200 mesh (75–125 μm) and mean pore size 85.5 nm or of particle diam 200/400 mesh (36–75 μm) and mean pore size 35.5 nm.

Lactase coupling

Lactase was coupled to porous glass by diazolinkages using silane after cleaning with 0.1N HNO_3 and heating at 600°C over night in the presence of air according to Li et al. (1972) and Messing and Weetall, 1970. The following major sequential reaction steps resulted:

1. Silanization of glass using 10% γ-aminopropyltriethoxysilane in toluene.
2. Conversion of alkylamino-glass derivative to an arylnitro form.
3. Reduction of arylnitro-glass derivative to arylamino form.
4. Conversion of arylamino-glass derivative to diazonium salt.
5. Coupling of enzyme to diazo-glass derivative.

In the final step, the enzyme preparation was coupled to diazo groups of glass in pH 6.8 phosphate buffer under continuous rotary shaking at 4°C for 24 hr. The ratios of enzyme preparation to glass in the coupling mixtures were 300 mg/g glass in the commercial preparations and 800 mg/g glass in the Cornell preparations. Under these conditions, the diazo groups should covalently bond to exposed tyrosine residues of the enzyme, residues with ϵ-amino groups (e.g., lysine residues) and histidine residues. Unbound protein and enzyme were removed from the glass particles by filtration. The particles were then washed alternately with distilled water and 2M NaCl until no free protein could be detected in the washes by the Lowry method or by assay for lactase activity.

Before the enzyme assay, 0.5g of filtered and air-dried preparations of lactase bound to porous glass (lactase-BG) were washed with 25 ml of milk or whey for 1 min. The particles were then separated from the liquid by filtration. Several ml of the filtrate were heated to 100°C for 1 min and analyzed for monosugars. The remaining filtrate was incubated for 5 hr at the optimum pH and temperature of the enzyme and then assayed for monosugars. Similar levels of monosugars were observed in the two samples, showing that no detectable enzymic activity was released by these glass particles during 1 min of contact with acid whey. Subsequent work shows that these lactase-BG preparations are stable for periods of months in semi-continuous flow column experiments at 55°C (Wierzbicki et al. 1973a, b).

Enzyme assay

Approximately 0.5g of filtered and air-dried lactase-BG preparation was mixed with 25 ml of acid whey or skim milk to which several drops of toluene had been added to inhibit microbial growth and to which potassium or sodium hydroxide had been added if necessary to adjust the pH of the acid whey to a higher value. These mixtures were then shaken for 5 hr at the optimum pH and temperature of the soluble lactase used to prepare the lactase-BG sample. The pH and temperature optima of the *A. niger* lactases are not affected by immobilization on diazotized glass (Wierzbicki et al., 1973a, b), or on glutaraldehyde glass (Woychik and Wondolowski, 1972, 1973). After incubation, 5 ml of supernatant was heated to 80°C for 5 min to inactivate any enzyme present and the amount of lactose hydrolyzed was determined either by thin-layer chromatography (Randerath, 1964) or by a glucose oxidase procedure (Glucostat assay, Worthington Biochemical Corp., 1972). Thin-layer chromatography (TLC) is accurate to ±5% when wet combustion analysis is used, but visual comparison with standard solutions was used for speed without great sacrifice in accuracy. The TLC method can detect as little as 1 μg of sugars in the 1 μl samples used here. The Glucostat (GS) method has a reported coefficient of variation of ±1.5% for the analysis of blood glucose (Worthington Biochemical Corp., 1972).

Determination of bound protein

Estimates of the amounts of protein bound to porous glass were made by an adaptation of the Kjeldahl method for total nitrogen (Wierzbicki, 1971). The values reported may be only semiquantitative because of difficulty in the acid digestion of the lactase-BG preparations and because of nonprotein nitrogen present in molecular linkages coupling protein to the porous glass. Unrounded values are reported because the quantitative limitations of the method applied to protein-BG have not yet been determined.

Because difficulties with the Kjeldahl method were not anticipated, all lactase-BG samples assayed were also used for the Kjeldahl analysis and no effort was made to quantitatively determine residual protein in the washes during preparation of the lactase-BG samples. The yields of

the glass-enzyme coupling reactions were clearly low because of the large excess of enzyme preparation added to the coupling mixture and because of the strong Lowry protein reaction in the supernatant from the coupling reaction and the initial washes of the lactase-BG derivatives.

Enzyme stability

Stabilities of lactase-BG preparations were tested by periodic assays with storage between assays at 5°C. Stabilities were calculated as the percent lactose hydrolysis observed at the final assay, divided by the percent lactose hydrolysis observed on the first assay of the preparation. The calculation method thus neglects the effect of lactose concentration on the rate of lactose hydrolysis.

RESULTS & DISCUSSION

Initial activity of lactase-BG preparations

Several lactase preparations were successfully coupled to porous glass with 35.5 nm pore diameter and particle sizes of 200/400 mesh (Table 1). The preparations initially hydrolyzed 5–100% of the lactose in whey in 5 hr, depending upon the preparation. The range of values for each preparation results from multiple assays on each preparation and the limited accuracy of the TLC used. Subsequent preparations of lactase-BG from the corresponding crude preparations showed that the immobilization procedure gave equally reproducible activities (Wierzbicki et al., 1973a).

Three of the four commercial preparations tested (*A. niger* lots No. 2 and No. 4 and *S. lactis*) showed the highest initial total activities, but also had much higher specific activities in crude form than the Cornell isolates (Table 1). The Cornell isolates contain considerable particulate matter and have undergone fewer purification steps (Wierzbicki, 1971). As shown by analyses of comparable preparations in Table 2, the amount of protein coupled in each case (81–125 mg/g glass) was less than the amount of enzyme preparation initially added to the coupling mixture by three- to eight-fold, so that the differences in the initial bound lactase activity may reflect primarily differences in initial lactase purity. The low initial activity observed for the *A. saitoi* preparation may be due to partial degradation of lactases present by the high proteolytic activity of the preparation (Sugimoto and Van Buren, 1970).

Table 1 also summarizes the percent hydrolysis that would be expected under identical conditions from the addition of 20 mg of the original glass-free enzyme preparations to 25 ml of whey solutions incubated under comparable conditions (Kosikowski et al., 1972; Wierzbicki and Kosikowski, 1973). The two exceptions are the *S. lactis* preparation (based on 1 mg/25 ml of skim milk) and the *A. niger* preparation (based on 10 mg/25 ml of acid whey). As shown in Table 2, the amount of bound protein in the lactase-BG preparations added to the assay mixtures in about 40–60 mg/25 ml of substrate.

Four major factors are likely explanations for the lower initial specific activity of the lactase-BG preparations:

1. Different temperature and pH optima of the lactase and corresponding lactase-BG preparations. (This factor has been subsequently shown not to be important for the *A. niger* preparations).
2. Loss in specific lactase activity during coupling to glass by denaturation of some lactase molecules during the coupling reaction and by preferential competive coupling to other materials present in these crude lactase preparations (Li et al., 1972; Messing and Weetall, 1970; Zaborsky, 1973).
3. Diffusional hindrance of contact between lactose and the lactase molecules bound inside the porous glass particles (Jackson and Edwards, 1973; Li et al., 1972; Wingard, 1972; Zaborsky, 1973).
4. Systematically high total nitrogen assays for protein-BG due to nitrogen in the molecules coupling the protein to the glass and other possible systematic errors in the Kjeldhal procedure applied to the protein bound to porous glass.

For trypsin-BG prepared by the same procedures in our laboratories, only about 1/5 to 1/10 as much protein is

Table 1–Initial activity of immobilized microbial lactases in acid whey

Source enzyme	pH	Temp °C	Lactose hydrolysis in 5 hr, %	
			Lactase-BG[a]	Soluble enzyme[b]
L. helveticus	6.8[c]	55	20–30	80
L. bulgaricus	6.8[c]	55	10–15	60
S. thermophilus[d]	6.8[c]	55	10–20	50
S. lactis	6.8[c]	35	30–35	90
S. fragilis	6.5[c]	40	10–15	57
A. niger No. 2	4.5	55	90–100	95
A. niger No. 4	4.5	55	80–90	–
A. phoenicis ATCC 11362	4.5	55	2–7	15
A. saitoi (Molsin)	4.5	55	5–10	25

[a] 0.5g of lactase-BG in 25 ml of whey containing 4% lactose
[b] Values for 20 mg original enzyme preparation per 25 ml of whey (Wierzbicki and Kosikowski, 1973; Kosikowski et al., 1972), except for S. lactis preparation, which was based on 1 mg preparation/25 ml skim milk and 10 mg of A. niger preparation/25 ml
[c] pH adjusted if necessary by addition of concentrated potassium or sodium hydroxide
[d] Streptococcus thermophilus

Table 2–Stability of the immobilized lactases stored at 4°C and periodically assayed at optimum pH and temperature of soluble enzyme

Source of lactose	Glass[a]	Protein bound mg/g of glass	Age of lactase-BG (days)	No. of assay	Retention of % initial activity
L. helveticus	A	114	10	3	<10%[b]
L. helveticus	B	105	25	2	90–100[b]
L. bulgaricus	B	–	25	2	95–100[b]
S. thermophilus	B	–	25	2	90–100[b]
S. lactis	B	116	15	2	90–100[b]
S. lactis	A	85	20	3	7–9[c]
S. fragilis	A	101	3	3	88–90[c]
A. niger No. 4	A	81	90	10	73–75[c]
A. niger No. 3	B	125	47	10	88–90[c]
A. phoenicis	B	105	20	4	70–72[c]
A. saitoi	B	107	20	4	72–74[c]

[a] A = Particle size 200/400 mesh, and mean pore diameter 35.5 nm; B = Particle size 120/200 mesh, and mean pore diameter 86.5 nm
[b] Based on TLC assay for lactose
[c] Based on GS assay for lactose

bound per gram of 35.5 nm glass (Li et al., 1972) and Kjeldahl method gives values for glass-bound protein as much as five times higher than the actual values (K.T. Tam and V.H. Edwards, unpublished results, 1972). However, lactases that have been characterized (*E. coli*, Wallenfels and Weil, 1972; *Sacch. fragilis*, Kulikova et al., 1972) have a molecular weight (MW) in excess of 500,000 daltons and dimensions of about 5 by 15 nm (*E. coli* lactase) and are thus much larger than trypsin (MW = 23,800 daltons, molecular diameter of about 4 nm). Also, only about 10% of the available glass surface would be covered by trypsin if it is deposited as a monolayer (Li et al., 1972). It is thus possible that the much higher protein-to-glass ratios reported here are reliable. Except for the *A. niger* and *S. lactis* preparations, the crude lactases contained significant amounts of cellular debris that is visible in aqueous suspensions when examined under the light microscope. Some of these particles may contain a significant fraction of lactase activity and may be bound to the glass, also accounting for high protein loadings.

Three other reports of lactase-BG have been published for porous glass silanized and then modified with glutaraldehyde. Woychik and Wondolowski (1972, 1973) repectively report coupling only 3 and 7 mg of *A. niger* lactase/glutaraldehyde glass, while Robinson et al., (1971) report coupling 12 mg of *E. coli* lactase/g glass. However, Jackson and Edwards (1973) showed that only about half of the glass surface is silanized by refluxing in a 10% solution of γ-aminopropyltriethoxysilane in acetone and still less (10–20%) of the glass surface is silanized by refluxing with 2% solutions of the silane. The lower concentration was used by the other two groups. Our subsequent work with acetone-silanized lactase-BG derivatives show no significant differences from the values reported here for toluene-silanized glass (Wierzbicki et al., 1973a). The glutaraldehyde-glass lactase derivative of Robinson et al. (1971) has an initial specific activity of 36% of the value for the soluble lactase, while Woychik and Wondolowski (1972, 1973) reported initial specific activities of their lactase-BG preparations of about 75% of their soluble lactases.

Stability of lactase-BG preparations

Table 2 shows the stability of various lactase-BG preparations for the two different types of glass particles used and stored at 5°C between assays. The most striking result is that in the two cases (*L. helveticus* and *S. lactis*) where the stability of lactase activities on different types of glass were compared, the lactase-BG derivatives bound to larger particles with larger pores had much greater stability. In each of the seven cases where particles with 86.5 nm diameter pores were used, good retention of stability was observed with the intermittent assay procedure used here. Poor activity retention was observed with the 35.5 nm pore glass in two of the four cases tested. Surprisingly, the total amount of protein (as 6.25 × total nitrogen) bound to glass did not vary greatly with initial enzyme preparation, and was about 15% higher for the 86.5 nm diameter pore glass than for the glass with 35.5 nm pores. Because the 86.5 nm glass would only have less than half as much reactive surface area per unit volume as an equal weight of the 35.5 nm glass, either strong adsorption or covalent binding of particulate cellular debris along with soluble material again appears to be a consistent explanation. Dissociation of multisubunit lactases is also a possible explanation for activity losses. Wierzbicki (1971) showed that most of the total lactase activity was usually found in the water insoluble fractions of solvent-treated and freeze-dried microbial lactase preparations studied here. However, the total and specific lactase activities were occasionally higher in the soluble fraction, depending on the microorganism. The very large Michaelis constant reported by Wierzbicki (1971) for soluble *A. niger* preparations also suggest that particle bound, rather than soluble lactases are responsible for most of the lactase activity in crude cell preparations. Also, this lactase is competitively inhibited by the reaction product, D-galactose (Woychik and Wondolowski, 1972, 1973), thus requiring a modification of the kinetic model and increasing further the reduction in reaction rate caused by diffusional limitations (Jackson and Edwards, 1973; Wierzbicki et al., 1973a, b).

All lactase-BG preparations had less total activity than the lactase preparations originally added to the coupling mixture, but the specific activities of lactase-BG were occasionally greater (Table 1). High enzyme purity is thus not necessarily essential for successful enzyme immobilization, and some purification may be achieved during immobilization. Broun et al. (1973) have shown that when cross-linking is used to immobilize a number of enzymes, higher specific activities are obtained with mixtures of the

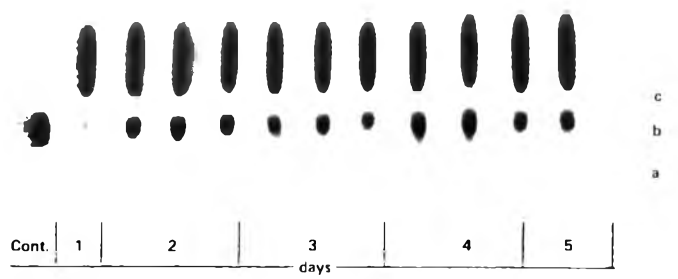

Fig. 1—TC Chromotogram: Activity of immobilized lactase from A. niger incubated 5 days at 55° C in acid whey. (a—lactose; b—galactose; c—glucose)

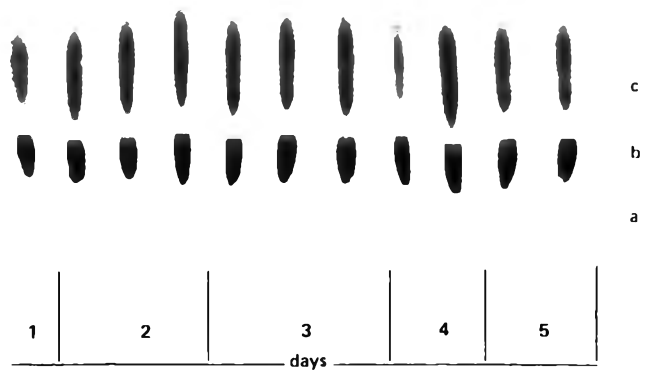

Fig. 2—TC Chromotogram: Activity of immobilized lactase from A. niger incubated 5 days at 55° C in skim milk. (a—lactose; b—galactose; c—glucose)

pure enzyme and an enzymatically inactive protein such as bovine serum albumin than by cross-linking of the purified enzyme alone.

Crude lactase preparations from yeast and molds were relatively stable and hydrolyzed from 5–30% of the lactose in whey under the standard assay conditions (Table 2). Lactase-BG preparations from lactic acid bacteria coupled to 86.5 nm glass retained their initial activity in deproteinized whey for 25 days at 5°C and converted from 10–30% of the lactose in whey under the standard assay conditions (Table 2).

An immobilized *A. niger* lactase preparation was used to produce low-lactose milk and acid whey semicontinuously by mixing 0.3-g aliquots of the lactase-BG preparation with 25 ml milk and with 25 ml acid whey and incubating at 55°C. At daily intervals, about 20 ml of the low-lactose milk or whey was removed and replaced with an equal volume of fresh milk or whey. The extent of lactose hydrolysis during the 5-hr test period was then determined (Fig. 1 and 2). A drop of toluene was added daily to each flask to inhibit microbial growth. Lactose conversion was higher in acid cottage cheese whey than in milk, because the optimum pH of the soluble and immobilized *A. niger* lactase is 3.5–4.5 (Wierzbicki et al., 1973a; Woychik and Wondolowski, 1972, 1973). No oligosaccharides from the nonhydrolytic transglycosidation reactions of lactases are detectable in Figures 1 and 2 or other TLC chromatograms at high lactose conversions.

The *A. niger* lactase-BG was relatively stable on storage at 5°C and periodic assay at 55°C during the 90-day test period (Table 2). Subsequent storage experiments at 55°C and experiments with continuous flow of deproteinized acid whey through a 1.6 cm diameter glass column filled with lactase-BG preparations from *A. niger* have shown good stability for 75 days of operation at high lactose conversions (Wierzbicki et al., 1973a, b).

The present study suggests that several immobilized lactases have sufficiently high initial activity and stability to be useful in the economic production of low-lactose foods.

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JFS RESEARCH NOTES

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A Research Note
GROWTH OF TWO GENERA OF PSYCHROTROPHS ON BEEF ADIPOSE TISSUE

INTRODUCTION

BACTERIAL contamination which occurs during the handling and processing involved in fabrication, trimming and packaging of retail cuts is closely related to ultimate caselife. Processing equipment often provides the means for introducing bacteria onto the surfaces of retail cuts since this equipment becomes contaminated with bacteria from contact with the surfaces of beef cuts. Correspondingly, it is of concern to identify storage conditions and genera of bacteria that are associated with microbial growth on the external fat surfaces of beef carcasses.

Goldman and Rayman (1952) reported that *Pseudomonas fluorescens* was capable of hydrolyzing tallow. Since Alford and Elliot (1960) and Ayres (1960) reported that lipolytic bacteria can grow at low temperatures, psychrotrophic bacteria which are lipolytic can grow on the external fat surfaces of beef carcasses and wholesale cuts. Bacterial counts ($\log_{10}$) of 4.1 and 5.1, respectively, have been reported on the external fat cover of beef ribs following shipment from a packaging plant (Rea et al., 1972) and on the external fat of short loins at a retail store (Stringer et al., 1969). The present study determined the growth of species of *Pseudomonas* and *Flavobacterium* on adipose tissue from beef rib and brisket tissue.

EXPERIMENTAL

ADIPOSE TISSUE samples (13 × 13 × 6 cm) were derived from the wholesale ribs and briskets of 10 beef carcasses. The external surfaces of the cuts and cutting utensils were sprayed with 95% ethyl alcohol and flamed prior to removal of the fat samples. Each sample was temporarily placed in a sterile petri dish prior to inoculation with the appropriate bacteria.

The bacteria used in this study (*P. fluorescens* and a *Flavobacterium* sp.) were originally isolated from meat products. Bacterial cultures of each genera were grown at 20°C for 24 hr, suspended in phosphate buffer and subsequently used for inoculation. The inoculum for each genera was adjusted turbidometrically at 660 mμ to obtain a cell/volume concentration such that an area concentration of approximately 1.2×10^3 bacteria per 6.45 cm^2 was applied (by means of a sterile syringe) to each adipose tissue sample. The internal side of each adipose tissue sample was inoculated to avoid the use of surfaces that had been subjected to alcohol rinsing and flaming. Bacterial analyses performed on the adipose tissue samples indicated a complete absence of bacteria, and those performed after inoculation verified that the desired concentration was achieved (assuming 40–60% recovery via swabbing).

After inoculation, samples were immediately (a) vacuum packaged in polyvinylidene chloride bags, (b) tightly wrapped in extruded polyvinyl chloride film (PVC), or (c) stored in a petri dish. Storage in a petri dish prevented airborne bacteria contamination without necessitating actual film contact with the surface of the adipose tissue.

Each fat sample was randomly allotted to one of three storage intervals (4, 8 or 12 days) in a 2°C cooler. At the end of the appropriate storage periods, sterile aluminum templates and cotton swabs were used to collect bacterial samples from the surface of the adipose tissue samples. Total psychrotrophic counts were determined on standard plate count agar after incubation at 5°C for 10 days. Data were ana-

Table 1—Bacterial counts on beef adipose tissue as affected by storage method

Genera of bacteria	Bacterial count ($\log_{10}$)[a] Vacuum package	PVC film	Petri dish
Pseudomonas	6.2[b]	7.0[c]	6.9[c]
Flavobacterium	4.8[b]	5.0[b]	4.5[b]

[a] Mean bacterial counts ($\log_{10}$) per 6.45 cm^2; means on the same line bearing different superscripts are significantly different ($P < 0.05$).

Table 2—Bacterial counts on rib and brisket adipose tissue after 4, 8 and 12 days of storage[a]

Genera of bacteria	Bacterial count ($\log_{10}$)[a] Storage for 4 days: Rib adipose tissue	Brisket adipose tissue	Storage for 8 days: Rib adipose tissue	Brisket adipose tissue	Storage for 12 days: Rib adipose tissue	Brisket adipose tissue
Pseudomonas	4.6[b]	4.9[b]	7.1[e]	7.3[e]	8.2[h]	8.0[h]
Flavobacterium	2.6[c]	4.1[d]	4.0[f]	5.9[g]	5.1[i]	7.0[j]

[a] Mean bacterial counts ($\log_{10}$) per 6.45 cm^2; means on the same line or in the same column within a storage time period bearing different superscripts are significantly different ($P < 0.05$).

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lyzed using analysis of variance (Snedecor and Cochran, 1967) and the mean separation technique of Duncan (1955).

RESULTS & DISCUSSION

BACTERIAL COUNTS for adipose tissue samples stored in vacuum packages, polyvinyl chloride film or petri dishes are presented in Table 1. *Pseudomonas* counts for vacuum packaged samples were significantly ($P < 0.05$) lower than those for samples packaged in PVC film or stored in petri dishes. However, *Flavobacterium* counts did not differ among samples stored in vacuum packages, PVC film or petri dishes. Rea et al. (1972) observed no difference in bacterial counts on the subcutaneous fat cover of rounds and chucks which were stored in polyethylene bags, PVC film, paper bags or left unprotected.

When analyses of variance were performed within storage time periods, a significant ($P < 0.05$) interaction was noted between bacterial genera and adipose tissue location. Mean bacterial counts at both adipose tissue locations for all three storage intervals are presented in Table 2. *Pseudomonas* counts were significantly ($P < 0.05$) higher than those for *Flavobacterium* on both rib and brisket adipose tissue after all three periods of storage. Greater ($P < 0.05$) numbers of *Flavobacterium* were observed on brisket than on rib samples after all three storage intervals, but no difference in *Pseudomonas* count was detected between rib and brisket samples. Differences in *Flavobacterium* counts between brisket and rib samples may have resulted from differences in Aw since brisket samples contained 29% moisture while rib samples contained only 6% moisture. This explanation does not account for the fact that *Pseudomonas* have a very high Aw requirement, yet *Pseudomonas* counts did not differ between brisket and rib samples. It is possible that differences in fatty acid composition between brisket and rib samples may have affected bacterial counts. Dahl (1962) reported that brisket fat contained greater quantities of unsaturated fatty acids than did subcutaneous fat from the round. Den Dooren de Jong (1926) reported that *P. fluorescens* was capable of utilizing saturated and unsaturated fatty acids as a source of carbon. Davidson et al. (1973) found that many species of *Pseudomonas* were capable of using short chain fatty acids as the sole source of carbon and energy.

Pseudomonas and *Flavobacterium* counts were averaged across adipose tissue location and storage method for 4, 8 and 12-day storage intervals. The number of *Pseudomonas* colonies increased more between the 4th ($\log_{10} = 4.7$) and 8th ($\log_{10} = 7.2$) day of storage than was the case for *Flavobacterium* ($\log_{10} = 3.4$ and 4.9 for 4 and 8 days, respectively). Increases in counts between the 8th and 12th days of storage were similar for both genera of bacteria.

Off-odors and surface stickiness were apparent on fat samples inoculated with both genera after the 4th day of storage. Odors typical of putrefaction were noticed after 8 and 12 days of storage for samples inoculated with *Pseudomonas* while samples inoculated with *Flavobacterium* displayed a fruit-like odor after 8 and 12 days of storage. Jeremiah et al. (1971) concluded that the off-odors associated with spoilage of lamb are probably a result of proteolysis, while off-flavors associated with spoilage are probably a result of lipolysis. In the present study, brisket samples frequently exhibited a greenish discoloration after 12 days of storage and occasionally evidenced slime formation.

Based on the results of the present study: (a) Both *Pseudomonas* and *Flavobacterium* are capable of substantial growth on bovine adipose tissue even under the microaerophilic conditions that exist in vacuum packages; (b) It is possible that the compositional characteristics of adipose tissue affect the growth of *Flavobacterium* to a greater extent than is the case for *Pseudomonas*; and (c) Swab samples obtained from the wholesale rib region should provide an adequate estimate of *Pseudomonas* growth on both moist and dry areas of the beef carcass, but may underestimate the extent of *Flavobacterium* growth on the brisket.

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A Research Note

EFFECT OF ELECTRONIC, CONVECTION AND CONVENTIONAL OVEN ROASTING ON THE ACCEPTABILITY OF PORK LOIN ROASTS

INTRODUCTION

TIME SAVING METHODS of cooking have become popular with the use of the electronic and convection ovens. Among the problems encountered in roasting meat with the newer equipment was increased moisture loss from pork roasts cooked by microwave (electronic) technique, as reported by Kylen et al. (1964) and Moore et al. (1966). Marshall (1960), working with beef roasts, found appearance, tenderness, juiciness and flavor to be less desirable following cooking in an electronic than in a conventional oven. The new convection ovens with fan-forced air to circulate the heated air within the oven chamber produces more uniform heating and shortens the cooking time (Anon. 1966, 1967). Apparently roasted meats have a tendency to dry, as one author suggested placing a pan of water in the bottom of the oven to avoid drying out (Anon. 1968).

The objectives of these experiments were to study the acceptability and palatability of pork loins roasted in conventional, convection and electronic ovens and in the electronic oven with a browning unit.

EXPERIMENTAL

THE ANTERIOR portions (tenth thoracic vertebrae forward) from paired pork loins of 30 animals were made available through the meat laboratory of South Dakota State University (Gee, 1970). The roasts were held in frozen storage for 8 months. The left side was used as the control and roasted in Jr. broiler pans without racks in a constant temperature oven at 177°C to an internal temperature of 77°C. This was cooking method one. The loin pieces from the right side were randomized for three cooking methods. Method two utilized a convection oven with the roasts in Jr. broiler pans. In compliance with the manufacturers' recommendations, the oven was not preheated, the temperature was set at 177°C with the fan turned on, and the meat was roasted to an internal temperature of 77°C. An electronic oven (G.E. Versatronic) with 15.24 × 25.40 × 3.81 cm pyrex baking dishes was used for cooking method three. The roast was placed fat side down and heated on high power (2450 MH_Z) for 3 min, then turned fat side up and cooked to an internal temperature of approximately 65°C. The temperature of most roasts rose to 77°C or more after removal from the oven. The final temperature varied considerably, making it hard to maintain a constant end-point, as noted by Noble and Gomez (1962). Cooking method four duplicated method three but added a browning unit set at 232°C.

Sampling was started at the posterior end of the cooked roast, utilizing seven slices, 3.2 mm thick for a six member taste panel. The seventh slice was rotated as a paired sampling. The taste panel scored on a 7-point scale (7 as highest or best and 1 as lowest or unacceptable) for texture, juiciness, tenderness and flavor. Two slices, 1.5 cm thick, were trimmed for tenderness measurements of the L.E.E. Kramer shear press with a 1364 kg proving ring and cell speed of 20 sec. The maximum readings were converted to kilograms of force per 100g of meat. Another slice was cut for the water-holding capacity (WHC) ratio and extractable fluids to be determined on the Carver press (Deethardt and Tuma, 1971). WHC was determined on a 0.5g sample taken from a 1.3 cm core and pressed between Plexiglas plates on the Carver press. The ratio of pressed sample area to moisture ring area was calculated by use of a planimeter (Miller and Harrison, 1965). Extractable fluids were determined from two weighed cores (2.54 cm diam) in the 5.08 cm cell on the Carver press with warming plates at 50°C the extracted fluid was transferred to graduated centrifuge tubes to determine the amounts of serum and fat for each sample. The data were subjected to least square analysis of variance.

RESULTS & DISCUSSION

HIGHLY SIGNIFICANT differences between cooking methods were observed in the percentage of drip loss and the percentage of volatile loss (Table 1). The means indicate that the volatile loss reduction (one-half as much) in the electronic oven was compensated for by increased drip loss. Total cooking loss means were nearly the same for all four roasting methods.

Significant ($p < 0.05$) differences between cooking methods were noted in total extractable fluid and juice from the cooked meat on the Carver press (Table 2). A reduction in the amount of juice extracted from samples cooked in the electronic oven resulted in a lesser amount of total fluids.

Table 1—Analysis of variance and means for 30 animals and four cooking methods[a] with cooking losses and panel evaluations

Variables	df[c]	Cooking loss %	Drip loss %	Volatile loss %	Panel scores[b] Texture	Juiciness	Tenderness	Flavor
				Mean Square				
Animal	29	22	6	12	0.35	0.47	0.79	0.32
Method	3	14	318**	245**	0.38	0.23	0.29	0.30
Error	27	8	3	8	0.19	0.25	0.29	0.12
Cooking method[a]				Means				
1		23	5	18	5.7	4.3	5.4	4.5
2		25	5	20	5.4	4.5	5.4	4.6
3		23	15[d]	9	5.5	4.5	5.2	4.6
4		25	15	10	5.5	4.4	5.1	4.1

[a] 1 Conventional oven, left side as control; 2 Convection oven; 3 Electronic oven; 4 Electronic oven with browning unit
[b] Panel score 7 excellent to 1 poor
[c] df = degrees of freedom
[d] Some numbers do not add to the total due to rounding
** $p < 0.01$

[1] Deceased

Table 2–Analysis of variance and means for 30 animals and four cooking methods with objective measurements

Variable	df	Extractable fluid %	Total fluid ml	Juice ml	Fat ml	Moisture ring cm^2	WHC ratio	Shear press kg/100 gm	Cooking time mn/lb
					Mean square				
Animal	29	64	0.46	0.46	0.48	920	3.57*	128953*	139
Method	3	59	2.29*	2.29*	0.16	684	1.17	17602	21532**
Error	27	24	0.58	0.58	0.17	582	0.89	37640	1383
Cooking method[a]					Means				
1		39	4.9	3.5	1.4	328	0.62	610[b]	104
2		39	4.8	3.4	1.4	326	0.63	590	95
3		35	4.3	3.0	1.4	312	0.54	579	23
4		35	3.6	2.5	1.1	315	0.59	638	24
					Range and means among animals				
low							0.26	350	
high							0.79	840	
mean							0.59	605	

[a] See footnote Table 1
[b] Converted from lb to kg (2.2)
* $p < 0.05$
** $p < 0.01$

There were no significant differences between the cooking methods in the taste panel evaluations for texture, juiciness and tenderness; however, flavor did approach a significant difference with the electronic oven plus the browning unit having the lowest score (Table 1). There was a highly significant ($p < 0.01$) difference in the roasting time in minutes per pound, with the electronic methods taking a much shorter time (Table 2) than roasting in the conventional and convectional ovens.

The WHC ratio and the shear press values for tenderness were significantly ($p < 0.05$) different among animals. The mean values for the WHC ratio ranged from 0.26 to 0.79, and the shear press values for tenderness on the cooked meat ranged from 350 to 840 kg of force per 100g (Table 2).

The general appearance of the roasts from the electronic oven was not as desirable, and often the fat on the edges did not appear cooked. The browning unit had little effect on general appearance of the roast, as the length of time in the oven was not adequate to brown the meat. However, the meats (pork loin) as served to the panel were all acceptable with no significant difference in the characteristics (texture, juiciness, tenderness and flavor) related to the different cooking techniques utilized in the study.

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A Research Note
ACCELERATED PORK PROCESSING: CURED COLOR STABILITY OF HAMS

INTRODUCTION

PROCESSING of pork prior to carcass chilling has created considerable interest in the packing industry. Processing costs could be reduced by application of accelerated processing techniques. Present methods of processing require 2 days to 2 wk before finished products are moved from the packing house to retail trade. With accelerated processing, hams can be successfully processed in 15 hr following slaughter (Mandigo and Henrickson, 1966). However, research in this area is limited, and most investigations have been completed on a laboratory basis. In this study, accelerated processing was evaluated under packing house conditions.

Development of the boneless, cured ham has resulted in a more uniform product with increased consumer acceptance. Since color development and color stability of the cured ham are important factors for sales appeal, the effect of accelerated processing on cured ham color was questioned. Injecting hams while hot improved cured color according to Mullins et al. (1957). Henrickson et al. (1969) reported that cure diffused most rapidly through warm muscles and that cured pigment from pre-chilled hams was more stable when exposed to 100 ft-c of light for a period up to 24 hr than the post-chilled muscle.

This study was designed to investigate the color stability of accelerated and conventional processed hams under "packing house" conditions.

MATERIALS & METHODS

48 BUTCHER HOGS of unknown origin were selected at random from a commercial slaughter plant operation. Six animals were slaughtered daily, on eight different processing days. Following slaughter, carcasses were split with one-half assigned to the conventional treatment (cold) and the other half assigned to the rapid accelerated treatment (hot).

Conventional treatment involved a 24-hr chilling period at 1.7°C prior to separation of the ham from the carcass. With the accelerated or hot treatment, the ham was separated within 1 hr after slaughter.

Following separation from the carcass, all hams were skinned, outside fat removed and boned. A multiple stitch needle process was used to stitch pump the hams with brine (salt, sugar, nitrate, nitrite, ascorbates and phosphates). The brine was prepared daily from these ingredients in a manner consistent with accepted industry practices. The pumped hams were stuffed into individual fibrous casings, placed in stockinettes and steel molds, and then smoked. After a 1-hr initial drying period, smoking was accomplished over an 8-hr period followed by cooking to an internal temperature of 68°C. The smoked, fully cooked hams were removed from the smoke house and cooled. Conventionally processed hams were chilled in coolers at 1.7°C, whereas accelerated hams were chilled in an experimental chilling chamber at a −37°C until the mass average temperature reached 4°C. All hams were maintained at 1.7°C prior to sampling.

After chilling, hams were transported to the University of Nebraska Meat Lab. for evaluation of nitroso- and total-pigment content. All pigment extraction procedures were carried out in a darkened, refrigerated laboratory cooler (1.7°C). Samples were obtained as a 7.5 cm section from the center of each ham, followed by removing outside edge portions and grinding the trimmed core through a 0.32 cm grinder plate and mixed thoroughly. Twenty 10-g samples were weighed from each ground section and spread uniformly over the surface of 20 individual petri dishes.

The samples were placed in a light chamber for 0, 30, 60, 120, or 180 min and were exposed to 200 ft-c of cool white deluxe fluorescent light. After the various periods of exposure, the nitroso and total pigments were extracted with acetone-water or acetone-acid solutions respectively, as described by Hornsey

Table 1—Influence of light exposure time upon cured ham nitroso and total pigment[a]

	Light exposure time (min)				
	0	30	60	120	180
Nitroso pigment (ppm)					
Hot	40.6	30.0	21.3	20.3	15.8
Cold	42.7	31.0	25.1	21.4	16.6
$\bar{X}$	41.7^{a}	30.5^{b}	23.2^{c}	20.9^{c}	16.2^{d}
Total pigment (ppm)					
Hot	57.5	54.5	53.1	52.2	49.8
Cold	57.8	53.7	52.1	49.1	46.1
$\bar{X}$	57.6^{a}	$54.1^{a,b}$	$52.6^{b,c}$	$50.6^{b,c}$	48.0^{d}

[a] Means with different superscripts are significantly different ($P < 0.01$).

Table 2—Influence of processing periods upon curred ham nitroso and total pigment[a]

	Processing periods (days)							
	1	2	3	4	5	6	7	8
Nitroso pigment (ppm)								
Hot	22.7	29.1	27.5	33.3	25.3	24.1	28.9	14.0
Cold	40.0	31.5	25.9	35.6	24.4	20.4	25.8	15.2
$\bar{X}$	$31.4^{a,b}$	$30.3^{b,c}$	$26.7^{c,d,e}$	34.5^{a}	$24.9^{d,e,g}$	22.3^{g}	$27.3^{c,d}$	14.6^{h}
Total pigment (ppm)								
Hot	65.3	52.8	53.7	49.3	54.8	52.1	67.8	31.7
Cold	71.4	59.3	44.9	58.4	49.7	45.4	53.0	31.8
$\bar{X}$	69.4^{a}	56.1^{b}	$49.3^{d,e}$	$53.8^{b,c}$	$52.2^{c,d}$	48.8^{e}	60.4^{f}	31.8^{g}

[a] Means with different superscripts are significantly different ($P < 0.05$).

(1956). The nitroso pigments were read at 540 mμ and the total pigments at 512 and 612 mμ on a spectrophotometer.

Data were analyzed by the analysis of variance method (Steel and Torrie, 1960). Significant differences were determined by F-tests and were separated by the Duncan New Multiple Range Test.

RESULTS & DISCUSSION

TABLE 1 contains the mean nitroso- and total-pigment values for accelerated and conventionally processed hams at the different light exposure periods. No significant differences existed between accelerated and conventionally processed ham for nitroso or total pigments. With increased light exposure, or time, nitroso and total pigment decreased significantly ($P < 0.01$) indicating an apparent enhanced pigment degradation. Nitroso- and total-pigment concentrations were significantly ($P < 0.01$) lower after 180 min of exposure than comparable concentrations after 0, 30, 60 or 120 min of exposure to light. A major portion of the nitroso-pigment loss was during the first 60 min of light exposure. Lawrie (1966) reported that light disassociates the nitric oxide from the cured meat pigment, and the discoloration may take place in as little as 1 hr of exposure.

Total pigments are composed of hematin derived from any uncombined pigments present, together with those resulting from the oxidation of the nitric-oxide pigment. Total pigments were extracted to determine the efficiency of the nitroso-pigment extractions, as described by Hornsey (1956).

Results from the eight processing days were significantly different ($P < 0.01$) for nitroso and total pigments (Table 2). A processing method x processing period interaction revealed significant differences in pigment content. Hot or accelerated processed hams had higher nitroso pigment for period 3, 5, 6 and 7. Total-pigment content followed a similar pattern. These differences probably reflect processing differences beyond the control of the study. Bressler (1954) found that slices from hams treated alike and slices from the same ham differed in color stability. No apparent accelerated or conventional processing influences on nitroso and total pigments were found in this investigation.

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A Research Note

USE OF CHEMICAL COMPOUNDS AND A ROSEMARY SPICE EXTRACT IN QUALITY MAINTENANCE OF DEBONED POULTRY MEAT

INTRODUCTION

WHEN BROILER NECKS and backs or turkey racks are used to produce mechanically deboned poultry meat (MDPM), there is severe physical and chemical stress placed on the material during the deboning operation. Because of exertion of high pressures and violent tissue disruption, the MDPM is prone to chemical and microbial action which in turn may adversely affect the shelflife and flavor of the product.

Grunden et al. (1972), Vadehra and Baker (1970) and Essary and Ritchey (1968) outlined important chemical and physical characteristics of MDPM from several sources (i.e., broiler necks and backs, turkey racks and whole spent layers). Storage stability problems with this material under refrigerated and frozen conditions have been reported by Dimick et al. (1972), Froning et al. (1971), Cunningham et al. (1971) and Maxon and Marion (1970). Ostovar et al. (1971) observed high initial microbial populations in MDPM due mainly to the presence of psychrophilic and psychrotolerant microorganisms. Other investigations in this laboratory (unpublished data) showed that a trained taste panel could detect a reduction in flavor quality after three months in frozen storage.

Preliminary experiments conducted in two commercial processing plants have shown that materials such as polyphosphates, butylated hydroxyanisole (BHA) plus citric acid, and a rosemary spice extractive (RSE) are effective in maintaining lower thiobarbituric acid (TBA) values as well as reduced bacterial numbers compared to untreated controls. One of the problems encountered in these preliminary studies was the inability to determine actual levels of the treatment materials in the final MDPM since some of this material was expelled from the machine along with the bone waste fraction.

To verify the industrial information a laboratory study was designed to evaluate the above compounds for their ability to maintain the initial quality in simulated MDPM.

EXPERIMENTAL

A SIMULATED MDPM was produced by separately grinding turkey breast meat and skin through a 3/8-in. die. Meat and skin were combined (85% meat and 15% skin) and placed in a Hobart silent cutter along with a poultry meat flavoring compound since this material would normally be used in products such as frankfurters or luncheon loaf. After chopping for 1.5 min in the silent cutter the material appeared to be similar to commercial MDPM. Treatments used in this study were RSE at levels of 0.01% and 0.05%; BHA plus citric acid at 0.075% by weight of fat in the meat; polyphosphates at 0.5%; and a control group with no quality maintenance compounds added. All samples were stored at 3°C for 11 days. Each treatment was replicated three times.

TBA values were determined by the method of Tarladgis et al. (1960). Changes in microbial population were monitored using the procedure described by Thatcher and Clark (1968). Sensory panel evaluations were performed by a trained taste panel on cooked samples according to the procedure outlined by MacNeil and Dimick (1970).

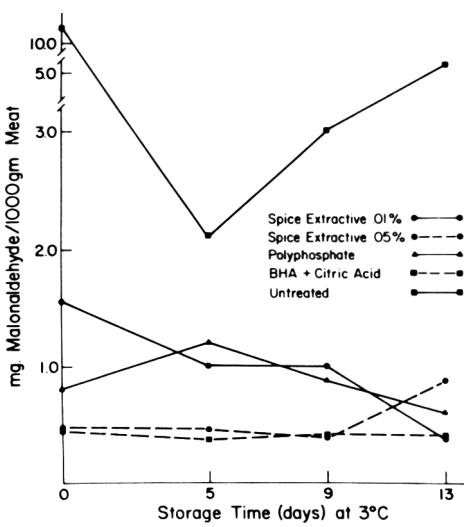

Fig. 1—TBA values of cooked simulated mechanically deboned meat product.

Table 1—Duncan's multiple range test for treatment means (Multiple comparison test)

Treatments	Means[a]
Untreated (no maintenance compounds added)	4.99[b]
Rosemary spice extractive 0.01%	4.27[a]
Rosemary spice extractive 0.05%	5.99[c]
Polyphosphate	6.22[c]
BHA + citric acid	5.03[b]

[a] Means having different superscripts are significantly different ($P < 0.01$). Panelists rank samples on a scale from 1 to 9: 1 = extremely better than control, 5 = no difference from control, 9 = extremely inferior to control.

RESULTS & DISCUSSION

TBA VALUES for cooked simulated MDPM are presented in Figure 1. The meat, which had the compounds added, exhibited substantially lower TBA values than the untreated sample. RSE 0.05% and BHA + citric acid were the most effective followed closely by polyphosphate and RSE 0.01%. It is interesting to note that the high initial value of 10.53 for the untreated sample represents a TBA value from meat taken from a freshly deboned turkey carcass under laboratory conditions. This phenomenon has been observed in other experiments in this laboratory.

All of the simulated MDPM which had been treated with chemical compounds had lower total plate counts (bacteria per gram) for the first week of storage at 3°C than did the untreated meat. However, this beneficial effect was not evident in storage beyond a week.

Normally MDPM would be used as an ingredient in the manufacture of frankfurters, various loaf products, fermented sausages, spreads and other comparable products. While the storage stability of the MDPM would depend to some extent on how it was used in these products, it is, however, important to measure the changes in flavor which occur in the MDPM itself during storage. The flavor changes were measured by a sensory panel using the multiple comparison test. The untreated material was included as a treatment and compared to itself. Data

from these tests showed significant differences ($P < 0.01$) among treatments, storage times and judges. Duncan's Multiple Range Test (Table 1) showed that the material treated with RSE 0.01% had a better flavor rating than any of the other treatments, including the untreated control.

The results of this study show that the compounds tested were effective in maintaining lower TBA values, lower total plate counts for a limited period, and in one case actually improving the flavor of the simulated mechanically deboned poultry meat. These data verify results obtained in two other experiments using MDPM from turkey racks and broiler backs and necks obtained from processing plants in Pennsylvania.

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A Research Note

MOISTURE AND CALORIC CONTENT OF MALE TURKEY BREAST AND THIGH

INTRODUCTION

TYPICAL of the food industry, the poultry industry faces the challenge of nutritional labeling. The assignment of accurate caloric values to turkey products is compounded by the relatively large number of convenience products now available to the consumer. Previously published data (Scott, 1956; Watt and Merrill, 1963) show that cooked turkey ranges from about 175–250 calories/100g sample (Table 1). The purpose of this research was to determine the heat of combustion for turkey breast and thigh, raw and cooked, with and without natural proportions of skin and subcutaneous lipid.

EXPERIMENTAL

10 LARGE WHITE male turkeys were randomly selected from a group of 24-wk-old birds reared in confinement and fed a commercial corn-soy ration. The birds were sacrificed, chilled in ice slush for 24 hr, frozen at −25°C and stored at −15°C only as long as necessary to complete the experiment. The frozen birds were defrosted at 4°C for 24 hr before samples were taken.

Half the breast and an entire leg were removed from each bird for cooking, leaving the remaining half for raw samples. The samples to be cooked were wrapped in aluminum foil and roasted at 185°C to an internal temperature of 85°C. They were kept wrapped until cooled to room temperature and then sampled. The uncooked halves also were allowed to adjust to room temperature before samples were taken.

A 2 × 2 × 2 factorial experiment involving two types of meat (breast and thigh), raw and cooked, and two methods of sampling was used. First, a sample was taken from each turkey to include skin and all deposited fat in a cross section from the surface to the bone. Second, muscles were excised from both breast and thigh samples, with all skin and obvious deposited fat being removed. The resulting four samples of tissue were ground individually in a Hobart grinder (4-mm plate perforations) and duplicate 7.0 ± 1.0g samples were weighed to the nearest 0.1 mg. The samples were dried at 95°C in vacuo at 30 in. Hg for 24 hr, cooled in a desiccator and weighed for moisture determination.

The dried samples were finely ground in a high-speed Wiley mill. Samples were pelleted (1.0 ± 0.1g), and the caloric value of the pellets determined by a PARR Oxygen Bomb, 1230 Adiabatic calorimeter (Parr Instrument Company, 1960).

RESULTS & DISCUSSION

A STATISTICAL analysis of the data, using analysis of variance techniques, indicated that treatment (cooking), type of meat (breast or thigh) and method of preparation significantly influenced the caloric content of turkey (Tables 2 and 3). In addition, the moisture content of meat was significantly influenced by cooking and method of preparation.

On the average, moisture values were lowered by cooking by 9.5%. Cooking, therefore, had a significant effect on caloric content. As shown in Table 2, breast calories increased from an average of 156 calories/100g of raw meat to 203 calories/100g of cooked meat, and thigh increased accordingly from 155 to 220 calories/100g of meat.

The removal of skin and obvious de-

Table 1—Food energy (calories/100g) of cooked (roasted) turkey

Sample	Male	Female	All Classes[a]
Breast	194	215	176
Leg	224	230	203
Edible viscera	200	254	233

[a] Source: Watt and Merrill (1963); all other data reported by Scott (1956)

Table 2—Moisture (%) and caloric content (per 100g meat) of raw and cooked turkey breast and thigh

Treatment[a]	Meat type	Preparation method[b]	Moisture[c]	Calories
C	Breast	−b	65.3 ± 1.4	180 ± 9
C	Thigh	−	64.1 ± 1.5	202 ± 11
C	Breast	+	60.9 ± 2.6	226 ± 26
C	Thigh	+	61.5 ± 2.6	238 ± 17
R	Breast	−	73.1 ± 0.8	140 ± 4
R	Thigh	−	75.9 ± 1.7	130 ± 13
R	Breast	+	70.2 ± 1.2	172 ± 13
R	Thigh	+	70.8 ± 1.3	180 ± 13

[a] C, cooked; R, raw
[b] Absence (−) or presence (+) of natural proportions of skin and accompanying fat in the meat sample
[c] Mean and standard deviation are based on 10 observations

Table 3—Mean squares associated with the analysis of variance of moisture and calorie values

Source	df	Mean Squares: Moisture	Calories
Treatment (T)	1	1819.18**	62,512**
Preparation method (P)	1	278.82**	32,953**
T × P	1	1.20	5
Meat type (M)	1	9.61	1,242**
T × M	1	18.27**	1,601**
P × M	1	0.21	972
T × P × M	1	20.55**	1,065*
Error	72	3.04	213

* Significant at the 0.05 level
** Significant at the 0.01 level

posited fat affected both the caloric and moisture values significantly. As would be expected, the removal of skin and deposited fat resulted in increased moisture values; it also decreased the relative caloric value of the sample. Thus, one can speculate that additions of fats or oils to whole turkey or turkey parts, as commonly used in programs yielding self-basting products, would increase their caloric content.

The results of this investigation generally confirm that the caloric content of cooked turkey falls within the range of published data. The data on raw meat differ somewhat, however, from those in the literature. Raw breast and thigh contained 140–130 calories/100g, respectively. One normally would expect thigh meat because of its greater intramuscular lipid content to have a higher caloric value than breast meat. Since the opposite was observed, there is the probability of greater variation among thigh muscles than among breast muscles. Indeed, the standard deviation of values for thigh was three times greater than that for breast. Thus, the method of sampling is critical with thigh muscles, and suggests that a comparison of breast with thigh meat should be made between individual muscles, such as Biceps femoris and Pectoralis superficialis.

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A Research Note

USE OF SODIUM ASCORBATE OR ERYTHORBATE TO INHIBIT FORMATION OF N-NITROSODIMETHYLAMINE IN FRANKFURTERS

INTRODUCTION

RECENTLY our Laboratory confirmed the presence of 11 to 84 ppb of dimethylnitrosamine (DMNA) in 3 of 40 commercial samples of frankfurters (Wasserman et al., 1972). While DMNA has been shown to produce malignant tumors in animals (Magee and Barnes, 1956; Druckrey et al., 1967), the carcinogenic effect in man of DMNA and nitrosamines in general has not been demonstrated, but is a matter of concern. Since more than 16 billion frankfurters were consumed in the United States last year, the presence of even a few samples containing nitrosamines required an investigation into the cause of their formation.

In a study of frankfurters using varying concentrations of sodium nitrite ($NaNO_2$) alone, we found that the legally permissible level of approximately 150 ppm failed to give measurable amounts of DMNA. Under our pilot plant conditions, it appeared that using approximately 10 times this amount of nitrite in the preparation of frankfurters would yield a final product containing at least 10 ppb DMNA (Fiddler et al., 1972). This paper reports the effect of other cure ingredients—sodium nitrate ($NaNO_3$), sodium ascorbate (NaAsc), and sodium erythorbate (NaEry)—on the formation of DMNA in frankfurters.

EXPERIMENTAL

FRANKFURTERS were prepared in the same manner as described previously (Fiddler et al., 1972) using a conventional 2-hr smoking and cooking schedule to bring the frankfurters to an internal temperature of 71°C. An additional 2-hr smoking and heating treatment was used to maintain the frankfurters at 71°C to assess the effect of an extreme condition on DMNA formation. Sodium nitrate, NaAsc or NaEry were added, in separate experiments, to frankfurter emulsions containing $NaNO_2$ at approximately the maximum permissible concentrations (Code of Federal Regulations, 1971) for each ingredient, and at 10 times these amounts. The methodology for the isolation, determination and confirmation of DMNA has been described by Fiddler et al. (1972). Depending on the level of interfering or background components, approximately 10 ppb DMNA or greater were confirmed using a gas-liquid chromatography-low resolution mass spectrometry system. Where DMNA could not be confirmed by mass spectrometry, values are given as the apparent amount of DMNA present as indicated by its GLC retention time alone.

RESULTS & DISCUSSION

RESULTS of the analyses of frankfurters prepared with 150 ppm $NaNO_2$ alone and with $NaNO_3$, NaAsc or NaEry at either the legally permissible level or 10 times these amounts indicated no DMNA to be present with either a 2- or 4-hr processing time. Representative data from analyses of frankfurters prepared with 1500 ppm $NaNO_2$ from several experiments are shown in Table 1. Frankfurters prepared according to the normal 2-hr processing schedule with 1500 ppm $NaNO_2$ alone or in combination with $NaNO_3$ contained approximately the same amount of DMNA: 10 ppb. The results of the 4-hr processing indicate that in general nitrate appears to have little or no effect on DMNA formation.

Sodium ascorbate, or its isomer erythorbate, is used in cure mixtures to speed cure color formation. These compounds act by reducing NO_2^- to nitric oxide which reacts with the meat pigment myoglobin and forms the stable pink nitric oxide hemochrome upon heating. The amount allowable in comminuted meat products is 7/8 oz per 100 lb or 547 ppm with respect to the meat (Code of Federal Regulations, 1971). Frankfurters prepared with either 550 or 5500 ppm NaAsc or NaEry and processed for 2 hr had no DMNA present, in comparison with the approximately 10 ppb present in the samples made with NO_2^- alone. In the case of the frankfurters cooked and smoked an additional 2 hr where a larger amount of DMNA was produced with 1500 ppm $NaNO_2$ alone, addition of Asc^- or Ery^- significantly reduced the amount of nitrosamine formed. Under the conditions used in these studies both reductants behaved similarly and exhibited the same inhibitory activity. While the mechanism for the blocking of the nitrosation reaction is not completely understood, it appears that these reductants compete for the NO_2^- thereby making it less available for the nitrosation of the secondary amine. Ascorbate and Ery^- are also known to reduce the amount of residual NO_2^- in the finished cured product.

The results of the use of the two reductants, NaAsc and NaEry on the inhibition of DMNA formation in frankfurters are similar to those obtained in our model system work (Fiddler et al., 1973).

It appears that the use of a reductant like Asc^- or Ery^- in concentrations greater than that now permitted offers a potential for the preparation of nitrosamine-free cured meat products.

Further research in this area is necessary.

Table 1—Formation of dimethylnitrosamine (DMNA) in frankfurters prepared with 1500 ppm $NaNO_2$ and with $NaNO_3$ or reductants

Cure ingredient	Amount added (ppm)	DMNA, ppb[a] Processing time 2 hr	4 hr
None		11	22[b]
$NaNO_3$	1,700	10	15[b]
$NaNO_3$	17,000	10	32[b]
None		11	22
NaAsc	550	0	7
NaAsc	5,500	0	4
None		10	11[b]
NaEry	550	0	6
NaEry	5,500	0	0

[a] Corrected using the recovery of an aliquot of the same sample with 20 ppb DMNA added
[b] Confirmed by mass spectrometry

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A Research Note

PARTIAL RECOVERY OF NITRITE NITROGEN BY THE KJELDAHL PROCEDURE IN MEAT PRODUCTS

INTRODUCTION

THE KJELDAHL PROCEDURE has been employed routinely to determine crude protein in meat products (AOAC, 1970). Digestion of the sample with acid converts carbon sources to carbon dioxide and hydrogen, oxygen to water, and nitrogen compounds to ammonia (Bradstreet, 1965). The ammonia liberated is distilled, collected and titrated and a value for crude protein is calculated (AOAC, 1970).

It is recognized (Dakin and Dudley, 1914; Bradstreet, 1965) that some forms of nitrogen are difficult, if not impossible, to convert to ammonia. Accordingly, several modifications of the Kjeldahl procedure have been published (Deb, 1925; Weizmann et al., 1930; McCutchan and Roth, 1952; Bradstreet, 1954; Bradstreet, 1965). Generally, these modifications depend on the type of nitrogen present. For oxides of nitrogen (nitrite and nitrate) a reduction step, such as adding salicylic acid or thiosalicylic acid, is often employed (AOAC, 1970; Bradstreet, 1965; McCutchan and Roth, 1952).

Cured meat products contain residual nitrite and/or nitrate. There is, however, little information available concerning the conventional Kjeldahl procedure in such a circumstance, except that a modification is necessary for quantitative recovery of total nitrogen. The implication seems to be one of total loss and no recovery of nitrogen oxides by a conventional Kjeldahl procedure. However, oat straw digested by the Kjeldahl procedure with varying amounts of nitrate has been shown to give partial recovery (40–50%) of the nitrate nitrogen as ammonia (Whitehead and Olson, 1942). Glucose in combination with nitrate also led to partial recoveries of the nitrate-N as ammonia and the percent recovered decreased with increasing nitrate levels (Bremner and Shaw, 1958).

It, therefore, seems likely that use of the conventional Kjeldahl procedure for crude protein in cured meats would include some nitrogen from nitrite and/or nitrate but the recovery would not be quantitative. The purpose of this investigation was to examine the effect of including nitrite with meat in a conventional Kjeldahl procedure.

MATERIALS & METHODS

THE FRESH PORK used for this study was pulverized by first freezing it in liquid nitrogen and then powdering it in a Waring Blendor. The meat was kept in a −20°C freezer until analyzed. Approximately 0.2g of meat was weighed accurately in a 30 ml micro Kjeldahl flask with a boiling chip and cupric selenite as the catalyst. Sodium nitrite was added in 1 ml of water at concentrations ranging from 0.106 mg/ml (0.46 mg/g meat) to 2.56 mg/ml (11.6 mg/g meat). The 2.0 ml of sulphuric acid for digestion was added immediately (within 1 min) after the nitrite. Samples were digested for about 30 min after clearing. Six samples were analyzed for each level of nitrite addition as well as the control and results are expressed as an average value.

RESULTS & DISCUSSION

RESULTS are diagrammed in Figure 1. Nitrite in meat products is at least partially recovered by the conventional Kjeldahl procedure, but the amount recovered depends on the amount present. Higher nitrite concentrations gave increasing quantities of N recovered as Kjeldahl N, but the percent of the nitrite–N recovered was decreased. Low levels of nitrite gave 80–90% recovery and high levels gave 25–30% recovery.

Bradstreet (1965) has suggested that when salicylic acid is used to reduce nitro compounds, its most important function is to supply free carbon which, upon heating, will reduce sulphuric acid to sulphur dioxide which is the active reducing agent. Other compounds such as sucrose have also been found to have this effect (Bradstreet, 1965). Thus, it seems likely that, in meat, the same mechanism is involved to produce partial reduction of nitrite by various carbon sources. Complete reduction is not likely because the sample is immediately heated to a high temperature for digestion and some compounds may have a relatively high volatility at the temperature used. In addition, nitrite is quite unstable under acid conditions and decomposes to NO which in turn combines with oxygen to give NO_2 (Bard and Townsend, 1971). The generation of NO_2 as a brown gas could be observed when sulphuric acid was added to samples of high nitrite concentrations.

The nitrogen recovered from nitrite as

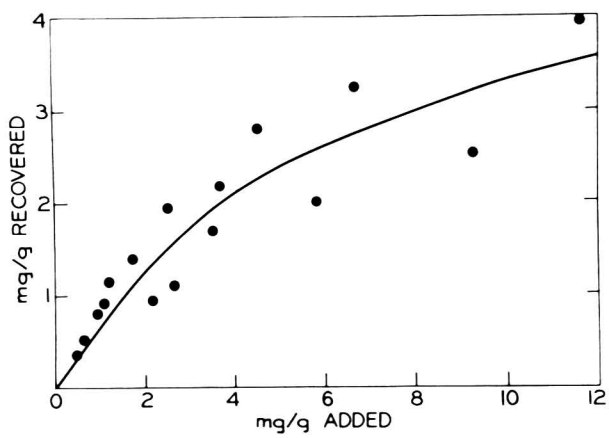

Fig. 1—The recovery of nitrite nitrogen from a meat mixture following various levels of addition of nitrite.

Kjeldahl N should not lead to a significant error in the Kjeldahl procedure for crude protein in meat, because the nitrogen of nitrite in commercial meats is a very low proportion of the total nitrogen. However, as in our experience, results of experiments involving an ^{15}N label for nitrite are very significantly altered if this effect is not considered.

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A Research Note

ULTRAVIOLET SPECTROPHOTOMETRIC DETERMINATION OF PROTEIN IN MEAT AND MEAT PRODUCTS

INTRODUCTION

ULTRAVIOLET absorption spectrophotometry has been widely used as a simple method for quantitating protein in biological materials (Webster, 1970). The complexity of food products and interferences derived from compounds other than protein in foods present difficulties in the utilization of this technique. This procedure has been used for protein determination in whole milk (Nakai and Le, 1970) and for other food items such as beans, egg yolk, flour and corned beef (Toma and Nakai, 1971). An application of this method for meat products was suggested by Toma and Nakai (1971).

This study evaluated the ultraviolet method for rapidly quantitating protein in meat and meat products and suggested the use of another solvent system for meat products. A solution of 8M urea containing 0.86M (5%) sodium chloride was employed for dissolving meat products prepared in a uniform meat suspension with 0.1M citric acid (Ashworth, 1971).

EXPERIMENTAL

REAGENT GRADE chemicals were used for preparing the following: 0.1M citric acid, 8M urea in 0.86M sodium chloride, 8M urea and 8M urea in 2N sodium hydroxide.

A Perkin-Elmer Model 139 spectrophotometer equipped with a photomultiplier attachment was used for measuring absorbances.

A 15g homogenous meat sample was blended with 250/ml of 0.1M citric acid in a Sorvall Omni Mixer for 2½ min (Toma and Nakai, 1971). To a 0.5g portion of this slurry in a test tube was added 9.5 ml of either 8M urea in 0.86M sodium chloride, or 8M urea, or 8M urea in 2N sodium hydroxide. This mixture was shaken, centrifuged at 5000 rpm for 7 min, filtered and the absorbance measured from 195 nm to 280 nm.

RESULTS & DISCUSSION

SOLUBILIZATION of meat samples was tried with several different solvents (0.2M sodium chloride, 0.1N sodium hydroxide, 2N sodium hydroxide, 0.1M citric acid, 8M urea, 8M urea in 2N sodium hydroxide and 8M urea in 0.86M sodium chloride) until complete dissolution without adverse color development and reasonable stability (for 10–15 min) of the absorbance was developed from a solvent.

The wavelength at 200–230 nm was used for amino acid determination by Saidel et al.(1952). At 260–280 nm, proteins absorb heavily due to their aromatic properties. Because of high and uniform sensitivity of absorbance of proteins of different origins, the wavelength of 200–220 nm was recommended for protein determination by Wrigley and Webster (1968). For these reasons, evaluations were made at 195, 200, 205, 210, 215, 220, 225, 230, 235, 240, 243, 245, 250, 260, 270 and 280 nm in this research.

Table 1–Correlation coefficients between absorbance values and Kjeldahl protein, standard error of estimate and regression equation for each meat product

Food product	Wavelength of measurement	Correlation coefficient	Standard error of estimate	Regression equation[a]
Cooked Beef	280	0.80	2.52	y= 46.00x– 35.55
Cooked Pork	270	0.89	2.08	y=113.52x–104.14
Cured Pork	280	0.90	2.15	y= 28.02x– 15.61

[a] y = Kjeldahl protein; x = optical density or absorbance values.

Meat samples (10 for each product) of varying protein content were prepared by adjusting the fat content. The absorbance values at each wavelength were correlated to the values obtained with the Kjeldahl method.

Fresh meat samples of beef and pork resulted in highly significant negative correlations when 8M urea in 0.86M sodium chloride was used as a solvent. Upon further investigation it was found that the citric acid caused foaming of the uncooked samples (not observed in cooked samples). As the protein percentage increased more protein was incorporated into the foam. When 8M urea in 2N sodium hydroxide was used as a solvent for uncooked meat, no significant relationship was found. These results are similar to those found by Nakai (1972), who stated that a large standard deviation was found with uncooked meat.

Cooked and cured meat samples gave highly significant positive correlation coefficients (Fig. 1). The correlation coefficient between the two methods, the standard error of estimate, wavelength of measurement and regression equation for each meat product are shown in Table 1.

A standard curve for calculation of protein for each meat product must be drawn separately.

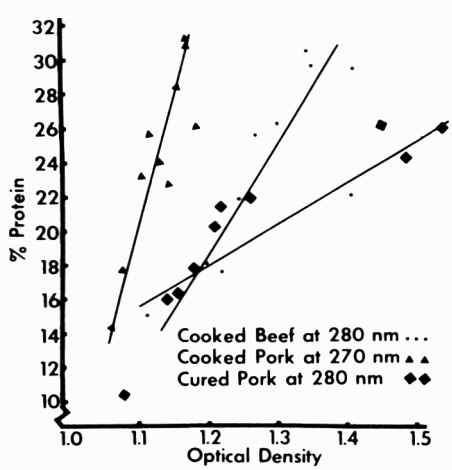

Fig. 1–Optical density vs. % protein of cooked beef and pork and cured pork.

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A Research Note

PHENOLASES AND BLUE DISCOLORATION IN WHOLE COOKED DUNGENESS CRAB (Cancer magister)

INTRODUCTION

THE OCCASIONAL OCCURRENCE of a blue to blue-black discoloration greatly decreases the quality of whole cooked Dungeness crab. The formation of "black spot" in shrimp (*Penaeus setiferus*) has been related to the formation of melanins by phenolases (Bailey et al., 1960). Pinhey (1930) has related the blackening of blood in clots of wounds in spider crab (*Maia squinado*) to the oxidation of tyrosine to melanin by tyrosinase. The phenolase system (Summers, 1967) involved in the hardening and darkening (sclerotization) of the cuticle of fiddler crab (*Uca pugnax*) appears to be very similar to systems present in insects (Malek, 1961) and amphibians (Miller et al., 1970). The purpose of this investigation was to evaluate the blueing mechanism in whole cooked Dungeness crab.

EXPERIMENTAL

Analytical methods

Methods for determining phenolase activities have been previously described (Bailey et al., 1960; Summers, 1967). Crude enzyme preparations were prepared by cuttting the lower joint of a rear crab leg and collecting the clear serum (blood) in an ice bath. For enzymatic studies filtered blood was used. Other studies utilized 10g of crab tissue and 90 ml of a 1.0 mM pyrocatechol solution buffered at pH 7.0 with 0.1M phosphate buffer. Colorimetric methods for tyrosine (Sweeney and Simandle, 1966), dopa (Drell, 1970) and "total phenols" (Malek, 1961), and ascending thin-layer silica gel chromatography described by Malek (1961) and Summers (1967) were used to detect the presence of phenolic compounds.

Processing crab

Freshly caught crab were purchased from local processors. Common industrial practices were followed for selecting and handling crab intended to be marketed as whole cooked crab. Instead of the commonly used 25 min cook, lots of 15 crab were cooked in boiling water for 15 or 30 min. Following cooking, the crab were cooled with a cold-water spray and held on ice at 1–3°C until examined. Crab prior to cooking were covered with wet towels and held at 1–3°C.

Table 1—Effect of ascorbic acid, phenylthiourea and boiling on phenolase activity[a]

Treatment	Tyrosine μg/ml[b]	Dopa μg/ml	Total phenols μg/ml	Visual color
Boiled blood	50.0	3.0	54.2	Colorless
Blood	67.5	11.5	18.0	Blue-black
Blood and 4% ascorbic acid	62.5	12.5	60.1	Colorless
Blood and phenylthiourea	96.1	1.2	54.8	Colorless

[a] After 1 hr at 37°C, visual color of the reaction mixture was noted and tyrosine, dopa and total phenols remaining in solution were determined.

[b] μg/ml of reaction mixture. Reaction mixture consisted of 1.0 ml 1 mM tyrosine, 0.2 ml distilled water or 4% ascorbic acid or 0.2g phenylthiourea, 1.8 ml 0.1M phosphate buffer (pH 7.0) and 1.0 ml filtered crab blood.

Table 2—Extent of blue discoloration in whole cooked crab held for 72 hr at 1–3°C[a]

Cooking time (min)	Holding time of live crab before cooking: 16 hr	96 hr
15	Moderate	Extensive
30	None-slight	Moderate

[a] Extent of blueing: Extensive—dark blue-black discoloration of gills, body cavity and meat; Moderate—blue to blue-black discoloration of gills, some discoloration in body cavity and meat; None—no discoloration.

RESULTS & DISCUSSION

THE PRESENCE of phenols in crab tissue and blood was confirmed. The Folin-Ciocalteau reagent is not specific for any particular type of phenol, and the development of blue-green colored spots on the chromatographic plates indicates ortho-dihydroxyphenols while the formation of blue spots after an overspray of alkali indicates the presence of para-dihydroxyphenols (Malek, 1961). Of the more than nine chromatographic spots, tyrosine, dopa (3,4-dihydroxyphenylalanine) and 3-hydroxytyramine were tentatively identified when compared to known standards.

Phenolases from crab exhibited similar properties of other known phenolase systems (Malek, 1961; Mathew and Parpia, 1971). A mono-phenolase (tyrosinase) was detected in Dungeness crab and particularly in the blood (Table 1). After 1 hr at 37°C, the formation of melanins from tyrosine was visually detected by the development of a blue-black color and a decrease in total phenols. Heating the crab blood in a boiling water bath for 20 min completely destroyed phenolase activity. However, there was only a slight loss in enzymatic activity after 2 min at 85°C. The increase in dopa in the presence of active phenolase (Table 1) may be explained by the enzymatic kinetics of phenolases (Mason, 1957). The phenolases in crab were inhibited by antioxidants (ascorbic acid) and metal chelating-substances (phenylthiourea). Apparently, phenylthiourea blocks tyrosinase activity completely by chelating copper that is required as its prosthetic group (Mathew and Parpia, 1971). The increase in dopa by tyrosinase in the presence of ascorbic acid (Table 1) suggests that the oxidation of tyrosine to melanin is blocked by the smaller oxidation-reduction potential of ascorbic acid which keeps dopa in the reduced state.

The phenolic content of crab held at 1–3°C increased from 140.8 ± 15.1 μg/g to 322.5 ± 80.6 μg/g after 4 days. The pH of the crab meat increased from 6.7 ± 0.3

to 7.7 ± 0.2 indicating that the crab were in poor condition after 4 days. When crab in "excellent" and "poor" condition were processed in a manner simulating the production of whole cooked crab, both length of holding and cooking times were apparent factors in the formation of blue discoloration (Table 2). When whole uncooked crab were frozen and subsequently allowed to thaw at 1–3°C for 20 hr, extreme blue-black discoloration formed throughout the entire crab. When the blue discolored meat was immersed in a strong reducing agent (sodium hydrosulfite) no change in discoloration occurred indicating that the blueing involved a more complex system than the oxidation of the copper bound haemocyanin.

Further experiments indicated that although antioxidants prevented the oxidation of certain phenols (dopa, pyrocatechol, and 3-hydroxytyramine), heat denaturation of the crab blood did not. These phenols were able to form colored chromophores particularly under alkaline conditions and in the presence of metals (copper and iron). These colored chromophores may be similar to the phenolic-metal complexes discussed by Mathew and Parpia (1971).

The results indicate that the blue discoloration in whole cooked crab was related to the condition of the crab, handling and holding during processing, and under-cooking. The presence of metals (iron and copper) may catalyze the oxidation of polyphenols to melanins via enzymatic as well as nonenzymatic pathways particularly under alkaline conditions.

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A Research Note
NUTRITIONAL STUDIES ON SOYBEAN CURD PRODUCED BY CALCIUM SULFATE PRECIPITATION OF SOYBEAN MILK

INTRODUCTION

SOYBEAN PROTEIN is unique among plant proteins by virtue of its relatively high biological value. As a result this oil-rich legume has been the subject of extensive investigation as a source of protein for the human diet. To this end many processes for utilization of soybeans have been developed. A feature common to these processes is some form of heat treatment. The heat treatment is necessary to destroy the antidigestive and growth depressant factors that occur naturally in soybeans (Klose et al. 1948). The heat treatment may also be an important determinant of the flavor of the product (Wilkens et al. 1967). Further processing of the soybeans or soymilk may include extraction, separation and precipitation. All of these treatments may affect the nutritional value of the finished product. With the increasing value of soybean products on the market it is important that we have a complete picture of the implications of processing as they affect human nutrition. Hackler recognized the significance of this problem and the data on processing of soybean milk are quite extensive (Hackler et al. 1965; Hackler and Stillings, 1967). They concluded that heat processing of soybean milk could markedly affect its nutritional value. The nutritive value of many other soybean products, including soybean curd, is covered in an extensive review by Liener (1972). In view of the considerable variation in nutritive value that can result from processing an evaluation was made of the soybean curd produced by calcium sulfate precipitation of heated soybean milk (Schroder and Jackson, 1972).

MATERIALS & METHODS

40 weanling Sprague-Dawley rats of the University of Alberta strain, equalized with respect to sex and randomized with respect to litter origin, were allocated to two dietary groups at an average age of 21 days and an average weight of 50.2g. The control diet contained casein as the sole source of protein while the test diet contained soybean curd as the sole source of protein. The soybean curd was prepared according to Schroder and Jackson (1972), freeze dried in a RePP, 42-FFD-WS Sublimator (The Virtis Co., Inc., Gardiner, N.Y.) and ground to a fine powder. The rats were housed individually in stainless steel cages, 7 in. wide, 10 in. deep, 7 in. high. The cages were in banks in an air conditioned room maintained at 23°C and a relative humidity of 45–50%. The diets were fed ad libitum and water was available at all times.

The formulation and composition of the experimental diets are given in Table 1. The diets were formulated to meet or exceed the minimal nutrient requirements for growth of the weanling rat as set down by the National Academy of Sciences-National Research Council (1962). The diets were further formulated to be isonitrogenous and isocaloric. Formulation was based on analysis of the major ingredients. Nitrogen was determined by the Kjeldahl method (AOAC, 1965) and gross energy determined with the aid of a Parr oxygen bomb calorimeter (Parr Instrument Co., Moline, Ill.). The crude fat was determined according to the official method for crude fat determination in soy flour (AACC, 1962). Amino acid analysis on the isolated proteins was carried out in the Beckman Spinco model 120-B amino acid analyzer (Beckman Instruments Inc., Fullerton, Calif.) according to Spackman et al. (1958). Hydrolysis of the protein was effected in 6N HCl at 110°C for 24 hr and corrections were made for hydrolytic losses by extrapolation of values back to zero hydrolysis time. All results are reported as percentage of total hydrolysate; however, no measurement of tryptophane was conducted.

The rats were fed the experimental diets for a period of 28 days during which time they were weighed at weekly intervals and their feed consumption recorded. At the time of weekly weighings, wasted feed was collected and recorded, feed consumption being corrected accordingly. Average daily gain, average daily feed, feed conversion (g feed/g gain) and the protein efficiency ratio (PER) were calculated. The data were analyzed statistically by the analysis of variance as described by Steele and Torrie (1962).

Table 1—Formulation and composition of diets used to evaluate the nutritional value of soybean curd

Ingredients	Control %	Test %
Sucrose	48.88	43.38
Casein[a]	25.00	
Soybean curd		42.50
Cellulose	5.00	5.00
Corn oil	15.00	3.00
Mineral mix[b]	5.00	5.00
Fat soluble vitamin mix[c]	0.50	0.50
Water soluble vitamin mix[d]	0.50	0.50
Choline Chloride	0.12	0.12
Composition (by analysis)		
Crude protein (N x 6.25)%	22.94	22.31
Gross energy Kcals/g	4.91	4.67

[a] Vitamin free

[b] Composition, g/100g mix: $CaCO_3$, 30.0; KH_2PO_4, 34·1; NaCl (iodized), 25.0; $MgSO_4 \cdot 7H_2O$, 10.0; $FeCl_3 \cdot 4H_2O$, 0.60; $CuSO_4 \cdot 5H_2O$, 0.157; $MnSO_4 \cdot H_2O$, 0.12; $ZnCl_2$, 0.02; $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, 0.003.

[c] Composition, g/100g mix: Vitamin A (10,000 IU/g), 20.0; Vitamin D_3 (35,000 IU/g), 4.0; Myvamix (20,000 IU Vitamin E/lb), 20.0; Corn starch, 56.0.

[d] Composition, g/100g mix: Thiamine HCl, 0.20; Riboflavin, 0.20; Niacin, 1.00; Calcium pantothenate, 0.80; Pyridoxine HCl, 0.10; Vitamin B_{12} 0.001; Biotin, 0.004; Vitamin K (menadione), 0.02; Sucrose, 97.675.

RESULTS & DISCUSSION

THE DATA for the individual rats were averaged and are presented in Table 2. With either diet the males consumed more feed, gained weight faster and were more efficient than the females. All differences attributable to sex were significant ($P < 0.05$). A significant ($P \leq .05$) sex x diet interaction noted for average daily consumption, average daily gain and protein efficiency ratio shows there is an interaction between the diet and the sex of the rat. Recognizing the mentioned differences, the rats fed the diet containing casein as the sole source of protein consumed more feed, had higher gains, were more efficient both in terms of feed conversion and protein efficiency ratios than were the rats fed the test diet containing soybean curd as the sole source of protein. All differences were significant ($P \leq 0.5$). The feed wastage of soybean diet may reflect a palatability factor in the test diet. The low values of PER of both casein and soybean curd diets are no

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Table 2—Feed intake, gains, feed conversion and PER of rats fed diets containing casein or soybean curd

	Control			Test		
	M	F	M & F	M	F	M & F
Average daily feed g	13.75	11.38	12.57	10.76	10.70	10.73
Average daily gain g	6.88	4.84	5.87	4.37	3.88	4.13
Feed conversion g	2.00	2.35	2.18	2.46	2.76	2.60
Total feed wastage g	13.00	30.00	21.50	322.00	97.00	209.50
PER $\left(\frac{\text{g gained}}{\text{g protein consumed}}\right)$	2.19	1.86	2.02	1.80	1.62	1.71
PER[a]				2.06	2.18	2.12
Protein quality $\left(\frac{\text{Test PER}}{\text{Casein PER}} \times 100\right)$				82.2	87.1	84.6

[a] Corrected to PER for casein of 2.50

Table 3—Amino acid composition (% of diet)

Amino acid	Control (casein) %	Test soybean curd %	NRC minimum requirement %
L-tryptophan			0.15
L-histidine	0.72	0.53	0.30
L-lysine	2.03	1.38	0.90
L-leucine	2.50	1.79	0.80
L-isoleucine	1.66	1.10	0.50
L-phenylalanine	1.34	1.19	0.90
L-methionine	0.78	0.22	0.60
L-threonine	1.10	0.80	0.50
L-valine	1.98	1.18	0.70

doubt partly due to the high protein content (approximately 22%) of the diet.

Hackler et al. (1963) fed acid precipitated soybean curd to rats at levels of approximately 10, 20 and 30% dietary protein. The resulting PER values were 2.20. 1.94 and 1.64 respectively. In the present experiment the level of dietary protein of the soy curd diet was 22.31% and the PER 1.71. The average protein quality (PER soybean curd/PER casein x 100) in this study was 84.6. This is similar to the protein quality of soybean curd calculated from the results of Hackler et al. (1963). The protein quality in their study was 76.9, 80.5 and 87.7 when fed at dietary protein levels of approximately 10%, 20% and 30% respectively. The PER for the soybean curd in this study, 1.71, is also comparable to a PER of ca 1.67 for soybean milk heated at 93°C for 30 min (Hackler and Stillings, 1967) and a PER of ca 1.70 for soybean milk heated at 121°C for 5 min (Hackler and Stillings, 1967). The heat treatment of the soybean curd used in the present work was equivalent to 100°C for 30 min.

The poorer performance of the rats fed the diet containing the soybean curd may have been attributable to an unfavorable balance of certain essential amino acids. Using the figures from the amino acid analysis of the soybean curd and the amino acid composition of casein taken from Crampton and Harris (1969), the amino acid composition of the diets was calculated (Table 3) and compared with the amino acid requirements of the growing rat as set down by the National Academy of Sciences-National Research Council (1962). The test diet meets the amino acid requirements of the rat for all amino acids except methionine. The deficiency of an essential amino acid will result in reduced food intake and concomitantly reduced growth of the rat. The deficiency will also result in an increased feed conversion ratio and a decreased PER, resulting from less efficient utilization of dietary proteins. However, the cost of supplementation of the soybean precipitate with methionine would be very small and the resulting product would be a good source of dietary protein.

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A Research Note

UTILIZATION OF PLASTIC BAGS FOR CONCENTRATION OF DILUTE SOLUTIONS IN CONVENTIONAL FREEZE DRYERS

INTRODUCTION

THE CONCENTRATION of highly dilute extracts of water soluble fractions of foods, which may contain heat labile components, may be most satisfactorily accomplished by freeze dehydration. However, since the technique of laboratory freeze dehydration was first described by Shakel (1909), little change in the procedure has occurred.

Laboratory practice consists of dispersing small volumes (up to 2½ liters) among several round bottom flasks, shell freezing and then vacuum processing for 18–24 hr. Volumes on the order of 25 liters would require up to 2 wk to desiccate by this method. In addition, the technique requires large quantities of dry ice and alcohol and their frequent replenishment in order to provide a means for condensing the water vapor generated during the drying process.

Cabinet freeze dryers, providing the advantage of mechanical refrigeration for the condensation of water vapor, are designed primarily for laboratory food dehydration and did not appear to be suitable for the preparation of pure extracts because of the high probability of interaction with the equipment. However, a simple means of freeze drying large volumes without contamination was obtained by utilizing high density polyethylene bags (Cendanco, P.O. Box 89, Framingham, MA 01701) in conjunction with a cabinet type freeze dryer of 25 liter capacity (Model 2003 F-2, Stokes Machine Co., Philadelphia, PA.)

High density polyethylene has excellent properties for investigative use as a container for freeze dehydration: it is nontoxic, virtually chemically inert, remains pliable at low temperatures and can be autoclave sterilized at 121°C if container sterility is required.

EXPERIMENTAL

THE SOLUTION to be dried was poured into the plastic bags in 2–5 liter aliquots, making an even layer not more than 1½ cm thick, and subsequently set in the metal trays which are components of the freeze-drying cabinet. A simple suspension system, consisting of 8–10 mm diam rods and small "binder" type paper clips, attached to the outside of the bags prevented the plastic from settling on the surface of the solution. The mouth of the bag was gathered around a short section of 40 mm diam glass tubing and secured around the tubing with a piece of tape. These expedients assured transfer of water vapor to the freeze-dryer condenser during the drying period (Fig. 1 and 2).

RESULTS

BAGS containing the solution were laid horizontally in the freeze-dryer trays, quick frozen and placed on the shelves of the unit. Approximately 18–36 hr at 0.2 mm mercury were needed to dry up to 25 liters depending on the volume per container, layer thickness and temperature of the heating platens.

This procedure has been used routinely for a variety of extracts and has proven to be fully satisfactory while providing a significant savings in time.

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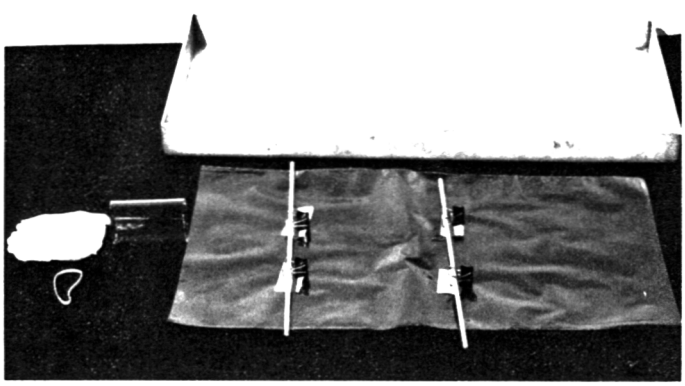

Fig. 1—Components for freeze dehydration using plastic bags.

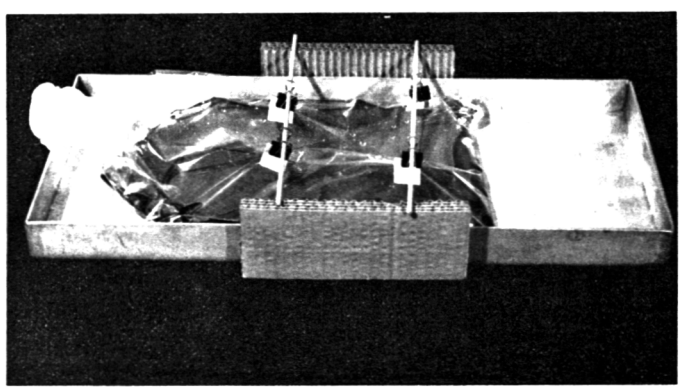

Fig. 2—Assembled components.

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A Research Note

EFFECT OF pH ON THE THERMAL DESTRUCTION KINETICS OF PATULIN IN AQUEOUS SOLUTION

INTRODUCTION

Patulin [4-hydroxy-4H-furo [3,2c]-pyran-2-(6H)-one] is one of the carcinogenic (Dickens and Jones, 1961; and Dustin, 1963) metabolites of *Aspergillus, Gymnoascus* and *Penicillium* species. It was discovered as a broad spectrum antibiotic and has been called claviformin, clavicin, clavatin, expansin, leucopin, mycoin, penicidin and tercinin. Fungal species with patulin-producing potential are common to a wide variety of foods (Karow and Foster, 1944; Ukai et al., 1954; Kraybill and Shimkin, 1964; Scott and Somers, 1968).

Patulin reportedly is stable as an antibiotic in serum, pus, urine and aqueous solutions in the pH range 3.3–6.3 (Wiesner, 1942; Stansfield et al., 1944; Jefferys, 1952). Peptone and sulfhydryl compounds such as cysteine, thioglycolate and and thiosulfate are known to inactivate patulin (Cavallito and Bailey, 1944; Geiger and Conn, 1945; Rinderknect et al., 1947). This compound's affinity for the sulfhydryl groups of enzymes has been the most often advanced theory for its toxicity (Atkinson and Stanley; Cavallito and Bailey, 1944; Geiger and Conn, 1945; and Rinderknecht et al., 1947).

In chemical stability studies, patulin was rapidly destroyed in wet grains but persisted in dry corn and apple juice held at room temperature (Pohland and Allen, 1970). Scott and Somers (1968) found that more than 50% of the patulin added to fresh and canned apple juice and grape juice remained after 3 wk. Chemical destruction in canned orange juice and whole wheat and bleached flours was more rapid, but some identifiable patulin remained for 2 wk or longer.

Wiesner (1942) was the first to report the thermal stability of patulin in aqueous solution. Scott and Somers (1968) reported that heating of fruit juices to 80°C for 10 and 20 min was inadequate for complete destruction of experimentally added patulin.

The objects of this study were to estimate the thermal destruction parameters for patulin in aqueous solution and to determine the effect of pH on thermal destruction kinetics.

EXPERIMENTAL

Experimental procedure

Crystalline patulin, determined to be pure by UV and IR spectroscopy and TLC, was dissolved in pH 3.5, 4.5 and 5.5 McIlvaine's buffers at concentrations of 100–150 μg/ml. The solutions were dispensed in 2.5-ml amounts into borosilicate tubes, sealed with a torch and heated at 5-degree intervals from 105–125°C. Twelve time intervals, including t = 0 min, were observed at each of the five temperatures. The time intervals chosen were based on short preliminary runs and were designed to observe a 2-log reduction in patulin concentration.

After heating, 2 ml of solution from each tube was extracted with two equal volumes of ethyl acetate. The extracts were dried over anhydrous sodium sulfate and evaporated in vacuo. The residue, dissolved in chloroform, and standards in chloroform were spotted on silica-gel-GF thin-layer plates and developed in benzene:acetic acid:methanol (90:5:5). The plates were dried and visualized by spraying with 3% ammonium hydroxide followed by 4% phenylhydrazine and heating at 110°C for 3 min. Results were calculated as μg/ml patulin remaining at each time interval.

Estimation of thermal destruction parameters

Patulin is a purified compound with a known molecular weight. Thus, estimates [393.16°K (120°C) taken as standard state] of the free energy increase (ΔF), the change in enthalpy (ΔH) and entropy (ΔS), associated with the degradation of this compound can be computed. These computations are outlined by Eyring and Stearn (1939). The temperature of standard state is taken as 393.16°K in these experiments. A knowledge of entropy of activation can be useful in determining the reaction mechanism.

Thermal destruction of spores and vegetative cells is often characterized by "D" and "z" as described by Schmidt (1961). Read and Bradshaw (1966) used these estimates to summarize thermal inactivation results for a microbial toxin. We estimated D and z values for patulin. The observed heating time was corrected for "come-up" and "cool-down" of the experimental heating system (Bigelow et al., 1920).

An estimate for experimental energy of activation (E_{exp}) can be obtained from the Arrhenius equation. A measurement of the specific rate constant (k') is obtained over a range of temperatures (T), and the slope of the $\log_{10} k'$ versus 1/T is used to obtain E_{exp}. Eyring and Urry (1965) have discussed this calculation in terms of biological systems.

A comparison of the effect of pH can be made by computing confidence limits for E_{exp}. Equation (1) is used to estimate the specific reaction rate (k'). Tests for normality of $\log_{10} Y$ have indicated that ε can be assumed to be normally distributed with mean = 0 and variance = σ^2. Thus, the estimator of k', is distributed N ($\mu k'$, $\sigma^2 k'$). Equation (2) which relates k' to E_{exp} is nonlinear. The method of Hartley (1961) was used in estimating E_{exp} and the standard error of E_{exp}. This requires that the estimates be obtained by successive iterations.

$$\log_{10} Y = d_0 + k' t + \epsilon \text{ (constant T)} \quad (1)$$

$$k = Ae^{-E_{exp}/RT} + \epsilon_r \quad (2)$$

where: Y = patulin concentration in μg; t = time of heating at constant T in minutes; T = absolute temperature; E_{exp} = experimental energy of activation, calories/mole; A = a frequency factor (1.0); R = 1.9872 cal deg^{-1} moles^{-1}; and ϵ, ϵ_r = experimental error.

Table 1—Patulin thermal inactivation parameters: D values (min) and z values (°C)

Temperature (°C)	pH 3.5	pH 4.5	pH 5.5
105	1058	695	167
110	744	444	109
115	527	334	71
120	378	236	48
125	268	167	33
z values	33.4	31.6	27.7

RESULTS & DISCUSSION

THE EFFECT of pH on the thermal destruction rates of patulin in aqueous solution is shown hy the D values in Table 1. As the pH increased, the time required to reduce patulin concentration by 90% decreased for all five temperatures. Values for z decreased with increasing pH. This means that the D value for destruction of

Table 2—Patulin thermal inactivation parameters: thermodynamic estimates

Source	pH 3.5	pH 4.5	pH 5.5
ΔH^a (cal/mole)	20,200	20,600	24,100
ΔF^a (cal/mole)	31,100	30,700	29,500
ΔS^a (cal/mole °F)	−27.7	−25.7	−13.7
E_{exp} (cal/mole)	20,900	20,900	24,000
99% CL - lower	20,000	19,000	23,200
99% CL - upper	21,800	23,200	24,700
% coefficient variation	1.3	3.0	0.9

[a]393.16°K (120°C) taken as standard state

patulin decreased more rapidly with temperature at pH 5.5 than at pH 3.5. The time to reduce the concentration 90% at pH 3.5 was about 10 times the value at pH 5.5.

Estimates of experimental error shown in Table 2 were determined from duplicate measurements at each point. Approximate confidence estimates for E_{exp} are arrived at using the error estimated by nonlinear technique (Hartley, 1961), and the percent coefficients of variation for these estimates are less than 6. The proportion of sums of squares due to linear regression (R^2) was greater than 0.97 for all sets of data.

Use of z and D values gives the experimenter an overall idea of how a system reacts, without examining the mechanism. Values for ΔH, ΔF and ΔS are related to the chemical reactions involved in patulin destruction. An interpretation of these quantities presumes some knowledge of the chemical reaction when patulin is heated in the buffer system. The present study was not extended to examine these associated chemical reactions. Our estimates of ΔH, ΔF, ΔS and E_{exp}, however, may be a value to future investigators seeking to identify patulin degradation products.

SUMMARY & CONCLUSIONS

PATULIN in McIlvaine's buffers of pH 3.5, 4.5 and 5.5 was heated at 105, 110, 115, 120 and 125°C. Ethyl acetate extracts of heated solutions were assayed for patulin remaining by TLC. Thermal destruction parameters (D and z values) were estimated to determine the effect of pH on thermal destruction kinetics. The classical thermodynamic estimates (ΔH, ΔF, ΔS and E_{exp}) were also made from the data obtained.

These data show patulin to be resistant to thermal destruction at all pH's from 3.5 to 5.5. Both D and z values increased, however, as the pH decreased. Destruction times at all temperatures were greater for pH 3.5 than for 5.5, i.e., patulin is more stable in more acid solutions.

Food characteristics other than pH undoubtedly contribute to patulin destruction. Scott and Somers (1968) speculated that the sulfhydryl content of fruit juices was related to the rate of patulin's disappearance at room and elevated temperatures. Our data indicate that thermal processing of low-acid foods, particularly those low in sulfhydryl content, may not destroy patulin. This work is being extended to selected foods to determine patulin thermal destruction kinetics and to shed some light on the role of food components in the inactivation of this mycotoxin.

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A Research Note

A CONTAMINANT IN N-NITROSODIMETHYLAMINE CONFIRMATION BY HIGH RESOLUTION MASS SPECTROMETRY

INTRODUCTION

THE CARCINOGENICITY of N-nitrosamines poses a potential health hazard to humans. Reports of the presence of these compounds in foods, therefore, should be carefully validated, and evaluated, in order to reduce the possibility of spreading misleading information. Current indications are that N-nitrosamines, when found in foods, are present only in the μg/kg range. Analysis of such low concentrations requires precise methods for the recovery of the nitrosamines from the food product and specific, sensitive quantitative procedures. Although sensitive procedures, such as thin-layer and gas chromatography are available, contaminants from the solvents or from the food product may lead to erroneous identification of nitrosamines based on retention values or response to indicator sprays. Confirmation by other methods is desirable. Mass spectrometry, which gives a unique fragmentation pattern for a particular compound, appeared to be, at this time at least, the ultimate, specific procedure for the identification of trace amounts of nitrosamines. We are now reporting the possibility of confusing the identification of N-nitrosodimethylamine with the ^{13}C and ^{29}Si isotopes of trimethylsilyl ion when using high resolution mass spectrometry.

EXPERIMENTAL

IN A STUDY of the microbial formation of nitrosamines, the bacterial culture medium was shaken with methylene chloride to extract N-nitrosodimethylamine (DMNA) which had been produced. Severe foaming and emulsion formation interfered with the separation. A drop of Dow-Corning Anti-foam B was added. The final methylene chloride extracts, after washing with 0.1N HCl to remove basic interfering substances, were concentrated to 0.1–0.5 ml in a Kuderna-Danish concentrator and submitted for analysis by gas chromatography using a Carbowax 20M column and an alkali flame ionization detector as described previously (Wasserman et al., 1972).

RESULTS

WHEN A POSITIVE response for apparent DMNA was obtained the sample was examined by gas chromatography-mass spectrometry in order to confirm the presence of the nitrosamine. The DuPont MS 21-492, adjusted to a resolution of 1:12000, was used in the peak matching mode in which the mass of an unknown ion is compared to that of a standard and the mass of the unknown is measured very accurately. Two mass spectral peaks were obtained from the extracts of the culture medium; the first, a small peak, reached a maximum and diminished slightly, then was followed immediately by a very large peak. Initially, it was not known which response could be attributed to DMNA, since only one peak was discernible by either the alkali flame ionization or the flame ionization detectors of the gas chromatograph, apparently indicating the presence of a single compound. Upon resorting to peak matching with the mass spectrometer we determined that the first peak had an m/e = 74.048 and the large, second peak an m/e = 74.046. When the material was re-run in the mass spectrometer at low resolution the readily recognizable isotopic pattern of a silicon-containing compound was evident at m/e 73, 74 and 75. DMNA has an m/e = 74.0480; the ^{13}C isotope of the trimethylsilyl ion has an m/e = 74.0502, and the ^{29}Si isotope has an m/e = 74.0469. The range among them is three parts in 74000.

The Dow-Corning Anti-foam B used in the extraction procedure contains a silicone compound; however, the identity of the silicon-containing compound that has essentially the same GLC retention time as DMNA was not established.

The presence of an ion with a mass: charge ratio so similar to that of DMNA may be misleading in high resolution confirmation studies for trace quantities of DMNA particularly when it is realized that trimeth· l silyl derivatives are used to silanize GC columns or the GC-MS interfacing lines to prevent adsorbtion. Silicone compounds are also used in food packaging materials and may become contaminants of the packaged product. Fragmentation of these compounds in the mass spectrometer then can result in the formation of trimethylsilyl ions, as detailed by Biemann (1962).

False positive identification of DMNA could possibly be avoided by resorting to one or more of the following: (1) Utilizing gas chromatographic column coatings other than Carbowax 20M to achieve separation of DMNA from the silicon-containing compound; (2) Use the alkali flame ionization detector to differentiate N-containing compounds; (3) Low resolution mass spectral analysis of fairly pure preparations to obtain the entire fragmentation pattern, and (4) Monitor two specific ion peaks for DMNA, i.e., m/e = 74.048 and 42.034, in the high resolution mode.

During the preparation of this paper Gough and Webb (1973) also reported observing fragment ions corresponding to the trimethylsilyl group in some food extracts. Under their conditions, however, this material was not eluted from the GC at the same time as N-nitrosodimethylamine, and would not be mistaken for the nitrosamine if a nitrogen-specific detector was used.

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

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A Research Note
WATER ACTIVITY DETERMINATION IN FOODS IN THE RANGE 0.80 TO 0.99

INTRODUCTION

DUNMORE-TYPE electric hygrometers, having moisture sensitive salt-coated probes, have been used with good success in measuring water activities (A_ws) of food products and grains at low and intermediate water activity levels (Mossel and Van Kuijk, 1955; Hubbard et al., 1957). However, these instruments lose accuracy in the upper A_w range (> 0.90), often giving slow response, and/or low sensitivity.

Because of a need for A_w measurements in doughs, batters and baked goods, accurate to within $\pm$ 0.005 A_w units, a method was sought having greater accuracy than that attainable with an electric hygrometer. Hagenmaier (1972) showed that oilseed proteins adsorb a given percent moisture at a specified A_w and temperature. The moisture adsorption is characteristic for a given protein and in the A_w range > 0.80 there is essentially no hysteresis in the moisture sorption isotherm. Hagenmaier found that at 84% relative humidity, 0.2g of protein will attain its equilibrium moisture adsorption in 24 hr under reduced pressure (ca 0.1 atmosphere) at 25°C. The A_w measurement procedure described in this report is based on Hagenmaier's observations.

EXPERIMENTAL

Preparation of standard curve

Sulfuric acid solutions in the range 11–28% H_2SO_4 are prepared for use as A_w standards (Stokes, 1949). 250–300 ml of each of the standard solutions are poured into the well of a 7-in. desiccator which serves as humidity chamber and the well is covered with a perforated porcelain plate. 0.18–0.30g of a reference protein (soy isolate or sodium caseinate) are weighed to within 0.1 mg into a tared 15 ml weighing bottle. The bottle (with stopper removed) is placed in a 50 ml beaker in the humidity chamber. The chamber is evacuated to ca 0.1 atmosphere and the protein allowed to equilibrate with the head space gas at 25 $\pm$ 0.1°C for 24 hr. The gain in weight is determined gravimetrically.

Rather than dry the proteins prior to equilibration over standard A_w solutions, moistures are determined on separate samples by drying in an air oven for 2 hr at 115°C. Total moisture adsorbed by a protein at a given A_w is equivalent to that initially present plus that adsorbed during equilibration.

For the A_w range 0.95–0.99 rennet casein is used as reference protein and sodium chloride solutions for the A_w standards (Robinson and Stokes, 1955). Figure 1 shows the relationship between equilibrium moisture adsorption and A_w for the three reference proteins.

Measurement of A_w of food products

100–200g of the food material to be analyzed are placed in the well of the humidity chamber and covered with a perforated porcelain plate. Weighed protein samples are allowed to equilibrate in the head space gas of the evacuated chamber at 25 $\pm$ 0.1°C for 24 hr in the same manner as described above. The percent total moisture adsorbed by the protein at equilibrium conditions is calculated and A_w is ascertained by reference to the standard curve (Fig. 1).

RESULTS AND DISCUSSION

IN SETTING UP the standard curves, 21 equilibrium moisture determinations were made for each of the reference proteins, soy isolate and sodium caseinate in the range 0.79–0.90 A_w. Precision at the 95% confidence level based on this sampling was $\pm$ 0.012 A_w. In the A_w range 0.90–0.95, where there is a sharp increase in the slope of the percent moisture, vs. A_w curve, the precision, based on 21 determinations for each of the proteins, is improved, being $\pm$ 0.005 A_w. Above 0.95 A_w the sodium caseinate, which is water soluble, requires more than 28 hr to reach equilibrium adsorption. The soy isolate gives $\pm$ 0.005 A_w precision in 25 hr through 0.96 A_w, but again requires more than 28 hr to reach equilibrium adsorption at A_ws > 0.96. Rennet casein will equilibrate in 24 hr giving a precision of $\pm$ 0.003 A_w in the range of 0.96–0.99 (20 determinations). It is not satisfactory in A_w ranges < 0.90.

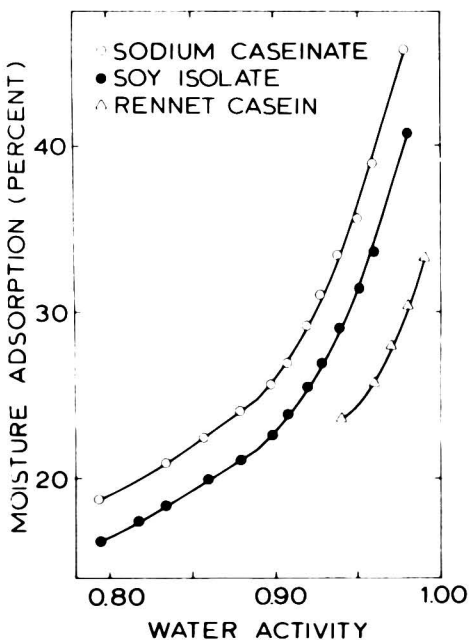

Fig. 1—Equilibrium moisture adsorption of proteins at 25 ± 0.1°C as a function of water activity.

Sulfuric acid and sodium chloride solutions were chosen for A_w standards in this work because their A_w values have been well documented in the literature (Stokes, 1949; Robinson and Stokes, 1955).

As a check on the accuracy of the described A_w measurement procedure, soy isolate and sodium caseinate were equilibrated over (1) saturated KCl solution; (2) saturated $BaCl_2 \cdot 2H_2O$ solution; and (3) saturated K_2CrO_4 solution. A_w values obtained at 25°C were: (1) 0.845 and 0.849 for KCl vs. literature value of 0.843 (Stokes, 1949); (2) 0.912 and 0.906 for $BaCl_2 \cdot 2H_2O$ vs. literature value of 0.902 (Stokes, 1949); and (3) 0.872 and 0.869 for K_2CrO_4 vs. literature value of 0.87 (Rockland, 1960).

In measuring A_ws in foods by the equilibrium moisture adsorption procedure accuracy could possibly be affected by: (1) adsorption by the reference protein of nonaqueous volatiles in the head space gas above the sample; (2) change in A_w of the food sample due to moisture adsorption by the reference protein (this should not occur if the ratio of sample to protein is of the order of 500); and (3) microbial deterioration of either the product being analyzed or the reference protein. To date there has been no evidence that any of the above situations have occurred, even when equilibration

Table 1—Comparison of sinascope and protein equilibrium moisture adsorption A_w determinations in foods

Food product[a]	A_w Value	
	Sinascope	Protein equilibrium moisture adsorption
Baked cake — 1	0.90 ± 0.01	0.924 ± 0.005
2	0.93 ± 0.01	0.944 ± 0.005
3	0.94 ± 0.01	0.918 ± 0.005
Cake icing — 1	0.79	0.841 ± 0.011
2	0.76	0.805 ± 0.011
Refrig. biscuit dough — 1	—	0.942 ± 0.005
2	—	0.940 ± 0.005
Pork sausage — 1	0.99 ± 0.01	0.973 ± 0.003
2	0.97 ± 0.01	0.973 ± 0.003
White bread — 1	0.94 ± 0.01	0.942 ± 0.005
2	0.97 ± 0.01	0.950 ± 0.005
Vanilla pudding	0.97 ± 0.01	0.991[b] ± 0.003

[a]The numerals 1, 2, and 3 represent different products rather than replicates of the same product.
[b]Required 4 hr to attain equilibrium, 25 hr A_w being 0.984.

time has been extended from 25 to 48 hr at 25°C. Accuracy, then, can essentially be equated with precision.

Choosing a variety of food products having high water activities, A_w measurements were made on a model WP 612 EB sinascope with EZFBA-4W sensors (Sina, Ltd., Zurich, Switzerland) for comparison with A_ws determined by the protein equilibrium moisture adsorption procedure. Analyses results are shown in Table 1. Precision of the sinascope procedure in the A_w range > 0.90 was no better than $\pm$ 0.01 A_w.

Based on analyses data it has been concluded that when precision (and accuracy) of the order of $\pm$ 0.005 A_w is required in the analysis of a food product having high water activity, the protein equilibrium moisture adsorption procedure is preferable to that of an electric hygrometer, even though it requires 25—26 hr analysis time vs. 20—30 min for the electric hygrometer.

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A Research Note
ENZYMATIC GLUCOSYLATION OF SOLANIDINE

INTRODUCTION

POTATO (*Solanum tuberosum* L.) tubers synthesize an excess of α-solanine and α-chaconine, steroidal glycoalkaloids, under the conditions of bud activity and stress such as exposure to light and mechanical injury. The results of Guseva and Paseshnichenko (1958) and Guseva et al. (1961) indicated that the biogenesis of glycoalkaloids of *Solanum* goes from acetate through mevalonic acid. Recently, Jadhav et al. (1973) reported the incorporation of label from structurally appropriate precursors such as β-hydroxy-β-methylglutaric acid (HMG), L-alanine, L-leucine and D-glucose.

Solanidine, an aglycone of α-solanine or α-chaconine, is derived from cholesterol (Tschesche and Hulpke, 1967). Biogenesis of cholesterol is known to follow an isoprenoid pathway. Therefore, the distribution of carbon atoms in all the steroidal rings of solanidine, synthesized from acetate or mevalonate, may agree with that expected on the basis of the known biosynthetic and cyclization scheme of squalene. However, the origin of the nitrogen atom in potato alkaloids remained an unsolved biosynthetic problem. According to the hypothesis of Heftmann (1967), cholesterol may be undergoing cyclization in the side chain subsequent to the formation of 27-hydroxycholesterol followed by a direct replacement of the hydroxyl group by an amino function.

In earlier investigations, we found that the distribution of label from D-glucose-U-C^{14} administered to potato sprouts was more in the glycoside moiety than that in the aglycone part of the glycoalkaloids (Jadhav et al., 1973). However, information concerning the mechanism of glycosylation of solanidine is meager. The present work was, therefore, initiated to demonstrate enzymatic glucosylation of solanidine by potato slices and by the extracts from potato sprouts.

EXPERIMENTAL

UDP-GLUCOSE-U-C^{14} (50–150 mC/mM) was purchased from ICN Chemical and Radioisotope Div., Irvine, Calif.

In the early stage of this study, thin slices (7 mm diam) of potato tubers (cv. Norgold Russet) were used according to the procedure of Procházka (1971) with some modifications. The tuber slices (10g) in a 25 ml aqueous medium containing 0.4 μmole ATP, 1 μmole solanidine and 0.5 μC UDPG-U-C^{14} were incubated (25°C) under light with constant shaking. At the end of 16 hr, the aqueous medium was basified with ammonium hydroxide, the precipitate washed with 1% ammonium hydroxide, dissolved in 1% H_2SO_4 in MeOH, and radioactivity in aliquots was measured with a Unilux II-A Scintillation Counting System, Nuclear-Chicago by using 15 ml scintillation liquid (5g PPO, 0.3g dimethyl POPOP and 333 ml Triton X-100 in toluene made to 1000 ml).

In the second stage, chlorophyll void potato sprouts (3.5–4 in. in length) were utilized as the source of enzymes. The sprouts (25g) were cut into small pieces, soaked in a 0.5% sodium sulphite solution, washed with distilled water after 30 min, mixed with 12.5g insoluble polyvinylpolypyrrolidone (PVP), and homogenized in a mortar containing a phosphate buffer (pH 7, 0.05M). The homogenate was passed through four layers of cheesecloth and the filtrate was centrifuged at 10,000 × G for 10 min. The supernatant was fractionated between 10 and 60% saturation by $(NH_4)_2SO_4$, the precipitate collected by centrifugation (10,000 × G, 30 min), dissolved in 20 ml phosphate buffer, centrifuged and the soluble fraction was dialyzed against the same buffer for 16 hr. This enzyme preparation was diluted fourfold and subsequently 2 ml were added to a reaction mixture of 0.1 μmole ATP, 0.25 μmole solanidine, and 0.2 μC UDPG-U-C^{14} in 1 ml phosphate buffer (pH 7, 0.05M). The reaction mixture was inactivated by boiling in water and the product was treated as above.

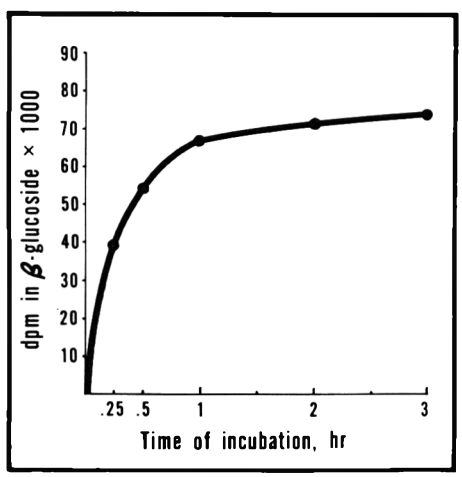

Fig. 1—Conversion of solanidine and UDP-glucose-U-C^{14} to β-glucoside by the extracts from potato sprouts.

RESULTS & DISCUSSION

THE ALKALOID fraction isolated from the suspension of potato slices contained nearly 0.56% (6224 dpm) of the total radioactivity administered. The incorporation of label indicated that the enzymatic system of potato tubers was capable of glucosylating the 3β-hydroxyl group of solanidine when UDPG-U-C^{14} served as a donor. Similar investigations by Procházka (1971) on the specificity of the enzymatic system of potato tubers led to the conclusion that a sterically unhindered 3β-hydroxyl group of steroids could be glucosylated if the steroids belong to the 5α-H or Δ^5-series.

The formation of β-glucoside also occurred in enzyme extracts from sprouts. The results are presented in Figure 1. The rate of synthesis was faster in the early period of incubation than in the later. Glucosylation of solasodine by enzyme extracts from *Solanum laciniatum* has been reported by Liljegren (1971). On the basis of his results and on sequential synthesis (Barber, 1962; Harborne, 1963; Miles and Hagen, 1968; Hahlbrock and Conn, 1970) and degradation (Guseva and Paseshnichenko, 1959) of secondary metabolites such as glycosides and flavonoids in certain plants, he supported the theory that glycosylation is the last step in the synthesis of solasonine and solamargine. This hypothesis may be similarly applied to the synthesis of α-solanine and α-chaconine because of genetical factors existing in the same *Solanum* species. Since γ, β and α forms of solanine and chaconine (1, 2 and 3 sugars in the glycosidic part, respectively) occur in potato tubers as well as sprouts, stepwise synthesis of α-solanine and α-chaconine from solanidine seems possible. The formation of β-glucoside in both the cases indicated the presence of β-glucosyltransferase in *Solanum tuberosum* L.

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