3 April 1971
Volume 36 : Number 3
Pages 363-544

JOURNAL of FOOD SCIENCE

PPLIED SCIENCE and ENGINEERING

63 Flavor enhancement of potato products—*D. G. Guadagni, R. G. Buttery, R. M. Seifert and D. W. Venstrom*

67 Orange peel color extract: its use and stability in citrus products—*R. E. Berry, O. W. Bissett and T. J. Kew*

70 Nutritive value of opaque-2 corn and its mixture with hybrid corn wheat flour—*J. E. Dutra de Oliveira and M. L. Pereira da Silva*

72 Effect of processing method and pH of precipitation on the yields and functional properties of protein isolates from glandless cottonseed—*J. T. Lawhon and C. M. Cater*

78 Estimating food temperatures during various processing or handling treatments—*K. Hayakawa*

86 Microbial lethality during logarithmic cooling—*R. W. Dickerson Jr.*

88 Pore size effect in the freeze drying process—*E. B. Stuart and G. Closset*

92 Frozen French-fried potatoes. Effects of thawing and holding before finish frying and their nonrelation to starch retrogradation—*H. K. Burr*

Reverse osmosis of cottage cheese whey. 2. Influence of flow conditions—*C. Peri and W. L. Dunkley*

7 Field processing of tomatoes. 1. Process and design—*W. G. Schultz, R. P. Graham, W. C. Rockwell, J. L. Bomben, J. C. Miers and J. R. Wagner*

0 Field processing of tomatoes. 2. Product quality and composition—*J. C. Miers, J. R. Wagner, M-D. Nutting, W. G. Schultz, R. Becker, H. J. Neumann, W. C. Dietrich and D. W. Sanshuck*

5 Device to measure ease of skin removal from peanuts—*P. C. Barnes Jr., C. E. Holaday and J. L. Pearson*

8 Alternate storage systems for the production of canned black ripe olives—*J. W. Ralls, H. J. Maagdenberg, G. Lemoine and W. A. Mercer*

3 Variability of increase in α-amylase and sugars during storage of goldrush and centennial sweetpotatoes—*H. J. Deobald, V. C. Hasling and E. A. Catalano*

416 Effects on the alkaloid content of potatoes grown from seeds subjected to low-dose gamma irradiation—*W. R. Sutton, O. P. Agarwala and G. M. Pigott*

419 Effect of partial replacement of sucrose by corn syrup on quality and stability of canned apple sauce—*H. N. Daoud and B. S. Luh*

423 Influence of emulsifier type and solubility on the stability of milk fat-water emulsions—*J. B. Mickle, W. Smith, J. M. Tietz, T. C. Titus and M. Johnston*

426 Relationship between composition and stability of sausage-type emulsions—*G. S. Morrison, N. B. Webb, T. N. Blumer, F. J. Ivey and A. Haq*

431 Fatty acid composition of lipids from broilers fed saturated and unsaturated fats—*G. A. Schuler and E. O. Essary*

435 Effect of ultimate pH upon the water-holding capacity and tenderness of mutton—*P. E. Bouton, P. V. Harris and W. R. Shorthose*

440 Effects of physiological maturity of beef and marbling of rib steaks on eating quality—*H. L. Norris, D. L. Harrison, L. L. Anderson, B. von Welck and H. J. Tuma*

445 Effects of physical and mechanical treatments on the tenderness of the beef longissimus—*G. C. Smith, T. C. Arango and Z. L. Carpenter*

450 Influence of controlled microbial curing on porcine muscle—*R. J. Bothast, P. P. Graham and R. F. Kelly*

454 Effect of freezing and packaging methods on survival and biochemical activity of spoilage organisms on chicken—*C. R. Rey and A. A. Kraft*

BASIC SCIENCE

459 Potato flavor as related to chemical composition. 1. Polyphenols and ascorbic acid—*N. I. Mondy, C. Metcalf and R. L. Plaisted*

—CONTENTS CONTINUED (on the inside of the front cover) . . . . .

A PUBLICATION OF THE INSTITUTE OF FOOD TECHNOLOGISTS

–CONTENTS CONTINUED–
from front cover

462 Phenolic compounds in rum—*R. Timmer, R. ter Heide, H. J. Wobben and P. J. de Valois*

464 Nitrogen compounds in rum and whiskey—*H. J. Wobben, R. Timmer, R. ter Heide and P. J. de Valois*

466 Carotenoids in juice of Shamouti orange—*J. Gross, M. Gabai and A. Lifshitz*

474 Metabolism of solanine and chlorophyll in potato tubers as affected by light and specific chemicals—*B. C. Patil, D. K. Salunkhe and B. Singh*

477 Basis of stability of amine salts of linoleic acid. 1. Generality of the oxidation protection and effect of physical state—*S. D. Koch, A. A. Hyatt and D. V. Lopiekes*

482 Basis of stability of amine salts of linoleic acid. 2. Structure-property correlations on lysinium linoleate—*D. V. Lopiekes and S. D. Koch*

486 Effect of substrate size on the activity of tomato polygalacturonase—*R. Pressey and J. K. Avants*

490 Factors affecting chlorogenic, quinic and caffeic acid levels in sunflower kernels—*J. V. Pomenta and E. E. Burns*

493 Chemical composition and amino acid contents of Brazilian beans (Phaseolus vulgaris)—*R. M. de Moraes and E. Angelucci*

495 Influence of sucrose, glucose and lactose on loss of water from solutions—*K. N. Patel and T. A. Nickerson*

498 Bound water in fruit products by the freezing method—*J. H. Moy, K. C. Chan and A. M. Dollar*

500 Histological and physical changes in carrots as affected by blanching, cooking, freezing, freeze drying and compression—*A. R. Rahman, W. L. Henning and D. E. Westcott*

503 Protein degradation in Cheddar cheese slurries—*W. J. Harper, A. Carmona and T. Kristoffersen*

507 Ultraviolet spectrophotometric determination of protein in some food products—*S. J. Toma and S. Nakai*

509 Proteins in meat and egg products determined by dye binding—*U. S. Ashworth*

511 Effect of malathion on nutrient composition of eggs and flavor of meat from laying hens—*E. W. Toepfer and K. Morgareidge*

515 Quantitative determination of metmyoglobin and total pigment in an intact meat sample using reflectance spectrophotometry—*W. C. Franke and M. Solberg*

520 Mechanism of bacterial penetration through the eggs of Gallus gallus. 2. Effect of penetration and growth on permeability of inner shell membrane—*E. M. Wedral, D. V. Vadehra and R. C. Baker*

523 Interpretation of nonlogarithmic survivor curves of heated bacteria—*W. A. Moats, R. Dabbah and V. M. Edwards*

527 Poultry product quality. 4. Levels of carbonyl compounds in fresh, uncooked chicken and turkey skin—*C. P. Thomas, P. S. Dimick and J. H. MacNeil*

532 Quality and stability of some freeze-dried foods in "zero" oxygen headspace—*S. J. Bishov, A. S. Henick, J. W. Giffee, I. T. Nii, P. A. Prell and M. Wolf*

RESEARCH NOTES

536 Occurrence of 6-pentyl-α-pyrone in peach essence—*M. R. Sevenants and W. G. Jennings*

537 Ascorbic acid content of the developing tomato fruit—*W. Malewski and P. Markakis*

538 Roasted peanut flavor and its relation to growth environment—*W. Y. Cobb and H. E. Swaisgood*

540 Carbon-14-concentrations in recent wines and spirits—*M. S. Baxter and A. Walton*

542 Chemically defined liquid medium for the growth and sporulation of Bacillus stearothermophilus 1518 rough variant—*S. M. Tandon and K. G. Gollakota*

544 Author guideline to IFT research papers reporting sensory evaluation data

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Volume 36 : Number 3

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ABSTRACTS:
IN THIS ISSUE

FLAVOR ENHANCEMENT OF POTATO PRODUCTS. D. G. GUADAGNI, R. G. BUTTERY, R. M. SEIFERT & D. W. VENSTROM. *J. Food Sci.* **36,** 363-366 (1971)—The effect of selected compounds on flavor of various potato products was studied by sensory panels. The minimum detectable amount of each of six compounds in four brands of dehydrated mashed potatoes was determined. The difference thresholds of the six compounds in the reconstituted potato products varied from 0.05-3.1 ppm. Only 2-methoxy-3-ethylpyrazine (0.1-0.2 ppm) was effective in increasing the flavor level of all four brands of dehydrated potatoes; it also proved to be effective in increasing the potato flavor level of potato salad, dehydrated scalloped potatoes and potato soup. Potato salad stored at 3°C for 1 wk required at least 0.2 ppm of this compound to maintain its initial flavor difference from the control sample.

ORANGE PEEL COLOR EXTRACT: ITS USE AND STABILITY IN CITRUS PRODUCTS. R. E. BERRY, O. W. BISSETT & T. J. KEW. *J. Food Sci.* **36,** 367-369 (1971)—Storage stability of the color was tested alone and in various citrus products using an extraction process which had been developed for obtaining a highly colored concentrated extract from orange peel. When concentrated color extract was added to single-strength orange juice in tin-lined or enamel-lined cans or clear glass bottles, color stability was good during storage at 35°F; except for some fading in glass-packed samples, no notable changes in color were observed after 10 wk. However, at 70°F and 85°F color changes occurred, mainly from darkening due to browning. These storage changes were more predominant in glass-packed juice and enamel-lined cans. Color in frozen concentrated orange juice remained stable more than 10 wk, showing no notable change in color during storage at −5°F. Stored in the concentrated form the color extract required protection from light and heat. Color was stable for more than 6 months when stored at −5°F in the dark.

NUTRITIVE VALUE OF OPAQUE-2 CORN AND ITS MIXTURE WITH HYBRID CORN AND WHEAT FLOUR. J. E. DUTRA de OLIVEIRA & M. L. PEREIRA da SILVA. *J. Food Sci.* **36,** 370-371 (1971)—A study was carried out to evaluate the nutritive value of opaque-2 corn grown in Brazil and the nutritional effects of its mixture with hybrid corn and wheat flour. These studies were conducted with rats to determine the growth rate, feed efficiency and protein efficiency ratio. Amino acid analyses of Brazilian opaque-2 corn confirmed its high lysine content and other differences in amino acid content; nutritive value was shown to be similar to the American variety. There were small variations among samples of opaque-2 corn grown in different regions and crop years, but all had high nutritive value. The nutritive value of the hybrid corn was little improved by its mixture with the high lysine opaque-2 corn. Better results were found when the opaque-2 was mixed with the wheat flour.

EFFECT OF PROCESSING METHOD AND pH OF PRECIPITATION ON THE YIELDS AND FUNCTIONAL PROPERTIES OF PROTEIN ISOLATES FROM GLANDLESS COTTONSEED. J. T. LAWHON & C. M. CATER. *J. Food Sci.* **36,** 372-377 (1971)—Glandless cottonseed meals were prepared under controlled conditions in a pilot plant by three different processing methods. These meals along with a glandless cottonseed meal produced at a commercial oil mill were used as source meals for protein isolates. Two protein fractions differing in composition and characteristics were isolated from each type meal using a two-step, two-solvent isolation procedure developed at the USDA Southern Utilization R&D Div. The effect of meal processing method and pH of precipitation on the yield and functional properties of each isolate was measured. Functional properties tested included whippability, heat gelation, solubility and foaming properties. Meal processing method was found to significantly affect the yield of Isolate I, the minor isolate. pH of precipitation was found to significantly affect the yield of Isolate II, the major isolate. The functional properties of isolates from meals processed without heat were superior to those of isolates from heated meals. Data collected indicated the need for a new practice in evaluating the extent of denaturation of cottonseed protein products. The present practice of determining nitrogen solubility at one point was shown to be inadequate.

ESTIMATING FOOD TEMPERATURE DURING VARIOUS PROCESSING TREATMENTS. K. HAYAKAWA. *J. Food Sci.* **36,** 378-385 (1971)—A procedure was developed for predicting the transient temperature of a solid food exposed to time variable ambient temperatures. Duhamel's theorem was applied to the empirical formulas used for calculating the temperature of a food subjected to a step change in its ambient temperature. The formulas contain two constants f, which is the slope index of a heating or cooling curve of a food, and j, which is the intercept coefficient of the heating or cooling curve. With the procedure developed transient temperature can be estimated without regard to the geometric shape of the food provided the f and j values are known. A table is presented to reduce computations involved in the procedure. Sample calculations are given for a fresh orange, a fresh carrot and a canned food product.

MICROBIAL LETHALITY DURING LOGARITHMIC COOLING. R. W. DICKERSON, JR. *J. Food Sci.* **36,** 386-387 (1971)—By applying the normal restrictions of thermal inactivation experiments, Ball's general equation for lethality during logarithmic cooling was reduced to a form requiring no exponential integrals. The computation is brief within the limits of two restrictions, and accuracy is ± 1% of Ball's general equation. The equation is limited to thermal inactivation experiments where the minor dimension of the sample is less than 15 mm; it cannot be applied to commercial sterilization processes.

PORE SIZE EFFECT IN THE FREEZE DRYING PROCESS. E. B. STUART & G. CLOSSET. *J. Food Sci.* **36,** 388-391 (1971)—Experiments were conducted to determine the influence and/or effect of the structure of the dried material itself on transport rates. Drying rates were measured in the 0.2-3.0 mm Hg pressure range, the usual operating range of commercial freeze driers. Thermal conductivities of dried beef and heat and mass transfer were calculated from drying rate data and the structure of the freeze-dried material was studied by optical means. A simplified relationship between thermal conductivity and pore structure was derived. Drying rates were related to basic transport properties, thermal conductivity and pore structure.

FROZEN FRENCH-FRIED POTATOES. EFFECTS OF THAWING AND HOLDING BEFORE FINISH FRYING AND THEIR NONRELATION TO STARCH RETROGRADATION. HORACE K. BURR. *J. Food Sci.* **36,** 392-394 (1971)—Frozen par-fried French-fry cuts allowed to thaw and held several hours before being finish-fried lost more weight (due to loss of moisture) and absorbed more fat than if not held. The yield of finished product decreased about 7% and fat uptake increased about 25% when par-fries were held 3 days at 50°F. Smaller but economically significant changes occurred in only 1-1/2 hr at this temperature or in 5 hr at 40°F. The rate of the change in par-fries which causes lower yields and greater fat absorption increases with increasing temperature. The time course of the change at 50°F is similar to that of starch retrogradation in par-fries but the rate of retrogradation decreases with increasing temperature; therefore, these appear to be unrelated phenomena. There is no combination of time and temperature for frying par-fries from the thawed state which is equivalent in all respects to a given combination for frying them from the frozen state.

REVERSE OSMOSIS OF COTTAGE CHEESE WHEY. 2. Influence of Flow Conditions. C. PERI & W. L. DUNKLEY. *J. Food Sci.* **36,** 395-396 (1971)—Flow conditions in tubular, cellulose acetate reverse osmosis membranes were varied by changing the flow rate and by inserting turbulence promoters (rods with intermittently-spaced rings cemented to them) in the tubes. In general, increasing the flow velocity increased the permeation rate. Turbulence promoters were most effective in increasing permeation rate under conditions that, in their absence, permitted fouling of the membrane surface by macrosolutes from the feed. When the feed contained macrosolutes, increasing the flow velocity or use of turbulence promoters increased retention of lactose and potassium. Modifying the flow conditions to increase turbulence improves performance of reverse osmosis membranes by increasing both permeation rate and retention.

FIELD PROCESSING OF TOMATOES. 1. Process and Design. W. G. SCHULTZ, R. P. GRAHAM, W. C. ROCKWELL, J. L. BOMBEN, J. C. MIERS & J. R. WAGNER. *J. Food Sci.* **36,** 397-399 (1971)—Among the advantages of processing tomatoes in the field, compared with processing solely at a cannery, are increased yield and reduced waste. Mechanically harvested tomatoes were processed at 500 lb/hr through a mobile field unit within 6 hr after harvest during the 1969 season. Processing within 6 hr from harvest prevented a loss of about 9.5% of the ripe tomatoes that occurred when processing was delayed 24 hr. An increase of 4.3% in solids recovery was obtained by acid treatment of the hot macerate, bringing the total increase to 13.8% for field processing. Either single strength or concentrated material is suitable for bulk transport to a cannery for storage, additional concentration, or immediate formulation into sauces, catsup and other tomato products.

FIELD PROCESSING OF TOMATOES. 2. Product Quality and Composition. J. C. MIERS, J. R. WAGNER, M-D. NUTTING, W. G. SCHULTZ, R. BECKER, H. J. NEUMANN, W. C. DIETRICH & D. W. SANSHUCK. *J. Food Sci.* **36,** 400-404 (1971)—Tomatoes were macerated, heated by steam injection to several temperatures from 170-240°F and held in a treatment pot from 0-4.6 min at several pH levels. Treated macerates were neutralized to the original pH, put through a finisher screen and pumped through a deaerator and chiller. The optimum processing conditions based on highest consistency were 212°F breaking temperature, and a macerate treatment time of 3.3 min at pH 2.75. Optimum conditions increased juice solids yield 4.3% and consistency by 90% for VF-145 variety. The neutralized acid products could contain 13% less tomato solids and still have a consistency equal to that of the products extracted at natural pH. Dry waste solids decreased 33% and total wet waste 47%. A delay of 1 day caused 9.5% loss in recovered tomato juice solids.

DEVICE TO MEASURE EASE OF SKIN REMOVAL FROM PEANUTS. P. C. BARNES JR., C. E. HOLADAY & J. L. PEARSON. *J. Food Sci.* **36,** 405-407 (1971)—A new procedure for measuring the blanchability of peanuts is described. A simple device, made from readily available materials, will blanch a 250g sample in 2 min. Tests showed that the procedure has a standard deviation of less than 1% and compares favorably with two commercial blanchers tested.

ALTERNATE STORAGE SYSTEMS FOR THE PRODUCTION OF CANNED BLACK RIPE OLIVES. J. W. RALLS, H. J. MAAGDENBERG, G. LEMOINE & W. A. MERCER. *J. Food Sci.* **36,** 408-412 (1971)—The storage of freshly harvested olives in systems other than the traditional NaCl brine was investigated in an attempt to provide an alternative storage system which would reduce the present saline pollution potential of the olive canning industry. Chemical salts used as fertilizers were partially successful in holding olives for several months without spoilage. The difficulties in completely removing ammonium salts from the olives limited the commercial potential for this type of storage. Food grade glycols containing bacteriostats can be used to store olives for periods of over 6 mo. Olives submerged in propylene glycol-diethyl pyrocarbonate mixtures showed severe shriveling. The most promising storage system developed in this study was a mixture of water-propylene glycol-benzoic acid-potassium-sorbate-HCl. By using KOH in place of NaOH for the chemical curing preparation steps, it was possible to prepare canned olives with essentially no generation of liquid waste containing sodium ions.

VARIABILITY OF INCREASES IN α-AMYLASE AND SUGARS DURING STORAGE OF GOLDRUSH AND CENTENNIAL SWEETPOTATOES. H. J. DEOBALD, V. C. HASLING & E. A. CATALANO. *J. Food Sci.*, **36,** 413-415 (1970)—α-amylase, glucose and sucrose contents were determined to compare the potential processing characteristics of Centennial and Goldrush sweetpotatoes having similar storage histories. The extent and nature of the increases in these constituents in both varieties were also compared to reveal any relationship between sugar formation and α-amylase activity during storage. The two varieties differed greatly in the extent of α-amylase increase and to a lesser degree in the rate of glucose formation. The pattern of α-amylase increase was very erratic for all samples, but the increase was less for the Centennial samples. Glucose content increased at a more uniform rate and was also lower in the comparable Centennial roots. No correlations were apparent in the patterns of the increases in α-amylase and those of the sugars. Sucrose content increased at varying rates in all samples reaching a plateau of about 20% (dry basis) in from 9-35 days. The erratic rise in α-amylase contents in the different samples emphasizes the need for knowing the α-amylase content immediately prior to processing to regulate the rate of starch conversion during processing for flake production to enable better quality control.

EFFECTS ON THE ALKALOID CONTENT OF POTATOES GROWN FROM SEEDS SUBJECTED TO LOW-DOSE GAMMA IRRADIATION. W. R. SUTTON, O. P. AGARWALA & G. M. PIGOTT. *J. Food Sci.* **36,** 416-418 (1971)—Effects of low dose gamma irradiation of seeds on alkaloid content, proximate composition and yield of the potato tubers were studied. Increase in yield with irradiation of seeds was inconclusive. Changes in proximate composition of Russet potatoes grown from tubers irradiated at 1,500 rads were found to be insignificant. Varietal differences in solanine content were more pronounced than those due to gamma irradiation. In all cases the solanine content in potatoes grown from irradiated seeds was found to be far below the toxic level for human beings.

ABSTRACTS:
IN THIS ISSUE

EFFECT OF PARTIAL REPLACEMENT OF SUCROSE BY CORN SYRUP ON QUALITY AND STABILITY OF CANNED APPLE SAUCE. H. N. DAOUD & B. S. LUH. *J. Food Sci.* **36,** 419-422 (1971)—Apple sauces were made from Gravenstein apples under normal commercial processing conditions, using pure sucrose and combinations of sucrose and 62 D.E. corn syrup as sweeteners. Products were tested for storage stability at 68 and 86°F. Undesirable chemical and physical changes occurred rapidly at 86°F as indicated by the increase of hydroxymethyl furfural, darkening of serum color, decrease in consistency and increased can corrosion. This was accompanied by lower organoleptic color and flavor scores. It appeared that the quality and storage stability of apple sauce were influenced more by higher storage temperatures and longer storage duration than by the type of sweeteners used in making the apple sauce. For a longer shelf life and better quality retention, canned apple sauce should be stored at 68°F or lower. Results of this investigation indicate that corn syrup can be used to replace 25% of the added sucrose in processing apple sauce.

INFLUENCE OF EMULSIFIER TYPE AND SOLUBILITY ON THE STABILITY OF MILK FAT-WATER EMULSIONS. J. B. MICKLE, W. SMITH, J. M. TIETZ, T. C. TITUS & M. JOHNSTON. *J. Food Sci.* **36,** 423-425 (1971)—The purpose was to measure the amount of change in emulsion stability caused by different chemical types of emulsifiers in relation to the amount of change caused by emulsifier HLB. Seven emulsifiers used as 12 different binary mixtures were evaluated in model systems containing 10, 25 and 40% fat in water. Each emulsifier mixture was used at HLB numbers of 7, 10 and 13. The effect of chemical type on emulsion stability was minor in relation to the large changes caused by the fat percentage in the model system and the HLB of the emulsifier. A method was developed, using gas-liquid chromatography, to accurately measure the HLB numbers of the emulsifiers used in this work. With these measurements it was learned that any differences in emulsion stability which could be traced to the chemical type of emulsifier were probably caused by errors in the original measurement of HLB numbers.

RELATIONSHIP BETWEEN COMPOSITION AND STABILITY OF SAUSAGE-TYPE EMULSIONS. G. S. MORRISON, N. B. WEBB, T. N. BLUMER, F. J. IVEY & A. HAQ. *J. Food Sci.* **36,** 426-430 (1971)—Evaluation of emulsions prepared from fresh and frozen meat over widely varying compositions by a specially developed laboratory technique revealed that lean or fat percentages could be altered widely without significantly affecting emulsion stability. However, the percentage of water was critical to stability for various compositions and temperatures of meat storage. For example, at 30% fat level, there was a sharp drop in stability as the added water level was reduced below 16% for fresh beef; whereas, an equivalent drop in stability was found as the added water was reduced below 21% for frozen beef. The generally accepted theory that emulsion instability is caused by lack of soluble protein is not borne out by the results of this investigation.

FATTY ACID COMPOSITION OF LIPIDS FROM BROILERS FED STAURATED AND UNSATURATED FATS. G. A. SCHULER & E. O. ESSARY. *J. Food Sci.* **36,** 431-434 (1971)—The fatty acid composition of lipids from broiler-type chicks fed a corn-soybean type of diet from 1 day to 10 wk of age was determined by gas chromatography and compared with that from broilers fed the same diet with either 8% saturated fat (tallow) or 8% unsaturated fat (safflower oil) substituted for an equal weight of corn. Fat was extracted from the raw skin, breast, thigh, and abdominal fat at bi-weekly intervals from 4-10 wk of age and from the cooked tissue and the water in which the broilers were cooked at 8 and 10 wk. The fatty acid composition of fatty acids in these tissues was influenced by the degree of unsaturated fatty acids in the diet. Fatty acids from cooked tissues and cooking water contained a large amount of 18-carbon unsaturated fatty acids. Presence of 13- and 25-carbon saturated fatty acids were noted in the skin of 4 wk-old broilers. Futher research is needed to substantiate this finding.

EFFECT OF ULTIMATE pH UPON THE WATER-HOLDING CAPACITY AND TENDERNESS OF MUTTON. P. E. BOUTON, P.V. HARRIS & W. R. SHORTHOSE. *J. Food Sci.* **36,** 435-439 (1971)—Pre-slaughter injections of epinephrine were used to induce a range of ultimate pH, 5.6-7.0, in sheep muscles. The water-holding capacity of the uncooked meat was closely correlated with ultimate pH. Cooking losses, at 65°C, decreased linearly with increased pH. At a cooking temperature of 90°C there was little change in cooking loss until the pH of the raw meat reached ca. 5.9 when loss declined linearly with pH. The amount of juice expressed from the cooked meat by centrifugation increased linearly as pH increased. There were large differences in the tenderness of the M. semimembranosus and biceps femoris associated with an increase in ultimate pH.

EFFECTS OF PHYSIOLOGICAL MATURITY OF BEEF AND MARBLING OF RIB STEAKS ON EATING QUALITY. H. L. NORRIS, D. L. HARRISON, L. L. ANDERSON, B. von WELCK & H. J. TUMA. *J. Food Sci.* **36,** 440-444 (1971)—Paired rib steaks from the 7th and 8th and the 11th thoracic vertebrae were used to study the effects of three levels of physiological maturity and two levels of marbling on selected measurements of beef longissimus dorsi. Maturity and marbling affected total moisture ($P < 0.001$), histological measurement of fat in raw muscle ($P < 0.01$), ether extract ($P < 0.001$) and dripping losses (7th and 8th thoracic vertebrae, $P < 0.001$), but had little effect on palatability of the steaks. Correlation coefficients for overall acceptability and histological measurements were low. Tenderness had more influence on overall acceptability of the meat than flavor or juiciness.

EFFECTS OF PHYSICAL AND MECHANICAL TREATMENTS ON THE TENDERNESS OF THE BEEF LONGISSIMUS. G. C. SMITH, T. C. ARANGO & Z. L. CARPENTER. *J. Food Sci.* **36,** 445-449 (1971)—280 steaks from 56 beef carcasses were used to evaluate various mechanical and physical methods for increasing the tenderness of the longissimus muscle. Changes in method of suspension, carcass integrity or chilling procedures resulted in increases in tenderness approximating 47.5% for shear force and 53.6% for taste panel ratings. Of the methods studied, a combination of suspension via the obturator foramen, severance of vertebrae in five locations, severance of the ligamentum nuchae and the attachment of 68 kg weights to the neck gave the greatest positive response in panel tenderness ratings. However, chilling the carcass for the first 16-20 hr post-mortem in a 16°C cooler appears to enhance tenderness by 28-47% and would be most feasible for industry use.

INFLUENCE OF CONTROLLED MICROBIAL CURING ON PORCINE MUSCLE. R. J. BOTHAST, P. P. GRAHAM & R. F. KELLY. *J. Food Sci.* **36,** 450-453 (1971)—A previously developed technique was adapted to study the influence of certain microbiological populations and their effects on processed meat. The technique consisted of an initial reduction of surface bacteria on conventionally handled muscle tissue via a hot water dip, followed by processing at 28°C in a sterile plastic isolator where *Pediococcus cerevisiae* was introduced into the curing solution. This treatment was compared to reduced initial count and conventional samples. Identification of the bacteria in the curing solution of each treatment indicated that a *Lactobacillus* spp. was predominant in the reduced initial count treatment. The inoculated *Pediococcus cerevisiae* was predominant in the reduced initial count inoculated treatment while *Staphylococcus epidermidis* and *Flavobacterium diffusum* were predominant in the conventional treatment depending upon the trial. Tenderness and bacterial load were significantly ($P < .01$) increased by treatment while pH was significantly ($P < .01$) decreased. Oxidation and muscle composition were not affected by treatment. Samples from all treatments were acceptable organoleptically.

EFFECT OF FREEZING AND PACKAGING METHODS ON SURVIVAL & BIOCHEMICAL ACTIVITY OF SPOILAGE ORGANISMS ON CHICKEN. C. R. REY & A. A. KRAFT. *J. Food Sci.* **36,** 454-458 (1971)—A study was conducted on effects of freezing, thawing, and subsequent holding at 5°C on survival or growth and biochemical activity of aerobic bacteria on chicken packaged with various materials. Fluorescent pigment production, proteolytic and lipolytic spoilage was directly related to the availability of oxygen provided by the packaging procedure. Bacterial numbers paralleled increases in biochemical indices of deterioration. Freezing of chicken at −29°C for 35 days followed by defrosting and refrigerated storage increased the proportion of biochemically active psychrophiles. Vacuum packaging generally limited amount of spoilage. Total aerobic bacterial counts were not greatly influenced by alternate freezing and thawing.

POTATO FLAVOR AS RELATED TO CHEMICAL COMPOSITION. 1. Polyphenols and Ascorbic Acid. N. I. MONDY, C. METCALF & R. L. PLAISTED. *J. Food Sci.* **36,** 459-461 (1971)—A significant correlation was found between phenolic content and bitterness and a highly significant correlation between phenolic content and astringency in Ontario, Pontiac and Katahdin varieties of potatoes as well as in clones that were from a population of hybrids between varieties of *Solanum tuberosum* subsp. *tuberosum* grown in New York and varieties of *Solanum tuberosum* subsp. *andigenum* grown in Peru. No significant correlation was found between ascorbic acid content and potato flavor.

PHENOLIC COMPOUNDS IN RUM. R. TIMMER, R. ter HEIDE, H. J. WOBBEN & P. J. de VALOIS, *J. Food Sci.* **36,** 462-463 (1971)—A study was made of the phenolic fraction of a Jamaica rum containing 75% ethanol. Identification of the phenols was performed by coupled gas chromatography and mass spectrometry and further by infrared spectrometry and thin layer chromatography. Phenol, p-ethylphenol, o-cresol, p-cresol, guaiacol, p-ethylguaiacol and dihydroeugenol were found in Jamaica rum for the first time; the presence of p-methylguaiacol and eugenol was confirmed.

NITROGEN COMPOUNDS IN RUM AND WHISKEY. H. J. WOBBEN, R. TIMMER, R. ter HEIDE & P. J. de VALOIS. *J. Food Sci.* **36,** 464-465 (1971)—The nitrogen compounds from a Jamaica rum and a Scotch blended whiskey were isolated and identified by gas chromatography and mass spectrometry. In both spirits were found: pyridine, α-picoline, β-picoline, thiazole, methylpyrazine, 2,5-dimethylpyrazine, 2,6-dimethylpyrazine, 2,3-dimethylpyrazine, 2-methyl-6-ethylpyrazine, 2-methyl-5-ethylpyrazine, trimethylpyrazine and 2, 5-dimethyl-3-ethylpyrazine. 2-Methyl-6-vinylpyrazine was detected only in rum. Nine compounds in whiskey and eleven in rum have not previously been reported as constituents of these drinks or fusel oils.

CAROTENOIDS IN JUICE OF SHAMOUTI ORANGE. J. GROSS, M. GABAI & A. LIFSHITZ. *J. Food Sci.* **36,** 466-473 (1971)—More than 50 carotenoids were isolated from the juice of Shamouti orange *(Citrus sinensis* [L] Obs.) using a combination of column and thin layer chromatography. In addition to carotenoids usually found in citrus, others not previously detected in common orange varieties were identified. These include γ-carotene, rubixanthin, the new ketones sintaxanthin and its OH-derivative, as well as lutein 5,6-monoepoxide. The diol-polyol fraction was most predominant yielding about 70% of the total carotenoids. It was established that low pigmentation of Shamouti orange juice is due to its low total carotenoid content rather than the absence of colored carotenoids.

METABOLISM OF SOLANINE AND CHLOROPHYLL IN POTATO TUBERS AS AFFECTED BY LIGHT AND SPECIFIC CHEMICALS. B. C. PATIL, D. K. SALUNKHE & B. SINGH. *J. Food Sci.* **36,** 474-476 (1971)—Chlorophyll and solanine syntheses as influenced by cultivars, specific gravities, light intensities and chemicals were studied. When exposed to 100 ft-c of white fluorescent light for 5 days, tubers of Bounty, Kennebec, Norchip and Red Lasoda were most sensitive to greening and solanine development. LaChipper and Platte tubers were resistant to the light effects. Chlorophyll synthesis was inversely related to specific gravity of tubers, while specific gravity did not affect solanine synthesis. Chlorophyll contents of tubers exposed to 50 ft-c and 200 ft-c were significantly less than that of tubers exposed to 100 ft-c and 150 ft-c light intensities. The solanine synthesis was not influenced by the light intensities studied. Chemicals were applied at a concentration of 10,000 ppm by vacuum injection technique. Ethrel® and Alar® were effective in inhibiting chlorophyll and solanine formation. Ethrel was more effective in checking the chlorophyll formation and Alar was most effective in preventing solanine formation. Maleic hydrazide MH®-30 and Cycocel® were ineffective in controlling both chlorophyll and solanine formation.

ABSTRACTS:
IN THIS ISSUE

BASIS OF STABILITY OF AMINE SALTS OF LINOLEIC ACID. 1. Generality of the Oxidation Protection and Effect of Physical State. S. D. KOCH, A. A. HYATT & D. V. LOPIEKES. *J. Food Sci.* **36,** 477-481 (1971)—The mechanism and generality of the known stabilization against autoxidation conferred on linoleic acid by certain basic amino acids was investigated. Basic amino acids were the only class of compounds found to confer the effect, but 2,3-diaminopropionic and 3,6-diaminohexanoic acids were not effective. A large number of physical and chemical observations were made and correlated but is has not been possible to draw detailed conclusions about the mechanism of stabilization, nor can a detailed structure of the stabilized complex be suggested. The cause of the phenomenon appears to be closely associated with the physical arrangement of the ions in the crystal lattice.

BASIS OF STABILITY OF AMINE SALTS OF LINOLEIC ACID. 2. Structure-Property Correlations on Lysinium Linoleate. D. V. LOPIEKES & S. D. KOCH. *J. Food Sci.* **36,** 482-485 (1971)—The solid compound formed by treating L-lysine with linoleic acid has been examined by chemical stoichiometry, electrical conductivity, infrared and nuclear magnetic resonance spectroscopy. It is a true equimolar salt and not merely a mixture of the two acids. The diene system of the linoleic acid is not changed from that in the free fatty acid. Conductivity measurements show that the salt is a moderately strong electrolyte. Salts of other basic amino acids, ornithine and 2,4-diaminobutyric acid, with linoleic and oleic acids were also examined and showed similar evidence. Interpretation of the data and the significance of the findings to stability of unsaturated fatty acids are discussed.

EFFECT OF SUBSTRATE SIZE ON THE ACTIVITY OF TOMATO POLYGALACTURONASE. R. PRESSEY & J. K. AVANTS. *J. Food Sci.* **36,** 486-489 (1971)—Three polygalacturonic acid preparations with widely differing molecular weight distributions were obtained by controlled enzymatic hydrolysis of pectic acid. A study of the action of tomato polygalacturonase on the three polygalacturonic acids and pectic acid revealed that the activity is dependent on the molecular size of the substrate. Pectic acid was hydrolyzed optimally at pH 5, with little activity below pH 4. Decreasing the molecular weight of the substrate resulted in a progressive shift of the pH optimum to the acid side. For the smallest substrate, the activity extended to below pH 2. Monovalent cations enhanced the activity at low pH, and this effect was also dependent on the molecular weight of the substrate. Below pH 4, pectic acid inhibited 70% of the hydrolysis of low molecular weight substrates by tomato polygalacturonase. The incomplete inhibition is attributed to the presence of a polygalacturonase isoenzyme which is not inhibited by high molecular weight polygalacturonic acids.

FACTORS AFFECTING CHLOROGENIC, QUINIC AND CAFFEIC ACID LEVELS IN SUNFLOWER KERNELS. J. V. POMENTA & E. E. BURNS. *J. Food Sci.* **36,** 490-492 (1971)—Location of the seed on the sunflower head and storage temperatures were related to chlorogenic, caffeic, and quinic acid levels. Kernels of freshly harvested sunflower seeds located near the center of the head were higher in chlorogenic acid than seeds located near the margin. The opposite was obtained for the caffeic acid content, and quinic acid did not exhibit a positional effect. Chlorogenic acid content decreased during storage at 5°C, 15°C, and 40°C. After 120 days, the seeds attained similar levels of chlorogenic acid. Relatively small changes occurred in the caffeic and quinic acid content during storage.

CHEMICAL COMPOSITION AND AMINO ACID CONTENTS OF BRAZILIAN BEANS *(Phaseolus vulgaris).* R. M. de MORAES & E. ANGELUCCI. *J. Food Sci.* **36,** 493-494 (1971)—The chemical composition of 12 varieties of beans *(Phaseolus vulgaris)* most commonly found on Brazilian markets was determined. Average moisture content was 11%, ash 3.5%, fat 1%, protein 25%, starch 40%, crude fiber 4% and pentosans 7%. Minerals content in mg/100g sample was: phosphate 1000, iron 3.2, calcium 40 and magnesium 210. Essential amino acids content in mg/g protein, calculated on a dry basis, was: lysine 72-106, threonine 46-61, valine 29-54, methionine 3-18, isoleucine 28-49, phenylalanine 33-118 and tryptophane 103-138. The product was rich in lysine and threonine as compared to the FAO table for essential amino acids required in the human diet; however, it was poor in methionine, isoleucine, valine, tryptophane and leucine.

INFLUENCE OF SUCROSE, GLUCOSE AND LACTOSE ON LOSS OF WATER FROM SOLUTIONS. K. N. PATEL & T. A. NICKERSON. *J. Food Sci.* **36,** 495-497 (1971)—Moisture losses from solutions of 40, 50 and 60% lactose, sucrose, glucose, and combinations of these sugars were determined at 80°F and 27 in. vacuum. Differences in rates of water loss of 20-50% were observed depending on type of sugar and concentration. Rate of water loss from solution was in ascending order of sucrose, lactose, glucose, and pure water. In mixtures, the rate increased with increased proportion of lactose or glucose, and decreased with increased proportion of sucrose. In all cases, after lactose crystallization occurred, water loss first increased and then decreased.

BOUND WATER IN FRUIT PRODUCTS BY THE FREEZING METHOD. J. H. MOY, K. C. CHAN & A. M. DOLLAR. *J. Food Sci.* **36,** 498-499 (1971)—An insulated heat-sink containing Freon-12® (N.B.P.-21.6°F) provides a reproducible system for measurement of thermal properties of fruit products. The difference in the time required to remove the latent heat of fusion of the "eutectic" mixture in comparison with distilled water measures the "bound" water. The 34-37% "bound" water in papaya (var. solo) pulp is unaffected by varying pH in the range 3.0-6.0. Less than 10% water is bound in guava, passion fruit and pineapple juice products with up to 35% sucrose added. Thermal conductivity of the solid phase "eutectic" mixture in the 5-50% soluble solids range fits the regression $Y = AB^x$, $Y = (3.69)(0.96)^x$ where x = percent total soluble solids and Y = thermal conductivity $[(cal \times 10^{-3})/(cm^2)(sec)(°C/cm)]$.

HISTOLOGICAL AND PHYSICAL CHANGES IN CARROTS AS AFFECTED BY BLANCHING, COOKING, FREEZING, FREEZE-DRYING AND COMPRESSION. A. R. RAHMAN, W. L. HENNING & D. E. WESTCOTT. *J. Food Sci.* **36,** 500-502 (1971)—This study was initiated to determine the effect of processing variables on cell structure and physical characteristics of carrots. Fresh carrots were peeled, diced and subjected to one of the following treatments: blanching; cooking for 10 min; freezing at 0°F, −30°F; or −320°F; freeze drying, compressing after freeze drying at approximately 1500 psi. Carrots at each treatment were tested for texture, water holding capacity and histological changes. Results indicate that among all treatments, freezing temperature is the most critical factor affecting the cell structure of the carrots. Freezing at 0°F or −30°F results in considerable disruption of the cellular structure, whereas it was minimal at −320°F. Carrots frozen at −320°F showed firmer texture as well as higher water holding capacity than the rest.

PROTEIN DEGRADATION IN CHEDDAR CHEESE SLURRIES. W. J. HARPER, A. CARMONA & T. KRISTOFFERSEN. *J. Food Sci.* **36,** 503-506 (1971)—Preferential degradation of caseins was noted in the ripening of Cheddar curd slurries, with the degradation generally being in the order of para-κ, β-, α_s-caseins. The addition of reduced glutathione to the slurry caused an immediate release of peptides from the protein mass and decreased the initial rate of β-casein degradation. The peptides appeared to be preferentially utilized during the first 4 days of ripening; thereafter rapid degradation of β-casein occurred. In quiescent slurries, α_s-casein degradation exceeded that of β-casein, whereas periodic agitation reversed the degradation rate of the two caseins. The rate and extent of characteristic flavor development appeared to be related directly to β-casein degradation, but not to changes in concentration of para-κ or α_s-caseins.

ULTRAVIOLET SPECTROPHOTOMETRIC DETERMINATION OF PROTEIN IN SOME FOOD PRODUCTS. S. J. TOMA & S. NAKAI. *J. Food Sci.* **36,** 507-508 (1971)—A clear solution was obtained by addition of 50% sulfuric acid or 2N sodium hydroxide with and without 7-8M urea depending on solubility of food products from flour, bean, egg and meat. The protein content was calculated from the absorbance at 280, 243 or 215 nm on a spectrophotometer. The correlation coefficient between the absorbance and the protein by Kjeldahl method was 0.99, 0.99, 0.99 and 0.99 with the coefficient of variability of Kjeldahl protein for absorbance of 1.8, 4.1, 3.8 and 4.1% for bean, corned beef, egg yolk and flour, respectively.

PROTEINS IN MEAT AND EGG PRODUCTS DETERMINED BY DYE BINDING. U. S. ASHWORTH. *J. Food Sci.* **36,** 509-510 (1971)—Under optimum conditions Acid Orange 12 will bind to the proteins of meat and eggs. The dye-binding capacity is somewhat greater for these products than the previously reported values for wheat and milk products. A standard curve relating free dye concentration to mg of protein in the sample as determined by the Kjeldahl method can be readily prepared for each type of product. The method is rapid and useful for composition control in ground meats, eggs and prepared mixes. Cooking has little effect on the dye-binding capacity but hydrolysis of the proteins to the proteose-peptone stage reduces the dye bound. The coefficient of variation averaged about 3% of the protein content for the samples of meat and eggs tested.

EFFECT OF MALATHION ON NUTRIENT COMPOSITION OF EGGS AND FLAVOR OF MEAT FROM LAYING HENS. E. W. TOEPFER & K. MORGAREIDGE. *J. Food Sci.* **36,** 511-514 (1971)—Malathion in the feed of laying hens did not produce any indications of adverse effects on the flavor of the cooked meat or on the nutrient composition of the egg whites and egg yolks.

QUANTITATIVE DETERMINATION OF METMYOGLOBIN AND TOTAL PIGMENT IN AN INTACT MEAT SAMPLE USING REFLECTANCE SPECTROPHOTOMETRY. W. C. FRANKE & M. SOLBERG. *J. Food Sci.* **36,** 515-519 (1971)—A technique for determining the relative quantities of oxymyoglobin, metmyoglobin and total pigment concentration at the surface of an intact meat sample was developed. A Beckman DK-2 spectrophotometer with a modified reflectance attachment was used and spectra were recorded on the R_A scale. Known proportions of oxygenated and oxidized meat were simultaneously exposed to the light beam. A linear relation was obtained when ΔR_{A632} was plotted against percent metmyoglobin or against total pigment determined by the Hornsey method. One reading of the sample followed by one reading of the same sample after oxidation with $K_3Fe(CN)_6$ provides a quantitative evaluation of the metmyoglobin concentration and the total heme pigment concentration.

MECHANISM OF BACTERIAL PENETRATION THROUGH THE EGGS OF Gallus gallus. 2. Effect of Penetration and Growth on Permeability of Inner Shell Membrane. E. M. WEDRAL, D. V. VADEHRA & R. C. BAKER. *J. Food Sci.* **36,** 520-522 (1971)—The mechanism of penetration of bacteria into the eggs of domestic fowl *(Gallus gallus)* was investigated by determining the changes in permeability of the inner shell membrane (i.s.m.) to radioactive amino acids and viable *Salmonella typhimurium* LT2 cells after bacteria had penetrated the i.s.m. It was found that penetration and growth of bacteria in eggs did not cause any significant changes in the permeability of the i.s.m. for up to 8 days of incubation after exposure to spoilage. Bacteria, however, were found to penetrate the exterior structures within 36 hr after exposure. These results indicate that the actual penetration of bacteria through the i.s.m. does not permanently alter its permeability.

INTERPRETATION OF NONLOGARITHMIC SURVIVOR CURVES OF HEATED BACTERIA. W. A. MOATS, R. DABBAH & V. M. EDWARDS. *J. Food Sci.* **36,** 523-526 (1971)—Complex survivor curves of heated bacteria are interpreted to be composites of several convex survivor curves that represent populations of different heat resistances in a single culture of bacteria. The variation in heat resistance appears to be physiological rather than genetic since subcultures of heat-resistant cells were no more heat resistant than the parent culture. Composite curves can appear to be nearly exponential. Results support a multiple-site hypothesis of thermal death. Tailing of survivor curves, with small numbers of cells surviving extended heating, was frequently noted when curves were carried through 6-9 log cycles. Such tailing might be of practical importance because it would predict that small numbers of cells might survive much longer heating than would be predicted from D-values calculated from curves carried through 4-5 log cycles.

POULTRY PRODUCT QUALITY. 4. Levels of Carbonyl Compounds in Fresh, Uncooked Chicken and Turkey Skin. C. P. THOMAS, P. S. DIMICK & J. H. MacNEIL. *J. Food Sci.* **36,** 527-531 (1971)—Male and female chickens and turkeys were raised on standard diets to selected ages to study the influence of age, sex, and group of birds on the level and composition of carbonyl compounds. Carbonyls investigated were the total carbonyls, the total monocarbonyl fraction, and the specific aliphatic monocarbonyl classes. Data showed higher levels of total carbonyl and total monocarbonyl compounds in male turkeys than in females. The only methyl ketone found in any of the samples was acetone. The male turkeys had an increase in acetone concentration from 20 wk to a max at 24 wk, the same time at which lipid composition was the lowest. During the same time the female turkey maintained a relatively constant methyl ketone level. At 30 wk both sexes exhibited the lower concentration of acetone.

QUALITY AND STABILITY OF SOME FREEZE-DRIED FOODS IN "ZERO" OXYGEN HEADSPACE. S. J. BISHOV, A. S. HENICK, J. W. GIFFEE, I. T. NII, P. A. PRELL & M. WOLF. *J. Food Sci.* **36,** 532-535 (1971)—Fresh flavor quality was retained in freeze-dried foods of plant and animal origin in "zero" oxygen headspace, using an atmosphere of 5% hydrogen in nitrogen and palladium catalyst. Flavor quality was determined by a flavor profile panel and a technological panel; consumer acceptability by randomly selected untrained panels. Foods packed in the "zero" oxygen headspace had aroma and flavor profiles after storage at 100°F for 6 months comparable to the original products.

ABSTRACTS:
IN THIS ISSUE

OCCURRENCE OF 6-PENTYL-α-PYRONE IN PEACH ESSENCE. M. R. SEVENANTS & W. G. JENNINGS. *J. Food Sci.* **36,** 536 (1971)—A previously unidentified component in a steam distillate of Red Globe variety freestone peaches, characteristic of an α-pyrone, has been identified as 6-pentyl-α-pyrone. Nobuhara reported the substance exhibited a "butter or butter cake" aroma; several of our co-workers described the aroma as "coconut-like" or "lactonic."

ASCORBIC ACID CONTENT OF THE DEVELOPING TOMATO FRUIT. W. MALEWSKI & P. MARKAKIS. *J. Food Sci.* **36,** 537 (1971)—The tomato cultivars, Fireball, New Yorker, VF-13L and VF-145B were grown in the field and the ascorbic acid of the fruit was determined during the maturation period. A maximum of ascorbic acid concentration was noted 5-6 wk after anthesis and just before the fruit turned fully red.

ROASTED PEANUT FLAVOR AND ITS RELATION TO GROWTH ENVIRONMENT. W. Y. COBB & H. E. SWAISGOOD. *J. Food Sci.* **36,** 538-539 (1971)—Presents data on the amino acid and sugar composition of samples grown at two locations in different soil types under varying moisture conditions and the correlation with roasted flavor quality and growth environment. Concludes that environmental conditions may be primary determinants of year-to-year quality rather than genetics.

CARBON-14 CONCENTRATIONS IN RECENT WINES AND SPIRITS. M. S. BAXTER & A. WALTON. *J. Food Sci.* **36,** 540-541 (1971)—Analyses of malt whiskies and vintage wines have shown that a marked correlation exists between the concentration of carbon-14 in the sample alcohol and that prevailing in the atmosphere during original plant growth. This relationship permits the dating of post-1955 samples as a result of the variability of atmospheric carbon-14 levels caused by nuclear weapon testing.

CHEMICALLY DEFINED LIQUID MEDIUM FOR THE GROWTH AND SPORULATION OF Bacillus stearothermophilus 1518 ROUGH VARIANT. S. M. TANDON & K. G. GOLLAKOTA. *J. Food Sci.* **36,** 542-543 (1971)—The medium reported, consisting of minerals, glucose, 8 amino acids and 3 vitamins supports good vegetative growth and sporulation of *Bacillus stearothermophilus* 1518 rough variant. Lysine was necessary for building up an active culture.

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APPLIED SCIENCE and ENGINEERING

D. G. GUADAGNI, R. G. BUTTERY, R. M. SEIFERT and D. W. VENSTROM
USDA Western Regional Research Lab., ARS, Albany, Calif. 94710

FLAVOR ENHANCEMENT OF POTATO PRODUCTS

SUMMARY—The effect of selected compounds on flavor of various potato products was studied by sensory panels. The minimum detectable amount of each of six compounds in four brands of dehydrated mashed potatoes was determined. The difference thresholds of the six compounds in the reconstituted potato products varied from 0.05–3.1 ppm. Only 2-methoxy-3-ethylpyrazine (0.1–0.2 ppm) was effective in increasing the flavor level of all four brands of dehydrated potatoes; it also proved to be effective in increasing the potato flavor level of potato salad, dehydrated scalloped potatoes and potato soup. Potato salad stored at 3°C for 1 wk required at least 0.2 ppm of this compound to maintain its initial flavor difference from the control sample.

INTRODUCTION

FLAVOR of potatoes and potato products has been studied for some time (Ryder, 1966; Self, 1967). While considerable progress has been made, much remains to be learned about flavor improvement especially in dehydrated mashed potato products. Factors affecting off-flavor development in these products have been studied and procedures have been devised to monitor and control off-flavor development (Buttery et al., 1961; Burton, 1949; Hendel et al., 1951; Boggs et al., 1964). These procedures have generally taken advantage of hermetic packaging, inert atmosphere and the addition of antioxidants to improve stability or commercial shelf life of the products.

This paper is concerned with the possibility of enhancing flavor of potato products by constituents which have been identified in raw or cooked potatoes and by the use of other compounds possessing unusual flavor characteristics (Seifert et al., 1970). If some of these compounds could increase the natural potato flavor characteristics of the reconstituted products, it is possible that off-flavors developed during storage might not be as readily detectable in the presence of these flavor enhancing compounds.

MATERIALS & METHODS

Materials

The methoxy pyrazines were included in this study because of their unusually strong flavoring characteristics in addition to the fact that 2-methoxy-3-ethylpyrazine has been tentatively identified in potatoes (Buttery et al., 1970).

The other compounds were selected from the list of identified potato consituents roughly on the basis of their relative amounts in raw and cooked potatoes.

The compounds were obtained from reliable commercial sources or were synthesized by well-established procedures. All compounds were purified by preparative gas liquid chromatography before use in potato products.

The principal testing was done on dehydrated mashed potatoes. Commercial consumer-size samples of potato buds, granules and flakes were used. Each product was prepared according to manufacturer's instructions except for addition of varying amounts of the compounds to be tested. Stock water solutions of compounds were prepared and the proper amounts of these solutions were added to the liquid portion of the mixture at the same time that the dry potatoes were mixed in. Samples with and without additive were adjusted to the same water content to avoid texture differences.

Sensory Evaluation

A sensory panel of 20 trained tasters was used to evaluate the flavor effect of the various additives. The amount of each compound required for detection in the finished potato product was determined by plotting percentage of correct responses from the triangle test against concentration of each additive. The threshold concentration or detectable amount of the different compounds was taken at the point where correct responses corresponded to $P \lesssim 0.04$.

A paired comparison test was used to determine the effect of additive on potato flavor. Each judge was presented with a pair of samples and asked to check the sample with more potato flavor. Since this was a trained panel, the test is considered a difference rather than a preference test, but it is recognized that the decision of some panelists may be influenced by their preference. For some of the comparisons, a larger group of 40 untrained subjects were asked to indicate which sample in each pair had the best potato flavor. These tests would be expected to give a better indication of preference than difference.

All tests were conducted in a room equipped with individual booths supplied with conditioned odor-free air at 22° ± 1°C and 50% R.H. The tests were conducted under subdued lighting to eliminate any possible color differences. Each judge was given one coded pair or triangle per session and all samples were completely randomized to avoid positional or order effects. Statistical significance of the results were determined from the Tables of the Binominal Probability Distribution (National Bureau of Standards, 1950).

RESULTS & DISCUSSION

Dehydrated mashed potatoes

The relation between compound concentration and percentage of correct responses for the various dehydrated mashed potato products is shown in Figure 1. As expected, increasing concentration of the additives caused a progressive increase in percentage correct responses well beyond significant detection ($P \lesssim 0.04$). The curves for phenylacetaldehyde (I), methional (II), and 2-methoxy-3-methylpyrazine (III) are fairly well clustered together for the four different samples of potato products. This indicates that the compound–product interaction was fairly similar for each of the products. For 2-methoxy-3-isopropylpyrazine (IV), 2-methoxy-3-ethylpyrazine (V), and oct-1-ene-3-ol (VI), the concentration–response curves are widely scattered indicating a marked variation in the detectability of these compounds in different samples of dehydrated mashed potatoes.

The difference thresholds for the six compounds in four samples of reconstituted potato product are shown in Table 1. These data clearly show the superior

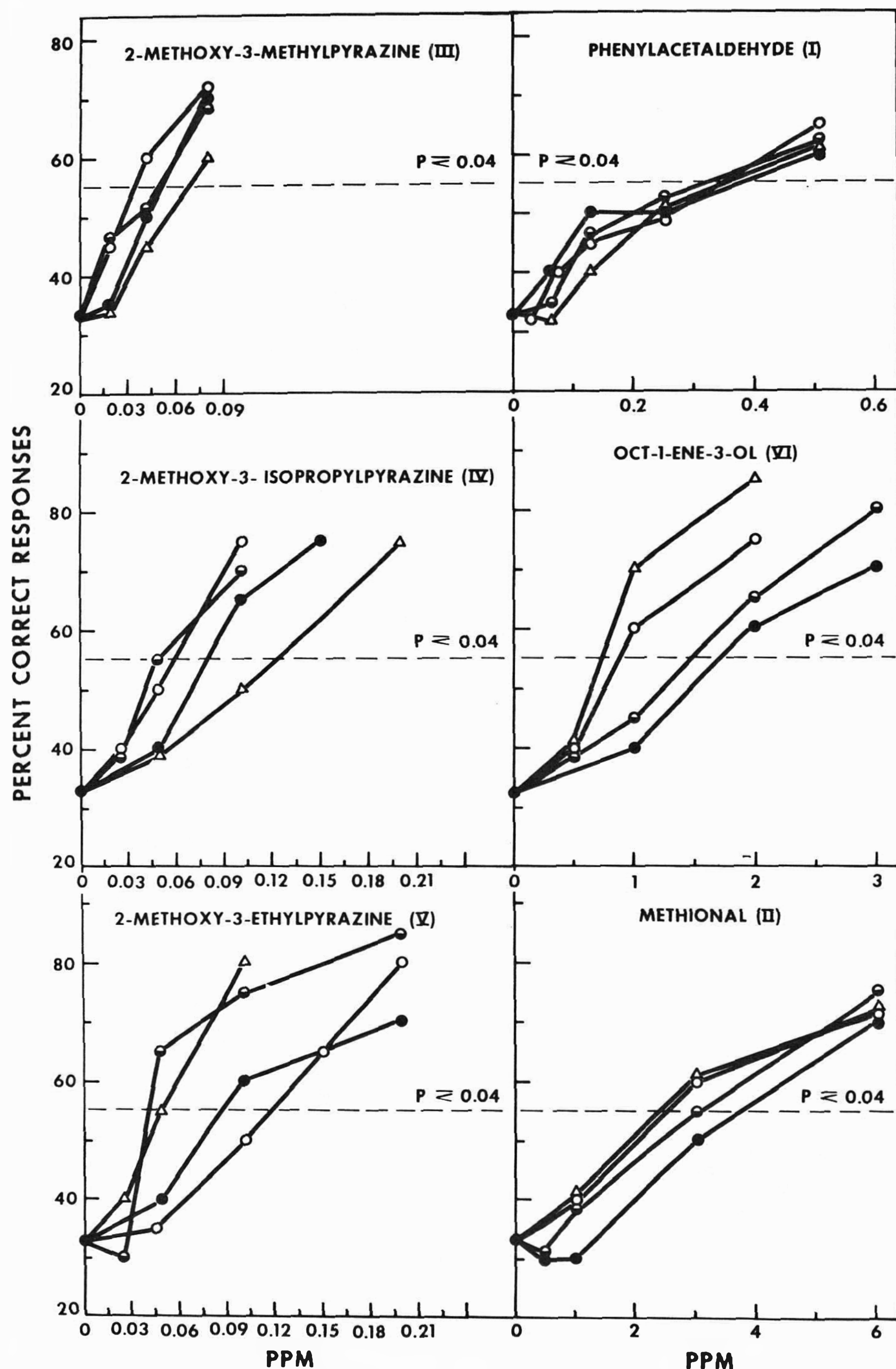

Fig. 1—Effect of compound concentration on detection by triangle test (N = 20) in dehydrated mashed potatoes: ● = "buds"; ○ = flakes #1; ◒ = flakes #2; △ = granules.

effectiveness of the methoxy-pyrazine compounds in changing the flavor of buds, granules or flakes. As will be seen later, concentrations of (V) near these levels significantly increased the level of potato flavor in all of these products. On the other hand, the identified constituents (I, II and VI) required much larger amounts for detectability and did not appear to improve the potato character of the reconstituted products with the possible exception of methional.

The paired comparison results for the six compounds and four potato products are shown in Table 2. It is clear that 2-methoxy-3-ethylpyrazine was the only compound tried which consistently gave a significant increase in potato flavor to all four of the products tested. In general, this was true for both trained and untrained tasters. As mentioned previously, the trained tasters made decisions largely on the basis of more or less natural potato flavor without regard to their personal preference, so that their results can be interpreted as indicating increases or decreases in potato flavor. While the untrained tasters were also asked to make decisions on the basis of potato flavor, their results are more inclined to reflect their personal preferences rather than an actual increase or decrease in potato flavor. In view of these differences in panel composition, it appears that 0.1–0.2 ppm of (V) significantly increased potato flavor and that the increase was generally liked by the untrained group of judges. The trained panel found no significant potato flavor increase in the four potato products as a result of adding (III). In flakes #1, the untrained group considered the sample with (III) at 0.04 ppm to be significantly better than the control, but reversed this decision at 0.08 ppm. None of the other results obtained with this compound were significant. Therefore, it does not appear that this compound would be useful for flavor improvement of these products. The only significant results obtained with (IV) were in favor of the control sample indicating that this compound detracts from natural potato flavor rather than improve it.

The addition of (I) to the four potato products caused no significant selection of either sample as having more potato flavor. On the other hand, addition of (VI) caused significant selection of the control sample as having more potato flavor in all four of the products. Thus it is clear that compounds (I), (IV), and (VI) serve no useful purpose for the enhancement of potato flavor in these products.

Methional (II) and other sulfur compounds have been reported as volatile constituents in cooked potatoes (Gumbmann and Burr, 1964; Ryder, 1966; Self, 1967). Although it has been considered of importance in cooked potato flavor, these results indicate that fairly large amounts of (II) failed to significantly increase potato flavor in all products except flakes #1. At a level of 3 ppm, the untrained group considered the sample with additive to have more potato flavor. It is interesting to note that flakes #1 was the only product of the four tested in which flavor was improved by compounds other than (V). A possible explanation may be associated with the observation that the control samples of this product were not particularly well liked. Consequently, changes which were not considered significant in other products may have been sufficient in flakes #1 to rate the sample with (II) and (III) as better than the control.

Other potato products

In view of the effectiveness of (V) in enhancing the flavor of dehydrated

Table 1—Difference thresholds of some pure compounds in various forms of dehydrated mashed potatoes

Compound	Products[a]			
	Potato buds	Flakes # 1	Flakes # 2	Granules
phenyl-acetaldehyde (I)	0.38	0.35	0.35	0.35
methional (II)	3.1	2.0	2.5	2.1
2-methoxy-3-methylpyrazine (III)	0.050	0.033	0.050	0.067
2-methoxy-3-isopropylpyrazine (IV)	0.080	0.060	0.050	0.123
2-methoxy-3-ethylpyrazine (V)	0.090	0.12	0.040	0.050
oct-1-ene-3-ol (VI)	1.8	0.9	1.5	0.7

[a]Data represent ppm of compound in each product detectable at $P \leqslant 0.04$ by triangle test.

Table 2—Effect of additives on flavor of dehydrated mashed potato products

Compound	Potato Buds				Flakes # 1				Flakes # 2				Granules			
	Conc (ppm)	N	Additive	Control[a]	Conc (ppm)	N	Additive	Control[a]	Conc (ppm)	N	Additive	Control[a]	Conc (ppm)	N	Additive	Control[a]
V	0.050	52[b]	33*	19	0.075	20	12	8	0.050	20	10	10	0.050	20	10	10
V	0.100	60[b]	36	24	0.125	20	15*	5	0.050	40[b]	28*	12	0.100	20	12	8
V	0.200	40	26*	14	0.150	40[b]	28*	12	0.100	20	15*	5	0.150	40[b]	27*	13
									0.100	40[b]	26*	14				
III	0.020	20	12	8	0.020	20	10	10	0.020	20	10	10	0.020	20	13	7
III	0.040	20	13	7	0.040	20	13	7	0.040	20	10	10	0.040	20	12	8
III	0.040	40[b]	22	18	0.040	40[b]	26*	14	0.080	20	7	13	0.040	40[b]	22	18
III	0.080	20	10	10	0.080	20	9	11					0.080	20	7	13
IV	0.050	20	9	11	0.025	20	10	10	0.025	20	10	10	0.050	20	8	12
IV	0.100	20	7	13	0.050	15	7	8	0.050	24	12	12	0.100	20	6	14
IV	0.150	15	0	15*	0.100	20	4	16*	0.100	14	4	10	0.200	15	4	11
I	0.064	20	10	10	0.064	20	10	10	0.064	20	10	10	0.064	20	13	7
I	0.128	20	10	10	0.128	20	10	10	0.128	20	12	8	0.128	20	10	10
I	0.256	20	12	8	0.256	20	13	7	0.256	20	11	9	0.256	20	12	8
I	0.512	20	9	11	0.512	20	7	13	0.512	20	8	12	0.512	20	6	14
VI	1.0	20	8	12	0.5	20	10	10	0.5	20	10	10	0.5	20	8	12
VI	2.0	20	4	16**	1.0	20	8	12	1.0	20	4	16**	1.0	20	6	14
VI	3.0	14	1	13***	3.0	17	4	13*	2.0	13	3	10*	3.0	17	3	14**
II	3.0	20	11	9	3.0	20	13	7	3.0	20	8	12	3.0	20	10	10
II	6.0	20	12	8	3.0	40[b]	29**	11	6.0	20	14	6	6.0	20	9	11
II	10.0	20	6	14	6.0	20	13	7	10.0	20	6	14	10.0	20	8	12
					6.0	40[b]	24	16								
					10.0	20	10	10								

[a]No. of judges indicating which sample had most potato flavor (Paired comparison test).
[b]Untrained panel.
*Significant at $P \leqslant 0.05$; **Significant at $P \leqslant 0.01$; ***Significant at $P \leqslant 0.001$.

mashed potato products, it seemed useful to investigate the flavor enhancing potential of this compound in other potato products. The products included potato salad, cream of potato soup, vegetable beef soup and scalloped potatoes. Results of paired comparison tests on these products with and without (V) are shown in Table 3. It is evident that small amounts of (V) were very effective in raising the potato flavor level of the different products. Most of the trained panelists also observed that the increased potato flavor improved the overall flavor of potato salad, cream of potato soup and scalloped potatoes. Thus it appears that (V) should be a generally useful compound for enhancing potato flavor in products containing potato.

Storage

Effectiveness of (V) during storage of commercially prepared potato salad is shown in Figure 2. It will be noted that the level of (V) which caused a significant initial increase (0.1 ppm) in potato flavor was not sufficient to maintain the increased flavor level during storage at 3°C. The percentage of the panel that considered the sample with 0.1 ppm of (V) as having more potato flavor steadily decreased with time.

Samples containing 0.2 ppm or more of (V) were selected by 75–90% of the panel as having more potato flavor than the control throughout the 1-wk storage period. Thus it appears that at least 0.2 ppm of (V) is required to maintain the higher level of potato flavor during storage at 3°C.

Samples containing 0.4 and 1 ppm of (V) were considered to have an undesirably strong flavor by most members of the panel, but samples with 0.1–0.2 ppm of (V) were considered improvements over the control samples.

Table 3—Effect of 2-methoxy-3-ethylpyrazine (V) on flavor of some potato containing products

Product	Conc (ppm)	N	Most potato flavor in[a] Control	Additive
Potato salad # 1	0.100	20	4	16**
Potato salad # 2	0.100	20	4	16**
Cream of potato soup	0.025	20	4	16**
Cream of potato soup	0.050	20	3	17**
Cream of potato soup	0.100	20	4	16**
Vegetable beef soup	0.015	20	9	11
Vegetable beef soup	0.025	20	5	15*
Vegetable beef soup	0.050	20	5	15*
Vegetable beef soup	0.100	20	5	15*
Scalloped potatoes	0.100	20	2	18***

[a]Paired comparison test. Judges asked to select sample in each pair with most potato flavor.
*P ⩽ 0.05; **P ⩽ 0.01; ***P ⩽ 0.001.

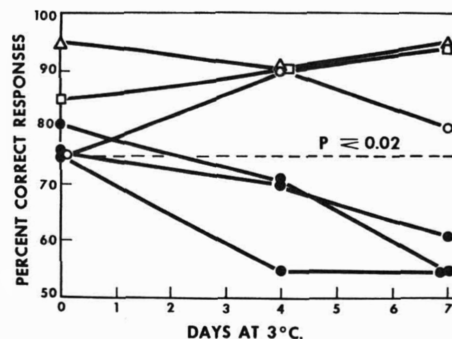

Fig. 2—Effect of storage on flavor enhancing effectiveness of various levels of 2-methoxy-3-ethylpyrazine in potato salad: □ = 1 ppm; △ = 0.4 ppm; ○ = 0.2 ppm; ● = 0.1 ppm. Paired Comparison Test, N = 20. Three different batches of product were tested at 0.1 ppm.

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R. E. BERRY, O. W. BISSETT and T. J. KEW
USDA Southern Utilization R& D Div., Fruit & Vegetable Products Lab, Winter Haven, Fla. 33880

ORANGE PEEL COLOR EXTRACT: ITS USE AND STABILITY IN CITRUS PRODUCTS

SUMMARY—Storage stability of the color was tested alone and in various citrus products using an extraction process which had been developed for obtaining a highly colored concentrated extract from orange peel. When concentrated color extract was added to single-strength orange juice in tin-lined or enamel-lined cans or clear glass bottles, color stability was good during storage at 35°F; except for some fading in glass-packed samples, no notable changes in color were observed after 10 wk. However, at 70°F and 85°F color changes occurred, mainly from darkening due to browning. These storage changes were more predominant in glass-packed juice and enamel-lined cans. Color in frozen concentrated orange juice remained stable more than 10 wk, showing no notable change in color during storage at −5°F. Stored in the concentrated form the color extract required protection from light and heat. Color was stable for more than 6 months when stored at −5°F in the dark.

INTRODUCTION

IMPORTANCE of color to acceptability of food products has long been recognized. This has been shown especially pertinent in orange juice products by a marketing research report (CECO Marketing Consulting Research, Inc., 1965) which indicated consumers prefer a dark orange color. Following recent public concern over the use of synthetic materials, food additives and the safety of foods being consumed by the general public, there is more interest than ever in the use of "natural" food sources.

Methods for extraction of coloring materials from citrus peel, treatment to remove color-interfering substances and flavor-influencing factors, and the incorporation of these color concentrates into citrus products have been described by Ting and Hendrickson (1968) and by Kew and Berry (1970). In the latter study, conducted on a relatively small laboratory scale, yields of color were found to vary depending upon the variety of citrus fruit used as a color source. Color concentrates from different sources also varied somewhat in degrees of redness or yellowness.

In order to be effective commercially, products treated with the color extract should be relatively stable. Thus in further development of the previously reported method, studies were made on the use of the color concentrates in commercial citrus products, particularly stability of the color-treated products stored at different temperatures and times. This paper reports these later developments.

EXPERIMENTAL

STABILITY during storage was determined both on products to which the color extract had been added and on the concentrated color extracts alone.

Storage tests on products

Color extracts from Valencia orange peel were checked for stability in concentrated and single-strength orange juice. Concentrated color extract prepared as described by Kew and Berry (1970) was added at 1 part/1000 to single-strength orange juice or at 4 parts/1000 to 45° Brix concentrated orange juice by blending the color into the product at about 120°F with gentle stirring. The color blended smoothly in a few minutes. Products were then canned and stored as follows: (A) 45° Brix concentrate in tin cans at −5°F and 35°F; (B) single strength in clear bottles at 35°F and 70°F; (C) single strength in tin-lined cans at 35°F and 85°F; and (D) single strength in enamel-lined cans at 35°F and 85° F.

Enamel-lined cans were included to check the possible effect of browning during high temperature storage and to avoid possible bleaching effects of tin. These samples were analyzed for Hunter citrus yellow (CY) and citrus red (CR) factors using the Hunter Lab D45 Citrus Colorimeter at 14-day intervals using the methods described by Kew and Berry (1970). Ranges and reproducibility of this method have been described by Huggart et al. (1966; 1969;) and Barron et al. (1967).

Storage studies on concentrated color extracts.

Samples of concentrated color extract prepared as described above were evaluated for storage stability using a Color Eye Reflectance Spectrophotometer Model D-1 (Instrument Development Laboratories, Kollmorgan Corporation, Attleboro, Massachusetts). Concentrated color extract was placed in small vials under nitrogen, 0.15 ml of color/vial. A standard method for reading the concentrated color on the Color Eye instrument was developed as follows: contents of the vial were diluted to 10 ml with hexane and a 4.25 cm dia disc of Whatman No. 1 filter paper was soaked in the vial for 10 min. The disc was air-dried for 2 min, placed in the instrument and read after 1 min. In the instrument, the disc was backed by two layers of Whatman filter paper and a white Vitrolite plate. A reference white Vitrolite plate was inserted in the other side of the instrument for comparative readings.

The Color Eye Spectrophotometer measures tristimulus color values X, Y and Z which are approximately degrees of redness, greenness, and blueness, respectively. Because there were slight variations in individual readings of a given sample, a range of experimental error had to be determined before evaluation could be effectively carried out. 30 samples of the color concentrate to be evaluated were prepared as described, and independent readings of X, Y and Z values made. Ranges were determined for each of the three values as follows: X = 102.0 − 100.5; Y = 93.7 − 90.2; Z = 36.4 − 25.9.

Stored samples of color concentrates were then evaluated at regular intervals until scores had changed to a degree that the probability of their being within the original mean was 1% or less. Samples of color concentrates were placed in storage and evaluated periodically as follows: clear vials of color concentrate prepared as above were placed at ambient temperature (about 80°F) on a laboratory bench about 3 ft from fluorescent light fixtures. Other samples were placed in the dark in a refrigerator at 35°F, and others in a freezer at −5°F. The samples at ambient temperature were evaluated daily until there was a color change. Then evaluation of 40°F stored samples was begun and they were evaluated at 4-wk intervals until a color change developed. Then samples stored at −5°F were evaluated at 30-day intervals until change developed.

RESULTS & DISCUSSION

RESULTS of storage studies of color stability in orange juice products are reported in Tables 1-4. Changes in CR and CY values of 45° Brix concentrates with added peel color extract and controls which did not contain added color, stored in tin cans at −5°F and 35°F, are shown in Table 1. After 10 wk at −5°F the experimental sample still had notably greater redness and yellowness values than controls although the color advantage had diminished some. After 10 wk at 35°F, which is above the temperature normally suggested for storing concentrated orange juice, the experimental sample had retained an advantage in yellowness, but had decreased in redness compared with the control sample.

Changes in color in single-strength orange juice with added color are compared with controls in Tables 2, 3, and 4 for samples stored in glass bottles, tin-lined steel cans and enamel-lined steel cans, respectively. Some browning occurred at the higher storage tempera-

Table 1—Changes in CR and CY values of 45° Brix orange juice concentrates with added peel color extract and controls, stored in tin cans at −5°F and 35°F

Storage		CR		CY	
Temp °F	Time wk	Control	Exp	Control	Exp
−5	0	37.8	42.3	81.2	83.7
	4	37.1	41.4	81.2	82.0
	8	37.4	41.0	81.8	82.6
	10	37.9	40.4	81.1	82.7
35	0	37.8	42.3	81.2	83.7
	4	37.2	39.4	80.4	82.0
	8	37.4	36.4	81.8	80.1
	10	37.4	37.2	80.3	81.8

Table 2—Changes in CR and CY values of orange juice samples with added peel color extract and controls, stored in glass bottles at 35°F and 70°F

Storage		CR		CY	
Temp °F	Time wk	Control	Exp	Control	Exp
35	0	34.9	35.8	80.5	84.8
	4	36.7	38.7	80.8	84.5
	8	37.3	38.1	80.1	84.1
	10	38.9	38.7	80.2	84.0
70	4	41.4	40.6	79.3	82.6
	8	46.7[a]	48.0[a]	77.4	79.3
	10	43.7[a]	46.2[a]	78.6	81.7

[a]These samples showed visible signs of browning.

Table 3—Changes in CR and CY values of orange juice samples with added peel color extract and controls, stored in tin cans at 35°F and 85°F

Storage		CR		CY	
Temp °F	Time wk	Control	Exp	Control	Exp
35	0	38.5	39.6	81.9	85.7
	4	38.3	39.0	81.3	85.2
	8	37.4	38.9	81.1	85.3
	10	38.1	39.6	81.2	85.0
85	4	38.4	39.0	81.2	85.2
	8	37.7	38.9	80.8	85.3
	10	38.2	39.6	81.0	85.0

Table 4—Changes in CR and CY values of orange juice samples with added peel color extract and controls, stored in enamel-lined cans at 35°F and 85°F

Storage		CR		CY	
Temp °F	Time wk	Control	Exp	Control	Exp
35	0	39.2	40.0	82.4	85.6
	4	38.5	39.5	81.6	84.7
	8	37.8	38.6	80.7	84.6
	10	38.5	39.6	81.5	84.7
85	4	42.0	43.4	80.6	82.8
	8	37.8	38.6	80.7	84.6
	10	44.0[a]	44.6[a]	80.2	82.8

[a]These samples showed visible signs of browning.

tures both in samples stored in glass and in cans, but this was partially suppressed in samples stored in tin-lined cans, possibly by bleaching action of the tin. In canned samples at 35°F the additional redness and yellowness of experimental samples was retained after 10 wk storage while with bottled juices the experimental samples had reverted in redness to about the CR values of the controls. This may have been an effect of fading due to light through the clear container. With juice in both types of cans (Tables 3 and 4) stored at 35°F, experimental sample CR and CY values were greater than controls to about the same extent after 10 wk storage as they had been initially.

Orange juice color is usually expressed in terms of color scores using standard color tubes and visual comparison under standard conditions (USDA 1963 and 1964). The following equivalents were determined based on studies by Huggart et al. (1969) of relationship between standard color scores and Hunter Citrus Colorimeter values. For the FCOJ and orange juice samples in glass, generally the controls were equivalent to a color score of 37 and experimentals were 38. Most of the samples in glass at 70°F were outside the color tube ranges due to darkening by browning. The remaining canned orange juice samples rated a color grade equivalent of 38 for controls and 39 for experimentals. The wide range of Hunter Colorimeter values included in a given color tube score points up the greater sensitivity of the colorimeter.

Several unexpected observations were made at 35°F and 70°F storage of the bottled juice samples; possibly due to browning reaction. As indicated in Table 2, although the CY values maintained about the same differential between experimental and control samples after 10 wk, the CR values changed considerably. An unexpected observation was the increase in CR values of both samples with storage time, especially at the higher temperature. This was probably due to browning reactions occurring in bottled juice at these storage temperatures. Although after 8 wk the experimentals still showed an increased CR value over controls, both samples appeared visually to have darkened. Similar observances were made on samples stored in enamel-lined cans at 85°F as indicated in Table 4. Again, after 10 wk the experimental samples showed some advantage over controls in CY value but little advantage in CR values. Also, very high CR values were noted in these samples as well as an increase in CR values of both control and experimental with storage time. These samples also appeared slightly darker by visual observation. This tendency was not so notable in juice samples stored in tin-lined steel cans as shown in Table 3. This might be due to a bleaching action of the tin-lined cans. Some advantage with respect to both CY and CR value was shown for the experimentals as compared to the control samples after 10 wk storage in tin-lined cans at 85°F. The degree of advantage with respect to both values was about the same after 10 wk as it had been initially.

Thus, these storage studies indicate the concentrated peel color extract imparts a relatively stable color in concentrated orange juice stored at −5°F and in canned single-strength juice stored at 35°F. For juice samples stored at 70°F or 85°F, darkening of product due to browning appeared to generally overshadow any effects of added color with the exception of those samples which were in tin-lined steel cans. Thus, this would be the container of choice for color-improved samples of this type if they were expected to encounter temperatures of 70°F or higher.

Because of the nature of the color concentrates, and because the Hunter Citrus Colorimeter was not designed to measure lightness and darkness without correction factors, a more precise specification of the color was required for studying their storage stability. The measurement of tristimulus values has been recorded as the most reliable means of specifying a color in absolute terms (Burham et al., 1963; Judd, 1952).

Using a reflectance spectrophotometer, the standard tristimulus X, Y and Z measurements can be made and these may be interpreted in common visual terms as degrees of redness, greenness, and blueness (or darkness), respectively.

The changes in X, Y and Z values of concentrated color extracts stored at ambient, refrigerator and freezer temperatures are indicated in Table 5. At ambient temperatures, which averaged about 80°F (70°-90°F), samples were beginning to show fading in 3 days and at the end of 7 days had reached a point of notable color fading. By the end of 9 days they were definitely visually faded and were out of the original color value range. At that point evaluations on samples which had been stored in the refrigerator (40°F) were begun. These samples remained in the acceptable range of values for 85 days but began to be slightly out of range and showed slight fading. By 127 days the samples were definitely out of range with respect to all tristimulus values. At that point those samples which had been stored at −5°F were evaluated. They remained within range until 197 days. At that point the tristimulus values were slightly out of range but no visible changes were apparent. After 243 days the sample still remained slightly within the original ranges. At this point, dilution comparisons with original values indicated these color concentrates had diminished in "coloring power" by about 20%. That is, about 20% additional color was required to achieve a given color score in a standard juice over that which had been required initially. Thus, it appears that these color extracts have suitable stability in juice products, particularly in juice samples stored at 35°F or concentrates at −5°F. Those treated products stored in cans have better color stability than those in bottles. The color extract itself should be stored at −5°F for long-term storage and at 40°F or lower at all times. If these precautions on use and storage are observed, the color concentrates and products to which they are added appear to be suitable for commercial use.

Table 5—Changes in tristimulus values X, Y and Z

Temp °F	Time days	Tristimulus values X (redness)	Y (greenness)	Z (blueness)
ambient 80°[a]	0	100.3	93.0	28.1
	3	102.0	93.0	33.0
	7	102.7	97.8[b]	56.7[b]
	9	103.0	99.9[b]	75.9[b]
40	16	102.5	94.0	35.0
	49	101.6	93.3	35.5
	85	102.3	92.8	29.7
	102	102.8[b]	96.1[b]	44.1[b]
	127	103.3[b]	96.0[b]	40.0[b]
−5	127	102.4	92.8	36.3
	161	102.6	93.6	36.3
	197	102.9[b]	94.7[b]	37.0[b]
	220	102.3	93.4	34.3
	243	102.4	93.4	34.3

[a]80°F average, range 70-90°F.
[b]Probability of these values being outside the range of original values is greater than 99%.

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ห้องสมุด กรมวิทยาศาสตร์

J. E. DUTRA DE OLIVEIRA[1] *and MARIA LEONINA PEREIRA DA SILVA*[2]
Faculdade de Medicina, Ribeirão Prêto, S.P., Brazil

NUTRITIVE VALUE OF OPAQUE-2 CORN AND ITS MIXTURE WITH HYBRID CORN AND WHEAT FLOUR

SUMMARY—A study was carried out to evaluate the nutritive value of opaque-2 corn grown in Brazil and the nutritional effects of its mixture with hybrid corn and wheat flour. These studies were conducted with rats to determine the growth rate, feed efficiency and protein efficiency ratio. Amino acid analyses of Brazilian opaque-2 corn confirmed its high lysine content and other differences in amino acid content; nutritive value was shown to be similar to the American variety. There were small variations among samples of opaque-2 corn grown in different regions and crop years, but all had high nutritive value. The nutritive value of the hybrid corn was little improved by its mixture with the high lysine opaque-2 corn. Better results were found when the opaque-2 was mixed with the wheat flour.

INTRODUCTION

DISCOVERY by Mertz et al. (1964) of a high lysine, high tryptophan corn widened the possibilities of the utilization of this cereal in the world fight against protein malnutrition. A nutritious corn could have beneficial effects on the protein nutrition of a large segment of the world population.

We have been interested in the opaque-2 since its introduction in Brazil in 1965 and have therefore compared the amino acid composition and biological value of the American opaque corn with Brazilian varieties. We have also been concerned with possible future uses for this cereal. Mixture of opaque corn and low lysine cereals such as normal corn and wheat have been tested as a preliminary step in the production of a more balanced food for animals and humans.

EXPERIMENTAL

AMERICAN opaque-2 was provided by Mertz, and Brazilian varieties were made available through the Agronomy School of Piracicaba (mixed genetic background) and the Agronomy Institute of Campinas (Mayadent type of corn background). Other generations of these corns were grown in different regions of the country and in successive years, while hybrid corn (HMD 79-74) was obtained locally. A native floury-type corn cultivated by Indians in Brazil was also studied.

The amino acid composition of the American opaque-2, the first Piracicaba opaque-2 variety, the floury Indian variety and the hybrid corn were determined with an Autoanalyzer at the Food Technology Institute of Campinas. Standard techniques were used throughout.

Biological assays were conducted with weanling male rats (Wistar strain) fed the different samples of corn for a 4-wk period (AOAC, 1965). Six rats were randomly allocated to each group, individually housed and given access to food and water ad libitum. These rats were weighed weekly and food consumption was determined twice a week. The nitrogen content of the diets was estimated by the Kjeldahl method and total protein intake for each animal was calculated at the end of the experiment.

All the available corn samples and combinations of hybrid corn and opaque-2 were biologically tested. The proportions tested included 25%, 50% and 75% of the protein of the diet for each corn (Table 1, Exp. 1). Wheat flour, obtained locally, was also mixed with opaque-2 corn flour in the same proportions as in the previous experiment and their efficiency was measured in the rat (Table 1, Exp. 2).

The experiments had a completely randomized block design. Multiple comparisons were analyzed by the Tukey test. Results were considered statistically significant when they were different at the 1% level.

RESULTS & DISCUSSION

AMINO ACID analyses of the first available opaque corn in our laboratory showed its high lysine content to be similar to the American sample and above the values found in the hybrid and Indian floury corns. These data and other amino acid values are shown in Table 2 and confirm previous studies (Mertz, 1968). The only exception was the low quantity of tryptophan. Technical difficulties with this estimation could be partly responsible for these findings (Bates, 1966).

Growth rate of the animals fed the Brazilian variety and American opaque-2 corns showed similarly high values,

Table 1—Composition of the corn and corn/wheat mixtures

Experiment 1—Opaque and hybrid corn				
	Protein distribution %		Grams in the diet	
Diet	Opaque-2	Hybrid	Opaque-2	Hybrid
1	100	0	69	0
2	75	25	52	21
3	50	50	34	43
4	25	75	17	65
5	0	100	0	87
Experiment 2—Opaque and wheat flour				
	Protein distribution %		Grams in the diet	
Diet	Opaque-2	Wheat flour	Opaque-2	Wheat flour
1	100	0	86	0
2	75	25	65	16
3	50	50	43	33
4	25	75	22	49
5	0	100	0	65

Table 2—Amino acid content of different corns (mg/100 g corn)

Variety	Lys.	Try.	Arg.	Asp.	Gly.	Ala.	Leu.
Opaque-2 USA	367	64	525	870	377	672	857
Opaque-2 Brazil (Piracicaba)	395	62	470	647	445	626	638
Hybrid corn	156	54	186	392	237	716	983
Flowry Brazilian Indians	228	57	363	485	313	835	1094

[1] Associate Professor of Medicine, Chief Nutrition Laboratory.
[2] Fellow in Nutrition, São Paulo Research Foundation (FAPESP). Present address: School of Animal Husbandry and Agriculture, Jaboticabal, São Paulo, Brazil.

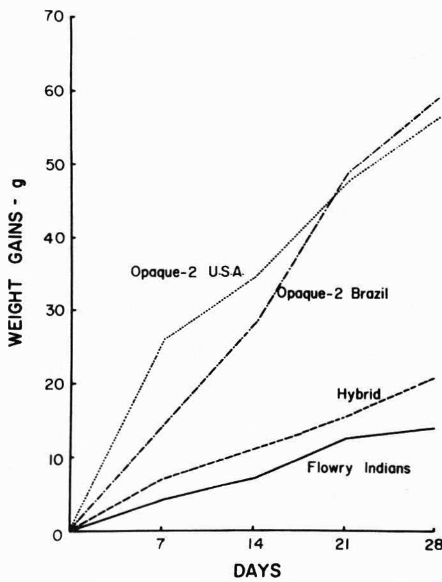

Fig. 1—Curves showing average weekly gains of rats fed on different corns.

Table 3—Nutritive value of Brazilians opaque-2 corns tested in rats

Line Background	Local grown	Crop year	Protein effic. ratio PER	Food effic. ratio FER
Piracicaba	Piracicaba	1967	2.35	0.18
Mixed	Piracicaba	1968	2.63	0.21
	Ituverava	1969	2.98	0.21
Campinas	Cravinhos	1968	2.08	0.18
Maya	Rib. Prêto	1968	2.17	0.19
	S. Joaquim	1969	2.34	0.20
	Campinas	1969	2.58	0.21
Purdue	USA	1967	2.39	0.20

Table 4—Protein quality of the mixtures of opaque-hybrid corn and opaque-wheat flour

Diet	Weight gain g	Food intake g	Feed efficiency ratio	Protein efficiency ratio
		Experiment 1—Opaque and hybrid		
1	44	243	0.18	2.35
2	29	217	0.13	1.79
3	20	175	0.11	1.49
4	15	163	0.09	1.22
5	16	154	0.10	1.28
		Experiment 2—Opaque and wheat		
1	57	262	0.22	2.98
2	55	286	0.19	2.47
3	25	199	0.13	1.58
4	21	196	0.10	1.34
5	9	154	0.06	0.79

much better than those obtained with the hybrid and Indian floury varieties (Fig. 1). Although the Indian corn had higher values for lysine and other amino acids than the hybrid, this did not result in a better growth in the rats.

The protein efficiency ratio obtained in the samples of the Brazilian opaque-2 corns grown in different places and years is shown in Table 3. Some variations were found among them, but all values were considered indicative of good nutritive value, and the results obtained were quite close to those observed from casein groups.

The nutritive value of the mixtures of hybrid and opaque corns did not show a statistically significant improvement over the hybrid corn alone, until the amount of opaque reached 75% of the diet protein (Table 4, Exp. 1). On the other hand, all the mixtures of opaque-2 and wheat flour showed statistically significant better protein quality than when wheat was the only source of protein in the diet (Table 4, Exp. 2). Considering that lysine is the limiting amino acid of both corn and wheat, it was surprising that the mixture of each with opaque-2 greatly improved the nutritive value of the latter. This is due, we believe, to an improvement in the overall amino acid balance of the wheat-opaque mixture rather than the lysine supplementation alone.

Our findings are important because in countries where there is a lack of good dietary protein there is a possibility of mixing opaque-2 with wheat to make bread, rolls and other products with better nutritive quality. Also, in countries where wheat flour is not readily available, the addition of opaque-2 could have importance from an economic point of view.

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J. T. LAWHON and C. M. CATER
Texas A&M University, Oilseed Products Research Center[1], College Station, Texas 77843

EFFECT OF PROCESSING METHOD AND pH OF PRECIPITATION ON THE YIELDS AND FUNCTIONAL PROPERTIES OF PROTEIN ISOLATES FROM GLANDLESS COTTONSEED

SUMMARY—Glandless cottonseed meals were prepared under controlled conditions in a pilot plant by three different processing methods. These meals along with a glandless cottonseed meal produced at a commercial oil mill were used as source meals for protein isolates. Two protein fractions differing in composition and characteristics were isolated from each type meal using a two-step, two-solvent isolation procedure developed at the USDA Southern Utilization R&D Div. Yields of each isolate precipitated at three different pH levels were determined on the pilot plant meals. Isolate yields from the commercial meal were determined near the respective isoelectric points of the two fractions. Functional properties including whippability, heat gelation, solubility and foaming properties, were measured on all isolates. Variation in measured values due to meal processing method and precipitation pH was statistically assessed in some instances. Meal processing method was found to significantly affect the yield of Isolate I, the minor isolate. pH of precipitation was found to significantly affect the yield of Isolate II, the major isolate. Also, it was shown that the pH-solubility profiles of both Isolates I and II could be altered by changing the pH at which they were precipitated. The functional properties of isolates from meals processed without heat were superior to those of isolates from heated meals. Data collected indicated the need for a new practice in evaluating the extent of denaturation of cottonseed protein products. The present practice of determining nitrogen solubility at one point was shown to be inadequate.

INTRODUCTION

THROUGH THE joint efforts of cotton breeders, oilseed researchers, and many others of related interests, production of glandless cotton is expanding and glandless cottonseed is increasingly being evaluated as a potentially important source of food protein. Since the introduction in 1969 of a high protein nut-like product (called Tamunuts) prepared from undefatted glandless cottonseed kernels, major food processors from all parts of the United States and several foreign countries have been investigating glandless cottonseed as a new low-cost source of nut meats (Lawhon et al., 1970). Prior to that time, cottonseed meals, protein concentrates, and protein isolates from glandless cottonseed had already been produced in pilot plants and in commercial processing trials. The meals had been tested in poultry and swine rations and the concentrates and isolates were under investigation for use in human foods.

It has been recognized by cottonseed processors through the years that processing methods affect the nutritional properties of cottonseed protein (Frampton, 1964). Other investigators have reported that meal processing conditions can affect the quantity of nitrogen that is solubilized in procedures used to isolate protein (Martinez, 1968).

In the work reported here, three glandless cottonseed meals were prepared under controlled conditions in the Texas A&M University Oilseed Products Research Center (OPRC) pilot plant by three different processing methods. Isolates were prepared from these meals and from a fourth meal produced in glandless cottonseed processing trials at a commercial oil mill.

The two-step, two-solvent protein isolation procedure developed at the USDA Southern Utilization R&D Div., New Orleans, was employed (Berardi et al., 1969). This procedure separates the protein into two fractions with widely differing chemical and physical properties. Isolate I is composed of low molecular weight, water-soluble proteins with a minimum solubility range of pH 3.8–4.2. It is normally precipitated at pH 4. This is the minor component of the protein, comprising roughly one-fourth of the protein isolatable by the procedure. Isolate II is composed of high molecular weight, alkali-soluble proteins with a minimum solubility range from pH 6.2–7.8. This isolate, the major component, is normally precipitated at pH 7.

Isolates I and II from the three pilot plant meals were each precipitated at three different pH levels and the relative yields from each meal were determined. Isolates I and II from the fourth meal were each precipitated at a single pH near their respective isoelectric points. Functional properties of the protein, including whipping, gelation, solubility, and foaming properties, were measured and the effect of meal processing and pH of precipitation on them statistically assessed in some instances.

[1] Operated by the Texas Engineering Experiment Station for the Cotton Research Committee of Texas.

EXPERIMENTAL

Cottonseed meal preparation

Three cottonseed meals were prepared from Watson GL-7 glandless cottonseed using OPRC pilot plant processing equipment. Whole and large kernel particles extremely low in hulls content were flaked without pretreatment with a Flak-All machine. Different processing procedures were then applied as follows:

Meal 1. Unheated flakes were direct-extracted with hexane at ambient temperature in a 55-gal batch extractor. Spent flakes were desolventized by warm air flowing upward through a stationary bed.

Meal 2. Flakes were placed in a 22-in. diameter steam-jacketed cooker in 75 lb. batch charges. Temperature inside the cooker was raised to 180°F within 10 min and water added to increase the flakes moisture from 7.5% to 12%. Cooking continued for an additional 20 min as flakes were agitated by a revolving sweep. Temperatures inside the cooker were controlled to a maximum of 225° F. Discharged flakes were screened to break up conglomerates formed in the cooker and to cool before extraction. Spent flakes were desolventized by indirect heat in a steam-jacketed kettle while under agitation. Desolventization temperature was controlled to a max of 190°F. Residence time did not exceed 15 min.

Meal 3. Flakes were cooked and screened as for Meal 2, then prepressed with a V.D. Anderson midget "expeller" screw press. The press cage was 8-1/4 in. long and 1-1/2 in. I. D. Capacity of the press was 9 lb/hr. Prepress cake was manually broken into particles less than 1 in. square and extracted and desolventized as for Meal 2.

Each meal was passed at a slow rate through a Fitzpatrick comminuting machine fitted with a coarse screen to shatter aggregated particles and then screened over a 20 mesh vibrating screen. Products passing through the screen were used for protein isolation.

Meal 4. Watson GL-16 glandless cottonseed was processed in an experimental run at Producers Cooperative Oil Mill, Oklahoma City (Smith, 1970). Coarse and fine kernels were conditioned in a seven-high stack cooker. Temperature of the kernels discharged from the cooker was 170°F. Kernels were flaked to a thickness of 0.010–0.012 in. with a Bauermeister flaker and hexane-extracted in a Crown 250-ton extractor running at half speed. Desolventization was in a 5-tray 102 in. dia Crown Desolventizer-Toaster. A max temperature of 150°F was used to prevent meal darkening. Sparge steam was not employed during desolventization.

Desolventized flakes were screened over a 12 mesh screen and the "through" fraction turbo-milled and rescreened over a 60 mesh screen. Meal 4 is a portion of the 60 mesh product.

Experimental design

A split-plot design was followed in making randomly ordered protein isolation runs using the three pilot plant meals. Meal processing methods corresponded to whole-unit treatments in the design and pH of precipitation corresponded to subunit treatments. Isolate I was extracted from Meals 1, 2, and 3 and randomly precipitated at pH 3, 4, and 5. Isolate II was extracted from each of these meals (after Isolate I had been extracted in step one of the two-step procedure) and randomly precipitated at pH 6, 7, and 8. Isolates I and II were each precipitated at only one pH level for each run, thus requiring a total of 9 runs per replication. Two replications were made of the run series.

Meal 4 was not included in the statistical design and analysis. Only two isolation runs were made using Meal 4. In these runs Isolate I was precipitated at pH 4 and Isolate II was precipitated at pH 7.

Protein isolation and drying

In each run, 6.95 lb of meal were extracted by continuous stirring for 30 min in 10 gal of tap water (Step I) at 25°C. The water was adjusted to pH 6.5 with 1N HCl. After separating the slurry by centrifugation into liquid extract (Extract A) and solids residue (Residue), Isolate I was precipitated at one of the test pH levels by HCl addition and separated by centrifuging. The Residue was reextracted (Step II) in 10 gal of .015N NaOH (around pH 10) at 25°C for 30 min. The second volume of slurry was then centrifuged to obtain a second extract (Extract B) and a second residue (Residue-R). Isolate II was precipitated from Extract B by HCl addition at the precipitation pH level chosen. The solidified isolate was recovered by centrifuging.

All isolates were dryed by spray drying after adjustment to neutral pH. An inlet air temperature of 275–280°F and an outlet air temperature of 200–205°F were used.

Dried isolates ranged in color from cream to light tan with Isolate I being somewhat lighter. The isolates were stored at 32–35°F for 2–3 days, usually, while awaiting drying.

Functional property measurements

Whipping properties. Whippability measurements were made on Isolates I and II as follows:

(a) 3g of dried isolate were dispersed in 100 ml of citrate-phosphate buffer (pH 7).
(b) The solution-suspension was whipped for 6 min at a speed for heavy beating (Mixmaster speed 9).
(c) Viscosity of the resulting whip was measured with a Model LVT-E Brookfield Viscometer, using a No. 3 and 4 spindle at 6 rpm. Volumes before and after whipping were recorded and the % volume increase due to whipping calculated as follows:

$$\% \text{ Vol. Increase} = \frac{\text{Vol. after whipping} - \text{Vol. before whipping}}{\text{Vol. before whipping}}$$

(d) 75g of sugar were added to whip and whipping resumed for an additional 2 min.
(e) Measurements in (c) were repeated.

Gelation properties. Measurements of gel strength were made at isolate concentrations of 6, 8, and 10% using a Precision Universal Penetrometer equipped with a miniature cone 1-3/8 in. dia and weighing 10.8g. The weight of the standard test rod without the cone was 47.5g. No weights were added to the loading bar. In measuring, the cone was allowed to penetrate the gels for 3 sec and then the depth of penetration read directly in tenths of millimeters.

After some developmental work a method which yielded measurable gels from Isolate II was derived for use as follows:

(a) 18g of dried isolate were suspended in 146.6 ml of distilled water and mixed well.
(b) 1M citric acid was added in the ratio of 0.8 ml/g of isolate. The resulting solution-suspension contained 10% isolate by weight and was at pH 3.5–3.9.
(c) 14 ml of distilled water were added to 56g of the solution-suspension to dilute it to 8% concentration.
(d) 28 ml of distilled water diluted 42g of the solution-suspension to give a 6% isolate concentration.
(e) The three concentrations were heated in boiling water for 10 min (being stirred while heating).
(f) Heated samples were cooled overnight in a refrigerator at 40°F.
(g) Penetrometer measurements were made the following day after samples had equilibrated to room temperature.

Gels were also prepared from gelatin and measured for comparative purposes.

Solubility characteristics. Nitrogen solubility was determined at either 5 or 6 points on one replication of the isolates. In addition, nitrogen solubilities were measured on 3 Isolate I samples and a glandless cottonseed meal which were available from other investigations. These 3 Isolate I samples labeled isolates A, B and C in Figure I, had been prepared using the same two-step, two-solvent isolation procedure used in this study, but were dryed by three different drying techniques which variously affected the solubility characteristics of the final product. The meal is designated Meal D.

Solubility characteristics were measured by the following method (Lyman et al., 1953):

a) 1g of dried isolate was dispersed in 100 ml of water already adjusted to pH of measurement.
b) After stirring 5 min, the slurry was readjusted to the original pH. After an additional 5 min of stirring the pH was rechecked and adjusted if necessary.
c) The slurry was heated for 30 min in a 37.5°C water bath and then shaken for 30 min at room temperature.
d) The slurry was centrifuged at 2000 rpm for 20 min, filtered, and a 70 ml aliquot removed for determining % soluble N by standard procedures (A.O.C.S., 1957).

pH adjustments were made with .01N HCl and .015N NaOH.

Foaming properties. Foaming capacity and foaming stability were measured on one replication of the isolates.

Isolates were tested by adding to 100 ml citrate-phosphate buffer (pH 4 for Isolate I and pH 6 for Isolate II) the amount of sample required to give 0.3 mg N/ml in the mixture. 50 ml of the resulting solution-suspension were pipetted into each of two stoppered 100 ml graduated cylinders and the cylinders shaken in a horizontal position for 1 min at the rate of 2 cycles per sec. The cylinders were then placed upright and the total volume of liquid plus foam in each cylinder read and recorded in ml after 30 sec. The mean value was taken as the foaming capacity of the protein solution.

After 10 min and again after 2 hr, the volume of foam only, i.e., the total volume minus the liquid, measured in ml, was recorded for each cylinder. The mean value of the volumes after each time period was taken as the foam stability.

RESULTS & DISCUSSION

ANALYTICAL DATA on the four source meals before protein extraction are shown in Table 1.

Meal 4 differed slightly from the pilot plant meals in oil, crude fiber and total gossypol contents. Higher crude fiber and gossypol may have resulted from the inclusion of both coarse and fine kernel particles in the material extracted at the commercial mill. The pilot plant meals were prepared from whole and large kernel particles which were lower in hulls content.

Table 2 shows the percentages of the total nitrogen present in the meals at the start of the first extraction which were extracted in Step 1 and the percentages of the total nitrogen present in the meals at the start of the first extraction which were precipitated at each of three pH levels to produce Isolate I. Also shown are the percentages of the total nitrogen present in the Residue at the start of the

Table 1—Analytical data on glandless cottonseed meals used for protein isolation

Source meals	Moist. & volatiles %	Oil %	Protein %	Soluble protein %	Crude fiber %	Gossypol Free %	Gossypol Total %
1	9.2	0.71	54.56	98.7	2.0	0.010	0.010
2	8.1	0.59	55.50	93.9	2.1	0.005	0.019
3	4.7	0.32	57.82	95.6	2.1	0.018	0.018
4	8.9	1.95	55.31	96.3	3.0	0.019	0.048

second extraction which were extracted in Step II and the percentages of the total nitrogen present in the Residue at the start of the second extraction which were precipitated at each of three pH levels to produce Isolate II.

The weight of nitrogen precipitated in each instance was obtained by taking the difference between the weight of nitrogen in solution in the extracts from Steps I and II and the weight of nitrogen in solution in the supernatants after precipitation and centrifugation.

An analysis of variance was performed to test the significance of observed differences in amounts of nitrogen precipitated. Variation between meals processed by different methods and between different pH of precipitation was tested for each type of isolate. Treatment means for the two main effects are included in Table 2.

Differences between meals, i.e., processing methods, proved to be statistically significant at the 5% level for Isolate I and differences between pH of precipitation proved to be significant for Isolate II. Using Duncan's multiple range test to make comparisons among the meal means for Isolate I, Meal 1 was found to differ significantly from Meal 2 and Meal 3. Meal 2 did not differ significantly from Meal 3. Meal 4 was not included in the statistical design and analysis.

Thus the significant differences in yields of Isolate I were obtained between meals processed with heat and meals processed without heat.

Duncan's range test showed yields of Isolate II when precipitated at pH 6 did not differ from yields at pH 7. But nitrogen precipitated at pH 6 and pH 7 differed from the yield of pH 8 at both the 5% and the 1% levels.

Interaction effects proved to be nonsignificant in all instances.

Because of the size of the extractions and equipment used yields of Isolate I were somewhat lower than those obtained in bench scale work. As shown in Table 2 approximately 2-1/2 times as much nitrogen was extracted from meal 1 as from Meal 2 or 3 in Step I. A higher percentage of nitrogen was also extracted from Meal 4 than from Meal 2 or 3. Meal 4 received a milder heat treatment in processing than did Meal 2 or 3.

The average nitrogen contents of Isolates I and II were 13.12% and 15.70%, respectively.

Data on whipping properties of Isolates I and II are presented in Table 3.

An analysis of variance was performed on whip viscosities measured after whippings with sugar added. For Isolate I whip viscosities associated with meals processed by different methods were barely significantly different at the

Table 2—Nitrogen extracted from source meals in Step I and residues in Step II and amounts precipitated at different pH levels

Source meals	Avg % of total meal N extr/run	pH of ppt	% of total meal N ppt from Extr A	Treatment means: Proc. methods %	Treatment means: pH of ppt %
			Isolate I		
1	27.0	3	9.1	8.2	5.9
		4	8.8		5.7
		5	6.8		3.9
2	10.0	3	3.5	2.7	5.9
		4	2.7		5.7
		5	1.7		3.9
3	12.4	3	5.2	4.6	5.9
		4	5.5		5.7
		5	3.1		3.9
4	17.0	4	5.2	5.2	5.2
Source meals	**Avg % of total residue N extr/run**	**pH of ppt**	**% of total N ppt from Extr B**	**Treatment means: Proc. methods %**	**Treatment means: pH of ppt %**
			Isolate II		
1	83.5	6	81.5	64.8	66.2
		7	65.0		62.0
		8	48.0		31.3
2	69.3	6	61.8	49.1	66.2
		7	62.7		62.0
		8	22.7		31.3
3	62.0	6	55.3	45.6	66.2
		7	58.2		62.0
		8	23.2		31.3
4	77.3	7	65.5	65.5	65.5

Table 3—Whipping properties of protein isolates from meals processed by different methods and precipitated at different pH levels

pH of ppt	Source meal	Viscosity, cps: w/o sugar	Viscosity, cps: w/sugar	Vol increase on whipping, %: w/o sugar	Vol increase on whipping, %: w/sugar
			Isolate I		
3	1	1460	3260	488	425
	2	2900	1900	438	350
	3	3990	2940	450	450
4	1	1310	3390	500	513
	2	3470	2230	438	363
	3	3480	2320	438	400
	4	3290	4270	438	488
5	1	1230	2660	500	538
	2	2650	1460	450	325
	3	3280	2790	450	388
			Isolate II		
6	1	1610	1430	488	513
	2	6180	2940	350	175
	3	3690	1250	400	225
7	1	2270	4960	438	475
	2	5490	830	225	175
	3	4110	2490	350	250
	4	3790	2830	425	338
8	1	6220	2780	488	388
	2	7160	1910	338	263
	3	5140	2500	388	288

5% level. No real difference in Isolate I whip viscosities was found to exist for different pH of precipitation.

For Isolate II neither meals processed by different methods nor different pH of precipitation caused significant viscosity differences.

Interaction effects were not significant in any instance. Volume change data were not statistically analyzed.

As shown in Table 3 the viscosity of Isolate I whips from Meal 1 tended to increase more than two-fold when whipped with sugar added. Isolate I whips from heat-processed meals tended to decrease in viscosity and volume when whipped with sugar added.

These viscosity data were obtained on isolates whipped at pH 7 and are suitable for comparative purposes. However, Isolate I from Meal 1 whipped in a buffer at pH 3 had more than twice the viscosity of a duplicate sample whipped at pH 7. For Isolate II the viscosity increase going from pH 7 to pH 3 was on the order of five times, to a viscosity of 25,000 cps.

Measurements made on the heat gelation properties of Isolate II are contained in Table 4.

The strengths of gels obtained are reflected by the depth of cone penetration in millimeters. Gels prepared from Isolate II from Meal 1 permitted less cone penetration at each of the protein concentrations than gels from Isolate II from either Meals 2 or 3. Isolates from precipitation pH 7 generally produced stronger gels than those from pH 6 or 8 for a given meal. The gels were opaque and heat reversible. Gels of measurable strength were not obtained using Isolate I.

Gels prepared from gelatin for control purposes, showed essentially the same strength as gels from Meals 1 and 4 isolates precipitated at pH 7. However, gelatin gels were somewhat stronger than isolate gels precipitated at pH 6 or 8 for all the meals.

Table 4—Strength of gels from glandless cottonseed protein measured by cone penetration (Isolate II)

pH of ppt	Source meal	Conc of protein Isolate II, %			pH of protein soln.
		6	8	10	
6	1	21.3 mm	17.5 mm	11.5 mm	3.49
	2	*	*	17.4	3.56
	3	*	*	*	3.65
7	1	20.3	11.1	6.4	3.45
	2	23.0	17.0	11.2	3.56
	3	23.0	21.2	20.1	3.65
	4	17.4	11.9	9.0	3.56
8	1	19.2	13.9	12.9	3.61
	2	*	*	*	3.87
	3	*	*	*	3.90

*Insufficient gel strength for measurements.

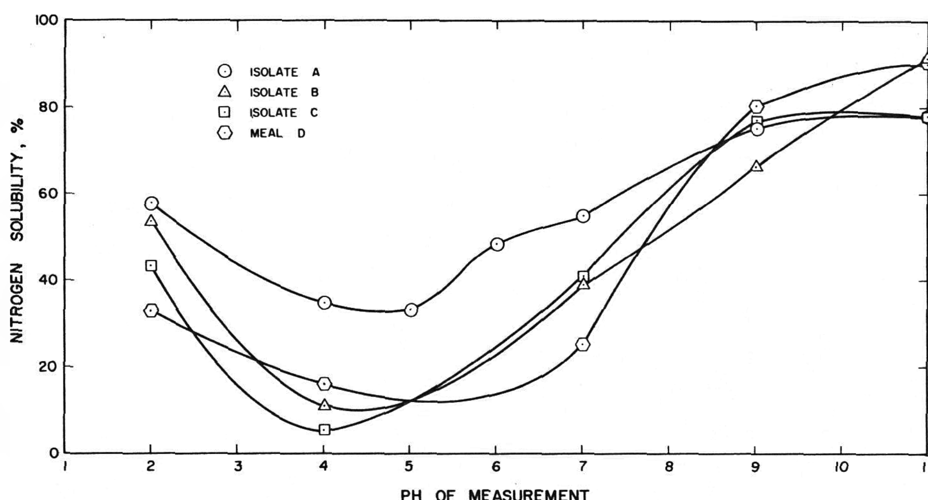

Fig. 1—Nitrogen solubility at different pH of measurement in Isolate I dried by three different methods and in a glandless cottonseed meal.

Figure 1 contrasts the nitrogen solubility characteristics of Isolates A, B, and C and Meal D, over a wide pH range.

Traditionally when the cottonseed processing industry has used nitrogen solubility as an indication of the protein quality of cottonseed meals, the nitrogen solubility has been determined at a single point. The solubility of nitrogen in

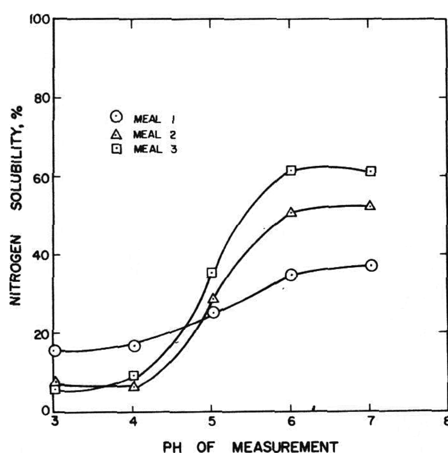

Fig. 2—Nitrogen solubility of Isolate I samples precipitated at pH 3 from Meals 1, 2, and 3.

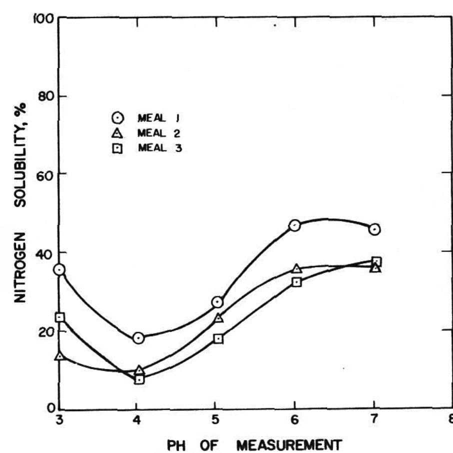

Fig. 3—Nitrogen solubility of Isolate I samples precipitated at pH 4 from Meals 1, 2, and 3.

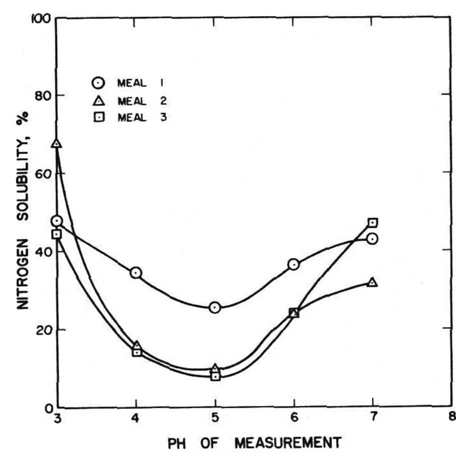

Fig. 4—Nitrogen solubility of Isolate I samples precipitated at pH 5 from Meals 1, 2, and 3.

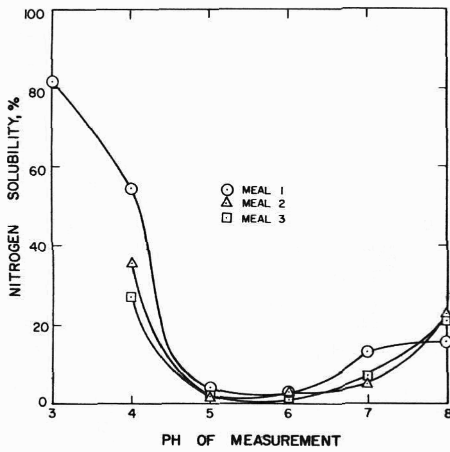

Fig. 5—Nitrogen solubility of Isolate II samples precipitated at pH 6 from Meals 1, 2, and 3.

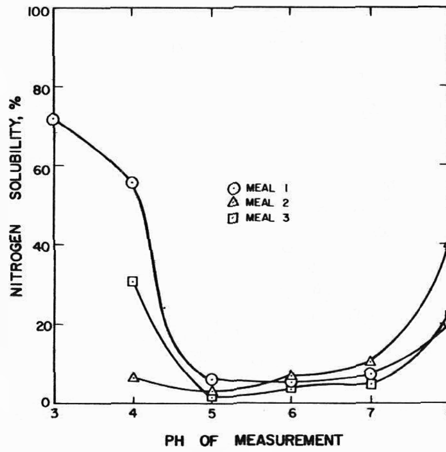

Fig. 6—Nitrogen solubility of Isolate II samples precipitated at pH 7 from Meals 1, 2, and 3.

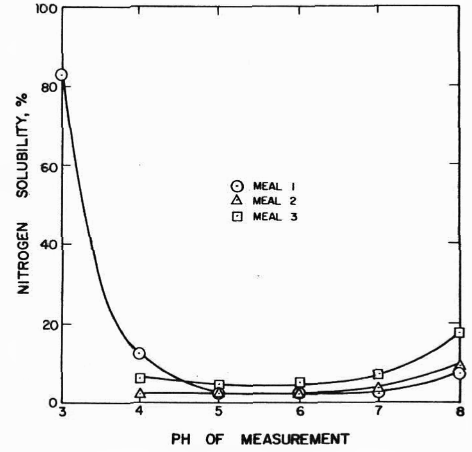

Fig. 7—Nitrogen solubility of Isolate II samples precipitated at pH 8 from Meals 1, 2, and 3.

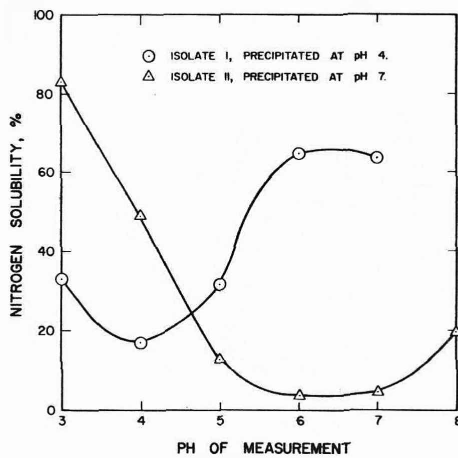

Fig. 8—Nitrogen solubility of Isolates I and II precipitated at pH 7 from Meal 4.

.02N NaOH (above pH 12) is conventionally determined. The data shown in Figure 1 illustrate the fact that meals or isolates may give similar results for nitrogen solubility at high pH levels and yet give widely varying results at lower pH levels. In Table 1, the protein solubilities of the four meals in .02N NaOH were not appreciably different, yet their functional properties were found to differ importantly.

Figures 2 through 8 depict the effects of meal processing method and precipitation pH on nitrogen solubilities of one replicate of the isolates prepared in this study.

Solubility characteristics of Isolate I from a particular meal were found to vary with pH of precipitation. This may be observed in Figures 2, 3, and 4.

Figures 5, 6, and 7 pertain to Isolate II. The pH-solubility of Isolate II was also altered when the pH at which it was precipitated was changed. Isolates from the unheated meal were more soluble than those from heat-treated meals below measurement pH 5 for all pH of precipitation. Nitrogen solubilities in excess of 80% were obtained at measurement pH 3 for Isolate II from Meal 1.

Figure 8 compares the solubility curves of Isolates I and II from the commercial meal. Again, the nitrogen of Isolate II was more than 80% soluble measured at pH 3.

Data on foaming properties are shown in Table 5.

Each entry in the table is the average of two points. Notable differences in foam stability are observable for Isolate II from Meal 1 at the different pH of precipitation. Foam from Isolate II precipitated at pH 7 and 8 was less stable than that obtained from Isolate II precipitated at pH 6. These data were not statistically analyzed.

Results obtained in this investigation confirm the desirability of minimizing tbe use of heat during meal processing and suggest that the end use intended for the protein isolates may determine the pH at which they are precipitated. Isolates could conceivably be custom made

Table 5—Foaming properties of glandless cottonseed protein from meals processed by different methods and precipitated at different pH levels

pH of ppt	Source meal	Foaming capacity, ml. 1/2 min.	Foam stability, ml. 10 min.	Foam stability, ml. 2 hrs.
		Isolate I		
3	1	76.5	24.5	15.5
	2	72.5	21.5	14.5
	3	73.5	24.8	18.5
4	1	82.0	32.0	29.5
	2	66.0	11.3	4.0
	3	74.0	23.8	15.0
	4	77.5	26.5	15.5
5	1	81.5	30.8	24.0
	2	64.0	15.0	10.0
	3	70.5	21.0	15.5
		Isolate II		
6	1	83.5	30.5	29.0
	2	76.5	27.5	23.5
	3	69.5	19.5	11.5
7	1	87.0	3.0	1.0
	2	86.0	37.0	23.0
	3	75.5	25.0	22.0
	4	73.0	4.0	2.5
8	1	82.0	14.5	6.5
	2	55.5	2.5	2.0
	3	63.5	4.5	3.5

to meet a particular food processor's individual needs.

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KAN-ICHI HAYAKAWA
Food Science Department, Rutgers The State University, New Brunswick, N.J. 08903

ESTIMATING FOOD TEMPERATURES DURING VARIOUS PROCESSING OR HANDLING TREATMENTS

SUMMARY—*A procedure was developed for predicting the transient temperature of a solid food exposed to time variable ambient temperatures. Duhamel's theorem was applied to the empirical formulas used for calculating the temperature of a food subjected to a step change in its ambient temperature. The formulas contain two constants: f, the slope index of a heating or cooling curve of a food; and j, the intercept coefficient of the heating or cooling curve. With the procedure developed transient temperature can be estimated without regard to the geometric shape of the food provided the f and j values are known. A table is presented to reduce computations involved in the procedure. Sample calculations are given for a fresh orange, a fresh carrot and a canned food product.*

INTRODUCTION

ACCURATE estimations of temperatures are important in determining proper procedures for processing or handling of food, since temperature plays a great role in determining nutritional and organoleptic quality. Many procedures are available for predicting transient temperatures in solid foods (Ball and Olson, 1957; Charm, 1963; Earle and Fleming, 1967; Gillespy, 1953; Hayakawa, 1969, 1970; Hayakawa and Ball, 1968, 1969a, 1969b; Hicks, 1951; Kopelman and Pflug, 1968; Pflug et al., 1965; Smith et al., 1967; Stumbo, 1965; Wadsworth and Spadaro, 1969). However, these procedures are applicable only when foods have been subjected to one or two constant ambient temperatures, except for the procedure presented by Gillespy, which is limited to foods whose shape can be approximated by a finite cylinder.

Theoretical formulas are available also for predicting temperatures in solid objects (Arpaci, 1966; Carslaw and Jaeger, 1959; Luikov, 1968). However, these formulas have limited value in predicting food temperatures since the conditions imposed during their derivation cannot be satisfied by many foods. Therefore, empirical expressions containing two experimental constants, f and j values (Ball, 1923) have been utilized for predicting food temperatures. The f and j values represent respectively the slope index and the intercept coefficient for a linear portion of the temperature history curve of a food. A classic on the use of these values was presented by Pflug and his coworkers (1965). They developed charts for estimating the f and j values for a solid food when its Biot number and thermal diffusivity are given. However, use of the charts is limited to geometrically simple bodies. Smith et al (1967) developed a procedure for estimating transient temperatures in foods of irregular shapes. Hayakawa (1970) obtained a set of formulas for estimating temperatures in an initial curvilinear portion of a temperature history curve.

During handling and processing of foods ambient temperatures change irregularly with time. Therefore, this study was designed to develop a procedure for predicting food temperatures during various treatments.

EXPERIMENTAL

Derivation of formulas

Duhamel's theorem (Carslaw and Jaeger, 1959) can be used to derive an analytical formula for the temperature in a conductive food subjected to time variable ambient temperatures. For this derivation any formula for estimating the temperature in a solid food subjected to a constant ambient temperature can be used. In the present investigation, this theorem was applied to the experimental formulas developed by Ball (1923) and by Hayakawa (1970).

A careful analysis of the Fourier's equation for transient heat conduction (Carslaw et al., 1959) revealed that the temperature in any solid subjected to a constant ambient temperature, T_a, can be represented by the following expression (Smith et al., 1967):

$$\frac{T_a - T}{T_a - T_o} = \sum_{n=1}^{\infty} \xi_n \cdot e^{-\gamma_n \alpha t/l^2} \quad (1)$$

When Duhamel's theorem is applied to eq. (1) together with the assumed variable ambient temperatures (Fig. 1), a curve, representing time variable ambient temperatures can be approximated with a combination of step changes by dividing the time axis into equal time intervals, Δt.

When Duhamel's theorem is applied to the approximated ambient temperatures, eq. (2) for calculating the food temperature, T (°F), is obtained:

$$\begin{aligned} T &= T_o + (T_{a1m} - T_o) \cdot \Delta U_n \\ &+ (T_{a2m} - T_o) \cdot \Delta U_{n-1} + \ldots\ldots \\ &+ (T_{aim} - T_o) \cdot \Delta U_{n-i+1} + \ldots\ldots \\ &+ (T_{a(n-1)m} - T_o) \cdot \Delta U_2 \\ &+ (T_{anm} - T_o) \cdot \Delta U_1 \\ &= T_o + \sum_{i=1}^{n} (T_{aim} - T_o) \cdot \Delta U_{n-i+1} \quad (2) \end{aligned}$$

For the derivation of eq. (2) see the Appendix. Although all symbols used are defined in the Appendix, Table 1 was prepared to assist in understanding eq. (2).

In this equation, ΔU_i ($i = 1, 2, \ldots\ldots, n$) represents a dimensionless temperature dif-

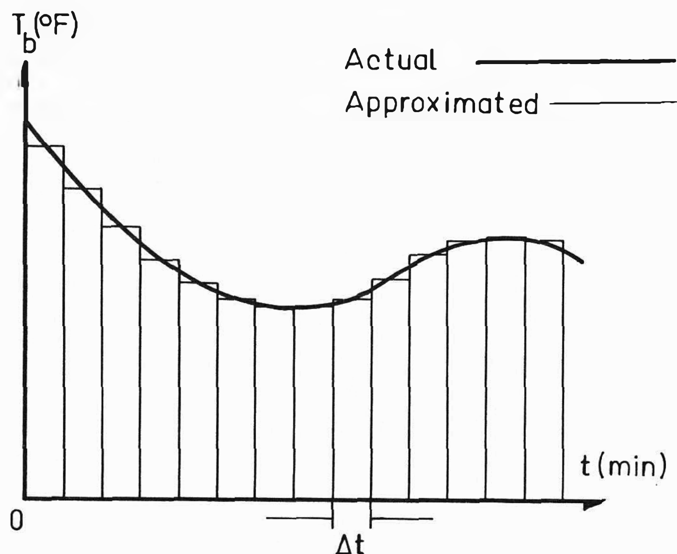

Fig. 1—Approximation of actual ambient temperatures with a series of step changes.

Table 1—Approximation of time variable ambient temperature

Time (min)	True ambient temp (°F)	Approximated ambient temp (°F)	Time interval
0	T_{a0}		
0.5 Δt	T_{a1m}	T_{a1m}	1
1.0 Δt	T_{a1}		
1.5 Δt	T_{a2m}	T_{a2m}	2
2.0 Δt	T_{a3}		
2.5 Δt	T_{a3m}	T_{a3m}	3
3.0 Δt	T_{a3}		
3.5 Δt	T_{a4m}	T_{a4m}	4
4.0 Δt	T_{a4}		
.	.	.	.
.	.	.	.
.	.	.	.
.	.	.	.
.	.	.	.
(i – 1) + 0.5 Δt	T_{aim}	T_{aim}	i
i Δt	T_{ai}		
.	.	.	.
.	.	.	.
.	.	.	.
.	.	.	.
.	.	.	.
[(n – 1) + 0.5] Δt	T_{anm}	T_{anm}	n
n Δt	T_{an}		

ference, which can be calculated from a dimensionless temperature history curve of a food subjected to a constant ambient temperature. To illustrate the estimation of ΔU_i, Figure 2 was prepared. The time axis in this figure is divided into equal intervals. The dimensionless temperature difference, ΔU_i, for ith time interval can be calculated as $\Delta U_i = U_{i-1} - U_i$. The temperature differences for other intervals can be estimated similarly.

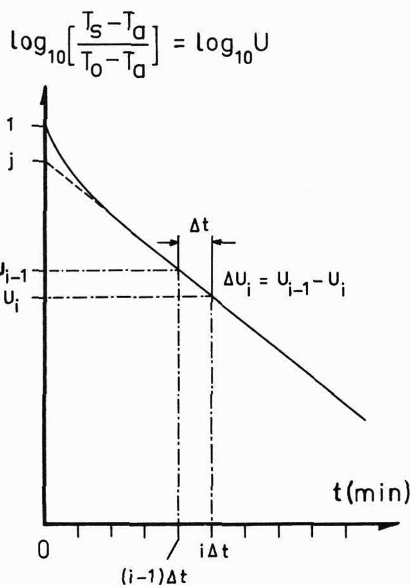

Fig. 2—Estimation of dimensionless temperature difference.

Development of procedure

In predicting a food temperature using eq. (2), a proper numerical magnitude for the time increment, Δt, should be determined, because an accumulation of round-off errors coupled with errors from approximated ambient temperatures are related closely to Δt. Trial calculations were run using temperature history curves for various samples obtained experimentally. The samples included fresh apples, cucumbers, ham, pears, and tomatoes. In addition a canned bentonite suspension and commercially canned foods were used. Each sample was exposed to a constant ambient temperature and also to time variable ambient temperatures. Temperatures at various locations in each sample were monitored with copper constantan thermocouples. From the data obtained from exposure to the constant ambient temperature, f and j values were estimated. From these values and eq. (2), temperatures of each sample, exposed to the variable ambient temperature treatment, were calculated. These values were compared with the experimental values. In the trial calculations various numerical magnitudes for Δt were used in order to obtain its proper magnitude. After careful analysis of the estimated and experimental temperatures, the following value for Δt, eq. (3), was most satisfactory:

$$\Delta t = f/20 \qquad (3)$$

Dimensionless temperature differences ΔU_i (i = 1, 2, . . ., n) were estimated with the formulas developed by Ball (1923) and Hayakawa (1970). Ball's formula was used to estimate the differences in the linear portion of the temperature history curves and Hayakawa's formulas were used to estimate those in the curvilinear portion. Formulas for the curvilinear portion, which were derived previously, are applicable when $0.4 \le j \le 3.0$. However, several j values obtained in the experimental temperature history curves were less than 0.4 especially when the temperatures were measured close to the surface of the sample. Therefore, a set of new experimental formulas, which could be applied to any j value in this range, $0.045 \le j \le 0.4$ was developed. These formulas are given in the Appendix.

Great computational effort is required for calculating the temperature differences, ΔU_i, for various time intervals. Therefore these temperature differences were calculated for various j values and also for various time intervals with an IBM 360 computer. In this calculation, eq. (3) was used to determine the time increment, Δt. Table 2 shows an abridged tabulation for ΔU_i.

By utilizing Table 2 and eq. (2), transient temperatures in any conductive food can be estimated when the initial temperature distribution in the food is uniform and when the food is exposed to time variable ambient temperatures by using the following series of computations.

(1) Locate a point in food, at which temperatures should be estimated during exposure to variable ambient temperatures, and then determine a temperature history curve at this point when the food is subjected to a constant ambient temperature. This curve is obtained by plotting the common logs of temperature differences between the food and the constant ambient temperature against heating or cooling times.

(2) Calculate the f and j values of the curve estimated in step (1).

(3) Determine dimensionless temperature differences, ΔU_i, by entering the j value into Table 2. Determine a value for a uniform time increment, Δt, by using eq. (3).

(4) Find the variable ambient temperatures, T_{aim}, at each time interval shown below.

Time	0.5 Δt	1.5 Δt	2.5 Δt	. . . .	[(n – 1) + 0.5] Δt
Temp.	T_{a1m}	T_{a2m}	T_{a3m}	. . . .	T_{anm}

(5) Estimate the food temperature, T, at each time interval by entering ΔU_i and T_{aim} into eq. (2).

The following condition must be satisfied in utilizing the proposed procedure since it was assumed in applying Duhamel's theorem. Thermophysical characteristics for both the surface of the food and for the heating or cooling medium during exposure to a constant ambient temperature should be identical to those characteristics obtained if the food is exposed to variable ambient temperatures. This condition can be stated as follows: The Biot number during the constant ambient temperature exposure should be approximately equal to the Biot number during the variable ambient temperature exposure.

Sample calculation

In order to illustrate the application of the computational procedure described, calculations of temperatures in a fresh orange, fresh carrot, and canned conductive food are given below. These foods were selected because they were typical of the foods used during these studies.

Fresh Valencia orange. Into each of Valencia oranges, purchased locally, were imbedded three copper-constantan thermocouple junctions. The locations of these junctions are 0.295 (a), 0.653 (b), or 0.927 (c), which represent ratios between the radial distances of the thermocouple junctions and the radius of the orange. The thermocouples were made from 30 gauge thermocouple wire with paper and fiberglass insulation. In order to obtain uniform temperature distributions in the oranges, they were placed in a 60°F incubator for 12 hr at 70–80% relative humidity. When approximately uniform temperature distributions were monitored through a recording potentiometer, the samples were subjected to a constant ambient temperature treatment by placing them in a forced air high-low temperature test chamber, whose temperature was maintained at 37°F and whose relative humidity was approximately 70–80%. Temperatures in the samples were monitored by the recording potentiometer during this treatment in order to determine f and j values.

When the temperature of each sample became approximately equal to the ambient temperature, each sample was subjected to a variable ambient temperature treatment by manually regulating the temperatures in the test chamber. The resultant ambient temperatures are given in Figure 3.

Calculations for only location (c) in one orange are given below. The diameter of this orange was 2.75 in. when measured from top to bottom and 3.15 in. when measured from side to side.

Table 2—Dimensionless temperature differences, ΔU_i, for various j values and for various time intervals[a]

Multiple of Δt	ΔU, Values for the following j values 0.20	0.40	0.60	0.80	1.00	1.20	1.40	1.60
1	0.73894E 00	0.52478E 00	0.19376E 00	0.17118E 00	0.10875E 00	0.17797E-01	0.99085E-02	0.71720E-02
2	0.69736E-01	0.10815E 00	0.14888E 00	0.13595E 00	0.96923E-01	0.51357E-01	0.29089E-01	0.21181E-01
3	0.36881E-01	0.63765E-01	0.11597E 00	0.10911E 00	0.86382E-01	0.81312E-01	0.46441E-01	0.34220E-01
4	0.23956E-01	0.44078E-01	0.91456E-01	0.79000E-01	0.76988E-01	0.92386E-01	0.60971E-01	0.45728E-01
5	0.17177E-01	0.32941E-01	0.72946E-01	0.54893E-01	0.68616E-01	0.82339E-01	0.71984E-01	0.55266E-01
6	0.13069E-01	0.25817E-01	0.58786E-01	0.48923E-01	0.61154E-01	0.73385E-01	0.79945E-01	0.62545E-01
7	0.10901E-01	0.21801E-01	0.47826E-01	0.43603E-01	0.54503E-01	0.65404E-01	0.76305E-01	0.67435E-01
8	0.97153E-02	0.19431E-01	0.31516E-01	0.38861E-01	0.48576E-01	0.58292E-01	0.68007E-01	0.69962E-01
9	0.86587E-02	0.17317E-01	0.25976E-01	0.34635E-01	0.43294E-01	0.51952E-01	0.60611E-01	0.68791E-01
10	0.77171E-02	0.15434E-01	0.23151E-01	0.30869E-01	0.38585E-01	0.46303E-01	0.54020E-01	0.61737E-01
11	0.68779E-02	0.13756E-01	0.20634E-01	0.27512E-01	0.34390E-01	0.41267E-01	0.48145E-01	0.55023E-01
12	0.61299E-02	0.12260E-01	0.18390E-01	0.24520E-01	0.30649E-01	0.36779E-01	0.42909E-01	0.49039E-01
13	0.54633E-02	0.10927E-01	0.16390E-01	0.21853E-01	0.27317E-01	0.32780E-01	0.38243E-01	0.43706E-01
14	0.48692E-02	0.97384E-02	0.14608E-01	0.19477E-01	0.24346E-01	0.29215E-01	0.34084E-01	0.38953E-01
15	0.43397E-02	0.86793E-02	0.13019E-01	0.17359E-01	0.21698E-01	0.26038E-01	0.30378E-01	0.34717E-01
16	0.38677E-02	0.77354E-02	0.11603E-01	0.15471E-01	0.19338E-01	0.23206E-01	0.27074E-01	0.30942E-01
17	0.34471E-02	0.68942E-02	0.10341E-01	0.13789E-01	0.17236E-01	0.20683E-01	0.24130E-01	0.27577E-01
18	0.30722E-02	0.61445E-02	0.92168E-02	0.12289E-01	0.15361E-01	0.18434E-01	0.21506E-01	0.24578E-01
19	0.27381E-02	0.54763E-02	0.82144E-02	0.10953E-01	0.13691E-01	0.16429E-01	0.19167E-01	0.21905E-01
20	0.24404E-02	0.48808E-02	0.73211E-02	0.97616E-02	0.12202E-01	0.14642E-01	0.17083E-01	0.19523E-01
21	0.21749E-02	0.43498E-02	0.65248E-02	0.86997E-02	0.10875E-01	0.13050E-01	0.15224E-01	0.17399E-01
22	0.19385E-02	0.38770E-02	0.58155E-02	0.77540E-02	0.96924E-02	0.11631E-01	0.13569E-01	0.15508E-01
23	0.17276E-02	0.34553E-02	0.51830E-02	0.69106E-02	0.86383E-02	0.10366E-01	0.12094E-01	0.13821E-01
24	0.15398E-02	0.30796E-02	0.46193E-02	0.61591E-02	0.76988E-02	0.92387E-02	0.10778E-01	0.12318E-01
25	0.13723E-02	0.27446E-02	0.41170E-02	0.54893E-02	0.68616E-02	0.82339E-02	0.96062E-02	0.10979E-01
26	0.12231E-02	0.24461E-02	0.36692E-02	0.48923E-02	0.61153E-02	0.73383E-02	0.85615E-02	0.97845E-02
27	0.10901E-02	0.21802E-02	0.32702E-02	0.43603E-02	0.54504E-02	0.65405E-02	0.76305E-02	0.87206E-02
28	0.97154E-03	0.19431E-02	0.29146E-02	0.38862E-02	0.48577E-02	0.58292E-02	0.68007E-02	0.77723E-02
29	0.86588E-03	0.17318E-02	0.25976E-02	0.34635E-02	0.43294E-02	0.51953E-02	0.60611E-02	0.69270E-02
30	0.77171E-03	0.15434E-02	0.23151E-02	0.30869E-02	0.38586E-02	0.46303E-02	0.54020E-02	0.61737E-02
31	0.68778E-03	0.13756E-02	0.20633E-02	0.27511E-02	0.34389E-02	0.41267E-02	0.48145E-02	0.55022E-02
32	0.61300E-03	0.12260E-02	0.18390E-02	0.24520E-02	0.30650E-02	0.36780E-02	0.42910E-02	0.49040E-02
33	0.54633E-03	0.10927E-02	0.16390E-02	0.21853E-02	0.27317E-02	0.32780E-02	0.38243E-02	0.43707E-02
34	0.48692E-03	0.97385E-03	0.14608E-02	0.19477E-02	0.24346E-02	0.29215E-02	0.34085E-02	0.38954E-02
35	0.43397E-03	0.86793E-03	0.13019E-02	0.17359E-02	0.21698E-02	0.26038E-02	0.30378E-02	0.34717E-02
36	0.38677E-03	0.77353E-03	0.11603E-02	0.15471E-02	0.19338E-02	0.23206E-02	0.27074E-02	0.30941E-02
37	0.34471E-03	0.68942E-03	0.10341E-02	0.13789E-02	0.17236E-02	0.20683E-02	0.24130E-02	0.27577E-02
38	0.30722E-03	0.61445E-03	0.92168E-03	0.12289E-02	0.15361E-02	0.18434E-02	0.21506E-02	0.24578E-02
39	0.27381E-03	0.54763E-03	0.82144E-03	0.10953E-02	0.13691E-02	0.16429E-02	0.19167E-02	0.21905E-02
40	0.24404E-03	0.48808E-03	0.73212E-03	0.97616E-03	0.12202E-02	0.14642E-02	0.17083E-02	0.19523E-02
41	0.21749E-03	0.43498E-03	0.65248E-03	0.86997E-03	0.10875E-02	0.13050E-02	0.15225E-02	0.17399E-02
42	0.19385E-03	0.38770E-03	0.58154E-03	0.77539E-03	0.96924E-03	0.11631E-02	0.13569E-02	0.15508E-02
43	0.17277E-03	0.34553E-03	0.51830E-03	0.69106E-03	0.86383E-03	0.10366E-02	0.12094E-02	0.13821E-02
44	0.15398E-03	0.30795E-03	0.46193E-03	0.61591E-03	0.76989E-03	0.92386E-03	0.10778E-02	0.12318E-02
45	0.13723E-03	0.27447E-03	0.41170E-03	0.54893E-03	0.68616E-03	0.82340E-03	0.96063E-03	0.10979E-02
46	0.12231E-03	0.24461E-03	0.36692E-03	0.48923E-03	0.61153E-03	0.73384E-03	0.85615E-03	0.97845E-03
47	0.10910E-03	0.21801E-03	0.32702E-03	0.43603E-03	0.54504E-03	0.65405E-03	0.76305E-03	0.87206E-03
48	0.97154E-04	0.19431E-03	0.29146E-03	0.38862E-03	0.48577E-03	0.58292E-03	0.68008E-03	0.77723E-03
49	0.86588E-04	0.17318E-03	0.25976E-03	0.34635E-03	0.43294E-03	0.51953E-03	0.60612E-03	0.69270E-03
50	0.77171E-04	0.15434E-03	0.23151E-03	0.30869E-03	0.38586E-03	0.46303E-03	0.54020E-03	0.61737E-03
51	0.68778E-04	0.13756E-03	0.20633E-03	0.27511E-03	0.34389E-03	0.41267E-03	0.48145E-03	0.55023E-03
52	0.61300E-04	0.12260E-03	0.18390E-03	0.24520E-03	0.30650E-03	0.36780E-03	0.42909E-03	0.49040E-03
53	0.54633E-04	0.10927E-03	0.16390E-03	0.21853E-03	0.27317E-03	0.32780E-03	0.38243E-03	0.43706E-03
57	0.48692E-04	0.97385E-04	0.14608E-03	0.19477E-03	0.24346E-03	0.29215E-03	0.34085E-03	0.38954E-03
55	0.43397E-04	0.86793E-04	0.13019E-03	0.17359E-03	0.21698E-03	0.26038E-03	0.30378E-03	0.34717E-03
56	0.38677E-04	0.77353E-04	0.11603E-03	0.15471E-03	0.19338E-03	0.23206E-03	0.27074E-03	0.30941E-03
57	0.34471E-04	0.68943E-04	0.10341E-03	0.13788E-03	0.17236E-03	0.20683E-03	0.24130E-03	0.27577E-03
58	0.30723E-04	0.61445E-04	0.92168E-04	0.12289E-03	0.15361E-03	0.18434E-03	0.21506E-03	0.24578E-03
59	0.27381E-04	0.54763E-04	0.82144E-04	0.10953E-03	0.13691E-03	0.16429E-03	0.19167E-03	0.21905E-03
60	0.24404E-04	0.48808E-04	0.73212E-04	0.97616E-04	0.12202E-03	0.14642E-03	0.17083E-03	0.19523E-03
61	0.21749E-04	0.43499E-04	0.65248E-04	0.86997E-04	0.10875E-03	0.13050E-03	0.15224E-03	0.17399E-03
62	0.19385E-04	0.38770E-04	0.58155E-04	0.77539E-04	0.96924E-04	0.11631E-03	0.13569E-03	0.15508E-03
63	0.17277E-04	0.34553E-04	0.51829E-04	0.69106E-04	0.86383E-04	0.10366E-03	0.12094E-03	0.13821E-03
64	0.15398E-04	0.30795E-04	0.46193E-04	0.61591E-04	0.76988E-04	0.92386E-04	0.10778E-03	0.12318E-03
65	0.13723E-04	0.27446E-04	0.41170E-04	0.54893E-04	0.68616E-04	0.82340E-04	0.96063E-04	0.10979E-03
66	0.12231E-04	0.24461E-04	0.36692E-04	0.48923E-04	0.61153E-04	0.73384E-04	0.85615E-04	0.97845E-04

Table 2—Dimensionless temperature differences, ΔU_i for various j values and for various time intervals[a] (Continued)

Multiple of Δt	ΔU_i Values for the following j values: 1.80	2.00	2.20	2.40	2.60	2.80	3.00
1	0.57517E-02	0.48704E-02	0.42658E-02	0.38220E-02	0.34821E-02	0.32129E-02	0.29953E-02
2	0.17040E-01	0.14457E-01	0.12675E-01	0.11368E-01	0.10364E-01	0.95691E-02	0.89239E-02
3	0.27699E-01	0.23589E-01	0.20740E-01	0.18636E-01	0.17016E-01	0.15729E-01	0.14682E-01
4	0.37356E-01	0.31997E-01	0.28241E-01	0.25450E-01	0.23289E-01	0.21565E-01	0.20158E-01
5	0.45702E-01	0.39444E-01	0.34995E-01	0.31657E-01	0.29054E-01	0.26966E-01	0.25255E-01
6	0.52508E-01	0.45744E-01	0.40850E-01	0.37130E-01	0.34201E-01	0.31835E-01	0.29884E-01
7	0.57637E-01	0.50778E-01	0.45696E-01	0.41770E-01	0.38643E-01	0.36093E-01	0.33977E-01
8	0.61043E-01	0.54478E-01	0.49464E-01	0.45512E-01	0.42317E-01	0.39684E-01	0.37478E-01
9	0.62770E-01	0.56841E-01	0.52132E-01	0.48325E-01	0.45190E-01	0.42569E-01	0.40352E-01
10	0.62933E-01	0.57917E-01	0.53718E-01	0.50208E-01	0.47250E-01	0.44736E-01	0.42580E-01
11	0.61711E-01	0.57807E-01	0.54278E-01	0.51193E-01	0.48515E-01	0.46189E-01	0.44163E-01
12	0.55709E-01	0.56642E-01	0.53896E-01	0.51337E-01	0.49023E-01	0.46956E-01	0.45117E-01
13	0.49170E-01	0.54582E-01	0.52685E-01	0.50716E-01	0.48828E-01	0.47075E-01	0.45472E-01
14	0.43823E-01	0.51801E-01	0.50767E-01	0.49424E-01	0.48002E-01	0.46602E-01	0.45273E-01
15	0.39057E-01	0.43397E-01	0.48278E-01	0.47566E-01	0.46627E-01	0.45604E-01	0.44570E-01
16	0.34809E-01	0.38677E-01	0.45354E-01	0.45250E-01	0.44791E-01	0.44151E-01	0.43427E-01
17	0.31024E-01	0.34471E-01	0.41206E-01	0.42583E-01	0.42584E-01	0.42321E-01	0.41907E-01
18	0.27650E-01	0.30722E-01	0.33795E-01	0.39670E-01	0.40096E-01	0.40190E-01	0.40076E-01
19	0.24643E-01	0.27381E-01	0.30120E-01	0.36608E-01	0.37412E-01	0.37834E-01	0.38003E-01
20	0.21963E-01	0.24404E-01	0.26844E-01	0.31775E-01	0.34609E-01	0.35323E-01	0.35749E-01
21	0.19574E-01	0.21749E-01	0.23924E-01	0.26099E-01	0.31761E-01	0.32723E-01	0.33377E-01
22	0.17446E-01	0.19385E-01	0.21323E-01	0.23262E-01	0.28925E-01	0.30093E-01	0.30941E-01
23	0.15549E-01	0.17277E-01	0.19004E-01	0.20732E-01	0.23953E-01	0.27482E-01	0.28489E-01
24	0.13858E-01	0.15398E-01	0.16938E-01	0.18477E-01	0.20017E-01	0.24933E-01	0.26065E-01
25	0.12351E-01	0.13723E-01	0.15095E-01	0.16468E-01	0.17840E-01	0.22481E-01	0.23703E-01
26	0.11008E-01	0.12231E-01	0.13454E-01	0.14677E-01	0.15900E-01	0.17751E-01	0.21431E-01
27	0.98107E-02	0.10901E-01	0.11991E-01	0.13081E-01	0.14171E-01	0.15261E-01	0.19273E-01
28	0.87439E-02	0.97154E-02	0.10687E-01	0.11658E-01	0.12630E-01	0.13602E-01	0.17244E-01
29	0.77929E-02	0.86588E-02	0.95246E-02	0.10391E-01	0.11256E-01	0.12122E-01	0.12988E-01
30	0.69454E-02	0.77171E-02	0.84888E-02	0.92606E-02	0.10032E-01	0.10804E-01	0.11576E-01
31	0.61900E-02	0.68778E-02	0.75656E-02	0.82533E-02	0.89412E-02	0.96289E-02	0.10317E-01
32	0.55170E-02	0.61300E-02	0.67430E-02	0.73559E-02	0.79690E-02	0.85819E-02	0.91949E-02
33	0.49170E-02	0.54633E-02	0.60096E-02	0.65560E-02	0.71023E-02	0.76487E-02	0.81950E-02
34	0.43823E-02	0.48692E-02	0.53562E-02	0.58431E-02	0.63300E-02	0.68169E-02	0.73038E-02
35	0.39057E-02	0.43397E-02	0.47736E-02	0.52076E-02	0.56416E-02	0.60755E-02	0.65095E-02
36	0.34809E-02	0.38677E-02	0.42544E-02	0.46412E-02	0.50280E-02	0.54147E-02	0.58015E-02
37	0.31024E-02	0.34471E-02	0.37918E-02	0.41366E-02	0.44813E-02	0.48260E-02	0.51707E-02
38	0.27650E-02	0.30722E-02	0.33795E-02	0.36867E-02	0.39939E-02	0.43011E-02	0.46084E-02
39	0.24643E-02	0.27382E-02	0.30120E-02	0.32858E-02	0.35596E-02	0.38334E-02	0.41072E-02
40	0.21964E-02	0.24404E-02	0.26844E-02	0.29285E-02	0.31725E-02	0.34166E-02	0.36606E-02
41	0.19574E-02	0.21749E-02	0.23924E-02	0.26099E-02	0.28274E-02	0.30449E-02	0.32624E-02
42	0.17446E-02	0.19385E-02	0.21323E-02	0.23262E-02	0.25200E-02	0.27139E-02	0.29077E-02
43	0.15549E-02	0.17277E-02	0.19004E-02	0.20732E-02	0.22459E-02	0.24187E-02	0.25915E-02
44	0.13858E-02	0.15398E-02	0.16938E-02	0.18477E-02	0.20017E-02	0.21557E-02	0.23097E-02
45	0.12351E-02	0.13723E-02	0.15096E-02	0.16468E-02	0.17840E-02	0.19213E-02	0.20585E-02
46	0.11008E-02	0.12231E-02	0.13454E-02	0.14677E-02	0.15900E-02	0.17123E-02	0.18346E-02
47	0.98107E-03	0.10901E-02	0.11991E-02	0.13081E-02	0.14171E-02	0.15261E-02	0.16351E-02
48	0.87439E-03	0.97154E-03	0.10687E-02	0.11658E-02	0.12630E-02	0.13602E-02	0.14573E-02
49	0.77929E-03	0.86588E-03	0.95247E-03	0.10391E-02	0.11256E-02	0.12122E-02	0.12988E-02
50	0.69454E-03	0.77171E-03	0.84889E-03	0.92606E-03	0.10032E-02	0.10804E-02	0.11576E-02
51	0.61900E-03	0.68778E-03	0.75656E-03	0.82533E-03	0.89411E-03	0.96289E-03	0.10317E-02
52	0.55170E-03	0.61300E-03	0.67430E-03	0.73560E-03	0.79690E-03	0.85820E-03	0.91949E-03
53	0.49170E-03	0.54633E-03	0.60097E-03	0.65560E-03	0.71023E-03	0.76487E-03	0.81950E-03
54	0.43823E-03	0.48692E-03	0.53561E-03	0.58431E-03	0.63300E-03	0.68169E-03	0.73038E-03
55	0.39057E-03	0.43397E-03	0.47737E-03	0.52076E-03	0.56416E-03	0.60755E-03	0.65095E-03
56	0.34809E-03	0.38677E-03	0.42544E-03	0.46412E-03	0.50280E-03	0.54147E-03	0.58015E-03
57	0.31024E-03	0.34471E-03	0.37918E-03	0.41366E-03	0.44812E-03	0.48260E-03	0.51707E-03
58	0.27650E-03	0.30723E-03	0.33795E-03	0.36867E-03	0.39939E-03	0.43011E-03	0.46084E-03
59	0.24643E-03	0.27381E-03	0.30120E-03	0.32858E-03	0.35596E-03	0.38334E-03	0.41072E-03
60	0.21964E-03	0.24404E-03	0.26844E-03	0.29285E-03	0.31725E-03	0.34166E-03	0.36606E-03
61	0.19574E-03	0.21749E-03	0.23924E-03	0.26099E-03	0.28274E-03	0.30449E-03	0.32624E-03
62	0.17446E-03	0.19385E-03	0.21323E-03	0.23262E-03	0.25200E-03	0.27139E-03	0.29077E-03
63	0.15549E-03	0.17277E-03	0.19004E-03	0.20732E-03	0.22459E-03	0.24187E-03	0.25915E-03
64	0.13858E-03	0.15398E-03	0.16937E-03	0.18477E-03	0.20017E-03	0.21557E-03	0.23097E-03
65	0.12351E-03	0.13723E-03	0.15096E-03	0.16468E-03	0.17840E-03	0.19212E-03	0.20585E-03
66	0.11008E-03	0.12231E-03	0.13454E-03	0.14677E-03	0.15900E-03	0.17123E-03	0.18346E-03

Table 2—Dimensionless temperature differences, ΔU_i, for various j values and for various time intervals[a] (Continued)

Multiple	ΔU_i Values for the following j values							
of Δt	0.20	0.40	0.60	0.80	1.00	1.20	1.40	1.60
67	0.10901E-04	0.21802E-04	0.32702E-04	0.43603E-04	0.54504E-04	0.65405E-04	0.76306E-04	0.87206E-04
68	0.97153E-05	0.19431E-04	0.29146E-04	0.38862E-04	0.48577E-04	0.58292E-04	0.68008E-04	0.77723E-04
69	0.86588E-05	0.17318E-04	0.25976E-04	0.34635E-04	0.43294E-04	0.51953E-04	0.60612E-04	0.69270E-04
70	0.77171E-05	0.15434E-04	0.23151E-04	0.30868E-04	0.38585E-04	0.46303E-04	0.54020E-04	0.61737E-04
71	0.68778E-05	0.13756E-04	0.20633E-04	0.27511E-04	0.34389E-04	0.41267E-04	0.48145E-04	0.55023E-04
72	0.61300E-05	0.12260E-04	0.18390E-04	0.24520E-04	0.30650E-04	0.36780E-04	0.42910E-04	0.49040E-04
73	0.54633E-05	0.10927E-04	0.16390E-04	0.21853E-04	0.27317E-04	0.32780E-04	0.38243E-04	0.43707E-04
74	0.48692E-05	0.97385E-05	0.14608E-04	0.19477E-04	0.24346E-04	0.29215E-04	0.34085E-04	0.38954E-04
75	0.43397E-05	0.86793E-05	0.13019E-04	0.17359E-04	0.21698E-04	0.26038E-04	0.30378E-04	0.34718E-04
76	0.38677E-05	0.77353E-05	0.11603E-04	0.15471E-04	0.19338E-04	0.23206E-04	0.27074E-04	0.30941E-04
77	0.34471E-05	0.68943E-05	0.10341E-04	0.13788E-04	0.17236E-04	0.20683E-04	0.24130E-04	0.27577E-04
78	0.30723E-05	0.61445E-05	0.92167E-05	0.12289E-04	0.15361E-04	0.18434E-04	0.21506E-04	0.24578E-04
79	0.27381E-05	0.54763E-05	0.82145E-05	0.10953E-04	0.13691E-04	0.16429E-04	0.19167E-04	0.21905E-04
80	0.24404E-05	0.48808E-05	0.73212E-05	0.97616E-05	0.12202E-04	0.14642E-04	0.17083E-04	0.19523E-04
81	0.21749E-05	0.43499E-05	0.65248E-05	0.86997E-05	0.10875E-04	0.13050E-04	0.15225E-04	0.17399E-04
82	0.19385E-05	0.38770E-05	0.58154E-05	0.77539E-05	0.96924E-05	0.11631E-04	0.13569E-04	0.15508E-04
83	0.17277E-05	0.34553E-05	0.51830E-05	0.69106E-05	0.86383E-05	0.10366E-04	0.12094E-04	0.13821E-04
84	0.15398E-05	0.30795E-05	0.46193E-05	0.61591E-05	0.76989E-05	0.92386E-05	0.10778E-04	0.12318E-04
85	0.13723E-05	0.27447E-05	0.41170E-05	0.54893E-05	0.68616E-05	0.82340E-05	0.96063E-05	0.10979E-04
86	0.12231E-05	0.24461E-05	0.36692E-05	0.48923E-05	0.61153E-05	0.73384E-05	0.85614E-05	0.97845E-05
87	0.10901E-05	0.21802E-05	0.32702E-05	0.43603E-05	0.54504E-05	0.65405E-05	0.76305E-05	0.87206E-05
88	0.97154E-05	0.19431E-05	0.29146E-05	0.38862E-05	0.48577E-05	0.58292E-05	0.68008E-05	0.77723E-05
89	0.86588E-06	0.17318E-05	0.25976E-05	0.34635E-05	0.43294E-05	0.51953E-05	0.60611E-05	0.69270E-05
90	0.77172E-06	0.15434E-05	0.23152E-05	0.30869E-05	0.38586E-05	0.46303E-05	0.54020E-05	0.61737E-05
91	0.68778E-06	0.13756E-05	0.20633E-05	0.27511E-05	0.34389E-05	0.41267E-05	0.48145E-05	0.55023E-05
92	0.61300E-06	0.12260E-05	0.18390E-05	0.24520E-05	0.30650E-05	0.36780E-05	0.42910E-05	0.49040E-05
93	0.54633E-06	0.10927E-05	0.16390E-05	0.21853E-05	0.27317E-05	0.32780E-05	0.38243E-05	0.43707E-05
94	0.48692E-06	0.97385E-06	0.14608E-05	0.19477E-05	0.24346E-05	0.29215E-05	0.34085E-05	0.38954E-05
95	0.43397E-06	0.86793E-06	0.13019E-05	0.17359E-05	0.21698E-05	0.26038E-05	0.30378E-05	0.34717E-05
96	0.38677E-06	0.77353E-06	0.11603E-05	0.15471E-05	0.19338E-05	0.23206E-05	0.27074E-05	0.30941E-05
97	0.34471E-06	0.68943E-06	0.10341E-05	0.13789E-05	0.17236E-05	0.20683E-05	0.24130E-05	0.27577E-05
98	0.30723E-06	0.61445E-06	0.92167E-06	0.12289E-05	0.15361E-05	0.18434E-05	0.21506E-05	0.24578E-05
99	0.27382E-06	0.54763E-06	0.82145E-06	0.10953E-05	0.13691E-05	0.16429E-05	0.19167E-05	0.21905E-05
100	0.24404E-06	0.48808E-06	0.73212E-06	0.97616E-06	0.12202E-05	0.14642E-05	0.17083E-05	0.19523E-05

(1) and (2) From the heat penetration data, f = 36.3 (1/6 hr) and j = 1.2454 are obtained. This unconventional time unit was used since the strip-chart speed for the recording potentiometer was 16 in./hr.

(3) By entering the j value obtained above in Table 2, values for the temperature differences are determined (Table 3). Note that ΔU_i values are recorded in the reverse order in this table since they are multiplied with ambient temperature data in this order. Δt is determined from eq. (3) as Δt = 36.3/20 = 1.815 (1/16 hr).

(4) The ambient temperature at each time interval is obtained from recorded data and then the temperature of the orange at the zero time of variable temperature treatment, T_o, is subtracted from the resultant ambient temperatures (Table 3).

(5) Temperatures of the orange at each time interval were estimated from the data given in Table 3 by using eq. (2). For example, the temperature at the 16th time interval, 29.0 (1/16 hr), is estimated as follows: $T = 38.2 + 0.02408 \times 7.9 + 0.02702 \times 16.4 + 0.03032 \times 16.1 + \ldots\ldots + 0.04470 \times 27.3 + 0.01543 \times 28.1 \simeq 55.9$ (°F).

The temperatures at other intervals can be estimated similarly. These temperatures are given in Figure 3 together with the tempertures determined experimentally. Sample temperatures at other locations are also shown. A difference of less than 2.5°F between the estimated and experimental temperatures was found. Similar results were obtained for the other oranges. Several things could cause these differences including:

1. A change in surface condition.
2. Slight errors involved in the experimental formulas.
3. Two sharp peaks in the ambient temperature history curve.

Among these factors, the last one would be a major cause for the observed deviations.

Fresh carrots. Similar experiments using locally purchased carrots were carried out. Copper-constantan thermocouples were placed at either 0.212, 0.519, or 0.911. Each value represents a ratio of the radial distance to the junction and the radius of carrot measured at the center of its length. Results obtained in only one experiment are presented.

The following conditions were used to determine f and j values: T_o = 73.7°F, T_a = 36.5°F, relative humidity = 70–80%. A thermocouple junction was placed at 0.212. The carrot was 9.45 in. long and 0.29 in. in diameter, which was measured at the center of its length. Values for f and j were 17.3 (1/16 hr) and 1.179 respectively.

The temperature of the carrot was 36.2°F when a variable ambient temperature treatment was initiated. The ambient and sample temperatures during this treatment are shown in Figure 4. The estimated sample temperatures are also shown in the same figure. There was close agreement between the predicted and experimental temperatures. Similar results were obtained in experiments for the other carrots.

Canned food. 211 × 400 cans of a solid food were processed commercially using a hydrostatic cooker. Curve A in Figure 5 shows the heating medium temperatures during processing; the initial and last portions of this curve represent temperature changes in water columns. In order to predict temperatures at the center of the product, f and j values were estimated from heat penetration data for the same product, which had been processed in a 245°F still retort: f = 43.0 min and j = 1.5. Using these experimental constants, the temperatures in the sample were estimated (Fig. 5). The experimentally determined product temperatures are shown in the same figure. There is fairly close agreement between the predicted and experimental temperatures dur-

Table 2—Dimensionless temperature differences, ΔU_i, for various j values and for various time intervals[a] (Continued)

Multiple of Δt	ΔU_i Values for the following j values: 1.80	2.00	2.20	2.40	2.60	2.80	3.00
67	0.98107E-04	0.10901E-03	0.11991E-03	0.13081E-03	0.14171E-03	0.15261E-03	0.16351E-03
68	0.87438E-04	0.97154E-04	0.10687E-03	0.11658E-03	0.12630E-03	0.13601E-03	0.14573E-03
69	0.77929E-04	0.86588E-04	0.95247E-04	0.10391E-03	0.11256E-03	0.12122E-03	0.12988E-03
70	0.69454E-04	0.77171E-04	0.84888E-04	0.92605E-04	0.10032E-03	0.10804E-03	0.11576E-03
71	0.61900E-04	0.68778E-04	0.75656E-04	0.82534E-04	0.89412E-04	0.96289E-04	0.10317E-03
72	0.55170E-04	0.61300E-04	0.67430E-04	0.73560E-04	0.79690E-04	0.85820E-04	0.91949E-04
73	0.49170E-04	0.54633E-04	0.60097E-04	0.65560E-04	0.71023E-04	0.76487E-04	0.81950E-04
74	0.43823E-04	0.48692E-04	0.53562E-04	0.58431E-04	0.63300E-04	0.68169E-04	0.73039E-04
75	0.39057E-04	0.43397E-04	0.47736E-04	0.52076E-04	0.56416E-04	0.60756E-04	0.65095E-04
76	0.34809E-04	0.38677E-04	0.42544E-04	0.46412E-04	0.50280E-04	0.54147E-04	0.58015E-04
77	0.31024E-04	0.34471E-04	0.37918E-04	0.41366E-04	0.44813E-04	0.48260E-04	0.51707E-04
78	0.27650E-04	0.30722E-04	0.33795E-04	0.36867E-04	0.39939E-04	0.43012E-04	0.46084E-04
79	0.24643E-04	0.27381E-04	0.30120E-04	0.32858E-04	0.35596E-04	0.38334E-04	0.41072E-04
80	0.21964E-04	0.24404E-04	0.26844E-04	0.29285E-04	0.31725E-04	0.34166E-04	0.36606E-04
81	0.19574E-04	0.21749E-04	0.23924E-04	0.26099E-04	0.28274E-04	0.30449E-04	0.32624E-04
82	0.17446E-04	0.19385E-04	0.21323E-04	0.23262E-04	0.25200E-04	0.27139E-04	0.29077E-04
83	0.15549E-04	0.17277E-04	0.19004E-04	0.20732E-04	0.22460E-04	0.24187E-04	0.25915E-04
84	0.13858E-04	0.15398E-04	0.16938E-04	0.18477E-04	0.20017E-04	0.21557E-04	0.23097E-04
85	0.12351E-04	0.13723E-04	0.15096E-04	0.16468E-04	0.17840E-04	0.19213E-04	0.20585E-04
86	0.11008E-04	0.12231E-04	0.13454E-04	0.14677E-04	0.15900E-04	0.17123E-04	0.18346E-04
87	0.98107E-05	0.10901E-04	0.11991E-04	0.13081E-04	0.14171E-04	0.15261E-04	0.16351E-04
88	0.87438E-05	0.97154E-05	0.10687E-04	0.11658E-04	0.12630E-04	0.13602E-04	0.14573E-04
89	0.77929E-05	0.86588E-05	0.95246E-05	0.10391E-04	0.11256E-04	0.12122E-04	0.12988E-04
90	0.69455E-05	0.77172E-05	0.84889E-05	0.92606E-05	0.10032E-04	0.10804E-04	0.11576E-04
91	0.61900E-05	0.68778E-05	0.75656E-05	0.82534E-05	0.89411E-05	0.96289E-05	0.10317E-04
92	0.55170E-05	0.61300E-05	0.67430E-05	0.73560E-05	0.79689E-05	0.85819E-05	0.91949E-05
93	0.49170E-05	0.54633E-05	0.60097E-05	0.65560E-05	0.71023E-05	0.76487E-05	0.81950E-05
94	0.43823E-05	0.48692E-05	0.53562E-05	0.58431E-05	0.63300E-05	0.68169E-05	0.73039E-05
95	0.39057E-05	0.43397E-05	0.47736E-05	0.52076E-05	0.56416E-05	0.60755E-05	0.65095E-05
96	0.34809E-05	0.38677E-05	0.42544E-05	0.46412E-05	0.50280E-05	0.54147E-05	0.58015E-05
97	0.31024E-05	0.34471E-05	0.37919E-05	0.41366E-05	0.44813E-05	0.48260E-05	0.51707E-05
98	0.27650E-05	0.30723E-05	0.33795E-05	0.36867E-05	0.39939E-05	0.43012E-05	0.46084E-05
99	0.24643E-05	0.27381E-05	0.30120E-05	0.32858E-05	0.35596E-05	0.38334E-05	0.41072E-05
100	0.21964E-05	0.24404E-05	0.26844E-05	0.29285E-05	0.31725E-05	0.34166E-05	0.36606E-05

[a]The last two digits in each value for the temperature difference given in this table represent a power of 10, by which each value should be multiplied in order to obtain a correct value. For example: 0.34553E-03 and 0.19376E 00 represents 0.00034553 and 0.19376 respectively. The symbol E is used to differentiate a mantissa part from an exponential part in each tabulated value as it is commonly used in computer-output.

ing the entire processing cycle except for the initial and last phases. These differences could have resulted from a nonconductive mode of heat transfer in the food and from slower heating or cooling rates in water columns in comparison with those in a steam dome.

DISCUSSION & CONCLUSION

DUHAMEL'S theorem has been utilized frequently for deriving analytical formulas for transient temperature distributions in solid objects subjected to time variable ambient temperatures. The resultant analytical formulas are both complicated and have limited practical value unless they are simplified. This theorem was applied to experimental formulas for conductive foods by approximating variable ambient temperature by a series of step changes. The resultant formula was used for estimating the transient temperature in the food. In the proposed procedure only simple arithmetic computations are necessary and much less effort is required

Table 3—Dimensionless temperature differences and variable ambient temperatures for orange used in sample calculation

Dimensionless temperature difference				Ambient temperature			
i	ΔU_i	i	ΔU_i	t (1/16 hr)[a]	$T_{aim} - T_o$(°F)	t (1/16 hr)[a]	$T_{aim} - T_o$(°F)
38	0.00191	19	0.01705	0.9	7.9	35.4	0.6
37	0.00215	18	0.01913	2.7	16.4	37.2	0.5
36	0.00241	17	0.02147	4.5	16.1	39.1	0.5
35	0.00270	16	0.02408	6.9	16.7	40.9	0.5
34	0.00303	15	0.02702	8.2	17.7	42.7	0.6
33	0.00340	14	0.03032	10.0	18.8	44.5	0.5
32	0.00382	13	0.03402	11.8	19.9	45.9	0.3
31	0.00429	12	0.03817	13.6	20.9	48.1	0.3
30	0.00481	11	0.04283	15.4	21.9	49.9	0.3
29	0.00539	10	0.04805	17.3	22.8	51.7	0.3
28	0.00605	9	0.05392	19.1	23.8	53.6	0.3
27	0.00679	8	0.06050	20.9	24.8	55.4	0.3
26	0.00762	7	0.06788	22.7	25.7	57.2	0.3
25	0.00855	6	0.07616	24.5	26.5	59.0	0.3
24	0.00959	5	0.08447	26.3	27.3	60.8	0.3
23	0.01076	4	0.08437	28.1	28.1	62.6	0.3
22	0.01207	3	0.07069	30.0	16.2	64.4	0.3
21	0.01581	2	0.04470	31.8	2.4	66.3	0.3
20	0.01520	1	0.01543	33.6	0.8	68.1	0.3

[a]The time listed in this table does not represent each time interval. However, it indicates the midpoint of each interval.

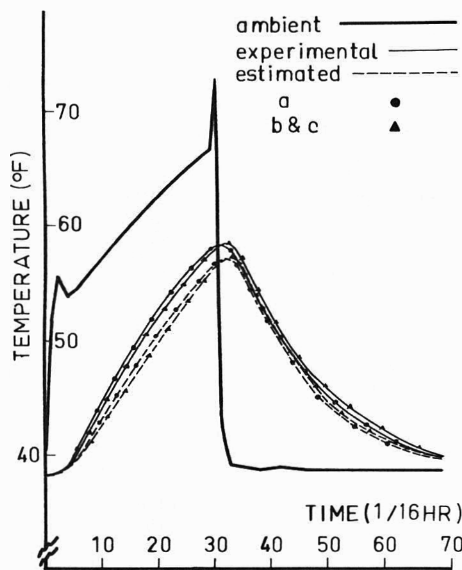

Fig. 3—Temperatures in an orange during a variable ambient temperature treatment. Curves labelled as a, b, and c respectively represent the temperatures measured at these points in the orange: 0.295, 0.653 and 0.927. These three values indicate ratios between radial distances to the points and the radius of the orange.

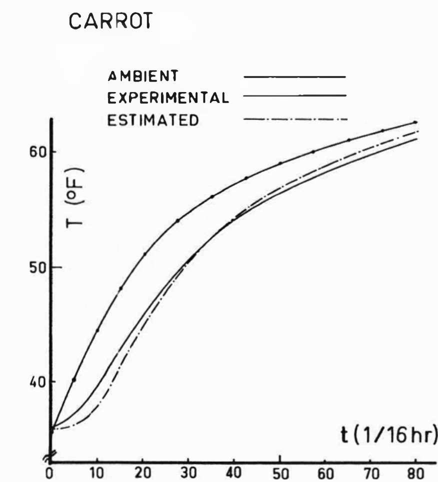

Fig. 4—Temperatures in a carrot during a variable ambient temperature treatment.

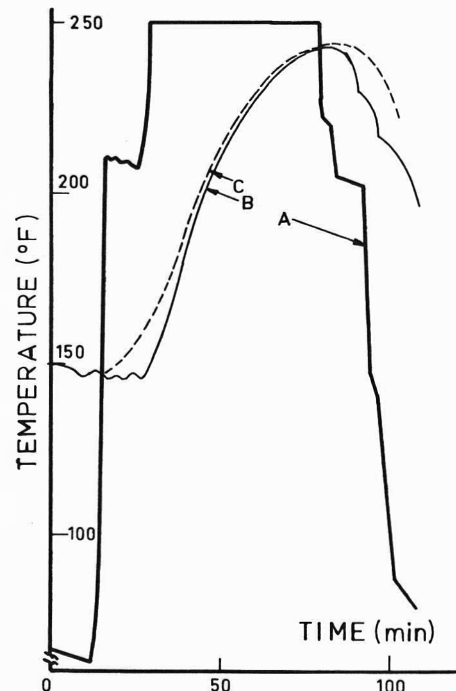

Fig. 5—Temperatures in a 211 × 400 can of thermally conductive food during a heat processing. A = temperature of heating medium; B = temperature of the food determined through an experimentation; C = temperature of the food estimated with the proposed procedure.

in comparison with using the analytical formulas.

Geometrical shapes for many foods are irregular and cannot be approximated by simple shapes such as spheres, slabs, or cylinders. Since most theoretical formulas were developed for geometrically simple objects they cannot be used to predict the transient temperatures of irregularly shaped foods. The proposed procedure can be used to predict transient temperatures since only the f and j values must be determined experimentally. These constants can be easily determined with a simple heat transfer experiment.

There are several published procedures for estimating f and j values for various foods (Ball and Olson, 1957; Kopelman and Pflug, 1968; Pflug et al., 1965; and Smith et al., 1967). These procedures can be utilized to determine f and j values without experimentation whenever they are applicable. The assumptions imposed during development of the procedures are as follows: The sample food is homogeneous and its thermophysical properties are constant. Methods developed by either Kopelman or by Pflug can be used when the geometric shape of the food is approximated by a cylinder, slab, or sphere. For irregularly shaped foods, the procedure developed by Smith and his coworkers can be used, although explicit formulas for estimating f and j values were not given. Expressions for evaluating these values can be obtained from other formulas given by them. For example, f and j values of a mass average temperature history curve for an irregularly shaped food can be estimated by eq. (4) and (5):

$$f = l^2 (\ln 10) / (\xi_1^2 \alpha) \quad (4)$$

$$j = 0.892 \cdot 10^{-0.0388 l^2 / (\alpha f)} \quad (5)$$

There are a number of temperature response charts available in the literature (Schneider, 1963) although most of them are geometrically simple objects. These charts can be used to determine the f and j values whenever assumptions imposed in obtaining these charts are satisfied.

In conclusion the transient temperatures of any conductive food, which is subjected to time variable ambient temperature, can be estimated by the proposed procedure when the f and j values are available.

NOMENCLATURE

A — Constant which can be estimated by eq. C2 (time).

f — Slope index of heating or cooling curve for food. This constant is geometrically defined as follows: A f value represents a heating or cooling time which is required for the linear portion of a temperature history curve to pass one log cycle. A procedure for obtaining this curve is discussed in the **Development of procedure** (time).

i — Dummy integer.

j — Intercept coefficient of heating or cooling curve for food. This coefficient is obtained by dividing a real temperature difference between food and constant ambient temperature at zero time of heating or cooling with a phantom temperature difference at the same time. The latter temperature difference is obtained by extrapolating the linear portion of a temperature history curve. A procedure for obtaining this curve is discussed in the **Development of procedure.**

l — Characteristic dimension of food (length).

N — Constant which can be estimated by eq. (C2).

n — Integer.

T — Food temperature when food is subjected to variable ambient temperature (°F).

T_a — Ambient temperature. This symbol is used in a step ambient temperature change treatment (°F).

$T_{a1m}, T_{a2m}, T_{aim}, T_{a(n-1)m}, T_{anm}$ — Variable ambient temperatures for 1st, 2nd, ith, (n − 1)st, and nth time intervals respectively (°F).

T_b — Ambient temperature. This symbol is used in Figure 1 (°F).

T_o — Initial food temperature (°F).

T_s — Food temperature when it is subjected to a step ambient temperature change treatment (°F).

t — Time variable (time).

t_l — Length of curvilinear portion for heating or cooling curve (time).

Δt — Uniform time interval (time).

U — $= (T_s - T_a)/(T_o - T_a)$. Dimensionless food temperature when it is subjected to a step ambient temperature change treatment.

U_o — $= 1$.

$U_1, U_2, U_{i-1}, U_i, U_{n-i}, U_{n-i+1}, U_{n-2}, U_{n-1}, U_n$ — Specific U values which are respective-

ly determined at the ends of 1st, 2nd, (i − 1)st, ith, (n − i)th, (n − i + 1)st, (n − 2)nd, (n − 1)st, and nth time intervals.

$\Delta U_1, \Delta U_2, \Delta U_i, \Delta U_{n-i+1}, \Delta U_{n-1}, \Delta U_n$
: Respectively defined as $1 - U_1$, $U_1 - U_2$, $U_{i-1} - U_i$, $U_{n-i} - U_{n-i+1}$, $U_{n-1} - U_{n-2}$ and $U_{n-1} - U_n$.

α
: Thermal diffusivity of food ($length^2$/time).

γ_n, ξ_n
: Expressions which contain Biot number, physical dimension of food, and location variable.

Θ
: $= (T_s - T_o)/(T_a - T_o)$.

Θ_{Bi}
: $= (T_{aim} - T_o)/(T_a - T_o)$.

ξ_i
: Constant, which is defined by Smith et al. (1967). This is identical to M_i in their paper.

APPENDIX

Derivation of eq. (2)

In applying Duhamel's theorem, the following solution for the heat conduction equation is needed. The solution which satisfies these conditions: initial temperature in the sample object is zero and its ambient temperature is unity. Because of this, eq. (1) should be transformed as follows:

$$\Theta(t) = 1 - \frac{T_a - T_s}{T_a - T_o} = \frac{T_s - T_o}{T_a - T_o}$$

$$= 1 = \sum_{n=1}^{\infty} \xi_n \cdot e^{-\gamma_n \alpha t/l^2} \qquad \text{(b1)}$$

The ambient temperature should also be transformed as follows:

$$\Theta_{Bi} = \frac{T_{aim} - T_o}{T_a - T_o} \qquad i = 1, 2, \ldots, n \qquad \text{(b2)}$$

Duhamel's theorem can be applied to eq. (b1) in order to derive a formula for estimating the sample temperature when it is subjected to the variable ambient temperatures, eq. (b2). A formula for estimating the food temperature, $(T - T_o)/(T_a - T_o)$, at the nth time interval can be derived as follows:

$$\frac{T - T_o}{T_a - T_o} = \sum_{i=1}^{n} \int_{(i-1)\Delta t}^{i\Delta t} \Theta_{Bi} \cdot \frac{\delta\Theta(t - \lambda)}{\delta t} \cdot d\lambda$$

$$= \sum_{i=1}^{n} \Theta_{Bi} \cdot \Big(U[(n - i)\Delta t] - U[(n - i + 1)\Delta t]\Big) \qquad \text{(b3)}$$

From eq. (b3), we can easily solve for T as follows:

$$T = T_o + \sum_{i=1}^{n} (T_{aim} - T_o) \cdot \Delta U_{n-1+1} \qquad \text{(b4)}$$

This is identical to eq. (2).

Experimental formulas

Experimental or theoretical temperature history curves for various conductive and convective foods were collected from data in published papers and also from our own data. After analyzing the geometrical characteristics of these curves, the following experimental formulas were obtained for estimating the transient temperature in the food when j values are in this range; $0.045 \le j \le 0.4$.

$$T - T_a = (T_o - T_a) \cdot 10^{-(t/A)^{1/N}} \qquad \text{(c1)}$$

when subscript $0 \le t \le t_l$

$$N = (0.3 - \log_{10} j)/0.3 \qquad \text{(c2)}$$

$$A/f = 0.3\,(0.3 - \log_{10} j)^{-N} \qquad \text{(c3)}$$

$$t_l/f = 0.3 \qquad \text{(c4)}$$

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R. W. DICKERSON JR.
Milk & Food Sanitation Research Branch, Div. of Microbiology, Bureau of Foods, FDA, PHS, U.S. Dept. of Health, Education, and Welfare, Cincinnati, Ohio 45226

MICROBIAL LETHALITY DURING LOGARITHMIC COOLING

SUMMARY—By applying the normal restrictions of thermal inactivation experiments, Ball's general equation for lethality during logarithmic cooling was reduced to a form requiring no exponential integrals. The computation is brief within the limits of two restrictions, and accuracy is ±1% of Ball's general equation. The equation is limited to thermal inactivation experiments where the minor dimension of the sample is less than 15 mm; it cannot be applied to commercial sterilization processes.

INTRODUCTION

HEAT is the most common method used to pasteurize or sterilize foods and thermal inactivation experiments are often employed to determine the necessary process. Usually, the most heat resistant pathogen is placed in a food sample with a minor dimension not larger than 15 mm, and the sample is heated to the exposure temperature, held for a specific lenth of time, cooled, and tested for survivors of the process. Because of the small sample size, heating and cooling is rapid, and most of the lethal effect occurs at the exposure temperature. Although the heating and cooling times are brief, their lethality must be evaluated.

The most generally used method of evaluating the lethality of heating and cooling phases is the graphical method of Bigelow (1920). More recently, numerical methods have been employed. Computer techniques have been applied to heating curves (Herndon et al., 1968; Griffin et al., 1969), and Hayakawa (1968) described a Gaussian integral technique that applies to both heating and cooling curves. For the specific conditions of logarithmic heating and cooling, Ball and Olson (1957) developed tables and equations for computing lethality of the process, which have been used extensively. Their tables for logarithmic cooling are limited, however, to the large food geometries of the canning industry, where there is a significant time lag at the beginning of the cooling process. Their tables were computed for the specific condition of $j_c = 1.41$.

In contrast to commercial sterilization processes, no significant time lag occurs with the small samples of thermal inactivation experiments ($j_c = 1.0$), and, although the tables of Ball and Olson (1957) are valid for logarithmic heating, a computation for logarithmic cooling will yield a value of lethality greater than that of the actual cooling process. Consequently, these tables, and derivations from them (Hicks, 1958; Pflug, 1968) cannot be used for computing lethality of the cooling process of most thermal inactivation experiments. Hicks (1958), Pflug (1968) and Ball and Olson (1957) recognized this and limited their tables to the specific condition where $j_c = 1.41$. Because of this limitation, Stumbo (1965) developed tables for logarithmic cooling for several different time lags ($0.4 \leqq j_c \leqq 2.0$). Stumbo's tables yield the total lethality of heating and cooling with the inherent assumption that the slope of the heating curve equals the slope of the cooling curve.

Thermal inactivation experiments are unique in that the heating and cooling phases are separated by a holding period at constant temperature, and when water bath techniques are used, the heating and cooling curves are simple logarithmic curves. By applying these restrictions, the equations for lethality during linear and logarithmic heating (Ball and Olson, 1957) have been reduced to forms that do not require the evaluation of logarithms or exponential integrals (Dickerson, 1969). The equations yield a single value for lethality, without the use of tables or nomograms. An equation has also been developed that determines the relative magnitude of lethality during logarithmic cooling (Dickerson, 1969; 1970), but it could be used only to determine when lethality of the cooling process was negligible. My objective was to develop an equation (without exponential integrals) for computing the precise lethality of a logarithmic cooling process.

THEORY

LETHALITY under a logarithmic cooling curve is given by (Ball and Olson, 1957)

$$E = \frac{0.435\,f_c}{F_{T_e}\left(10^{\frac{T_e - T_c}{z}}\right)}\left[\mathrm{Ei}\left(\frac{T_e - T_c}{0.435\,z}\right) - \mathrm{Ei}\left(\frac{T - T_c}{0.435\,z}\right)\right] \qquad (1)$$

Transposing equation (1) is not a straightforward step and the reader is referred to Dickerson (1970) for a related discussion. In many thermal inactivation studies, the lethal effect of the cooling process is computed as an equivalent time, t, at the exposure temperature and this value can be obtained by multiplying both sides of equation (1) with F_{T_e}:

$$t = EF_{T_e} = \frac{0.435\,f_c}{10^{\frac{T_e - T_c}{z}}}\left[\mathrm{Ei}\left(\frac{T_e - T_c}{0.435\,z}\right) - \mathrm{Ei}\left(\frac{T - T_c}{0.435\,z}\right)\right] \qquad (2)$$

When cooling is nearly complete, $T - T_c$ is small and, because of this the second exponential integral of equation (2) becomes negligible and may be eliminated as follows. Temperature during logarithmic cooling may be expressed as

$$T - T_c = (T_e - T_c)\,10^{-\frac{\tau}{f_c}} \qquad (3)$$

Since cooling is nearly complete when $\tau = 3f_c$, the temperature at the end of cooling is given by

$$T - T_c = 0.001\,(T_e - T_c) \qquad (4)$$

and substitution of equation (4) into (2) yields

$$t = \frac{0.435\,f_c}{10^{\frac{T_e - T_c}{z}}}\left[\mathrm{Ei}\left(\frac{T_e - T_c}{0.435\,z}\right) - \mathrm{Ei}\left(\frac{0.001\,(T_e - T_c)}{0.435\,z}\right)\right] \qquad (5)$$

When $(T_e - T_c)/z$ is large, the second exponential integral in equation (5) is negligible in comparison with the first. To treat the first exponential integral of equation (5), it is observed from tables of exponential integrals (Gautschi and Cahill, 1964) that

$$xe^{-x}\,\mathrm{Ei}(x) = 1.00 + \frac{1.33}{x} \qquad (6)$$

is accurate to within ±1% when $x > 8.3$. The straight line of equation (6) was obtained by taking values of $xe^{-x}\,\mathrm{Ei}(x)$ from the tables of Gautschi and Cahill (1964) and plotting them versus $1/x$, for values of $1/x$ between 0 and 0.3. Since

$$x = (T_e - T_c)/0.435\,z \qquad (7)$$

equation (6) is valid when $(T_e - T_c)/z > 3.6$. Furthermore, when $(T_e - T_c)/z > 3.6$, the second exponential integral of equation (5) may be neglected, and substituting equations (6) and (7) into (5) yields

$$t = \frac{0.19 f_c z}{T_e - T_c}\left[1.00 + \frac{0.58 z}{T_e - T_c}\right] \quad (8)$$

Equation (8) is valid to within ±1% of equation (2) (Fig. 1) provided the cooling period is equal to or greater than $3f_c$, and further provided that

$$T_e - T_c > 3.6 z \quad (9)$$

In thermal inactivation experiments, a difference of 12z between exposure temperature and cooling medium temperature is typical, and equation (8) will be applicable in most cases. The applicability of equation (8) is easily determined from equation (9).

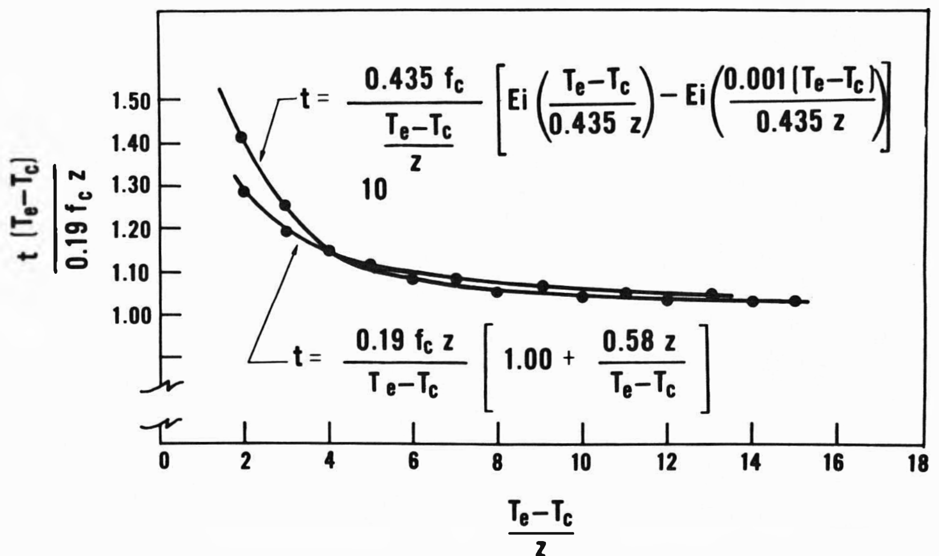

Fig. 1—Comparison of exact and approximate equations.

EXAMPLE

AT THE END of a thermal inactivation experiment, a microbial suspension was cooled by plunging it into a refrigerated water bath (5°C) and holding it in the bath for 10 min. The heat penetration curve indicated that cooling was logarithmic and that $f_c = 0.6$ min. The exposure temperature was 65°C and the z value was 6°C. As a first step, the example conditions were tested to determine whether equation (8) could be applied. In the first restriction, cooling time must be equal to or greater than $3f_c$; therefore, cooling time was computed in multiples of f_c

$$\text{cooling time} = \frac{10\ \text{min}}{0.6\ \text{min}} = 16.7\, f_c$$

and the restriction was satisfied. In the second restriction $T_e - T_c$ must be greater than 3.6z

$$65°C - 5°C > 3.6\,(6.0°C)$$
$$60°C > 21.6°C$$

therefore, the application of equation (8) was valid. Substituting into equation (8)

$$t = \frac{0.19\,(0.6\ \text{min})\,6°C}{65°C - 5°C}\left[1.00 + \frac{0.58\,(6°C)}{65°C - 5°C}\right]$$
$$t = 0.0120\ \text{min}$$

Using equation (2) (the general equation for logarithmic cooling), t = 0.0119 min, and using Bigelow's graphical method,

CONCLUSIONS

FOR THERMAL inactivation experiments, where the minor dimension of the sample is small (less than 15 mm) and the cooling curve is logarithmic from the start of cooling, equation (8) may be used to compute lethality of the cooling process. It is only necessary that cooling occurs for a time equal to or greater than $3f_c$, and the difference between exposure temperature and cooling medium temperature is greater than 3.6z. When these conditions are met, equation (8) is accurate to within ±1% of the result from equation (2). The computation is brief, and no mathematical tables or nomograms are required.

Because of the large size of commercial cans of food, and consequently, an initial deviation from logarithmic cooling, equation (8) cannot be applied to commercial sterilization processes.

NOMENCLATURE

E = lethality in multipliers of one equivalent treatment.

Ei (x) = $\int_{-\infty}^{x} \frac{e^n}{n}\, dn$

F = thermal death time of a known process (min).

F_{T_e} = thermal death time at T_e (min).

f_c = slope of heat penetration curve during logarithmic cooling (min).

j_c = $(T_o - T_c)/(T_e - T_c)$.

T = temperature (°C).

T_c = temperature of the cooling medium (°C).

T_e = temperature of the sample at the start of the cooling process (°C). T_e is also the exposure temperature.

T_o = artificial temperature (°C) obtained when cooling curve is extrapolated to zero time. For logarithmic cooling of small samples, $T_o = T_e$.

t = lethal effect of the cooling process expressed as an equivalent time (min) at the exposure temperature, T_e.

τ = time (min).

x = limit of integration of the exponential integral.

z = the temperature increase (°C) that yields a 10-fold reduction in thermal death time.

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EDWARD B. STUART and GERARD CLOSSET
Chemical and Petroleum Engineering Department, University of Pittsburgh, Pittsburgh, Pennsylvania 15213

PORE SIZE EFFECT IN THE FREEZE DRYING PROCESS

SUMMARY—Experiments were conducted to determine the influence and/or effect of the structure of the dried material itself on transport rates. Drying rates were measured in the 0.2–3.0 mm Hg pressure range, the usual operating range of commercial freeze driers. Thermal conductivities of dried beef and heat and mass transfer were calculated from drying rate data and the structure of the freeze-dried material was studied by optical means. A simplified relationship between thermal conductivity and pore structure was derived. Results show drying rates were related to basic transport properties, thermal conductivity and pore structure.

INTRODUCTION

ALTHOUGH extensively used for the preservation of biological materials, freeze drying has had a slow start in the food industry. Basically a low pressure food dehydration process, the advantage of freeze drying over other food preservation processes is that very little shrinkage, change in texture and loss of flavor takes place. The drawback however is the slowness of the process and nature of the equipment which make it a very costly process.

Research in the food industry and chemical engineering field has been oriented mainly toward understanding the transport properties of the dried material. A number of authors (Dyer and Sunderland, 1966; Harper, 1962; Lambert and Marshall, 1962; Massey and Sunderland, 1967; Sandall et al., 1967) have studied heat and mass transfer rates, drying rates and general properties of the freeze-dried products. However, little attention has been given to the influence of the structure of the dried material itself on transport rates. Since most freeze-dried materials are consolidated porous solids, the geometry of the system is a very significant factor.

This paper presents results of an experimental study of freeze drying of beef. Thermal conductivities and heat and mass transfer are calculated from drying rate data and a study of the structure of the freeze-dried material is performed by optical means. The major aim of this paper is to relate thermal conductivity and the structure of the dried material, thereby determining the influence of the individual conductivities of the solid tissue and pore space on the overall thermal conductivity which controls the drying rate.

THEORY

Thermal conductivity

The process of freeze drying involves a change of phase with a moving surface of separation between the two phases. As the ice surface moves inward, a porous layer of dried material gradually appears, surrounding the ice phase. The heat necessary for sublimation and the water vapor generated have to travel through this porous layer and it is found that at the low pressures used, the rate of drying is mainly controlled by heat transfer (Harper, 1962; Massey and Sunderland, 1967). In such a process, the thermal conductivity is the characteristic transport parameter.

In a gas filled porous solid, such as freeze-dried meat, the bulk or effective thermal conductivity depends upon the individual conductions of the gas and solid and on the geometry of the system (Carman, 1956; Strong et al., 1960). Since freeze-dried meats have a low bulk thermal conductivity and are highly porous, the contribution of the gas to the bulk conductivity is very significant. Furthermore, since the thermal conductivity of the gas and solid account respectively for the heat conduction in the pore space and solid tissues, it is of interest to determine what fraction of the meat surface is pore space. The thermal conductivity of the gas in the pore space varies with pressure, since the mean free path of the gas increases as the pressure decreases. As the pressure is reduced, the mean free path becomes significant compared with the dimensions of the confining space—in that case the pore size—and the so-called "temperature jump" phenomenon occurs (Kennard, 1929).

At still lower pressures, collisions among molecules become insignificant compared to collisions with solid surfaces and free molecule conditions prevail. Most commercial freeze driers have an operating pressure range corresponding to the transition region between slip and free molecule flow.

In a sample of freeze-dried tissue sectioned at right angles to the muscle fiber, it is possible to represent the meat structure as a network of fibers and pores oriented in a parallel direction. In such a model, heat conduction takes place to a great extent through solid and gas in parallel. In this case, equations representing the heat conduction in the dried layer may be set up as:

$$Q = q_T + q_p \quad (1)$$

$$Q = K_T A_T \Delta T/\Delta z + K_p A_p \Delta T/\Delta z \quad (2)$$

where q_T, K_T and A_T are respectively the heat conduction, thermal conductivity and cross sectional area of the solid material or tissue. The corresponding quantities respective to pore space are q_p, K_p and A_p. Δz is the width of the sample, and ΔT the temperature difference between the surface of the sample and its ice phase.

Equation (2) can be written as:

$$Q = (K_T A_T + K_p A_p)(\Delta T/\Delta z) \quad (3)$$

If the bulk or overall thermal conductivity is defined as:

$$K_b = (K_T A_T + K_p A_p)/A \quad (4)$$

equation (4) can be expressed as:

$$K_b = [K_T(1 - \phi) + K_p \phi] \quad (5)$$

where ϕ is the fraction of total cross sectional area that is pore space. K_T is constant, but K_p varies with pressure.

Equation (5) can be rearranged as:

$$K_b = K_T + \phi(K_p - K_T) \quad (6)$$

This expression shows that K_b will be directly dependent upon pressure and the pore structure of the material.

Furthermore, an expression for the thermal conductivity in the pore space can be written as:

$$K_p = (K_b - K_T)/\phi + K_T \quad (7)$$

An experimental bulk thermal conductivity can be determined from drying rate data. Sandall et al. (1967) developed the model of a uniformly retreating ice front and related basic transport properties to rates of drying by using the following equation:

$$(Z H_s/2 V_f)(-dx/d\theta) = K_b/[K_b/h + (1 - x) Z/2)](T_o - T_i) \quad (8)$$

Equation (8) may be integrated and used to calculate experimental bulk thermal conductivities:

$$(1 - x) = (8V_f K_b/Z^2 H_s)(T_o - T_i) \times [\theta/(1 - x)] - 4K_b/hZ \quad (9)$$

K_b may be determined from the slope of a plot of $(1 - x)$, the fraction of water removed, versus $\theta/(1 - x)$, the ratio of drying time to fraction of water removed.

Masamune and Smith (1963) proposed a model in which heat transfer through a solid-gas path in series as well as in parallel is considered. The following expression for the bulk thermal conductivity is obtained:

$$K_b = \phi K_p + \frac{(1 - \phi)(1 - \delta)}{(\gamma/K_p) + (1 - \nu)/K_T} + \delta\phi K_T \quad (10)$$

This equation will be used in this paper with the assumption that the mean free path remains essentially constant and equal to the diameter of the pore, therefore $\gamma = 1.0$. It is also arbitrarily assumed that the areas of contact between gas and pore are such that $\epsilon_2 = \epsilon_3$, and so $\delta = 1/2$. These assumptions have to be made since this equation will be used to fit data from the literature for which γ and δ are unknown.

EXPERIMENTAL

MEAT used in this experiment was a commercial type beef, part of the shoulder muscle. This was very adequate for this experiment because of its excellent connected fibrous structure and little amount of fat and connective tissues. In order to obtain comparable results, it was desirable to work with uniform samples; therefore, all samples were of the same thickness, cooked before freezing and cut in such a way that heat and mass transfer occurred in a direction parallel to the muscle fibers. Samples were slabs 1.4–1.8 cm wide, 0.9 cm thick.

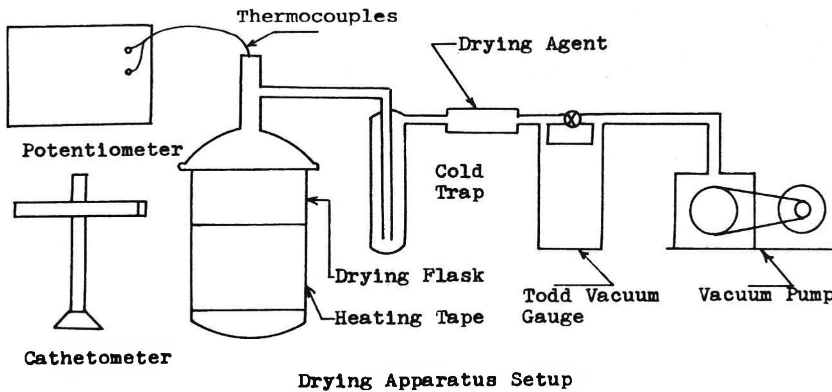
Iron constantan thermocouples were used to record the temperature changes at the surface and center of the samples. The drying rates were measured by hanging a sample to a quartz spring and recording its extension. Because the presence of thermocouple wires hanging from the sample considerably hampered the procedure, temperature recordings were made on a reference sample placed under the spring, at the center of the flask. It was found that this procedure did not introduce more than 2 or 3% error. After inserting a thermocouple in the center of the reference sample, both slabs were tightly wrapped in aluminum wrap and immersed in a mixture of acetone and dry ice. Freezing occurred very quickly, within 60 sec, and could be observed by reading the thermocouple. After freezing, a second thermocouple was placed on the surface of the reference sample. A thin layer of cellulose glue was then applied on the edge of the samples to avoid heat and mass transfer from the sides.

The experiment was designed to perform a series of runs at different sets of temperature and pressure. For that purpose, the apparatus illustrated in Figure 1 was used.

The loss of weight of the samples was recorded with a cathetometer at regular time intervals. The constant of the spring was found to be 0.0984 gm/mm; the cathetometer permitted readings of ±0.5 mm and weight differences could therefore be recorded within 0.005g.

Heat necessary for drying the samples was provided by a heating tape placed around the flask. Heat input could be controlled with a variable transformer, permitting a satisfactory control of the temperature at the surface of the sample, the temperature variation not exceeding ±3°C. Temperatures were read on a potentiometer. The pressure of the system was regulated by bleeding air into the system with a needle valve.

Photomicrographs of sections of the dried tissue were taken with a Carl Zeiss microscope in polarized light.



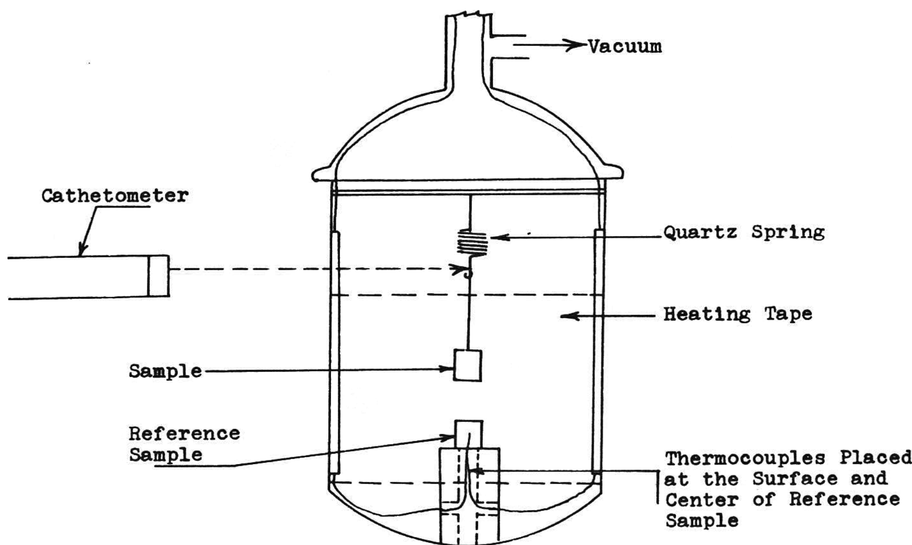


Fig. 1—Drying apparatus.

RESULTS

FIGURE 2 shows a typical weight loss curve for a sample of 0.9 mm thick and with grain orientation parallel to the direction of heat and mass transfer. Data for flask pressure P_T, external temperature T_o and total weight loss W_T are in-

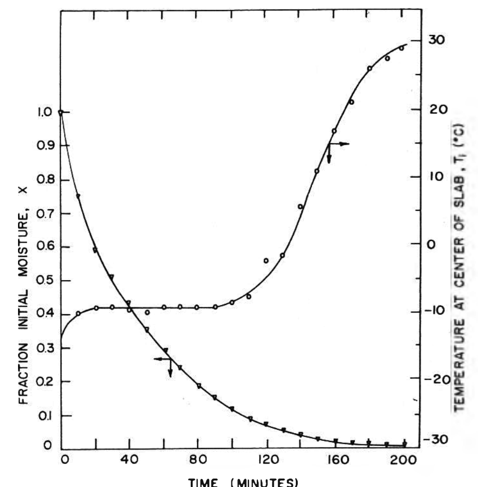


Fig. 2—Drying curve for run 5. Surface temperature, 30°C; flask pressure, 2.0 mm Hg; weight loss, 1.914g.

dicated on the graphs. It can be observed that the drying rate is at first very rapid and gradually slows down. A constant ice temperature is obtained during most of the run, then rises towards the value of the external temperature, indicating the disappearance of the ice phase and an end of the drying.

Drying to zero moisture content was assumed. Although this is not completely correct, it was observed, by further drying of the samples, that no more than 3–4% error was introduced by that assumption.

Table 1 gives heat and mass transfer results for several runs; it can be observed that the drying is largely heat transfer controlled. The thermal conductivity of the freeze-dried meat was calculated for each sample from data obtained in the drying runs. The values obtained are given in Table 1 and are plotted in Figure 3 as a function of pressure. The experimental thermal conductivity was calculated by using equation (9), and plots of $(1 - x)$ vs. $\theta/(1 - x)$ were made for each run. V_F is calculated by measuring the dimensions of the sample after drying and is the volume of food per unit weight of water. An average value of 0.0223 ft^3/lb was found for the type of beef used.

Figure 4 is a photomicrograph of freeze-dried tissues obtained at 2.0 mm Hg and an external temperature of 30°C. The magnification is 390. The tissue consists of a network of fibers (dark areas) and interstitial cavities or pore space (clear areas). The cavities are the spaces previously occupied by ice and emptied when sublimation took place. The width of the cavities, as measured by a light microscope, vary between 3.0–9.0μ. A few measure only 1μ, and a few may reach 15μ. The average is 6.0μ. If compared with the structure of a normal nonfrozen muscle, as described by Luyet (1962), the structure of the freeze-dried tissue shows very little shrinkage and deformation. The portion of the cross section that is pore space was determined for several samples by projecting the magnified image on a screen. An average value of 35% was found to be pore space. It is of interest to note that no appreciable difference in pore size or percentage of pore space was found between tissues freeze dried at different pressures, within the pressure range used (0.2–3.0 mm of Hg). Shrinkage and deformation are known to occur, however, when pressures higher than 5 mm Hg are used (Sandall et al., 1967).

Rinfret (1962) and Luyet (1962) reported that the rate of initial freezing plays a fundamental role in the structure of the freeze-dried material, and actually determines what the pore structures will be. It is interesting to compare the freezing rate and pore size obtained in this work with Luyet's results. Table 2 shows they are in general agreement. The thermal conductivity of the gas in the pore space was calculated using equation (7). K_T can be taken as the zero pressure value of the thermal conductivity and by interpolation was found to be 0.0220 Btu/ft°F. Results for K_p are plotted in Figure 5.

Massey and Sunderland (1967) measured the thermal conductivity during freeze drying of beef at pressures rang-

Table 1—Thermal conductivity and heat and mass transfer driving forces results

Run no.	P_T Flask pressure (mm Hg)	T_o External temp (°C)	T_i Ice temp (°C)	P_i Ice vapor pressure (mm Hg)	$(T_o - T_i)$ Heat transfer driving force (°C)	$(P_i - P_T)$ Mass transfer driving force (mm Hg)	$K \times 10^2$ Thermal conductivity Btu/hr°F ft
1	0.2	30	−25.6	0.448	55.6	0.248	2.28
2	0.5	30	−19.4	0.822	49.4	0.322	2.53
3	0.9	30	−16.1	1.121	46.1	0.221	2.67
4	1.5	30	−11.2	1.769	41.1	0.268	2.89
5	2.0	30	− 9.1	2.112	39.1	0.112	3.0
6	3.0	30	− 4.7	3.090	34.7	0.090	3.20
7	0.2	45	−26.7	0.401	51.7	0.201	2.38
8	0.5	45	−18.3	0.912	63.3	0.412	2.68
9	0.9	45	−16.3	1.101	61.3	0.201	2.72
10	1.5	45	−11.7	1.676	56.7	0.176	2.99
11	2.0	45	− 8.9	2.349	53.9	0.149	3.03
12	3.0	45	− 5.1	2.987	50.1	0	3.32
13	0.2	60	−27.1	0.385	87.1	0.185	2.30
14	0.5	60	−19.6	0.806	79.6	0.306	2.41
15	0.9	60	−15.9	1.142	75.9	0.242	2.71
16	1.5	60	−12.0	1.632	72.0	0.132	2.98
17	2.0	60	− 9.2	2.093	69.2	0.093	3.24
18	3.0	60	− 4.4	3.171	64.4	0.171	3.33

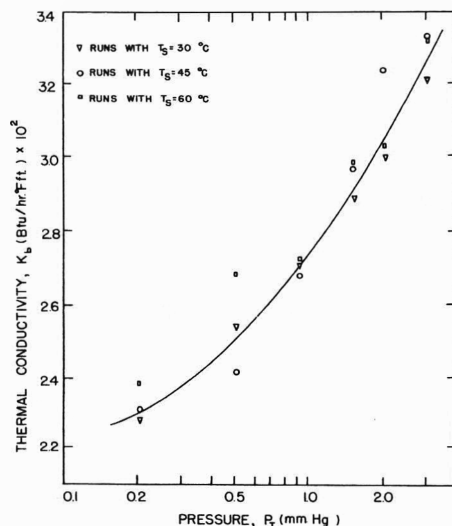


Fig. 3—Variation of bulk thermal conductivity of freeze-dried beef with pressure.

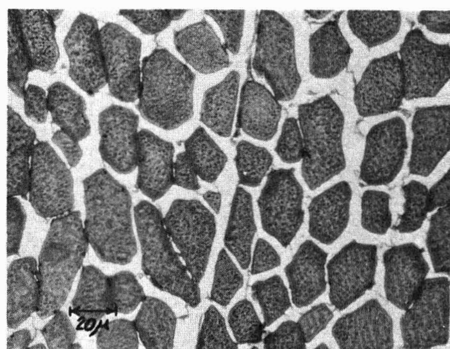


Fig. 4—Photomicrograph (390X) of freeze-dried tissues obtained at 2.0 mm Hg and an external temperature of 30°C. Tissue consists of a network of fibers (dark areas) and interstitial cavities or pore space (clear areas).

Table 2—Size of cavities in muscle tissue frozen at various rates

Thickness of piece of tissue (mm)	2[a]	2[a]	2[a]	100[b]	9[c]
Temp of cooling bath (°C)	−150	−50	−20	−29	−76
Duration of freezing (sec)	1/4	3/4	8	2 hr	50–60
Width of cavities (microns)	2	5	10	150	6

[a]Data from Luyet (1962).
[b]Data supplied by QmF&CI to Luyet.
[c]Data obtained in this work.

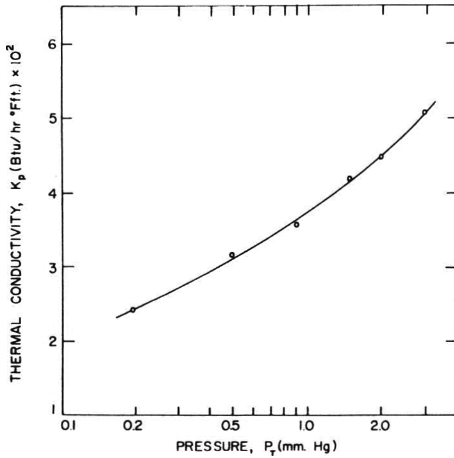


Fig. 5—Variation of pore thermal conductivity with pressure.

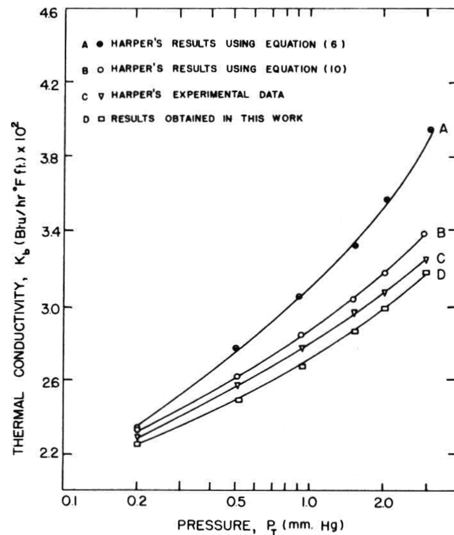


Fig. 6—Comparison of theoretical and experimental thermal conductivity of freeze-dried beef.

ing between 0.3–3.0 mm Hg. Their plot of thermal conductivity as a function of pressure shows an increase of conductivity with increasing pressure, and the shape of the curve is similar to that obtained in this work. However, their values for thermal conductivities are higher, resulting in an upward shift of their curve. A possible explanation for this result is that their samples were slow frozen (they used a domestic refrigerator) and therefore, as shown by Luyet (1962), a large porosity must have resulted, which would cause the higher overall thermal conductivity. Massey and Sunderland (1967) do not report porosity data and further comparison with the results obtained in this work is not possible.

Harper (1962) calculated the bulk thermal conductivity of beef by using kinetic relationships from the results of Pollard and Present (1948) on self diffusion in capillary tubes. His results are plotted in Figure 6 (curve C) and are in good agreement with those obtained in this work (curve D). When Harper's data are fitted with equation (6), a discrepancy with his experimental results is apparent, as can be seen from Figure 6 (curve A). Harper reported pore sizes in the range of 50–150μ, and a porosity of 64%. The large dimensions of the pores tend to indicate slow freezing rates, as indicated by Luyet's results. The shift between curves C and D is to be expected, since a more porous sample will have a higher bulk thermal conductivity. The discrepancy between Harper's experimental data (curve C) and the results obtained by fitting them with equation (6) (curve A) can be explained as follows:

1. Because of the wide difference in the dimensions of the pore space between those obtained in this work (6μ) and Harper's (50–150μ), it is to be expected that, because of mean free path effect, the thermal conductivity of the gas in the pores will not be the same in both cases. Curve A was obtained by using the pore thermal conductivity obtained in equation (6), which assumes no variation in mean free path with pore width.

2. Slow frozen materials undergo structural damages that alter the original ordered structure of the meat. Heat conduction then takes place through solid and gas in series as well as in parallel. For such a model equation (6) clearly does not apply. This assumption is confirmed by fitting Harper's data with a model developed by Masamune and Smith (1963) that takes into account heat conduction in series; the results are plotted in Figure 6 (curve B) and show a closer fit with Harper's data.

The simplified model developed in this work can be of use in the design of freeze drying of materials that have retained much of their original oriented structure and have pore widths in the 1–15μ range. The original structure of the material can be preserved by using rapid rates of freezing, thereby making it possible to predict the porosity. The design of such a process can then be worked out by relating heat input to thermal conductivity, thermal conductivity to porosity and pressure, and porosity and pore size to the rate of freezing.

NOMENCLATURE

- C = Specific heat
- D_p = Pore diameter
- h = External heat transfer coefficient
- H_s = Latent heat of sublimation of ice
- K = Thermal conductivity
- K_b = Bulk thermal conductivity
- L_g = Effective mean free path of gas
- P_T = Total flask pressure
- q = Heat flux
- T = Temperature
- V_f = Volume of food occupied by unit weight of water, initially
- X = Fraction of initial water still present in sample
- Z = Thickness of slab being dried
- Δz = Thickness of dried layer
- ΔW_T = Amount of moisture sublimed in one run

Greek Letters

- θ = Time since start of drying
- ϕ = Porosity
- γ = L_g/D_p
- δ = $\epsilon_3/(\epsilon_2 + \epsilon_3)$
- ϵ_2 = Area fraction for mechanism of heat transfer through solid-gas in series
- ϵ_3 = Area fraction for mechanism of heat transfer through solid phase

Subscripts

- $_T$ = Tissue
- $_p$ = Pore

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HORACE K. BURR
USDA, Western Regional Research Laboratory, ARS, Albany, Calif. 94710

FROZEN FRENCH-FRIED POTATOES. Effects of Thawing and Holding Before Finish Frying and Their Nonrelation to Starch Retrogradation

SUMMARY—Frozen par-fried French-fry cuts allowed to thaw and held several hours before being finish-fried lost more weight (due to loss of moisture) and absorbed more fat than if not held. The yield of finished product decreased about 7% and fat uptake increased about 25% when par-fries were held for 3 days at 50°F. Smaller but economically significant changes occurred in only 1-1/2 hr at this temperature or in 5 hr at 40°F. The rate of the change in par-fries which causes lower yields and greater fat absorption increases with increasing temperature. The time course of the change at 50°F is similar to that of starch retrogradation in par-fries but the rate of retrogradation decreases with increasing temperature; therefore, these appear to be unrelated phenomena. There is no combination of time and temperature for frying par-fries from the thawed state which is equivalent in all respects to a given combination for frying them from the frozen state.

INTRODUCTION

A FEW YEARS AGO a group at the Western Regional Research Laboratory studied the effects of thawing frozen French-fried potatoes (par-fries) and holding them for various times at 35°, 45° and 55°F before finish-frying (Boyle et al., 1964; Reeve et al., 1968a,b; Michener et al., 1968). They found that organoleptically detectable changes in flavor and texture did not occur until after 20, 8, and 4 days at these temperatures, respectively. Under these conditions the plate counts of aerobic bacteria on the par-fries had risen to about 10^8/g. They also found that the samples held in the thawed state lost more moisture and absorbed more fat during finish-frying than did control samples fried after being allowed to thaw for only an hour. In another paper, Notter et al. (1967) reported that oil absorption was maximal in samples held 9 hr or longer at 55°F. Potter (1954) studied the retrogradation (progressive loss of solubility) of starch in partially-dried potato cells and from his data one might estimate that in potatoes at the moisture content of par-fries (ca. 70%) retrogradation would be essentially complete in roughly 9 hr at 55°F. On this basis we speculated that the increase in oil absorbing tendency might be related to retrogradation of gelled starch in the par-fries.

The purpose of the present study was to learn in more detail the effects of time and temperature of holding par-fries in the thawed condition on the loss of water and uptake of fat during finish-frying, and on retrogradation of the starch in par-fries.

MATERIALS & METHODS

A SINGLE LOT of commercially frozen straight-cut French-fried Russet Burbank potatoes was used. The strips had been cut 7/16 in. square, blanched for 8.7 min in water at 160°-165°F, dipped for 15-20 sec in 1° Brix solution of glucose and tetrasodium pyrophosphate at 175°F, and par-fried 53 sec in oil at 365°F. Freezing in a blast tunnel required 10.5 min at −18°F. All strips were hand sorted to eliminate those shorter than 2-5/8 in. Each sample for frying consisted of 40 strips and weighed an average of 336g. The strips were arranged in a single layer on an 8 in. × 10 in. aluminum plate, covered with a sheet of 3-mil plastic and equilibrated at −20°F. Thawing was accomplished by placing the sample in a 2-mil polyethylene bag weighted at the bottom and submerging it in a stirred 50-gal tank of water at 70°F for exactly 5 min. It had been determined previously that this was just sufficient time to thaw all strips to their centers. The sample was then transferred to crushed ice (32°F) or to water at 40° or 50°F to bring it quickly to the desired holding temperature. The 32°F samples were left in crushed ice and the other samples were transferred to chambers maintained at 40° or 50°F ± 1° for the desired holding time of 1.5, 5 or 72 hr. The samples were fried in vegetable oil at 375°F for 2.25 min. The "zero time" samples were thawed as described above and immediately fried.

The finish-fried samples were drained for 1 min and frozen in powdered dry ice. They and the par-fry controls were then weighed, freeze-dried, weighed again, ground, and analyzed. Moisture content was determined by drying under vacuum at 70°C for 40 hr. Crude fat was determined by Soxhlet extraction with anhydrous diethyl ether for 16 hr. The entire experiment was run in quadruplicate.

For the starch retrogradation study frozen par-fries were ground with dry ice in a household blender. After evaporation of the CO_2 about 2g of the powder was packed into 12-ml conical centrifuge tubes precooled to −20°F. Thawing was accomplished by suspending duplicate tubes in stirred water at 70°F for 3 min, a time just sufficient to permit thawing of the entire mass. The tubes were then transferred to crushed ice or to water at 40° or 50°F. After 10 min equilibration, the 40° and 50°F samples were transferred to air chambers at the same temperatures. At the end of the desired holding time, the samples were refrozen by placing the tubes in powdered dry ice. Subsequently, all samples were freeze-dried and then largely defatted by leaching with petroleum ether, centrifuging, and decanting three times. The samples were freed of solvent in air and then under vacuum. To measure the extent of starch retrogradation approximately 0.1g of the powder was weighed and blended for 60 sec with 2000 times its weight of water. 10 ml of the suspension was centrifuged for 5 min at 2500g. A 1.0 ml aliquot of the supernatant was mixed with 10 ml of dilute Lugol's solution (0.10g I_2 and 0.20g KI per liter) and the absorbance measured at 605 nm.

RESULTS & DISCUSSIONS

FIGURE 1 shows the effect of time and temperature of holding the thawed par-fries on the yield of finish-fried product. A substantial loss in yield occurs in as little as 1-1/2 hr at 50°F or 5 hr at 40°F. This is long before any organoleptically detectable change occurs (Michener et al., 1968). The maximum loss in yield, after 72 hr at 50°F, is such that 7% more par-fries are required for a given size serving than if they were fried immediately after being thawed. The change that causes the lower yield has the direct rate-temperature relationship common to nearly all chemical reactions, i.e., the rate increases with increasing temperature.

From Figure 2 it is apparent that the decrease in yield with increasing holding time is due to increased loss of water during finish-frying.

Actually, in absolute terms the increased loss of moisture was greater than the loss in yield, the difference being made up by increased fat absorption, as shown in Figure 3.

Despite the considerable dispersion among replicate measurements, there is a clear tendency toward increased fat uptake with increased time of holding in the thawed state. The maximum increase amounts to about 25% greater uptake than in the "zero time" samples. In two preliminary experiments not described here, maximum increases in fat uptake were 28% and 37%. Increases of this magnitude would be economically significant to the restaurant owner but are far smaller than the two- to threefold increases reported by Reeve et al.

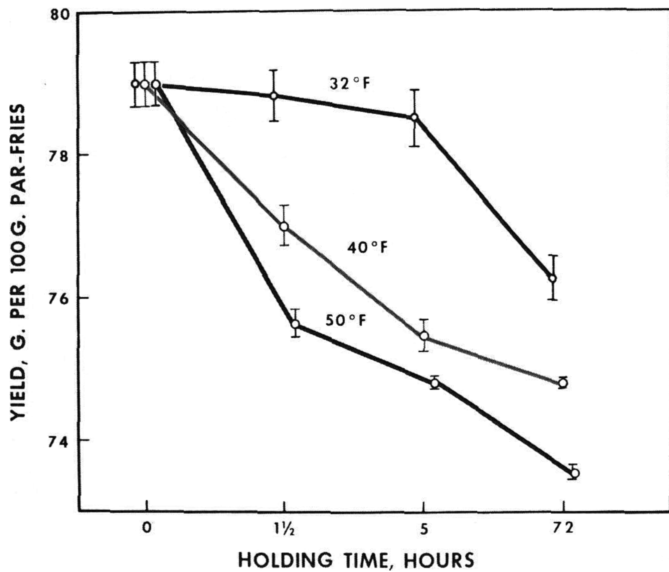

Fig. 1—Effect of time and temperature of holding par-fried potatoes on the yield of product finish-fried 2.25 min at 375°F. Bar represents plus/minus one standard error of mean; time scale is arbitrary.

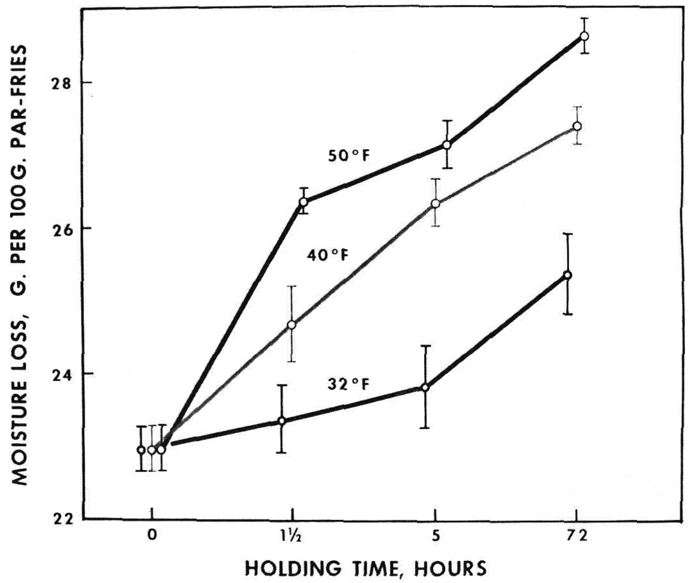

Fig. 2—Effect of time and temperature of holding par-fried potatoes on the loss of moisture when finish-fried 2.25 min at 375°F. Bar represents plus/minus one standard error of mean; time scale is arbitrary.

(1968 a,b). However their holding temperatures and times were somewhat greater than those reported here.

Results of the retrogradation study are given in Figure 4. The time course of retrogradation at 50°F is quite similar to that of declining yield and increasing moisture loss shown in Figures 1 and 2. This, of course, was the reason that we originally hypothesized that they might be causally related (Notter et al., 1967). However, Figure 4 shows the well-established inverse rate-temperature relationship characteristic of retrogradation of starches. At 32°F retrogradation proceeds much faster than the change which causes increased moisture loss and decreased yield. The basic nature of that change remains unknown.

The recommended practice in the use of frozen par-fries is to hold them at 0°F or below and to finish-fry them from the frozen state; therefore, it seemed desirable to include such controls in these experiments. In the retrogradation experiment two sets of controls were used, one thawed in water for 3 min and immediately refrozen and the other not thawed before being freeze-dried. They gave the same absorbances and hence showed no difference in degree of retrogradation.

However, there was a fundamental difficulty in providing a frozen control for the frying study. It is obvious that if one finish-fries two samples for the same length of time, one from the thawed state

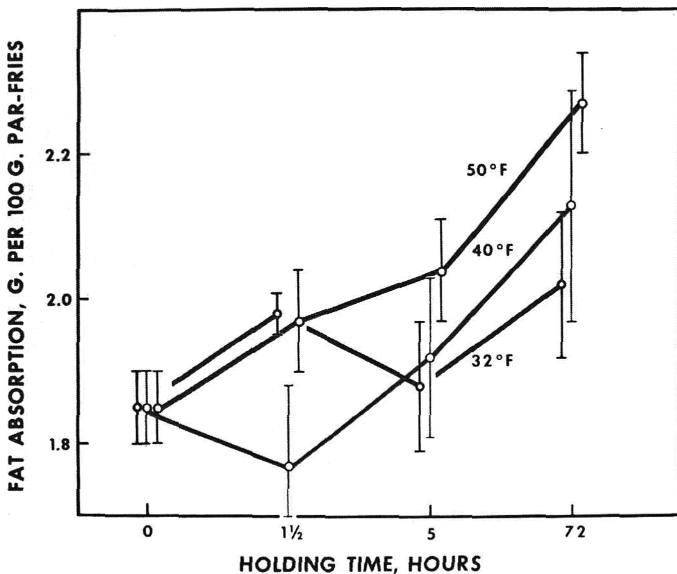

Fig. 3—Effect of time and temperature of holding par-fried potatoes on fat absorption when finish-fried 2.25 min at 375°F. Bar represents plus/minus one standard error of mean; time scale is arbitrary.

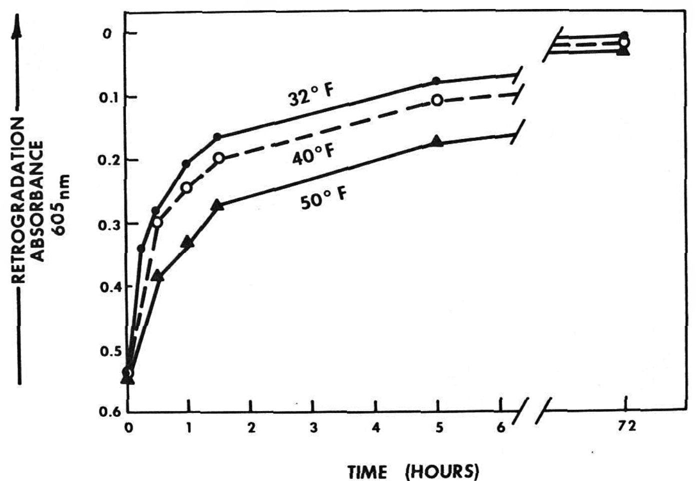

Fig. 4—Effect of time and temperature of holding ground par-fried potatoes on retrogradation of their gelled starch as measured by loss of solubility in water.

at say 32°F and the other from the frozen state at 0°F, the second will receive a less effective cook. Part of its cooking will be used up in melting the ice and in bringing the solids and water up to a mean temperature of 32°F. What is needed is to find an "equivalent cooking time," i.e., equivalent to the 2.25 min used for frying the thawed samples. One could fry to equal surface color, to equal interior texture, or to equal final center temperature, for example, but these "equivalent" frying times would all be different. Figure 5 illustrates the way heat penetrates par-fry strips during finish-frying. It is significant that the surface temperature rises to the boiling point of water almost immediately whether the strip starts out at 32° or 0°F. However, interior temperatures in the latter case lag behind those in the former, largely because of the need to supply the latent heat of fusion of ice. Visible browning is probably confined to the outer 0.01 in. or so; fat uptake occurs in, say, the outer 0.03-0.04 in.; moisture is lost to a somewhat deeper level; and the texture changes associated with cooking go to the center. For this reason there is no one time of frying from the frozen state which is in all ways equivalent to a given time of cooking from the thawed state. Consequently, controls fried from the frozen state were not included in the experiment.

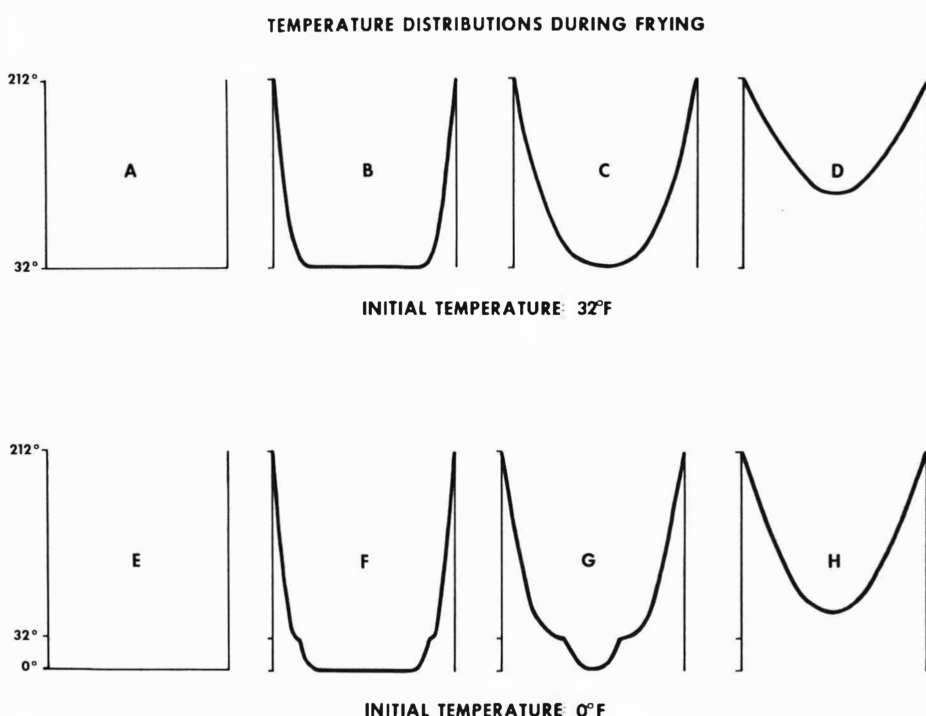

Fig. 5—Successive temperature distribution across the thickness of a par-fried potato strip during finish-frying. A–D, strip initially thawed at 32°F; E–H, strip initially at 0°F. Calculated by methods in Carslaw and Jaeger (1959) assuming all ice in strip melts at 32°F.

If the effects shown in Figures 1-3 are not caused by retrogradation of starch, what is the cause? The following is a possible explanation. During par-frying about 1/4 or more of the moisture in a potato strip will be driven off, primarily from the surface layers. If the strip is immediately frozen, the nonuniform distribution of moisture will be "frozen in." On subsequent thawing there will be a progressive diffusion of moisture from the center toward the surface and this diffusion will show a direct rate-temperature relationship. During finish-frying a strip with a relatively uniform moisture distribution would probably lose moisture faster than one in which the moisture was concentrated toward the center. It is also plausible that a greater loss of moisture would create more voids and permit a greater absorption of oil.

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C. PERI[1] and W. L. DUNKLEY
Dept. of Food Science & Technology, University of California, Davis, Calif. 95616

REVERSE OSMOSIS OF COTTAGE CHEESE WHEY
2. Influence of Flow Conditions

SUMMARY—Flow conditions in tubular, cellulose-acetate reverse osmosis membranes were varied by changing the flow rate and by inserting turbulence promoters (rods with intermittently-spaced rings cemented to them) in the tubes. In general, increasing the flow velocity increased the permeation rate. Turbulence promoters were most effective in increasing permeation rate under conditions that, in their absence, permitted fouling of the membrane surface by macrosolutes from the feed. When the feed contained macrosolutes, increasing the flow velocity or use of turbulence promoters increased retention of lactose and potassium. Modifying the flow conditions to increase turbulence improves performance of reverse osmosis membranes by increasing both permeation rate and retention.

INTRODUCTION

PART 1 of this study reported the influence of composition of the feed on the performance of cellulose acetate reverse osmosis membranes using varied operating pressures but constant flow conditions (Peri and Dunkley, 1971). In the present study flow conditions were varied by changing the flow rate and by inserting turbulence promoters into the tubular membranes.

Thomas and Watson (1968) studied the influence of turbulence promoters positioned away from the membrane surface on performance of dynamically formed reverse osmosis membranes. Selection of the turbulence promoters was based on an earlier study of effects of the devices on forced convection heat transfer (Thomas, 1967). The turbulence promoters increased permeation rates 10–50%, and also increased retention, particularly at the lowest Reynolds numbers.

EQUIPMENT & METHODS

REVERSE OSMOSIS membranes, apparatus and methods were described previously (Peri and Dunkley, 1971). In part of the experiments, turbulence promoters (Thomas, 1967) were mounted in the tubes. They were 0.80 cm dia plexiglas rods with 1.03 cm O.D. rings 0.15 cm wide cemented to them, with alternate spacings of 1.75 and 10.9 cm (center-to-center). Selected flow rates were obtained by varying the setting of the variable speed drive and the number of tubes connected in parallel in the manifold. For most experiments, the operating pressure was 28.2 kg/cm^2 (400 psi) for the type A membranes and 35.2 kg/cm^2 (500 psi) for the B membranes.

RESULTS & DISCUSSION

Flow conditions

Flow conditions are specified by giving mean velocities and indicating whether turbulence promoters were present. When the tubes contained turbulence promoters, flow of the circulating feed was through annuli with constant outside diameters, but with their inside diameters varied by rings cemented to rods mounted concentrically in the tubes. The turbulence promoters increased flow velocity at a given volumetric flow rate because of the fluid they displaced in the tubes, and increased turbulence because of the increased velocity and the varied flow channels created by the rings cemented to the central rods.

Calculated Reynolds numbers for the flow conditions included in the study ranged from about 300–3000. It is uncertain when the flow was turbulent. Turbulent flow could be expected at values of Reynolds number below that generally accepted as the critical value. The design of the turbulence promoters favored turbulent flow at low mean velocities. Even without turbulence promoters in the tubes, a constriction at the entrance probably caused turbulent flow through at least part of the tube when the calculated Reynolds number would indicate laminar flow.

Permeation rates

In general, changes in permeation rates during runs with varied flow conditions were similar to those reported previously for experiments with standardized flow velocity (Peri and Dunkley, 1971). The permeation rates were essentially constant during runs with lactose solution and simulated ultrafiltrate, but decreased during runs with whey and deproteinized whey. With turbulence promoters and especially at the higher flow velocities, flux leveled off to an essentially constant permeation rate within the first 3 hr, but without turbulence promoters flux was still decreasing at the end of 3-hr runs.

In experiments with whey protein solution as feed, the permeation rates decreased during runs without turbulence promoters and, at low flow velocity (11 cm/sec), with turbulence promoters. In contrast, with turbulence promoters and at high flow velocity (54 cm/sec), the permeation rate remained constant at a relatively high value (4.7–5.0 ml/cm^2 sec $\times$ 10^4 for membrane A).

In general, permeation rates increased with increases in mean flow velocity (Fig. 1). For membrane A without turbulence promoters and with whey as feed, however, increasing the velocity had little influence on permeation rate. Apparently, even the highest velocity used had little effect in reducing concentration polarization and fouling.

The turbulence promoters increased the permeation rates for both membranes with whey as feed, and for membrane A with deproteinized whey. In contrast, they did not increase flux with membrane B and deproteinized whey as feed, or with membrane A and simulated ultrafiltrate as feed. Thus, the effect of the turbulence promoters was

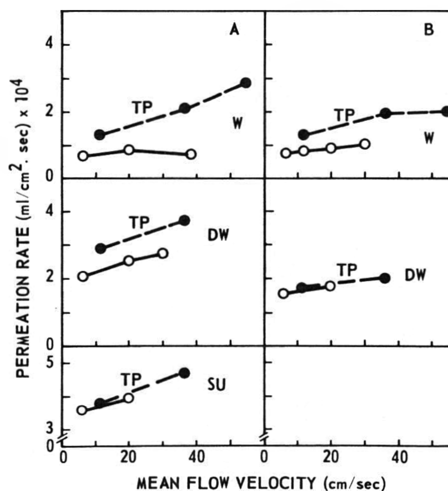

Fig. 1—Influence of mean flow velocity on permeation rate at end of runs for membranes A and B. Abbreviations: W, whey; DW, deproteinized whey; SU, simulated ultrafiltrate; TP, with turbulence promoters. Temperature of feed, 15–16°C. Respective available driving forces for membrane A with W, DW and SU as feed were 22.7, 22.9 and 24.3 atm, and for membrane B with W and DW as feed, 27.6 and 27.5 atm. (For conversion, 1 ml/cm^2.sec = 2.12 $\times$ 10^{4}gfd, gallons per square foot per day).

[1] On leave from Instituto di Industrie Agrarie, Universita di Perugia, Perugia, Italy.

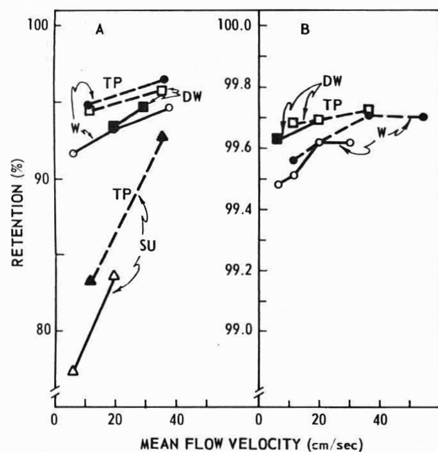

Fig. 2—Influence of mean flow velocity on retention of lactose for membranes A and B with selected feeds. (Abbreviations and operating conditions as for Fig. 1.)

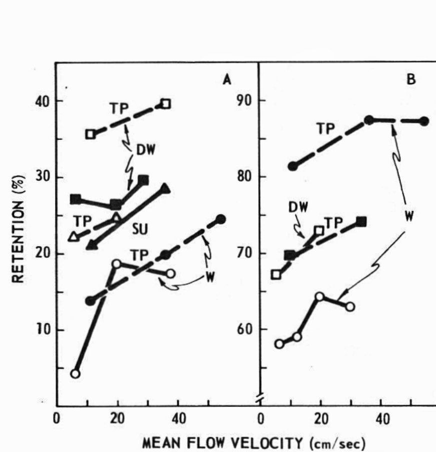

Fig. 3—Influence of mean flow velocity on retention of potassium for membranes A and B with selected feeds. (Abbreviations and operating conditions as for Fig. 1.)

greater with operating conditions that were conducive to concentration polarization and fouling.

Retention

For membrane A, retention of lactose increased with increasing flow velocity (Fig. 2). Also, turbulence promoters increased retention over that obtained at corresponding mean velocities without turbulence promoters, especially with whey as feed. For membrane B, because of its high intrinsic retentivity for lactose, changing the flow conditions had less effect on retention than for membrane A. Effects of changing flow conditions on retention of calcium were similar to those with lactose.

Retention of potassium also increased with flow velocity (Fig. 3). For membrane A, retention was higher and turbulence promoters had a greater influence with deproteinized whey as feed than with whey or simulated ultrafiltrate. For membrane B, turbulence promoters had a marked influence on retention with whey as feed, but not with deproteinized whey.

The data for retention of lactose in simulated ultrafiltrate (Fig. 2, membrane A) indicate the influence of flow conditions on concentration polarization. Increasing turbulence by increasing flow velocity and by inserting turbulence promoters reduces concentration polarization and thereby increases retention. The intrinsic retentivity of membrane A for potassium (Fig. 3) was so low that little concentration polarization could occur. Therefore, with simulated ultrafiltrate, changing the flow conditions had much less effect on retention of potassium (Fig. 3) than of lactose (Fig. 2).

The composition of the feed influenced the effects of changing flow conditions on retention of lactose. The increased retention of lactose in whey and deproteinized whey, compared with simulated ultrafiltrate (Fig. 2), may be explained on the basis of pore plugging and the formation on the membrane of a layer of gel or cake (Peri and Dunkley, 1971).

Explanation of the apparently anomalous data for retention of potassium is more complex than for lactose. For membrane A, retention of potassium in simulated ultrafiltrate is intermediate between that in whey and deproteinized whey.

In interpreting the results in Figure 3, we visualize that for membrane A, the operating conditions using turbulence promoters with deproteinized whey as feed gave the highest retention of potassium because macromolecules from the feed plugged pores and formed a thin fouling layer which increased retentivity but did not contribute seriously to microsolute concentration polarization. With simulated ultrafiltrate as feed, retention was lower because macromolecules were not available. With whey as feed, protein fouled the membrane and aggravated microsolute concentration polarization. In the absence of turbulence promoters, there was less turbulence at the membrane surface and therefore more concentration polarization of both macrosolutes and microsolutes.

For membrane B, turbulence promoters had little effect on retention of potassium in deproteinized whey, but a marked effect on retention in whey (Fig. 3). With whey as feed, the increase in turbulence caused by the turbulence promoters decreased accumulation of protein at the membrane surface and thereby reduced microsolute concentration polarization in the layer of gel or cake.

In processing complex feeds such as whey, microsolute concentration polarization is always detrimental, but macrosolute concentration polarization can have beneficial effects, depending on objectives of the process. In processing whey, objectives include concentration at minimum cost and fractionation to modify product composition. With some operating conditions, macrosolute concentration polarization (fouling) increases retention but decreases permeation rate. For example, when processing simulated ultrafiltrate, deproteinized whey and whey using membrane A operated with comparable flow velocity and available driving force, retentions of lactose for the three feeds were, respectively, 83.5, 93.2 and 93.2%, but corresponding permeation rates were 39.7, 25.5 and 8.8 ml/cm^2 sec × 10^4. Thus, macrosolutes in deproteinized whey increased retention markedly with only a 35% decrease in permeation rate. With whey, the same increase in retention was accompanied by 78% reduction in permeation rate. Fortunately, processing conditions can be selected to take advantage of controlled macrosolute concentration polarization. When turbulence promoters were inserted into membrane A and the equipment was operated with comparable flow velocity and available driving force, retention of lactose in whey increased from 93.2-94.6%, and permeation rate increased from 8.8-27.8 ml/cm^2 sec × 10^4. Thus, the turbulence promoters caused a three-fold increase in permeation rate, accompanied by a small increase in retention.

Macrosolutes in the feed can either increase or decrease retention of microsolutes, depending on operating conditions. Our results indicate that when macromolecules are present, increasing turbulence by increasing the flow velocity and especially by using turbulence promoters, increases the permeation rate without sacrificing the improvement in retention attributable to the macromolecules.

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W. G. SCHULTZ, R. P. GRAHAM, W. C. ROCKWELL, J. L. BOMBEN, J. C. MIERS and J. R. WAGNER
USDA Western Regional Research Lab., ARS, Albany, Calif. 94710

FIELD PROCESSING OF TOMATOES. 1. Process and Design

SUMMARY—Among the advantages of processing tomatoes in the field, compared with processing solely at a cannery, are increased yield and reduced waste. Mechanically harvested tomatoes were processed at 500 lb/hr through a mobile field unit within 6 hr after harvest during the 1969 season. Processing within 6 hr from harvest prevented a loss of about 9.5% of the ripe tomatoes that occurred when processing was delayed 24 hr. An increase of 4.3% in solids recovery was obtained by acid treatment of the hot macerate, bringing the total increase to 13.8% for field processing. Either the single strength or concentrated material is suitable for bulk transport to a cannery for storage, additional concentration, or immediate formulation into sauces, catsup and other tomato products.

INTRODUCTION

CALIFORNIA produces 6 billion pounds of processing tomatoes per year, or about 70% of U.S. production (Anon., 1970b; USDA, 1969); this generates about 400 million pounds of wet, solid waste, and 5 billion gallons of waste water per year (Water Resource Engineers, 1965; NCA, 1965). In California, most canneries are located near urban centers; because of their seasonal operation and high level of waste production, they find it difficult to dispose of this waste by using the municipal system. Some canneries are required to provide secondary and even tertiary waste treatment processes that add substantially to the processing cost.

In California, hand picking of tomatoes has given way to mechanical harvesting (NCA, 1966). Handling, washing and sorting operations have therefore been modified (May et al., 1970; Mercer and Olson, 1969; O'Brien et al., 1968). Mechanical harvesting is relatively nonselective compared with hand picking, so that green tomatoes are picked and are a sizeable waste material (Lipton and Uota, 1968). Mechanical harvesting then has aggravated the waste disposal problem and decreased harvest yields below the potential. Even though plant breeding research has developed plants that produce tomatoes with tough skins and firm shapes, the rough handling during mechanical harvesting still causes significant damage. Transportation to the cannery in 1000-lb bins further aggravates the problem. Once damaged, the tomato deteriorates because of microbiological and enzymatic actions. This causes a 10–20% loss in tomato solids during the usual 24-hr interval between harvest and factory processing, including an allowance for hauling and mold losses (Mercer and Olson, 1969). This damaged fruit ends up largely as waste at the urban cannery and is a direct yield loss.

If tomatoes were processed into a stable macerate or juice in the field and the macerate or juice transported in bulk to the cannery, the waste load at the municipal system would be reduced and the process yield increased. In addition, processing the tomatoes in the field provides an area for disposing of processing waste water by using it for irrigation either on unharvested tomatoes or other crops.

This report describes a project undertaken to explore the production of tomato juice and macerate near the harvesting area rather than at an urban cannery. Processing was integrated, from harvesting the tomatoes to the production of juice and concentrate as in commercial packing. During the 1969 tomato harvesting season, a mobile processing pilot plant was operated at a site adjacent to the fields in a typical northern California tomato growing area. The objectives were to provide information on overall yields, waste disposal and production during quick post-harvest processing. In addition, the stability of the juice in bulk transport from the field processing site to our laboratory in Albany, Calif. was measured. At the Albany laboratory, the juice was vacuum concentrated and further evaluated.

EXPERIMENTAL

Processing unit design

The mobile pilot plant was designed to process 500 lb of tomatoes per hour at a steady, continuous flow. The following steps were performed by this unit: (1) preparation of tomatoes—washing and sorting; (2) hot break—for rapid enzyme destruction; (3) acid treatment—an optional step to increase tomato solids extraction and consistency; (4) finish pulping—to separate tomato juice from the seeds, skins and fiber; and (5) deaeration and cooling—to stabilize the juice for holding and transporting.

It is generally considered that high-fluid shear and holding tomato material at elevated temperatures changes its texture, consistency, color and flavor (Hand et al., 1955; Marsh et al., 1968; Wagner et al., 1969). Therefore, in the design, holding time as well as agitation, pressure transitions, and the steam injection velocity were held to a minimum. 10 min elapsed between milling of tomatoes and cooling of juice to the final shipping temperature. The time could have been reduced further, but it was felt that such reduction would not appreciably improve the product and would not be commercially feasible. With the small flow rate of 500 lb of tomatoes per hour (1 gpm of macerate), it was more important to simulate the process than to use prototype equipment. True prototype equipment would require impractical small pipe and equipment size to achieve the proper flow velocities and conditions.

The processing unit consisted of three trailers. An 8 × 30 ft covered trailer contained the basic, permanently mounted process equipment. Steam was supplied from a self-contained boiler mounted on a 7 × 10 ft trailer. An 8 × 16 ft trailer housed an air-conditioned analytical laboratory. These trailers had interconnecting electrical, water, and steam supplies. The entire unit could be set up within 6 hr after its arrival at the field location.

Figure 1 is a schematic diagram of the equipment layout. Tomato bins were loaded onto the dump rack by a fork lift. Tomatoes were washed in a 6 × 3 × 3 ft tank and elevated from the wash tank through a water spray and onto an open mesh inspection belt.

After inspection and manual sorting, the tomatoes were macerated by a mill equipped with an 8-in. diameter bowl, a 1600 rpm impeller, and a 1/2-in. round-hole screen. From the mill, the tomato macerate dropped into the intake of a positive displacement pump which was the prime product flow-rate controller. The inlet of the pump had an overflow outlet so that the pump inlet was flooded, but excess tomato macerate could not collect. This insured a known, constant time between macerating and heating.

Hot-break heating was accomplished with a low-velocity injection and contact of steam and macerate, followed by a mechanical mixing (Fig. 2). Low-velocity contact, with subsequent mechanical mixing, has been used by others (Dietz, 1960; Marsh et al., 1966). A high velocity injection in the critical velocity range can be used to both heat and mix (Brown et al., 1951; Morgan and Carlson, 1960; Wasserman and Lazar, 1953), but this causes high shear which alters texture and consistency. In the steam injection system designed for this work, the steam flowed into a heating tee at about 100 fps. Tomato macerate flowed from the feed pump through a check valve at about 1.6 fps. The heating tee was a standard 1-1/2-in. sanitary tee, closely coupled to the feed pump and mixer. The mixer was a sanitary, 4-in. centrifugal pump in which the macerate flowed counter to the normal pumping direction. A thermocouple was installed at the mixer outlet. Hot break temperature was controlled by a three-mode electronic recording controller which sent a

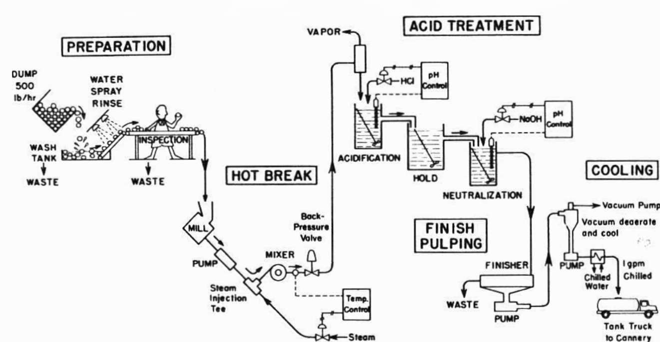

Fig. 1—Flow diagram of tomato field-processing unit.

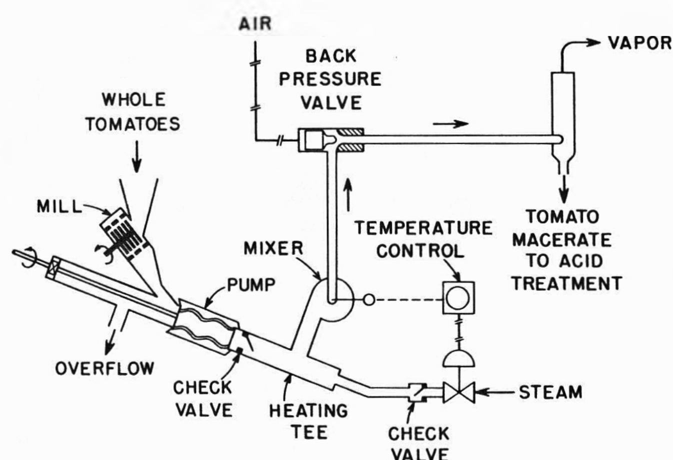

Fig. 2—Hot break diagram.

pneumatic output to the steam-modulating valve.

The steam injection system was held at 5 psig above the saturation vapor pressure of the water in the heated macerate by means of a pneumatically loaded back-pressure valve. From this back-pressure valve, the macerate flowed tangentially into a vapor separator.

From the vapor separator, the macerate entered either the optional acid-treatment section or went to the finish pulper. This acid-treatment section consisted of three tanks for acidification, hold, and neutralization; acid and caustic reservoirs; and two pH recording controllers. The acidification and neutralization tanks each held 1 gal of macerate for an average residence time of 1 min. Both tanks had thermally compensated pH probes, an agitator and a thermometer. The holding tank had an agitator and outlet taps corresponding to 0, 1.2, 3.3 and 4.6 min average macerate residence time. The pH controllers had pneumatic outputs to control valves for modulating the gravity flow of acid and caustic to their respective tanks.

Finish pulping was done with a 6-in. diameter paddle finisher having a 0.027-in. screen and a 1400 rpm paddle speed. The finish pulping, like the milling, was operated at constant conditions throughout these trials. Other scientists have reported on these operations (Hand et al., 1955).

The product was cooled in two stages. First, it was vacuum deaerated and flash cooled to 95°F. The vacuum was provided by a mechanical vacuum pump preceded by a shell and tube water-vapor condenser. The second stage of cooling was accomplished in a tubular heat exchanger having a 30-75°F cooling range.

Electricity was brought to the process trailer and distributed through a central power panel. Low pressure well water was supplied to the process trailer where it was filtered and pressurized to 50 psig with a booster pump. The steam generator produced up to 450 lb of steam per hour at 90 psig. This generator was self-contained and oil-fired; it had a water softener and a chemical additive system to control boiler water quality. Instrument air was supplied by a 60 psig compressor. Waste water was collected in a central drain under the trailer and piped to a settling pond.

Operating conditions

Tomatoes were mechanically harvested in the morning of each day of the trials, loaded into 1000-lb bins, and trucked 5 mi to the process equipment site. Here, the field-run tomatoes were rough washed and flotation graded (at a commercial central sorting station) to remove field debris and green tomatoes before being re-binned.

Each process run lasted about 4 hr and required 2-3 bins of tomatoes. In most runs, the VF-145 variety was processed as it is the predominant processing tomato in California. Some tomatoes of the San Marzano Napoli variety were also processed, since this variety is important in the production of tomato paste. For examining the effects of holding tomatoes in bins for 24 hr, a double quantity was obtained and half were held for processing the following day.

Finish washing and sorting was done on the process trailer. The tomatoes were dumped from the field bin into the wash tank where they were agitated for an average of 10 min before spray rinsing and elevating to the inspection belt. Sorting removed green or molding tomatoes and any remaining field debris. Tomato stems were not discarded, nor were split tomatoes that were otherwise sound.

Within 15 sec after the tomatoes were broken in the mill, steam injection had inactivated the enzymes. By testing hot-break temperatures between 170-240°F, it was found that 210-212°F gave optimum enzyme deactivation and product consistency.

The acid treatment extracts pectin from the seeds, fiber and skin that would otherwise end up as finisher pulping waste. It is an optional step and has been described by others (Miers et al., 1970; Wagner et al., 1968; Wagner et al., 1969). It consists of three steps—acidification, holding and neutralization at a temperature of 195°F. Several holding times and acidification levels were evaluated. A pH of 2.75 and a holding time of 3.3 min were optimum for maximum consistency and yield. The neutralization step was performed either immediately after the macerate left the acid-treatment holding tank or could have been done after transporting the juice to our laboratory in Albany for concentration. Acidification was done with hydrochloric acid; at pH 2.75, it required approximately 0.5 gal of 12N acid per 100 gal of tomato macerate. The macerate was always brought back to the fresh tomato pH of 4.3 ± 0.1 with a 50% solution of sodium hydroxide; this step consumed 0.33 gal of caustic per 100 gallons of tomato.

The wastes from sorting and finish pulping were collected and weighed. Samples of tomatoes, macerate, and juice were taken at various points along the process line: (1) before dumping; (2) after milling; (3) after heating; (4) after acidification; (5) after neutralization; and (6) after cooling. These samples were then analyzed in the field laboratory.

After each day's run, the process equipment was cleaned with hot water and detergent, followed by a water rinse. Following the rinse, a 50 ppm chlorine solution was run into the equipment and left overnight. Before starting the next day's operation, the equipment was thoroughly flushed with hot water.

DISCUSSION

IN COMMERCIAL field-processing operations, there should be a permanent installation, as in a central sorting-washing operation. The functions of a field receiving station, State Tomato Inspection, central sorter, and a processing line probably should be merged. Capacity would be 100 tons of tomatoes per hour, or more. All the equipment needed is now commercially available. The decision to apply a field-processing operation is largely an economic one. Thorough consideration should be given to the effects on associated tomato products, such as peeled tomatoes, if these were needed. If used, the acid treatment would best be handled by a first-in-first-out system using in-line mixers and acid injection. A holding line (tubing) could provide the necessary time lag following acidification of the tomato macerate. Neutralization of the acid, finish pulping, and vacuum concentration can be done either at the field site or the cannery.

Field processing of tomatoes helps to reduce the time when the tomato is in a perishable, fresh state. Tomato juice can

either be held temporarily for a day or two at reduced temperature or pH, or sterilized and held asceptically for several years. Results of these trials indicate that single-strength tomato juice at 36°F, pH 4.3, will be stable 4-6 days; juice at 36°F, pH 2.75, will be stable somewhat longer. Juice at 60°F, pH 4.3, will be stable 5-20 hr; at pH 2.75, for 24-48 hr. Juice at 85°F, pH 4.3, will be stable less than 6-10 hr; at pH 2.75, about 24 hr. Field processing could include concentration, sterilization and asceptic packing. Currently tomato paste is stored in bulk tanks holding 20,000 gal or more. It can be transported in tank trucks or railroad cars of similar size (Anon., 1961; Anon., 1970a; Dixon et al., 1963).

In these trials, processing tomatoes within 6 hr of harvest increased the yield of tomato solids 9.5% over that obtained when processing was delayed 24 hr. In addition to a tomato solids loss, this 24-hr delay allows mold growth and loss of fresh tomato flavor.

Acid treatment reduced the wet waste coming out of the finish pulper by 47% and in addition produced a juice of thicker consistency. This 36% solids-loss reduction (dry basis) increases the yield of tomato solids by 4.3% of the total tomatoes processed. When this extraction increase (4.3) is combined with the 6-hr field-processing increase (9.5), an overall solids yield increase of 13.8% is obtained. Details on this yield increase, and information on consistency, serum viscosity, vitamin retention, and color are described in Part 2 of this paper (Miers et al., 1971).

The National Canners Association (Berkeley, Calif.) took samples throughout the process line for microbiological checks. These tests were made during regular experimental runs and further checked during continuous 6-hr runs, to determine the nature of the microbial flora and whether there was a bacterial build-up during processing. At the start, the wash tank was inoculated with bacteria by introducing field dirt into the tank. Results indicate that the clean-up procedure and the hot-break heating were sufficient to pasteurize the tomato macerate and prevent bacterial build-up in the process line. The pasteurized product, without further treatment, was used for the time-temperature, holding-stability checks.

The wash water contained an average of 1000 ppm of tomato solids for all runs (ranging from 120 to 2000 ppm). This average is equivalent to 8 lb of COD (chemical oxygen demand) per ton of tomatoes processed. Assuming that the tomato soluble solids are sugars and acids, a COD of approximately 1100 ppm was in the waste wash water; this amount can easily be disposed of when used as irrigation water. The COD was probably lower than that of a commercial operation since the tomatos processed 6 hr after harvest were always in good condition (compared to those held 24 hr). Pre-washing at the central sorting station and the relatively short runs (4-6 hr) also contributed to the low wash-water solids. Significantly though, when tomatoes were held 24 hr, the soluble solids in the wash water doubled.

The solid waste, such as cull tomatoes, can be put back on the tomato field and plowed in during the post-harvest cultivation normally done a few days after harvest. This waste normally has too high a water content to dry economically for animal feed. The finish pulping waste contains about 20% protein and can be used as an animal feed supplement. In terms of dehydration cost to produce animal feed, this waste is 60% water if only a hot break is used, but only 52% water if both the hot break and acid treatment are used. The acid extracted waste is drier and would require about 16% less dehydration fuel.

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A 14-min, sound, 16-mm color movie covering this work is available for loan from the USDA Western Regional Research Lab., 800 Buchanan St., Albany, Calif. 94710.

Reference to a company or product name does not imply approval or recommendation of the product by the USDA to the exclusion of others that may be suitable.

J. C. MIERS, J. R. WAGNER, M-D. NUTTING, W. G. SCHULTZ,
R. BECKER, H. J. NEUMANN, W. C. DIETRICH and D. W. SANSHUCK
USDA Western Regional Research Lab., ARS, Albany, Calif. 94710

FIELD PROCESSING OF TOMATOES
2. Product Quality and Composition

SUMMARY—Mechanically harvested tomatoes were macerated, heated by steam injection to several constant temperatures from 170–240°F and held in a treatment pot from 0–4.6 min at pH levels (adjusted with conc. HCl) ranging from natural down to pH 2.0. Treated macerates were neutralized to original pH with conc NaOH, put through a finisher screen and pumped through a deaerator and chiller. The optimum processing conditions based on highest consistency (efflux-pipet flow time) in seven series of samples were 212°F breaking temperature and a macerate treatment time of 3.3 min at pH 2.75. With the optimum conditions, juice solids yield from VF-145 tomatoes was 4.3% greater and consistency 90% higher than from the natural pH juice. The neutralized acid products could contain 13% less tomato solids and still have a consistency equal to that of the products extracted at natural pH. Dry waste decreased 33% and total wet waste 47%. A 1-day delay before processing caused 9.5% loss in recovered juice solids. Ascorbic acid, reflectance and lycopene data showed no trends due to acid extraction. Water soluble color increased slightly in the acid extractions.

INTRODUCTION

THE INCREASING problems of land, air and water pollution in the last decade have affected many segments of the food industry including tomato processing plants. Waste disposal for many tomato processors has become increasingly more difficult and expensive; as a consequence, some processing plants have relocated away from centers of high population density.

The importance of these problems in food processing has encouraged governmental research agencies, university laboratories and trade associations to investigate and develop better ways of waste disposal (Graham et al., 1969; Mercer and Olson, 1967; California State Water Resources Control Board, 1968). Processing close to the growing area where the waste could be conveniently returned to the soil or used for livestock feed should help to simplify the problem. At the same time, the tomatoes, subjected to less transportation damage, would be much fresher when processed, thereby increasing yield considerably (Mercer and Olson, 1967).

Increased juice yields and less finisher wastes were also found in earlier laboratory experiments from using a very rapid hot break at 200°F and acid extraction; consistency increases were several fold over natural extraction (Miers et al., 1967; Wagner et al., 1968). If these benefits were significantly large in plant scale processing, low pH extraction could be used not only to increase consistency and solids, but also to decrease the waste disposal problem.

With the above ideas in mind, our research objectives were to find the optimum pH, breaking temperatures and macerate holding time to give the highest juice consistency possible and the highest yield of tomato solids with the least amount of loss due to leaching, finishing, and culls.

To study the aspects of field processing, this laboratory designed and built a mobile pilot plant at Albany which was operated at Dixon, Calif. during the 1969 tomato season (Schultz et al., 1971). This manuscript presents the data from the pilot plant experiments and discusses their importance to the tomato industry.

EXPERIMENTAL

Raw tomato sampling

Vine ripened tomatoes were mechanically harvested early in the morning, washed and sorted in a commercial washer and delivered to the field pilot plant. A bucket of tomatoes was selected at random from each of the 3–6 bins to be used in that day's processing. Four tomatoes were picked at random consecutively from each bucket and a one-quarter section was cut from each tomato. Each quarter was put into one of four 2-liter beakers. This quartering and distribution procedure was repeated until four equivalent 1-kg samples of tomato quarters (45–53) were obtained.

The potential consistencies of the above subsamples were determined in the manner outlined by Miers et al. (1967) after extraction with 15 ml of concentrated HCl. These extracts were also analyzed as outlined in a subsequent section.

Samples from the pilot plant

The tomatoes were washed, spray-rinsed and the culls removed. They were then macerated at 500 lb/hr, heated to selected temperatures (170–240°F) by steam injection, held in a treatment chamber for selected times (0–4.6 min) at various pH levels (adjusted with conc. HCl) from natural down to pH 2.0, neutralized if necessary with conc NaOH to the original pH, finished, deaerated and chilled.

The time between changing a processing condition and the complete replacement of macerate and juice in the line was 12–14 min. Therefore, no samples were collected until the control recorders had shown a steady state for at least 20 min. Samples included overflow macerate (feed in excess of the 500 lb/hr pump capacity), hot macerate from the treatment chamber, finisher waste and deaerated juice, chilled or unchilled. Samples taken were representative of 40–900 lb of tomatoes.

Four 2-day yield runs were made to study effects of 1-day delay in processing and of treating the macerate at natural pH or pH 2.75 (±0.1) for 3.3 min. 8–12 bins were obtained, using 4–6 bins each day. Tomatoes were macerated and heated to 212°F in all yield runs. Leaching losses due to washing were determined from samples of wash water taken near the beginning, at the middle and near the end of each sampling period. Total weights of juices, finisher wastes, wash water and cull tomatoes from the yield runs were determined.

Samples were sealed in enameled 211 × 304 cans or in fruit jars and held at 34 or 0°F until used. In the yield runs at least 1 doz enameled 211 × 304 cans of each juice were sealed, heated 35 min in boiling water and cooled in ice water.

In two yield runs, 2 doz plain body 211 × 414 cans of each natural and neutralized acid juice were sealed and heated as above. The remainder of these juices was concentrated 3–4 fold under reduced pressure at a product temperature of 100°F. The concentrates were sealed in 211 × 304 enameled cans, heated 35 min in boiling water and cooled in tap water.

Nonsterile juice, neutralized-acid juice and juice at pH 2.75 were stored at 85, 60 and 36°F. Samples were checked at intervals for changes in consistency and development of off-odors.

Laboratory analyses

Consistencies of the juices were measured at 25° ± 0.1°C usually by the efflux-pipet described by Wagner and Meirs (1967), but in cases of very high consistency, similar pipets having slightly larger I.D. tips were used. Consistencies were determined on four consecutive dilutions (by weight) of the nine pairs of natural and neutralized acid juice from the yield runs. Similar measurements were made on consecutive dilutions of the juices prepared by the potential consistency procedure.

The consistencies of the concentrates (duplicate cans) and several consecutive dilutions of each were determined in a Bostwick consistometer at 25°C. Two or more readings of the flow (cm) at 30 sec were recorded.

Levels of pH were determined with a

glass-electrode pH meter. Total solids were measured by the AOAC procedure (1960). Total nitrogen in finisher wastes was determined by the AOAC Kjeldahl procedure (1960).

Sodium chloride content of the samples was determined by diluting 20g to 100 ml with 10% acetic acid in 0.1N HNO_3, filtering and measuring Cl-ion content in an Aminco-Cotlove chloride titrator. The added NaCl was found by difference.

Fe, Cr, Ni and Cu contents were determined by wet ashing in conc H_2SO_4 and conc HNO_3 and analyzing by atomic absorption spectrophotometry.

Ascorbic acid content was determined by blending 50–100g of juice with 0.5% oxalic acid (5 ml/g of juice), adding 2% by weight of analytical Celite, stirring 1 min, and filtering through medium speed paper. Ascorbic acid in the filtrate was measured with indophenol dye at 520 mμ, using a 1.9 cm path in a Bausch and Lomb Spectronic 20 by the procedure of Loeffler and Ponting (1942).

Water soluble color in the tomato juices and concentrates was determined by either one or both of two procedures. First procedure—the optical density of the filtered 0.5% oxalic acid extract used in the analyses for ascorbic acid was determined in a Bausch and Lomb Spectronic 20 at 420 and 667 mμ. The longer wavelength was used as a blank for cloudiness or other color and the value obtained was substracted from the 420 mμ reading to give the O.D. of the water soluble color. Second procedure—50g of juice was blended 3 min at moderate speed with 50 ml of a 0.3M citrate buffer pH 4.5. After adding 2.5g analytical Celite, and blending 1 min, the extract was filtered through SS 604 paper; the first 10 ml was discarded. The O.D. was determined in the same manner as outlined for the first procedure. The color reflectance was determined with a Hunter Color Difference Meter (Judd, 1952). Lycopene content was determined by the procedure of Wong and Bohart (1957) but the addition of the Celite and filtration was omitted.

RESULTS & DISCUSSION

Optimum pH level

When the average consistency (flow time) of samples (macerated at 200°F and treated 3.3 min) from two runs was plotted against extraction pH level, a very significant increase in consistency at pH levels between 2.5–3.0 was evident (Fig. 1). This corroborates previous laboratory experiments with the drop-in procedure described by Wagner et al. (1968).

As the pH was decreased from natural to 3.0 or lower, wet waste decreased to about one half the weight of the natural pH waste. The dry solids content in one experiment increased from 41.6–50.0% at pH 3.0 and remained there for lower pH levels. The skin color in the waste changed progressively from pale reddish yellow to pale yellow; seeds became cleaner and whiter as the pH was lowered.

Based on the above data, macerates were treated at pH 2.75 for the remaining experiments.

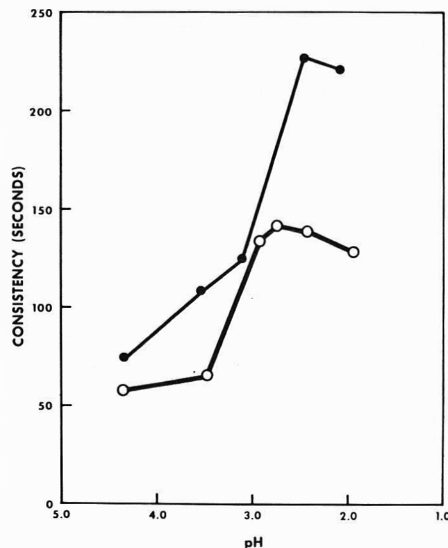

Fig. 1—Effect of extraction pH upon neutralized acid juice consistency from two runs; breaking temperature 200°F, macerate treatment time 3.3 min.

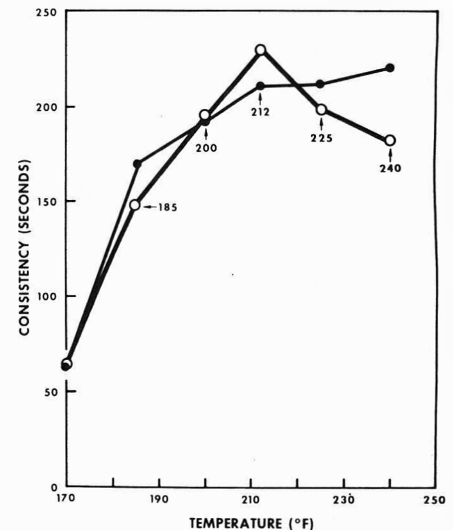

Fig. 2—Effect of breaking temperature upon neutralized acid juice consistency from two runs; macerate treatment time 3.3 min, pH 2.75.

Optimum breaking temperature

When the average consistency of samples treated at pH 2.75 for 3.3 min was plotted against several breaking temperatures, the highest consistency occurred at 212°F breaking temperature for one experiment (Fig. 2). The slight increase in consistency in the second experiment at 240°F was not considered sufficient compensation for the added risk of thermal damage to product quality (Miers et al., 1970; Kramer and El-Kattan, 1953) as shown by the first experiment (Fig. 2). Therefore 212°F was selected as the breaking temperature for the remaining runs.

Wet wastes tended to decrease one-quarter to one-half in weight. They became 2–4% drier as the temperature increased from 170–200°F above which the percent solids remained fairly constant. The color of the waste appeared less red and more yellow as temperature increased.

Optimum treatment time

When tomatoes were macerated at 212°F and treated at pH 2.75 for different time periods, maximum consistency was obtained at 3.3 min (Fig. 3). This closely approximated the time indicated by previous laboratory experiments. No changes in juice color or waste characteristics could be detected due to treatment time.

Yield of solids

Using optimum conditions (212°F and pH 2.75 for 3.3 min) acid extraction increased recovery of juice solids by 4.3% (Table 1) from the VF-145 tomatoes. Application of this method would have increased the 1967 combined yields of catsup, paste, puree, and sauce by more than 3,000,000 cases (USDA, 1969).

Delay of one day in processing caused almost 10% solids loss from the VF-145 juices due to a 75% increase in leaching losses and 6% loss of tomato solids from moldy and spoiled tomatoes. The losses in the Napoli San Marzano variety were considerably less even though they had been held one day when first processed. This variety is exceptionally firm and consequently not subject to as much loss as the VF-145 variety.

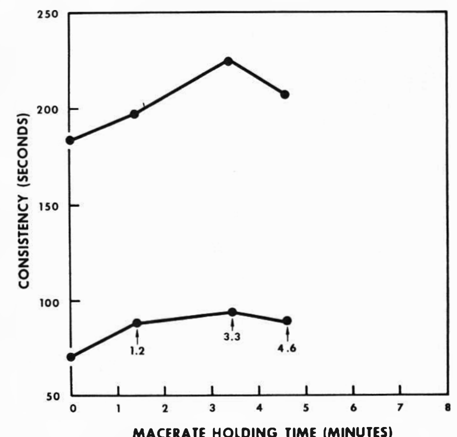

Fig. 3—Effect of macerate treatment time upon neutralized acid juice consistency from two runs; breaking temperature 212°F and pH 2.75.

Table 1—Effect of pH and delay on processing yields and losses

No. runs	Processing pH	Days delay	% Yield[a] Juice solids	% Loss of solids[a] Finisher waste	Wash H_2O	Culls
VF-145						
6	Natural	—	81.9	10.0	5.2	2.9
6	Acid	—	85.4	6.7	4.8	3.1
Δ due to acid extraction			+ 4.3[b]	−33[c]		
3	Natural	0	86.4	10.2	3.4	0
3	Natural	1	77.5	9.7	6.9	5.9
Δ due to delay			−10.3[d]		+103[e]	
3	Acid	0	89.3	6.9	3.8	0
3	Acid	1	81.5	6.5	5.7	6.3
Δ due to delay			− 8.7[d]		+ 50[e]	
Avg Δ due to delay all runs			− 9.5[g]		+ 75[f]	
Napoli San Marzano						
2	Natural	—	85.7	8.7	1.0	4.7
2	Acid	—	91.6	5.0	0.9	2.5
Δ due to acid extraction			+ 6.9[b]	−43[c]		
1	Natural	1	86.4	9.1	1.0	3.5
1	Natural	2	84.9	8.2	0.9	6.0
Δ due to delay			− 1.7[d]		− 10[e]	+ 71[f]
1	Acid	1	93.5	5.0	0.6	1.0
1	Acid	2	89.8	5.1	1.2	4.0
Δ due to delay			− 4.1[d]		+100[e]	+300[f]
Avg Δ due to delay all runs			− 2.9[g]		+ 31[g]	+122[g]

[a] Based on total tomato solids in juice, finisher waste, wash water and culls. Applies to all percentages except those with other footnotes.
[b] Based on % juice solids at natural pH.
[c] Based on % waste at natural pH.
[d] Based on % of all first day runs.
[e] Based on % leaching loss in H_2O of 1st day run.
[f] Based on % loss culls of 1st day run.
[g] Based on total % obtained at both pH levels on 1st day.

Statistical analyses of variance were made on the arc sine square root transformation of the percentages. The 4.3% increase in juice solids due to extraction at 2.75 pH was significant at the 0.05 level. The loss of almost 10% in juice solids due to a one day delay in processing was significant at the 0.05 level. The 33% decrease in finisher waste was significant at the 0.005 level.

Our losses did not include transportation damage which can be as much as 47% (Mercer and Olson 1967). Therefore, relocation of the first steps of processing near the growing areas would probably be even more advantageous than our figures indicate.

Acid extraction reduced the wet weight of the finisher waste by 47% (Table 2) and the dry weight by 35%. The acid extracted waste was drier (48.4% solids) than the natural pH waste (40.0% solids). Thus the disposal problem would be cut by almost one-half. The dried product would contain about 25% protein—considerably more than in waste from natural pH extraction. Tomato waste has been found satisfactory when fed to swine (Hays, 1919), dairy cows (Tomhave, 1931), steers (Chapman, et al., 1958), and chickens (Ammerman et al., 1965).

Consistency gains

Acid extraction increased consistency 1- to 2-fold (Table 3). The question arises as to what total benefit is derived from the increased solids and consistency of the neutralized acid tomato products? A measure of this benefit was obtained by diluting the neutralized acid juice to the same consistency as the original natural juice (Fig. 4). From these curves the percent solids difference between the original and the diluted neutralized acid juice having the same consistency as the original natural juice was determined. Table 3 shows results from two typical runs along with the average of seven runs using VF-145 and two runs using Napoli San Marzano. The total benefit due to acid extraction is quite high, averaging 13.6% for all nine runs. This is the percentage by which the original weight of a neutralized acid juice can be diluted and still have a

Table 2—Effect of extraction pH on finisher waste composition

Run	Extraction pH	Finisher waste lb/ton of juice	% Solids[a]	% Added salt from process[b]	% Protein Wet	Dry
1	Natural	28.8	43.0	0	9.9	23.0
	Acid	17.5	49.9	0.24	12.7	25.5
2	Natural	32.7	43.0	0	8.9	20.7
	Acid	17.0	50.2	0.26	12.9	25.7
3	Natural	—	41.6	0	8.9	21.4
	Acid	—	50.0	0.26	12.7	25.4
		Avg 9 runs				
	Natural	31.6	40.0			
	Acid	16.8	48.4			
	% Δ	47[c]	20[c]			

[a] Percent dry solids in wet waste.
[b] Dry basis.
[c] Based on averages for natural runs.

Table 3—Tomato solids and consistencies of natural, neutralized acid, and diluted neutralized acid juices

Juice	% Solids	% Δ Solids[a]	Consistency sec
VF-145			
Natural	5.16		38
Neut. acid	5.29	15.5	77
Dil. neut. acid	4.58		38
Natural	5.43		42
Neut. acid	5.51	10.6	68
Dil. neut. acid	4.98		42
Avg 7 runs			
Natural	5.50		51
Neut. acid	5.63	13.1	98
Dil. neut. acid	4.99		51
Napoli San Marzano			
Natural	5.36		52
Neut. acid	5.71	16.8	176
Dil. neut. acid	4.89		52
Natural	5.45		57
Neut. acid	5.40	13.9	155
Dil. neut. acid	4.74		57
Avg 2 runs			
Natural	5.40		54
Neut. acid	5.55	15.3	166
Dil. neut. acid	4.81		54

[a] Based on diluted neutralized acid juice.

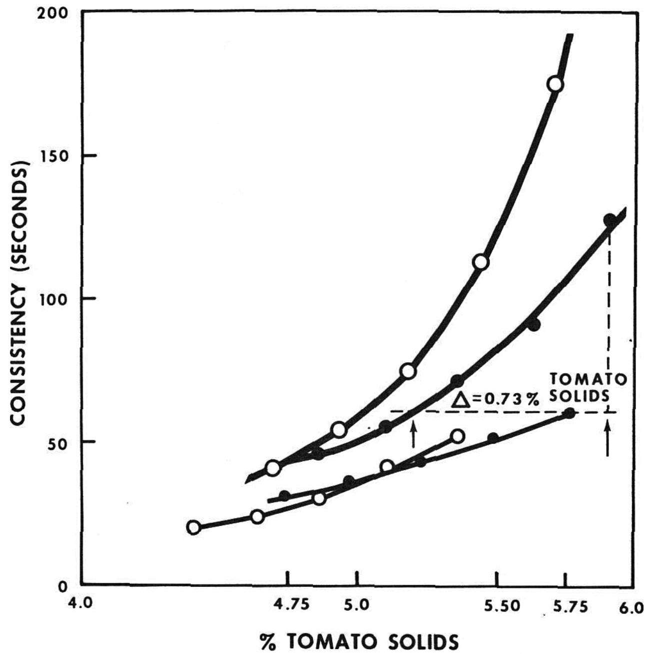

Fig. 4—The difference in percent solids between original and diluted neutralized acid juice having same consistency as original natural juice; —— natural juices; —— neutralized acid juices.

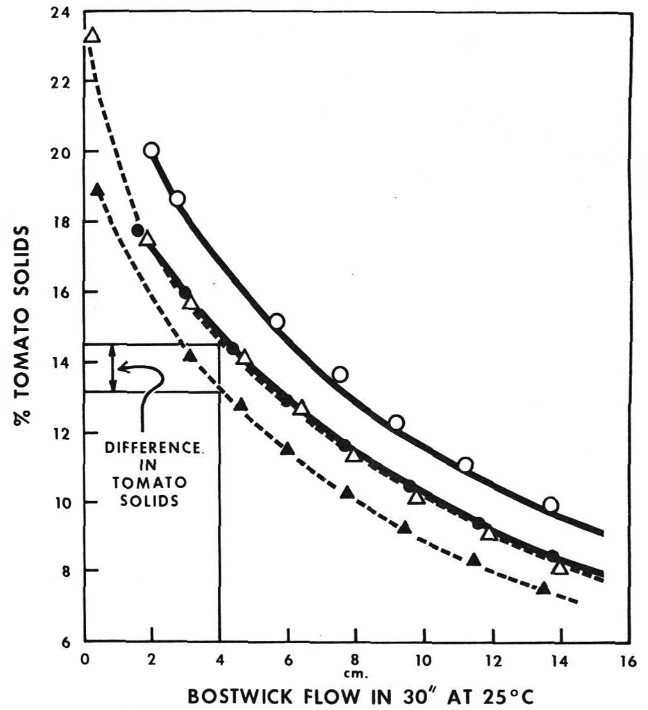

Fig. 5—Tomato solids in original or diluted concentrates vs. Bostwick flows. ——from VF-145's; ----from Napoli San Marzanos; ○ and △, made at natural pH; ● and ▲, acid treated.

consistency equivalent to that of the natural pH juice.

The retention of the total benefit due to increased consistency in concentrates was substantiated by plotting Bostwick flows (cm) against tomato solids in several consecutive dilutions of natural and neutralized acid paste (Fig. 5). One pair was from VF-145 and the second pair from Napoli San Marzano. From the curves the concentration differences between the natural and the neutralized acid paste were determined at seven Bostwick flow rates ranging from 2–14 cm. The average percent difference at seven flow rates show an over-all benefit of 13.0% for both varieties (Table 4). Such benefits from dilution would be of significant economic value to remanufacturers. The use of tomato pastes often depends in part upon the consistency given by tomato solids to the formulations.

The total benfit from dilution of pilot plant neutralized acid juices and concentrates is quite good. However, this benefit would have been much higher if the consistency of these juices had been as high as indicated by the potential consistency determined in the laboratory. In eight comparisons (Table 5) the consistency of the pilot plant neutralized acid juice averaged 30% less than the laboratory juice diluted to equivalent tomato solids.

Results in Table 6 do not show any trends in ascorbic acid, lycopene, and Hunter Color Difference Meter reflect-

Table 4—Percent tomato solids in natural and neutralized acid concentrates having equivalent Bostwick flows

Bostwick flow cm/30 sec	VF-145 % Solids pH 4.3[a]	VF-145 % Solids pH 2.75[a]	VF-145 % Difference[b]	Napoli San Marzano % Solids pH 4.3[a]	Napoli San Marzano % Solids pH 2.75[a]	Napoli San Marzano % Difference[b]
4	16.8	14.7	14.3	14.7	13.3	10.5
12	10.5	9.3	13.4	9.3	8.1	14.8
Total range used	9.6–19.9	8.4–17.2	12.6–16.3	8.3–17.2	7.4–15.9	7.9–14.8
Avg from 7 flow rates			13.9			12.1

[a]Extraction pH.
[b]Based on % solids in neutralized acid concentrates.

Table 5—Comparison of consistencies

Expt no.	Undiluted lab juice % Tomato solids	Undiluted lab juice Potential consistency (sec)	% Tomato solids	Consistency at same % of solids: Pilot plant neut. acid juice a	Consistency at same % of solids: Diluted lab juice b	% Diff. (a-b) 100 / b
1	6.11	359	5.63	138	213	−35
2	5.95	230	5.83	140	210	−33
3	6.57	478	6.18	211	330	−36
4	6.81	416	6.32	198	233	−15
5	5.47	172	5.11	79	105	−25
6	5.95	175	5.54	78	116	−33
7	6.08	123	5.53	123	169	−27
8	7.20	566	6.95	281	416	−32
					Avg =	−30
					s =	7

Table 6—Comparison of natural and neutralized-acid juices

	Ascorbic acid mg/100g					Metals			
	short[a] cans	tall[a] cans	Lycopene mg/100g	HCDM[b] reflectance a/b	Water soluble color O.D.[c]	μg/g of tomato solids Fe	Cr	Ni	Cu
9/8/69 Nat	6.3	—	9.8	1.85	0.12	84	1.2	15	14
Neut-acid	7.6	—	9.7	1.86	0.18	382	9.7	16	15
9/15/69 Nat	8.4	21.3	10.2	1.79	0.08	74	1.7	12	14
Neut-acid	9.5	19.7	10.2	1.80	0.14	65	6.7	13	15
9/25/69 Nat	8.6	—	11.0	1.86	0.11	145	1.5	11	12
Neut-acid	8.2	—	10.8	1.86	0.17	128	1.3	16	13
9/30/69 Nat	13.0	20.0	12.4	2.02	0.11	80	1.1	12	13
Neut-acid	14.2	21.0	12.5	2.05	0.15	55	1.3	16	12

[a]Short cans 211 × 304 fully enameled; tall cans 211 × 414 plain body, enameled ends.
[b]Hunter Color Difference Meter.
[c]Optical Density.

Table 7—Stability of juices at three holding temperatures

		Type of juice					
		Natural		Neutralized acid		Acid (pH = 2.75)	
Sample code	Holding temp °F	No off-odor	Off-odor	No off-odor	Off-odor	No off-odor	Off-odor
A	36	4 da	—	4 da	—	4 da	—
A	60	4.5 hr	Slight at 20 hr	5.5 hr	Slight at 22 hr	22 hr	—
A	85	6 hr	Slight at 9 hr	12 hr	Strong sour 27 hr	24 hr	—
B	36	6 da	—	6 da	—	6 da	—
B	60	24 hr	Strong 48 hr	21 hr	Strong 48 hr	48 hr	—
B	85	10 hr	Sour, gassy 23 hr	10 hr	Sour odor 23 hr	26 hr	—

ance due to acid extraction. In two experiments juice was canned in both short and tall cans. A much greater retention of ascorbic acid was found in the tall cans. Water soluble color (optical density) increased some, and erratic results in Fe, Cr, and Ni occurred in neutralized acid juices. The very large increase in Fe in the 9/8/69 neutralized acid sample is probably an artifact since all others contained much less Fe.

The juices prepared near the growing areas could be hauled to a central plant for further processing. The juices cannot be held at a temperature high enough to keep it sterile because such a temperature would lower the consistency (Miers et al., 1970). However, the holding stability would be increased considerably (Table 7) if the juice is cooled to 36°F or if the juice acidity is at the extraction pH of 2.75.

The preceding results show that excellent juices can be prepared at 2.75 pH with 4%-7% increase in yield of tomato solids, 1- to 2-fold increase in consistency, and 47% decrease in wet finisher waste. In addition fresher fruit could be processed, losses due to transportation damage decreased and preprocessing delays shortened, all increasing yield of tomato solids. The juices could then be hauled to a central plant for further processing without loss of quality.

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P. C. BARNES JR., CHARLES E. HOLADAY and JACK L. PEARSON
USDA Market Quality Research Div. ARS, Dawson, Ga. 31742

DEVICE TO MEASURE EASE OF SKIN REMOVAL FROM PEANUTS

SUMMARY—A new procedure for measuring the ease of skin removal from peanuts is described. A simple device was constructed from readily available materials and will blanch a 250g sample in 2 min. A series of tests showed that the procedure has excellent precision and compares favorably with two commercial dry-blanching operations.

INTRODUCTION

BLANCHING (skin removal) is a major step in processing peanuts for edible end uses (Anon., 1940). A high quality peanut butter should be as free as possible of skins and skin particles. Both skins and germs impart a bitter taste to peanut butter (Willich et al., 1952); skins also give peanut butter poor color and texture. Peanuts which have a low blanchability or poor blanching properties may make several passes through the blancher. In extreme cases, when the skins cannot be removed, the peanuts are often diverted to oil stock. This can mean an economic loss to the processor.

The most important methods of skin removal are dry blanching, spin blanching, water blanching and alkali blanching (Dieckert and Morris, 1958; Lawler, 1961; Reeve, 1962). Dry blanching removes both skin and germ, while the other methods remove only the skins (Woodroof, 1966). Varietal differences, maturity and curing are among the factors which affect the ease of skin removal.

This paper presents a simple method for measuring the ease of skin removal from peanuts. A pneumatic sample blancher was developed for making the test. It is simple in design and constructed of readily available materials.

APPARATUS & PROCEDURE

THE OUTER PART of the blancher is made of two gasoline funnels (No. 2F-123, General Metalware Company, Minneapolis, Minn.). A schematic diagram of the blancher is shown in Fig. 1. A funnel-shaped retainer screen made from 1/8-in. hardware cloth with a 1-in. sheet metal flange at the top is placed inside the lower funnel to hold the peanuts. The upper funnel has a piece of 1/8-in. hardware cloth soldered completely across the inside. This hardware cloth serves as the upper retainer screen. A small hand-type vacuum cleaner (Sears-Roebuck Model 208-6151) is connected to the upper funnel (Fig. 2). The vacuum is required to remove loose skins and dust and to maintain a tight seal between the upper and lower funnels during blanching. Foam rubber gaskets are cemented to the rim of the upper funnel and underneath the flange of the lower retainer screen to form the seal. An air nozzle (W. H. Curtin Company straight form, serrated, 3/8 in. I.P.S.) is soldered inside the spout of the lower funnel. The nozzle is connected to a source of compressed air with a static pressure of 50 lb.

To determine the blanchability of a lot of peanuts, heat a 250g sample uniformly at approximately 400°F for 25-30 min. The temperature is not critical. Willich et al., (1952) showed that uniform blanching is independent of the degree of heating. Place the peanuts in the lower retainer screen and set the top funnel in place. Turn on the vacuum cleaner, and after a seal has been established between the upper and lower funnels, turn on the compressed air. The

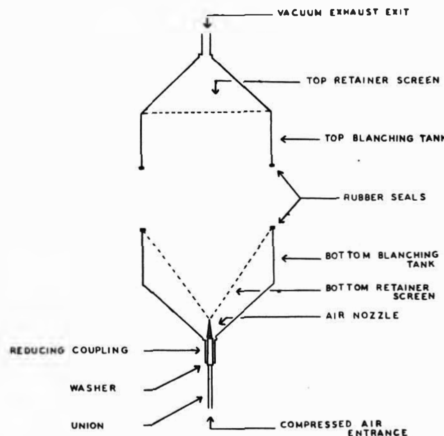

Fig. 1—Pneumatic peanut sample blancher.

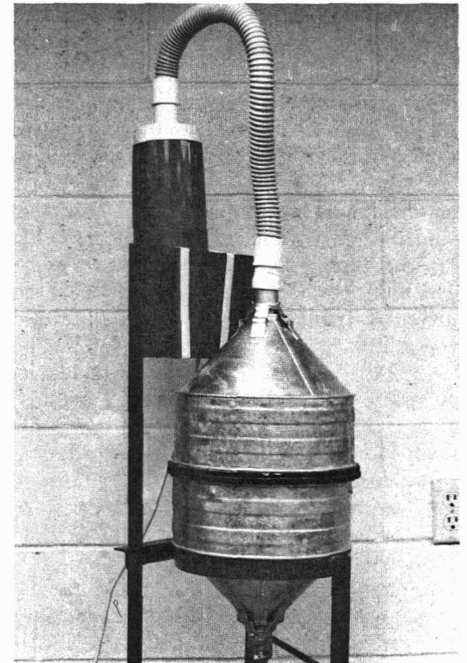

Fig. 2—Sample blancher completely assembled showing vacuum cleaner attached.

Table 1—Blanchability tests of 3 varieties of dry roasted peanuts

Spanish			Runner			Florigiant		
Initial sample weight (g)	Pick-out weight (g)	Blanchability (%)	Initial sample weight (g)	Pick-out weight (g)	Blanchability (%)	Initial sample weight (g)	Pick-out weight (g)	Blanchability (%)
250	10	96.0	250	34	86.5	250	9	96.5
250	10	96.0	250	36	85.5	250	13	94.5
250	11	96.0	250	34	86.5	250	8	97.0
250	13	94.5	250	37	85.0	250	6	97.5
250	10	96.0	250	32	87.5	250	6	97.5
250	12	95.0	250	37	85.0	250	11	96.0
250	9	96.5	250	40	84.0	250	7	97.0
250	10	96.0	250	34	86.5	250	10	96.0
250	11	96.0	250	34	86.5	250	6	97.5
250	15	94.0	250	37	85.0	250	4	98.0
Sample Mean		95.6%			85.8%			96.75%
Standard Deviation		0.768%			1.004%			0.981%
Coefficient of Variability		0.80%			1.17%			1.01%

peanuts immediately start a tumbling motion, rubbing against the retainer screens. This action, together with the stripping action of the air jet, removes the skins. After 2 min, turn off the compressed air and then the vacuum cleaner. Dump the peanuts from the lower retainer screen on to a white enameled pan to facilitate detection of unblanched particles. Shake the pan gently back and forth several times to turn the peanut halves over so that the flat side is facing down. Pick out all peanuts which have visible pieces of skin remaining on the kernels and weigh. Calculate the blanchability as follows:

% blanchability = (A-B)/A (100)
A = initial weight of sample
B = weight of unblanched pickouts

RESULTS & DISCUSSION

A SERIES of tests were made to test the precision of the blanchability method. Approximately 3500g of each of the major peanut types (Spanish, Runner and Virginia) were thoroughly blended and uniformly heated under the same conditions as mentioned above. Ten 250g samples were weighed from each 3500g lot and blanched in the pneumatic sample blancher. Results of these tests are given in Table 1. As shown in the table the standard deviations were ± 0.981%, ± 1.005% and ± 0.768% for Florigiant, Runner, and Spanish peanuts, respectively. The coefficient of variability for each of the tests indicates a high degree of precision.

Two additional series of tests were run to compare the pneumatic sample blancher with two commercial dry blanchers, since dry blanching is the most widely accepted method of skin removal from peanuts. Only the Florigiant and the Spanish types were used in tests with blancher No. 1, and in tests with blancher No. 2 a mixture of Runner and Spanish types (2:1) were used since these were the peanut types the two commercial blanchers were processing at the time. The peanut samples in each case were obtained from the commercial processor immediately before the peanuts entered the blancher and immediately after the peanuts left the blancher. The peanuts had been heated previously to about 300°F at the commercial blancher No. 1. The unblanched samples were blanched on the pneumatic sample blancher as described above. The results are given in Table 2. The mean weight of the blanched samples from the pneumatic sample blancher was 239g for the Florigiant and 236g for the Spanish. These same weights were then used for each sample of Florigiant and Spanish peanuts from the commercial blancher No. 1 in order that the blanchability test for the sample blancher and the commercial blancher would be on the same weight basis. Each individual sample from the commercially blanched peanuts was picked out by hand in the same manner as those from the sample blancher. The results show that both varieties had a higher blanchability in the sample blancher than they did in the commercial blancher. The same procedure was followed in the tests on samples from the commercial dry blancher No. 2. Peanuts had been previously heated to about 400°F. The results are given in Table 3. As in the case of the first commercial blancher, the sample

Table 2—Comparison of the pneumatic sample blancher with commercial dry blancher No. 1

	Pneumatic sample blancher				Commercial blancher		
Variety	Initial sample weight (g)	Weight after blanch (g)	Pick-out weight (g)	Blanchability (%)	Weight after blanch (g)	Pick-out weight (g)	Blanchability (%)
Florigiant	250	239	5	98.0	239	20	92.0
	250	239	3	99.0	239	20	92.0
	250	239	6	97.5	239	16	93.5
	250	240	5	98.0	239	14	94.5
	250	239	5	98.0	239	16	93.5
	250	240	3	99.0	239	15	94.0
	250	240	5	98.0	239	16	93.5
	250	239	9	96.5	239	18	93.0
	250	239	6	97.5	239	17	93.0
	250	239	3	99.0	239	13	95.0
	Sample Mean			98.05%			93.4%
	Standard Deviation			0.756%			0.916%
	Coefficient of Variability			0.77%			0.98%
Spanish	250	236	17	93.0	236	35	86.0
	250	235	16	93.5	236	27	89.0
	250	235	16	93.5	236	29	88.5
	250	236	15	94.0	236	31	87.5
	250	235	15	94.0	236	26	89.5
	250	237	16	93.5	236	27	89.0
	250	236	16	93.5	236	26	89.5
	250	236	17	93.0	236	30	88.0
	250	235	15	94.0	236	34	86.5
	250	236	16	93.5	236	29	88.5
	Sample Mean			93.55%			88.2%
	Standard Deviation			0.350%			1.144%
	Coefficient of Variability			0.37%			1.29%

Table 3—Comparison of the pneumatic sample blancher with commercial dry blancher No. 2

	Pneumatic sample blancher				Commercial blancher		
Variety	Initial sample weight (g)	Weight after blanched (g)	Pick-out weight (g)	Blanchability (%)	Weight after blanched (g)	Pick-out weight (g)	Blanchability (%)
Runner + Spanish (2:1)	250	225	22	91.0	224	40	84.0
	250	223	21	91.5	224	44	82.5
	250	223	25	90.0	224	49	80.5
	250	224	23	91.0	224	39	84.5
	250	224	20	92.0	224	42	83.0
	250	223	19	92.5	224	40	84.0
	250	224	21	91.5	224	45	82.0
	250	224	26	89.5	224	40	84.0
	250	224	27	89.0	224	42	83.0
	250	226	23	91.0	224	47	81.0
Sample Mean				91.0%			83.0%
Standard Deviation				1.044%			1.039%
Coefficient of Variability				1.140%			1.25%

blancher showed a higher percent of skin removal. This relationship will vary with the type and efficiency of the commercial blanching operations, but a few initial comparisons should provide the necessary correction factors. The sample blancher also has a lower standard deviation and coefficient of variation than both commercial blanchers used in these tests. With this simple procedure the blanchability of a lot of peanuts can be easily and rapidly determined before purchasing or processing.

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JACK W. RALLS, HARRY J. MAAGDENBERG, GARY LEMOINE and WALTER A. MERCER
Western Research Laboratory, National Canners Association, Berkeley, Calif. 94710

ALTERNATE STORAGE SYSTEMS FOR THE PRODUCTION OF CANNED BLACK RIPE OLIVES

SUMMARY—The storage of freshly harvested olives in systems other than the traditional sodium chloride brine was investigated in an attempt to provide an alternative storage system which would reduce the present saline pollution potential of the olive canning industry. Chemical salts used as fertilizers were partially successful in holding olives for several months without spoilage. Ammonium nitrate brines could be used for periods up to 15 wk; however, it was difficult to remove the salt completely during preparation for canning. Brines containing ammonium sulfate (with the pH controlled by the addition of H_2SO_4) held olives for 6 mo. The difficulties in completely removing ammonium sulfate from the olives limited the commercial potential for this type of storage. Food grade glycols containing bacteriostats can be used to store olives for periods of over 6 mo. Olives submerged in propylene glycol–diethyl pyrocarbonate mixtures showed severe shriveling. The recovery from shriveling on subsequent preparation was satisfactory only for the smaller-sized olives. A similar result was obtained for olives stored in a mixture of glycerol–benzoic acid–sorbic acid. The most promising storage system developed in this study was a mixture of water–propylene glycol–benzoic acid–potassium sorbate–HCl. By using KOH in place of NaOH for the chemical curing preparation steps, it was possible to prepare canned olives with essentially no generation of liquid waste containing sodium ions.

INTRODUCTION

THE FOOD processing industry is now entering a critical stage in its growth and development due to the problems associated with environmental pollution. The parallel increases in population, food production and urban development have created a situation which demands more efficient waste management. One of the more promising approaches to pollutional abatement in food processing operations is the modification of established practices to accomplish a reduction in, or the more economic disposal of, the by-product waste materials produced. This paper describes some exploratory work designed to provide a reduction in potential environmental pollution by evaluating the effect on final product quality of radical changes in the traditional production methods for canned, black, ripe olives.

A considerable portion of the annual crop of olives harvested in California during the past decade (27–66,000 tons) are stored for as much as 12 mo in 8–12% NaCl solutions. After storage, the olives are treated with several changes of 0.5–2% solutions of NaOH, aerated to develop the black color, washed, and canned in NaCl brine. These operations result in liquid wastes of high sodium, chloride and sulfate (after pH adjustment with H_2SO_4 ion content. The disposal of these brines is an increasingly difficult problem as requirements enforced by regulatory agencies become more stringent.

In recent years, the brine has been retained in ponds for gradual discharge into sewage treatment plants, or for solar evaporation; trucked to coastal processing plants where salty receiving waters can be used; or the storage and processing brines reused. All of these disposal methods are temporary expedients for most of the olive canneries due to economic or regulatory considerations.

The reuse of by-product liquids is a logical and useful route to pollution abatement, but it is not uniformly successful in the present state of technology. A commercial scale test of the use of activated carbon treatment to recondition storage and processing brines prior to reuse (Popper, et al., 1967) is now underway; this approach holds considerable promise in alleviating saline pollutional problems with minimum changes in production technology.

There is no certainty that any of the above mentioned disposal and reuse methods will provide a complete solution to the saline pollutional problems of the olive canning industry. Every promising alternative solution should be examined in enough detail to provide satisfactory input for management decisions. The alternative approach described in this paper is that of substituting reusable glycol–bacteriostat systems, solutions of chemical fertilizer salts, or acidified water–glycol mixtures for the conventional NaCl brines.

MATERIALS & METHODS

General

The olives used in the storage experiments were obtained from the sizing belts at receiving stations or canneries during 1965–1967. The olives were transported to the laboratory and put into previously prepared storage systems. The olives generally were placed in storage within 24 hr after picking. The Mission, Manzanillo and Sevillano varieties were used in medium, large and colossal sizes. There was no attempt to get a highly uniform maturity sample; only purple-colored olives were discarded.

The NaCl used for olive storage was kiln dried solar pond salt. The NaCl used in canning brine was vacuum canners grade. The NaOH and KOH used was reagent grade, as were the ammonium nitrate and ammonium sulfate salts used. The glycerol, propylene glycol, benzoic acid, potassium sorbate, sorbic acid, diethyl pyrocarbonate and HCl used were all food grade (Anon., 1966).

The olives were stored in 32-gal capacity polyethylene pails with lids. The majority of the samples were stored inside a building. The olives were submerged in the storage liquid by redwood disks of different diameter which could be wedged against the sides of the pails at different heights. The olives were prepared for canning after storage in plastic pails or in 20 gal stainless steel tanks.

The preparation of the olives took 7 days of soaking, treatment, aeration and washing. Generally, the stored olives were soaked in three changes of fresh water (10 gal for each 50 lb of olives on a fresh weight basis) over a period of 2 days. The rinsed olives were treated with a 1.5% solution of NaOH or KOH until the average depth of penetration of base was 1/16–1/8 in. After rinsing and aeration with stainless steel sparging tubes, the olives were treated for about 16 hr with a 0.75% solution NaOH or KOH. The olives were then washed and aerated to remove excess alkali and to develop color. The olives were soaked overnight in a water solution (5 gal vol) of 18 oz of NaCl and 16g of ferrous gluconate for each 5 lb of olives.

The olives were packed by hand into 301 × 411 cans and a hot brine (containing 6-3/4 lb of NaCl in each 30 gal of water) was added; the cans were closed and retorted for 47 min at 252°F, water cooled in the still retort, dried, and then stored on shelves at ambient temperature (55–85°F).

Olives in storage were sampled periodically and analyzed for juice pH, titratable acidity and reducing sugars (using Ames Company Reducing Sugar Test Kit, No. XA 6511.) The level of ammonium salts in olives was determined by steam distillation of an olive slurry (after the addition of strong NaOH solution) and the ammonia collected titrated with standard acid. The sulfate content of the olives was determined by a gravimetric method as barium sulfate using the slurried samples after steam distillation. Boiling with NaOH solutions was found necessary for good filtration of the olive flesh from the dissolved sodium sulfate.

Glycol storage of olives

Medium Mission olives (50 lb) were stored in 5 gal of propylene glycol which contained

12.5 ml of diethyl pyrocarbonate. These olives showed a moderate degree of shrivel after a storage period of 6 mo. Olives soaked in three 10 gal changes of water over 2 days showed a marked reduction in the degree of shrivel.

Two pails were each charged with 5 gal of U.S.P. propylene glycol, 12.5 ml of diethyl pyrocarbonate and 50 lb of colossal Sevillano olives. The olives became badly shriveled after 2 wk of storage. The propylene glycol-stored olives were carried through the standard process after 2 mo of storage.

Two pails were charged with 5 gal of U.S.P. glycerol, 23.8g of benzoic acid and 23.8g of sorbic acid. The pails were each charged with 50 lb of colossal Sevillano olives.

After 2-1/2 mo of storage, the olives in glycerol were badly shriveled (the shriveling took place within the first 2 wk of storage). The two 50-lb lots were combined and processed. The olives were put through a needle-board supplied through the courtesy of a local olive canner. The needling was done in one pass just before the final soaking in brine and ferrous gluconate solution.

Ammonium salt storage

In the preliminary studies on alternate salts, the same brine concentration in degrees salometer was used for the test brine and NaCl control brine. The brines were originally 16° salometer from the addition of 3 lb 9 oz of NaCl and 4 lb 7 oz of ammonium sulfate, respectively, in 10 gal of water.

The final concentration of brine after the build-up was adjusted to 32°S by the addition of 10 lb of ammonium sulfate and 7 lb 7 oz of NaCl, respectively, to each type of storage pail.

Three pails were prepared which contained 12°S ammonium nitrate brine (5 lb of reagent grade ammonium nitrate in 10 gal of water). 100 lb of colossal Sevillano olives were added to each pail.

Several samples of large Manzanillo olives were put down in ammonium sulfate brines of concentrations of 24–56°S. The length of storage before onset of spoilage and other brine characteristics are tabulated in Table 1.

The storage capabilities of ammonium sulfate brines acidified with sulfuric acid are summarized in Table 2.

Table 1—Storage conditions for Manzanillo olives in ammonium sulfate brines

	Pail number					
Date	7[a] °S, pH	9 °S, pH	11 °S, pH	13 °S, pH	15 °S, pH	16 °S, pH
10-12-67	22, 6.5	40, 6.8	—	—	—	—
10-15-67	30, —	40, 5.5	35, —	37, —	37, —	40, 5.5
10-18-67	28, —	38, —	36, —	35, —	33, 4.8	37, 4.8
10-21-67	30, 3.8	36, 4.8	40, 4.9	40, 4.8	41, —	44, —
10-24-67	34, 3.6	46, 4.1	40, 4.2	39, 3.9	45, 4.0	47, 4.1
10-27-67	38, 3.5	44, 4.1	47, 4.2	46, 3.9	50, 3.9	53, 4.0
11-1-67	42, 3.8	48, 4.2	50, 4.3	50, 4.0	53, 4.1	55, 4.2
11-8-67	39, 3.8	46, 3.7	46, 4.2	46, 4.2	49, 4.2	51, 4.3
11-15-67	41, 3.8	43, 3.7	50, 4.3	48, 4.0	51, 4.1	53, 4.2
11-23-67	41, 3.8	42, 3.7	48, 4.1	47, 4.0	51, 4.1	52, 4.2
11-27-67	—	D[b]	—	—	—	—
12-6-67	39, 3.8	—	44, 3.7	D	D	D
12-20-67	39, 3.8	—	45, 3.7	—	—	—
1-3-68	42, 3.7	—	45, 3.6	—	—	—
1-10-68	43, 3.9	—	46, 3.8	—	—	—
1-24-68	—	—	D	—	—	—
2-9-68	D	—	—	—	—	—

[a]10-gal brine volume.
[b]D = discarded.

Processing of ammonium salt stored olives

Colossal Sevillano olives stored in ammonium nitrate brine for 3 mo were put through a standard wash out and process. A parallel run was carried out on olives stored in NaCl brine for 3 mo.

The first sign of any difference in the olives from the two different types of brine was seen in the first lye cure.

After 2 hr in 1.5% NaOH solution, the olives from ammonium nitrate brine developed a strong odor of ammonia. After 5 hr of exposure to the 1.5% NaOH solution, the olives from NaCl storage had a normal 1/16–1/8 in. penetration by the base. After the same time, the olives from ammonium nitrate storage had no penetration by the lye.

After the second lye cure (0.75%) and aeration, the olives from the ammonium nitrate storage were yellow-green in color.

After canning, the olives from the ammonium nitrate storage had a light color. Of more serious consequence was the fact that the flesh had the consistency of a heavy puree. It was obvious that a radical change in processing would be required to produce a satisfactory product from olives stored in ammonium nitrate brine.

Modified processing to remove ammonium salts

An attempt was made to determine if the salt content of the olives stored in ammonium nitrate brines could be lowered by modified processing. Four 25-lb batches of ammonium nitrate-stored olives were taken after 14 wk of storage. They were subjected to the following treatment (all water volumes used were 5 gal):

1. Two hot water rinses each day;
2. Seven changes of boiling water each day;
3. Normal cold water soak, followed by 4.5% NaOH curing; and
4. 2 min live steam blanch, then two changes of hot water each day.

The ammonium nitrate content was measured three times a day for each of the four treatment samples.

Through the courtesy of a commercial olive canning company, 100 lb of olives stored in acidified ammonium sulfate brine were made available for study.

The storage history of the olives was

Table 2—Storage of Manzanillo olives in acidified ammonium sulfate brines

	Pail number			
Date	8 °S, pH	10 °S, pH	12 °S, pH	14 °S, pH
10-12-67	22, 6.9	22, 4.0	22, 3.7	22, 3.7
10-15-67	30, 4.1	21, 3.8	30, 3.9	33, 3.9
10-18-67	31, 3.3	31, 3.3	32, 3.4	31, 3.5
10-21-67	31, 3.7	38, 3.8	36, 3.7	39, 3.9
10-24-67	33, 3.4	37, 3.4	42, 3.4	37, 3.3
10-27-67	37, 3.4	44, 3.5	48, 3.5	46, 3.4
11-1-67	41, 3.6	48, 3.7	52, 3.7	50, 3.6
11-8-67	39, 3.4	44, 3.6	48, 3.6	46, 3.8
11-15-67	40, 3.7	42, 3.5	51, 3.5	47, 3.4
11-23-67	40, 3.6	41, 3.4	49, 3.6	47, 3.5
12-6-67	38, 3.6	39, 3.4	47, 3.6	44, 3.5
12-20-67	39, 3.8	40, 3.5	47, 3.7	45, 3.8
1-3-68	41, 3.6	42, 3.5	47, 3.8	45, 3.8
1-10-68	42, 3.7	42, 3.6	48, 3.7	45, 3.7
1-12-68	—	D[a]	—	D
1-24-68	—	—	D	—
3-8-68	D	—	—	—

[a]D = discarded.

described as follows by the production manager of the company.

"Large size Sevillano olives were put down on October 14, 1966, in a brine made from ammonium sulfate (Fertilizer grade) to a 12° salometer reading and a pH of 3.2. The pH was reached by adding equal volumes of 80% lactic acid and 80% acetic acid.

"On October 15, when the tank was filled with olives and covered, sufficient ammonium sulfate was added to bring the brine to a salometer reading of 18°. The salometer reading was maintained at 16-17° by six additions of ammonium sulfate.

"Three additions of lactic-acetic acid mixture were made to control the pH at 4.0 or less.

"On February 5, 1967, the tank (which contained about 1900 lb of olives) was opened and some olives removed. The olives were more shrivelled than those from an equal salometer value sodium chloride brine. Some of the olives were yellowish around the stem end and were a little more tender than the remainder of the olives. On processing, it was found that the olives took stronger lye and longer times to get penetration. There was a strong odor of ammonia during the lye treatment.

"The olives developed a desirable color, but the flavor was poor.

"On May 19, 1967, additional olives were removed from the storage. The color was good. Some were shrivelled and the fraction of soft stem end olives was about the same as in February. The brine was a brighter yellow-green than comparable sodium chloride storage brine. There was a grey pellicle on the surface of the ammonium sulfate storage tank; none of these was found on any of the sodium chloride storage vats at this time."

The Sevillano olives received from the commercial source were not from a high ammonium sulfate concentration storage. However, these olives did represent material which could be used to study the effect of different preparation stages on the reduction of sulfate level.

The Sevillano olives were hand-sorted to select two 25-lb portions of firm and uniformly colored olives. The two portions of olives were individually soaked in 2.5 gal of fresh water for 6 hr and the soaking repeated for 16 hr.

One 25-lb portion of the soaked olives was aerated in 2.5 gal of water containing 14 oz of potassium carbonate. After 5 hr, the suspension showed considerable foaming, but no odor of ammonia. After 6 hr of aeration, the suspension had a pH of 11.15. When the analysis of a sample of the olives from the aeration in the potassium carbonate solution showed only a slight reduction in the ammonium sulfate level (see Fig. 1) work on this sample was discontinued.

The second 25-lb portion of Sevillano olives was suspended in 2.5 gal of 2% KOH solution (pH = 13.8) for 7 hr. After 2 hr, there was no measurable alkali penetration, but a strong ammonia odor had developed. The same observation was made after 4 and 5 hr. After 5 hr, the pH of the soaking solution had dropped to 11.8. After 6 hr, a fresh 2.5 gal portion of 2% KOH solution was added to the drained olives. After a further period of 1 hr, 20% of olives samples showed alkali penetration. After a total alkali contact time of 11.8 hr, all the olives showed 1/16-1/8 in. alkali penetration. At this stage, the olives were rinsed with 2.5 gal portions of water and aerated for 11 hr in 2.5 gal of water.

A second lye cure for 5 hr with 2% KOH solution gave complete penetration of alkali to the pit. This curing was accompanied by a strong odor of ammonia and the final pH was 13.2. The olives were not canned due to their soft texture at the final stage of preparation.

Table 3—Storage of olives in salt-free acidified water systems

Date	Amt 12N HCl added, ml	pH 1A	pH 1B
11-27-67	0	3.3	4.5
11-27-67	5	2.9	—
	10	—	4.0
	20	—	2.7
11-28-67	0	2.6	2.5
12-6-67	—	2.9	2.7
12-15-67	—	3.1	3.0
12-26-67	10	3.4	—
	10	—	3.4
1-3-68	—	3.2	3.1
1-11-68	10	3.4	—
	10	—	3.3
1-24-68	0	3.1	3.0
1-31-68	0	3.2	3.1
2-8-68	0	3.4	3.4
3-15-68	0	3.4	3.4
Total	75		

Acidified water storage

Large Manzanillo olives which had been stored in NaCl brine for 43 days were washed free of salt by soaking and rinsing with fresh water. The olives were divided into two 25-lb portions. One portion was put into a pail containing a solution of 40g of benzoic acid and 5 gal of hot water (1A). The second portion was placed in a pail containing 20g of benzoic acid and 27g of potassium sorbate in 5 gal of hot water (1B). The storage solutions were allowed to cool to room temperature before 10g of propylene glycol and the olives were added to each pail. The pH of the storage solutions was controlled by addition of hydrochloric acid; the schedule of addition and the resulting pH are tabulated in Table 3.

Table 4—Flavor evaluation of medium Mission olives stored 6 mo in propylene glycol and NaCl solutions

Glycol	vs.	NaCl Control
Triangle presentation: 41/64 Correct judgments (Significant at the 0.1% level) Preference: 29 for Control; 12 for Glycol		

RESULTS & DISCUSSION

THE FINAL canned product from the storage of colossal Sevillano olives in glycerol containing benzoic and sorbic acids was inferior in taste and appearance to the control sample from olives stored in NaCl brine. The olives were wrinkled or showed crease lines on the skin of those olives which had filled out during processing. The olives had a stringy interior texture and a bland flavor. The final canned product from colossal Sevillano olives stored in propylene glycol containing diethyl pyrocarbonate consisted of badly shriveled olives of poor texture and inferior flavor.

Both the salt storage control and the propylene glycol-diethyl pyrocarbonate stored olives were soft in texture, poor in color and inferior to commercial samples in flavor. The olives from the stabilized

Table 5—Measurements on stored colossal Sevillano olives

Date	Brine temp °C	Brine pH	Brine titratable acidity g/lactic acid/100 ml	Ammonium nitrate content %	Reducing sugars %
9-28-66 (Raw)	—	(Juice) 4.95	0.83	0	10 ± 2
10-24-66 (Nitrate)	19.3	4.08	1.67	1.5	0.4
10-24-66 (Chloride)	20.5	4.00	0.310	—	0.2
11-22-66 (Nitrate)	19.2	4.07	1.68	4.99	0.6
11-22-66 (Chloride)	23.0	4.00	0.447	—	0.2
1-3-67 (Nitrate)	14.0	3.79	1.70	6.76	0.2
1-3-67 (Chloride)	14.0	3.73	0.77	0.2	0.2
1-26-67 (Nitrate)	13.2	2.72	1.90	6.16	0.2
1-26-67 (Chloride)	13.0	3.69	0.83	—	0.005

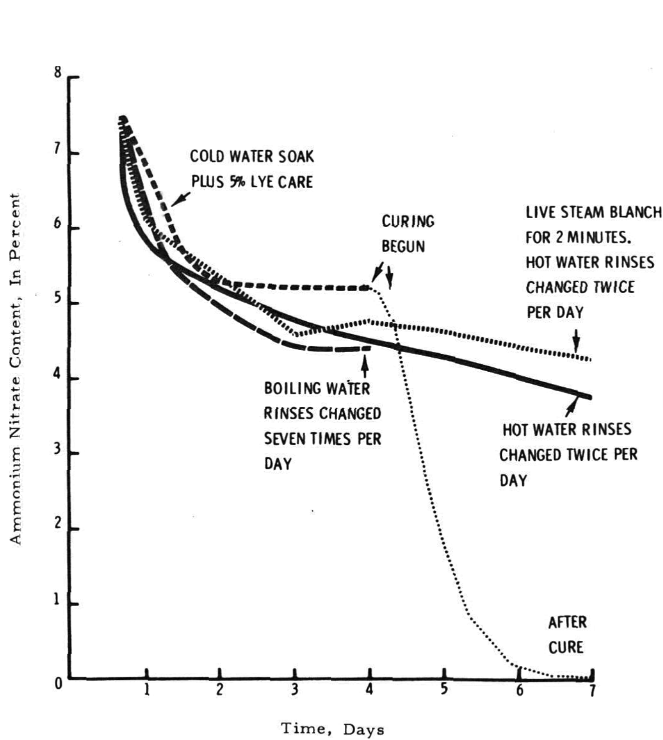

Fig. 1—Reduction in ammonium nitrate level in colossal Sevillano olives.

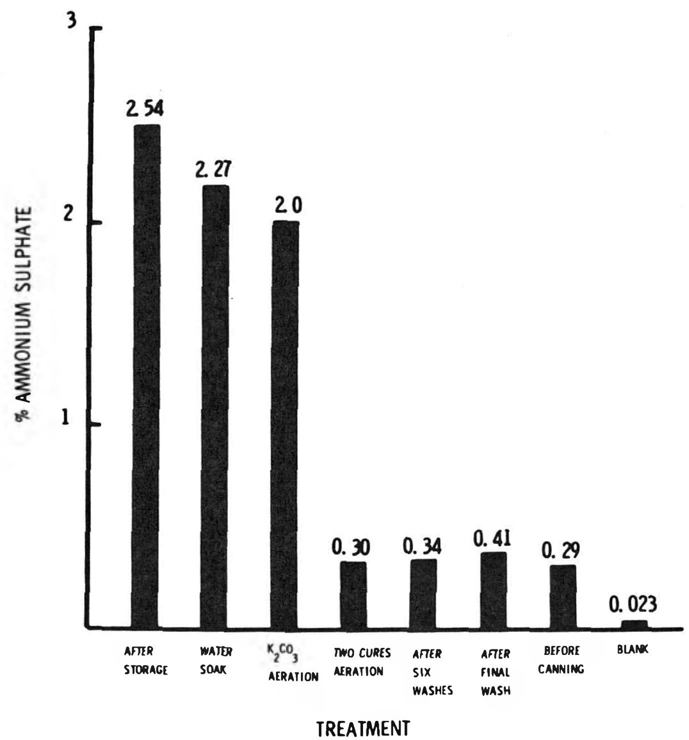

Fig. 2—Ammonium sulfate levels in olives at various stages of preparation for canning.

glycol storage systems had good appearance; there was no evidence for the shrivel which had occurred during storage. The taste panel evaluation of these samples is summarized in Table 4.

The measurements made on samples of olives stored in ammonium nitrate solutions are summarized in Table 5. The olives stored in ammonium nitrate brine were sound and firm after a period of 3 mo; they compared favorably in appearance with the control sample stored in NaCl brine. Some time between 3-3-1/2 mo of storage, the ammonium nitrate brined olives started to spoil rapidly. The olives became very soft, with wrinkles and a shrunken stem end. Measurements on Mission olives stored in ammonium sulfate solution are summarized in Table 6.

Figure 1 illustrates the results of various preparation treatments on the removal of ammonium nitrate from the olives; the only effective method found to reduce the level was to use rather strong NaOH solutions.

These experiments demonstrate that it is possible to store and process olives without the use of sodium compounds until the final stage of preparation. The poor quality of the final product could be improved by optimizing raw fruit maturity, washing procedures and curing conditions for this radically new storage system.

The storage of olives in glycol-bacteriostats systems could be considered a last resort possibility for smaller-sized olives and would require extensive development work before high quality olives could be produced.

The results of the analysis of olives stored in acidified ammonium sulfate solutions after various stages of preparation are summarized in Figure 2. These results show that washing of the olives with water before or after the alkali treatment does not lower the sulfate content. The only major reduction in sulfate level results from the treatment of the olives with alkali. It would be necessary to treat ammonium salt-stored olives with alkali of sufficient concentration and for enough times to reduce the alternate salt level to a point where it will not influence flavor. Only by the essentially complete removal of the ammonium sulfate from the olives would it be possible to produce olives of commercial quality.

Samples of olives canned after storage in acidified ammonium sulfate solutions were supplied for evaluation by an olive canner. The cans of olives exhibited normal head space vacuum, brine pH, texture and color. The flavor of the olives was evaluated by five experienced tasters from the laboratory staff. These tasters found the olives to have no immediate flavor of any kind, but an unpleasant after-taste. The ammonium sulfate content of these canned olives was found to be 0.16%.

The results of the experiments on ammonium sulfate brine storage show that even at high concentrations, spoilage of the olives can occur after periods as short as 55 days. The longest storage period of 104 days would not provide a

Table 6—Measurements on stored medium Mission olives

Date	Brine temp °C	Brine pH	Brine titratable acidity g/lactic acid/100 ml	Ammonium sulfate content %	Reducing sugars %
11-7-66	—	4.58	0.115	0	3
(Raw)		(Juice)	(Juice)		
12-9-66					
(Chloride)	17.8	4.70	0.39	0	1.5
(Sulfate)	19.5	3.53	1.85	6.97	0.2
1-10-67					
(Chloride)	18.6	5.22	0.45	0	0.8
(Sulfate)	19.2	4.10	1.58	4.62	0.1

Table 7—Analysis of olive storage stystems

System	pH	Total acidity (%)	BOD (ppm)	Chloride content (%)
Sodium chloride brine	4.1	0.12	11,270	8.1
Propylene glycol Water Benzoic acid Hydrochloric acid	3.4	0.16	10,486	1.4
Propylene glycol Water Benzoic acid Potassium sorbate Hydrochloric acid	3.5	0.15	10,594	1.4

long enough period for commercial application. The use of H_2SO_4 acidification did extend the storage period, but did not provide a system which held promise for the consistent 6 mo storage required as a minimum period for commercial utilization.

The olives canned after about 6 mo of storage in the water-propylene glycol-benzoic acid-potassium sorbate-hydrochloric acid system were of the same general quality as those from storage in conventional NaCl brine. The saline pollutional potential of this new storage system was 1/6 of that of the conventional system. The results of analysis of brines after about 6 mo of olive storage are tabulated in Table 7.

Of all the novel storage systems explored in this study, the water-propylene glycol-benzoic acid-potassium sorbate-hydrochloride acid system appeared to offer the best alternative to conventional practice. Although the use of this system does not completely eliminate the problem of chloride ion in waste discharges, the much lower level would reduce the difficulties in meeting discharge requirements.

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H. J. DEOBALD, VERA C. HASLING and E. A. CATALANO
USDA, Southern Utilization R& D Div., ARS, Southern Regional Research Laboratory, New Orleans, La. 70119

VARIABILITY OF INCREASES IN α-AMYLASE AND SUGARS DURING STORAGE OF GOLDRUSH AND CENTENNIAL SWEETPOTATOES

SUMMARY—Analyses for sugars and α-amylase were performed during 65 days' storage on five shipments of Goldrush and Centennial sweetpotatoes. Since shipments were harvested at 2 wk intervals, each represented the conditions existing at harvest time. Half the roots of each variety from every shipment were cured before being placed in storage and the effects of these treatments investigated. The patterns of α-amylase increases were so widely divergent in the various shipments that recognizable differences between the varieties in each shipment were overshadowed. Curing retarded α-amylase synthesis during subsequent storage. Glucose increases were quite consistent for each of the shipments, with the exception of the shipment that was wet and muddy when received. The Goldrush variety developed about twice as much glucose over the storage period, but curing had little, if any, influence on glucose synthesis. Sucrose development was apparently independent of the condition of the roots, varietal differences, or curing and reached a relatively constant level after 17 days. There were no obvious relationships between the increase in glucose, sucrose or α-amylase for either variety during the 65 day storage period.

INTRODUCTION

MANUFACTURERS of sweetpotato flakes who are primarily concerned with canning operations prefer to process roots as soon after harvesting as possible. However, for the fresh market, the roots are often cured then stored as long as 6 mo. Flakes are made from both sources.

In the production of precooked dehydrated sweetpotato flakes, both commercially and in the pilot plant, differences in processing characteristics of apparently comparable lots of sweetpototoes are frequently observed. Also roots analyzed 24 hr or less after harvesting and again after subsequent curing and storage do not consistently show the same α-amylase and sugar contents.

The increase in these constituents in Goldrush sweetpotatoes during storage and evidence for the lack of consistency in this rate of increase has been reported by Deobald et al. (1968; 1969) and Ikemiya and Deobald (1966). Methods for utilizing and controlling the activity of this enzyme in sweetpotatoes for the production of precooked dehydrated sweetpotato flakes have been described by Hoover (1967) and Deobald et al. (1968). Hoover (1967) introduced the "enzyme activation" method which makes possible the utilization, to the fullest extent, of the very low level of starch splitting enzymes in freshly harvested roots. Deobald et al. (1968) advocated controlling the activity of the enzyme by altering processing variables both when the enzyme content was in excess of requirement as well as when minimal for adequate starch conversion.

The present paper is a report of experiments designed to show whether any relationship exists between the degree and nature of the increases in these constituents and if possible to postulate a mechanism for sugar formation relating these changes. Such information should be of value to processors in providing a technical basis for control of sugar formation resulting in a more uniform product.

The newer Centennial variety now predominates in both the fresh market and in processing plants. These present studies were, therefore, also designed to compare increases in sugars and α-amylase activity characteristic of Centennial with Goldrush to ascertain any similarities or differences.

MATERIALS & METHODS

Goldrush and Centennial varieties of sweetpotatoes grown in the vicinity of St. Francisville, La., were delivered to the laboratory within 24 hr after harvesting at 2-wk intervals during September and October 1968. Upon arrival of each shipment consisting of 6 crates of each variety, 3 crates of each were removed to a cabinet for curing at 32°C and 85% RH and 2½ crates were placed in a 16°C storage room.

The remaining 1/2 crate-size samples were washed, hand peeled and ground in a Fitzpatrick Comminuting Machine Model D using 0.0625 in. round-hole screen. The resultant puree was used for α-amylase activity determinations in the first shipment and similar purees from subsequent shipments were used for determinations of sugars as well as for α-amylase values.

Juice equivalent to 30% of the weight of a puree aliquot was obtained by means of a Carver Press. The juice was centrifuged at 2500 rpm for 10 min and the supernatant analyzed for amylase activity by the procedure outlined by Ikemiya and Deobald (1966). The results are reported as sweetpotato dextrinizing units (SDU).

The aliquot of puree used for sugar analyses was weighed into 95% ETOH and brought to volume so that the solution was 80% with respect to ETOH. After the alcoholic solution equilibrated for at least 5 days, it was filtered and analyzed for glucose, sucrose and maltose by a method reported by Hoover and Harmon (1967) and Deobald et al. (1969). Moisture determinations were made on all raw ground purees and the sugar analyses calculated as percent of total dry solids. On the 9th day after arrival, 1/2 crates of the cured and uncured samples of both varieties were analyzed again for α-amylase and sugars as described above. On the 10th day the remaining 2½ crates of each variety of sweetpotatoes were removed from the curing cabinet and placed in the 16°C storage room. Similar half crates of roots were analyzed in the same manner on the 17th, 35th and 65th day after harvesting.

RESULTS & DISCUSSION

Alpha-amylase

All samples analyzed within 24-30 hr after harvesting contained less than 0.5 SDU. For Goldrush roots the initial values were 0.15, 0.21, 0.40, 0.43 and 0.05, respectively for the five shipments. Comparable values for Centennial varieties were 0.09, 0.10, 0.29, 0.18 and <0.03. The wide variations from less than 0.03-0.43 will result in wide differences in processing characteristics. The range of these α-amylase values for both varieties span the 0.20 SDU level below which it is almost impossible to attain adequate starch conversion using the "enzyme activation" procedure for flake production without the addition of supplemental amylolytic enzyme (Deobald et al., 1968).

The average α-amylase values during storage of the five shipments for each variety, cured and uncured, are shown in Figure 1. It is evident from the figure and verified by statistical analysis that there are significant differences (5% level) due to curing and variety. The post digging treatment resulted in significantly (5% level) lower levels of the enzyme during the 65-day storage period. Goldrush had higher enzyme levels at all storage time periods.

When all shipments were compared the results of the α-amylase determinations for a given variety, whether cured or uncured, showed a very large coefficient of variation—about 50% for each of the storage-time intervals.

This wide divergence of the different shipments is shown in Figures 2 and 3 to emphasize the unpredictability of α-amylase content of any sweetpotato

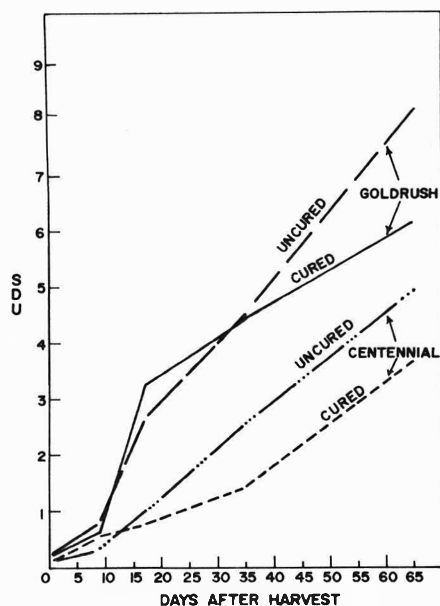

Fig. 1—Average increases in α-amylase during storage of cured and uncured Goldrush and Centennial sweetpotatoes.

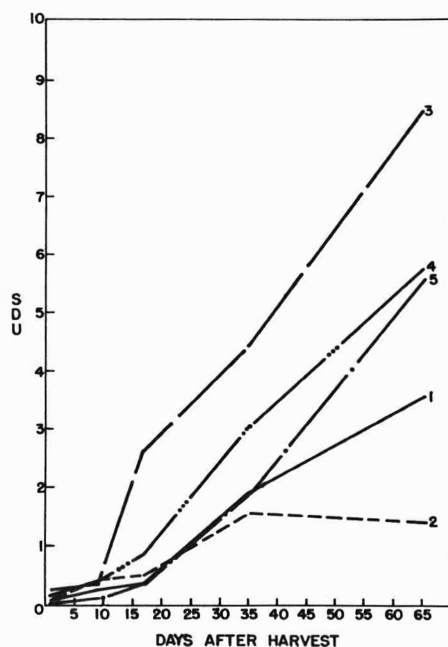

Fig. 2—Increases in α-amylase in the 5 shipments of Centennial sweetpotatoes during storage (without curing).

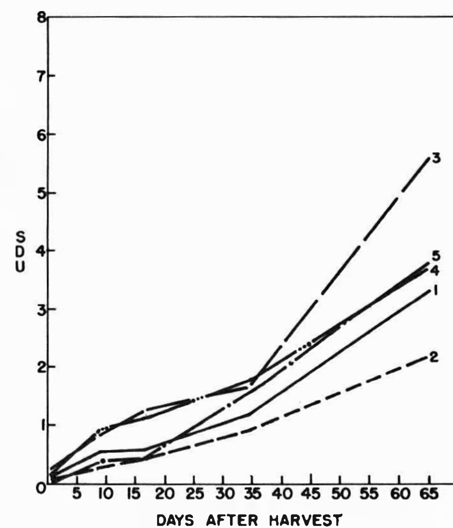

Fig. 3—Increases in α-amylase in the 5 shipments of Centennial sweetpotatoes during curing and subsequent storage.

sample based on the data averaged in Figure 1. Knowledge of the variety, treatment and time in storage would be a very poor criterion of potential processing characteristics of a given lot of roots. Statistical analysis for each variety-treatment combination indicates that there is a significant difference between the various diggings (5% level). However, the dates of digging could not be related to any sequential difference in the α-amylase development and no other data are available concerning the environment at the time of digging. Thus, even if the exact date of digging were known, the degree of variability would still be large—making it doubtful that one could predict effectively the enzyme level without actual measurement.

One of the reasons for the increase in sweetpotato storage stability caused by curing may be related to this inhibition of α-amylase synthesis. The data show that the effect of curing is probably influenced by the environmental conditions when the roots were harvested since both varieties in every way reacted similarly.

Sugars

Data presented in Table 1 show the sucrose and glucose contents of cured and uncured roots from shipments 2, 3, 4 and 5. Glucose increases in both cured and uncured roots during storage were approximately twice as great in Goldrush as in Centennial sweetpotatoes for any given shipment. This difference, therefore, may be the only one exclusively variety related.

Glucose values were quite consistent for each variety except in shipment 2 which was wet and muddy on arrival and possibly chilled. Glucose values in shipment 2 were generally higher, and this increased synthesis of glucose in both varieties during storage deserves further study.

Table 1—Variations in the increase of glucose and sucrose during storage of Goldrush and Centennial sweetpotatoes

	Cured				Uncured			
	Glucose[a]		Sucrose[a]		Glucose[a]		Sucrose[a]	
Days after harvest	Centennial	Goldrush	Centennial	Goldrush	Centennial	Goldrush	Centennial	Goldrush
Shipment 2								
1	1.1	1.1	9.8	12.0	1.1	1.1	9.8	12.0
9	1.4	2.8	16.9	14.8	2.0	2.5	14.6	16.7
17	1.2	4.6	18.8	18.1	1.6	5.3	17.7	17.1
35	2.0	4.9	22.1	25.3	2.9	9.6	20.2	25.9
65	4.2	7.4	23.8	18.8	4.0	9.5	21.2	20.0
Shipment 3								
1	0.9	1.6	9.6	11.5	0.9	1.6	9.6	11.5
9	3.6	5.0	17.3	14.9	1.7	2.6	16.4	16.6
17	2.0	5.6	17.3	18.1	5.3	6.0	15.6	19.2
35	1.2	5.3	18.7	19.6	2.5	5.8	18.7	20.3
65	2.3	5.1	17.6	19.3	2.5	5.0	20.8	22.2
Shipment 4								
1	1.5	1.7	13.8	11.6	1.5	1.7	13.8	11.6
9	0.9	3.7	16.0	16.5	0.7	3.0	18.1	16.1
17	1.4	3.7	19.0	19.5	1.1	4.2	18.6	18.1
35	1.5	4.1	20.0	19.5	2.1	5.8	19.4	14.6
65	2.5	5.2	19.6	18.4	2.6	5.7	20.8	18.0
Shipment 5								
1	0.8	0.7	10.6	11.7	0.8	0.7	10.6	11.7
9	0.9	3.3	15.1	16.3	0.8	2.9	16.3	16.1
17	1.1	2.1	16.1	16.2	1.2	5.5	16.4	15.3
35	1.2	3.4	17.2	17.8	1.5	6.8	18.4	17.4
65	1.6	2.4	18.0	17.3	2.2	5.1	17.8	16.3

[a]Percent dry basis.

The data on sucrose show almost no differences attributable to variety and all samples attained about 20% sucrose by the 65th day. It may be noteworthy, however, that though some roots attained nearly maximum sucrose content after 9 days, most of the other samples did not show near maximum values until the 17th day, indicating the advisability of retaining roots for at least 17 days to attain maximum sweet flavor. The maltose data were omitted from Table 1 because the values were extremely low (most less than 1.0%), did not increase during storage, and did not show any correlation with the other variables investigated.

Generally there were no obvious relationships between the pattern of increases in any of the three constituents reported in the data. α-amylase values differed so widely in the various shipments that both the effect of curing and of varietal differences were overshadowed. However, the great differences in α-amylase initially and during storage call attention again to the need for determination of α-amylase immediately prior to processing, if the degree of starch conversion is essential in the processing technique. Sucrose showed little variation between shipments or varieties. Glucose values apparently reflect the storage treatment of the roots at harvest and the varietal differences, but were not influenced by curing. Based on data presented, Centennial roots should have processing characteristics similar to Goldrush.

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It is not the policy of the Department to recommend the products of one company over those of any others engaged in the same business.

WILLIAM R. SUTTON[1], OM PRAKASH AGARWALA and GEORGE M. PIGOTT
Institute for Food Science & Technology, College of Fisheries, University of Washington, Seattle, Wash. 98105

EFFECTS ON THE ALKALOID CONTENT OF POTATOES GROWN FROM SEEDS SUBJECTED TO LOW-DOSE GAMMA IRRADIATION

SUMMARY—Effects of low dose gamma irradiation of seeds on alkaloid content, proximate composition and yield of the potato tubers were studied. Increase in yield with irradiation of seeds was inconclusive. Changes in proximate composition of Russet potatoes grown from tubers irradiated at 1,500 rads were found to be insignificant. Varietal differences in solanine content were more pronounced than those due to gamma irradiation. In all cases the solanine content in potatoes grown from irradiated seeds was found to be far below the toxic level for human beings.

INTRODUCTION

EVER SINCE the discovery of X-rays by Roentgen in 1895, research work has been reported claiming radiation induced stimulation in plants. However, there is general disagreement on this phenomenon, owing to the lack of research confirmation.

In numberous experiments small doses of ionizing radiation have given small but significant stimulation of seed germination, such as earlier flowering development and faster growth of seed crop plants. Owing to many controlling factors involved, these effects are inconsistent and often nonreproducible from one experiment to another. The quality, quantity, and duration of radiation, the physiological and biological conditions of seeds at the time of radiation, storage conditions before and after the irradiation, varieties and species of seeds, and such growing conditions as nutrition, temperature, and light are some of the probable variables involved in determination of optimal dose for seed stimulation.

Very little work has been reported on the effects of radiation on product composition, particularly alkaloid content and wholesomeness of tubers grown from irradiated seeds. An increased number of tubers but a decrease in average weights was reported in some of the early experiments (Heiken, 1960; Herrman and Raths, 1958; Johnson, 1928). However, many research workers (Sparrow and Christensen, 1950; Fishnich et al., 1961; Batygin and Skalinova, 1962; Suss, 1966) reported increases in both the number and average weight of the potatoes irradiated just after the sprouts appeared.

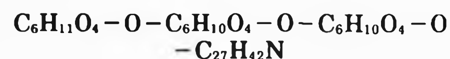
In general, better germination and stimulation of growth, and in some cases even an increase in yield, were observed by many others (Sparrow and Christensen, 1950; Smalik et al., 1962; Roze, 1963; Spragye and Lenz, 1929; Abramova et al., 1966; Avakyam et al., 1965, 1966; Lure et al., 1966; Berezina et al., 1962; Kuzin and Kasymov, 1963; Serebrenikov, 1960, 1965), the dose of radiation varying with varieties of the potatoes and growing conditions. Grechushnikov and Serebrenikov (1962; 1966) and Grechushnikov et al. (1964) observed that a dose of 150-500 rads hastened ripening of tubers, increased the number of eyes sprouted and the photosynthesis rate, while a dose of 1,000 rads supported the growth and development of plants. By the end of the vegetation period, sugar content decreased and protein nitrogen was low, while total nitrogen content was higher than in the controls.

The alkaloid poison solanine is present in potatoes and is potentially toxic to the consumer. The dose considered toxic for human beings is 20-25 mg per 100g (Wilson, 1959). The following symptoms were noted during the medical examination of an individual poisoned by solanine from whole potatoes baked in the jackets: abdominal pain and diarrhea 8 hr later, with complete recovery after 24 hr.

An assay of the batch of potatoes from which this meal was prepared showed 50 mg of solanine per 100g. Normal potatoes contain 2-10 mgs per 100g, although in certain potatoes as much as 55-100 mg per 100g has been found. The outer layers of the tubers, including the skin, are richer in solanine than the inner layers. Furthermore, unripe tubers have a higher proportion of solanine than mature ones, and small tubers contain more than larger ones (Bomer and Mattis, 1924).

Potato sprouts contain about 400 mg solanine per 100g (Arutiunyan, 1940), and the limited amount of data indicates that the development of sprouts from potatoes stored in the dark is accompanied by a decrease in the solanine content of the outer parts of the tubers. The amount of solanine in sprouted seed potatoes varies with the variety, and free solanidine occurs in the sprouts of some but not all varieties (Clemo et al., 1936).

Studies on the chemistry of solanine have centered mainly on its molecular structure. It has been established that solanine is a glycoalkaloid with emperical formula $C_{45}H_{73}NO_{15}$. It consists of a trisaccharide moiety linked to one molecule of its aglucon, solanidine, as follows:



$$C_6H_{11}O_4-O-C_6H_{10}O_4-O-C_6H_{10}O_4-O-C_{27}H_{42}N$$

On acid hydrolysis, solanine yields the aglycone, solanidine ($C_{27}H_{43}NO$), and one molecule each of rhamose ($C_6H_{12}O_5$), galactose ($C_6H_{12}O_6$) and glucose ($C_6H_{12}O_6$).

Intensive investigation during World War II led to a modification of Pfankuch's (1937) analytical method (Rooke et al., 1943). Dabbs and Hilton (1953) developed a shorter method for extraction and analytical determination of solanine. Baker et al. (1955) further improved the method which, with only slight modification, was chosen for this investigation.

For potatoes, results are reported as "total solanine" defined as solanine including solanidine.

The present research was carried out to investigate the effect of low-dose stimulation of seed potatoes and the effect on proximate composition and alkaloid content.

MATERIALS & METHODS

TO STUDY radiation effects on product yield from irradiated seed, four varieties of poatoes were grown through two generations. Seed for the first generation received a dose of 1500 rads and Table 1 shows the treatment received by the seed for the second generation. The Mark II food irradiator located in the Institute for Food Science and Technology, College of Fisheries, University of Washington was used in this work. The source is a double plaque of Cobalt 60, approximately 39,000 curies, located in a pool of water 11 ft in depth. The dose was measured by the Fricke dosimeter method as described in ASTM D 1671-63. The limited size of the potatoes made it possible to obtain the ratio of maximum to minimum dose of about 1.15. Only the maximum doses are reported in the results. Potatoes used in these experiments were grown at the Washington State Depart-

[1]Present address: 119 Nirvana Road, Brighton Beach, Durban, South Africa.

Table 1—Treatment of seed tubers prior to planting

Treatment Code	Treatment: First Generation	Treatment: Second Generation
CC	Control, no irradiation prior to planting	Control, no irradiation prior to planting
C T_{1000}	Control, no irradiation prior to planting	1,000 rads prior to planting
T_{1500} C	1,500 rads prior to planting	Control, no irradiation prior to planting
T_{1500} T_{1000}	1,500 rads prior to planting	1,000 rads prior to planting

Table 2—Effect of irradiating seeds on yield of potatoes grown from them

Variety	Treatment	No. of tubers per plant	Total yield per plant (g)	Avg wt of tuber per plant (g)
Netted Gem	CC	8	1095.07	136.88
	CT_{1000}	16	1071.95	67.00
	T_{1500} C	5[a]	0116.65	23.33
	T_{1500} T_{1500}	12	1068.44	89.04
Kennebec	CC	8	1609.54	20.12
	CT_{1000}	18	2796.76	155.38
	T_{1500} C	4	385.41	96.35
	T_{1500} T_{1500}	5	388.52	77.70
White Rose	CC	12	2265.29	188.77
	CT_{1000}	12	754.16	62.85
	T_{1500} C	26	2436.83	93.72
	T_{1500} T_{1500}	10	1145.66	114.57
Norgold	CC	11	552.17	50.20
	CT_{1000}	6[a]	364.52	60.75
	T_{1500} C	20	954.40	47.72
	T_{1500} T_{1500}	5	210.37	42.07

[a] Infected with leaf roll; hence adverse effect on yield.

ment of Agriculture Experimental Station, Bellingham, Washington.

Calibration of standard curve for colorimetric determination of solanine

Using purified solanine dissolved in 1% H_2SO_4, a standard solution containing 1 mg solanine per 1 ml was prepared. Aliquots of the standard solution were added to 10 ml volumetric flasks, followed by 5 ml conc H_2SO_4 over a period of 3 min 2.5 ml 1% formaldehyde over a period of 2 min, and brought to volume with 1% H_2SO_4. If either the conc H_2SO_4, or 1% formaldehyde solution was added too rapidly, the intensity of color was reduced greatly. A blank was prepared containing 2.5 ml 1% H_2SO_4, 5.0 ml conc H_2SO_4, and 2.5 ml 1% formaldehyde.

The relationship was obtained by plotting the concentration of solanine against absorbance, using a Hitachi Perkin-Elmer 139 UV-VIS spectrophotometer.

Extraction of solanine

Four potatoes were taken from each treatment and ground in a Waring Blendor for 1 min. A portion of the ground sample was weighed directly into an extraction thimble (50g for tubers and 5g for sprouts), and placed in a Soxhlet Extractor. To the Soxhlet flask, 100 ml alcohol (95% ethanol and 5% methanol) and 3 ml of glacial acetic acid were added. The extraction was carried on for 16 hr. The alcoholic extract was evaporated in a 150 ml beaker to a volume of 1-3 ml, to which 5 ml of 5% sulfuric acid solution was added. The suspension was filtered through No. 4 filter paper into a 15 ml tapered centrifuge tube and a further 5 ml of 5% sulfuric acid solution was used for washing the beaker and filter paper. The filtrate was neutralized by the addition of conc ammonium hydroxide, and a further 10 or more drops of ammonium hydroxide was added until a pH of at least 9.4 was obtained. The solution containing precipitated solanine was then heated in a water bath at 80°C to flocculate the solanine and kept overnight at 4°C. The following day the suspension was centrifuged, the supernatant solution discarded, and the residue washed with 10 ml of 1% ammonium hydroxide solution. After centrifuging, the supernatant solution was discarded, and the residue dissolved in 1% sulfuric acid, transferred and brought to volume in a 25 ml volumetric flask. From a suitable aliquot (approx 2.5 ml for tuber extracts and 0.5 ml for sprout extracts), the colorimetric determination of solanine was made.

RESULTS & DISCUSSION

TABLE 2 gives the number of tubers, yield, and average weight of tubers per plant for the four varieties and four treatments of each variety. All varieties did not exhibit similar results, so no definite conclusions could be made. In general, it was evident that irradiation effected an increase in the number but a decrease in the average weight of the tubers, with corresponding decrease in the yield in some cases and an increase in others.

Table 3 shows there was no significant change in proximate composition of Russet potatoes grown from tubers irradiated with 1,500 rads.

Table 4 gives results obtained for solanine content of the potato tubers. For the varietal differences, the data show that White Rose and Norgold varieties have a consistently higher alkaloid content than Netted Gem and Kennebec varieties. Generally, varietal differences are more pronounced than the effects between different treatments. The two varieties (White Rose and Norgold) appeared to be sensitive to irradiation, causing a net increase in solanine content with irradiation either in the first generation, second generation, or both. The effect of irradiation was carried over to the next generation. However, the other two varieties showed a decrease in solanine content of potato tubers with irradiation of the potato seeds, with one exception.

Table 3—Proximate composition of raw, whole potato tubers (Russet)

Analysis	Original material, %: Non-irradiated	Original material, %: Irradiated	Calculated on dry solids, %: Non-irradiated	Calculated on dry solids, %: Irradiated
Moisture	79.4	80.3	—	—
Fat	0.1	0.1	0.5	0.5
Fiber	0.6	0.5	2.9	2.5
Ash	1.1	0.9	5.4	4.6
Protein	2.8	2.5	13.6	12.7
Carbohydrates	16.0	15.7	77.7	79.7

Table 4—Effect of gamma rays on solanine content of potato tubers grown from irradiated seed

Variety	Treatment (rads)	Total solids (%)	Total solanine (mg/100g): Original material	Total solanine (mg/100g): Calc on dry solids
Netted Gem	C C	19.9	5.46	27.44
	C T_{1000}	17.6	3.90	22.16
	T_{1500} C	17.0	5.04	29.65
	T_{1500} T_{1000}	12.0	3.28	27.33
Kennebec	C C	17.4	5.04	28.96
	C T_{1000}	16.4	3.36	20.49
	T_{1500} C	13.8	3.18	23.04
	T_{1500} T_{1000}	16.8	4.46	26.55
White Rose	C C	18.4	8.10	44.02
	C T_{1000}	21.1	10.02	47.49
	T_{1500} C	16.5	9.88	59.88
	T_{1500} T_{1000}	17.6	10.00	56.82
Norgold	C C	17.4	7.44	42.76
	C T_{1000}	15.6	8.28	53.08
	T_{1500} C	17.6	7.86	44.66
	T_{1500} T_{1000}	16.9	8.00	47.34

CONCLUSIONS

IRRADIATION of potato seed tubers was found to give better germination and stimulated growth rate.

No definite conclusions as to the increase in yield with irradiation of seeds could be made for an experiment of this magnitude.

There did not appear to be any significant change in proximate composition of Russet potatoes grown from tubers irradiated at 1,500 rads.

There was a definite but insignificant increase in solanine content present in potatoes grown from irradiated seeds. Different varieties gave more pronounced effects than did the irradiation of seed.

In all instances the solanine content in potatoes grown from irradiated seed was found to be far below the toxic level for human beings.

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H. N. DAOUD and B. S. LUH
Department of Food Science & Technology, University of California, Davis, Calif. 95616

EFFECT OF PARTIAL REPLACEMENT OF SUCROSE BY CORN SYRUP ON QUALITY AND STABILITY OF CANNED APPLE SAUCE

SUMMARY—Apple sauces were made from Gravenstein apples under normal commercial processing conditions, using pure sucrose and combinations of sucrose and 62 D.E. corn syrup as sweeteners. Products were tested for storage stability at 68 and 86°F. Undesirable chemical and physical changes occurred rapidly at 86°F as indicated by the increase of hydroxymethyl furfural, darkening of serum color, decrease in consistency and increased can corrosion. This was accompanied by lower organoleptic color and flavor scores. It appeared that the quality and storage stability of apple sauce were influenced more by higher storage temperatures and longer storage duration than by the type of sweeteners used in making the apple sauce. For a longer shelf life and better quality retention, canned apple sauce should be stored at 68°F or lower. Results of this investigation indicate that corn syrup can be used to replace 25% of the added sucrose in processing apple sauce.

INTRODUCTION

QUALITY of canned apple sauce has been shown to be affected by the varietal characteristics and stage of maturity of the fresh apples and also by post-harvest storage conditions (LaBelle et al., 1960, 1961; Smock and Neubert, 1950; Wiley and Toldby, 1960). Allen and Torpen (1950) studied the effect of storage at 32°F on the quality of Gravenstein apples. The flavor of canned apple sauce can be improved by fortification with apple essence and citric acid (Buck, et al., 1955; Dyrden and Hills, 1957). The effects of packaging procedure on container performance were reported by Kohn and Fix (1956). Storage changes in apple sauce packed in baby food jars were investigated by Livingston et al. (1954). Luh and Kamber (1963) investigated the chemical and color changes in canned apple sauce during storage at different temperatures.

This paper reports the effect of partial replacement of sucrose with 62 D.E. corn syrup and post-canning storage on the chemical, physical and organoleptic properties of canned apple sauce made from Gravenstein apples.

MATERIALS & METHODS

Apple Sauce

Gravenstein apples that passed California grading standards were used in making apple sauce in a commercial plant in Sebastapol, California. The apples were passed through a roller grader to remove small fruits, washed with water, peeled and cored in a machine and dumped into a flume containing 1.5% NaCl solution. The product was sorted, trimmed, sliced with a mechanical cutter, and discharged directly into a continuous stainless steel thermoscrew cooker where it was heated rapidly to 210°F and held there for 3 min. The cooked apples were discharged directly into a Langsenkamp pulper with a 0.060-in screen and operating at 1000 rpm. The apple pulp was pumped into a holding tank where water and sweeteners were added to adjust the soluble solids to 21° Brix at 68°F. The apple sauce was pumped through a stainless steel tubular heat exchanger to reach 195°F, and then filled hot into No. 303 cans (303 × 406) made of differential electrolytic tinplate (100/25). The cans were sealed hot with steam injection, inverted, heated 5 min in a steam box, and then cooled in water to 110°F for 25 min. 48 cans of each sample were stored at 68 and 86°F respectively for stability studies.

Sweetener

The types of sweeteners used were (a) sucrose alone; (b) 75% sucrose + 25% corn syrup (62 dextrose equivalent); and (c) 50% sucrose + 50% corn syrup (62 D.E.) on solid basis. The chemical analyses of the 62 D.E. corn syrup were as follows: 44° Baume at 100° F, 16.3% moisture, 0.3% ash and 62% D.E. The distribution of carbohydrates were 35.0% monosaccharide, 28.0% disaccharide, 13.5% trisaccharide, and 23.3% higher saccharides, all on the dry basis.

Soluble solids

A Zeiss-Opton refractometer was used to determine the soluble solids content of the samples at 68°F.

Titratable acidity

A Beckman Model K automatic titrator was used. 10g of apple sauce was mixed with 150 ml of distilled water and then titrated with 0.1N sodium hydroxide solution to pH 8.0. Results are reported as percent malic acid.

pH

The pH of the canned apple sauce sample was measured with a Corning Research Model pH meter.

Gardner color difference meter

The color of apple sauce was measured with a Gardner automatic color difference meter. A light yellow porcelain plate (LYI) with Rd = 60.7, a = −2.1, and b = +22.3 was used as a reference.

Hydroxymethyl furfural (HMF)

The resorcinol colorimetric method described by Luh et al. (1958) was used for determining HMF in the apple sauce. The results are reported as parts HMF per million parts apple sauce.

Consistency

A Bostwick consistometer was used to measure the consistency of apple sauce at 68° F. Results are reported as cm/30 sec.

Serum color

The photoelectric colorimetric method described by Luh et al. (1958) and Luh and Kamber (1963) was used.

Can corrosion

Residual tin in the inner lining of the can was determined by the electrolytic tin-stripping method described by Kunze and Willey (1952). A 4-sq in. circular disc was cut from the can body. The tin was anodically removed from the steel plate in 1N HCl with the electric current maintained at 250 milliamperes. The voltage changes, which developed between a reference silver electrode as cathode and the tin plate specimen as anode, were transmitted to a Honeywell Electronick 19 lab recorder which plotted a time-potential curve with two end points. The first showed the end of the free tin removal and the second the end of the alloyed tin removal. By computing both end points, the total residual tin was determined. A normal can was run simultaneously as a control. Results are expressed as weight percent of tin retention.

Organoleptic evaluation

Flavor and color of the apple sauce samples were evaluated by a panel of 12 persons on a hedonic scale of 1 to 10; excellent, 9-10; good, 7-8; fair, 5-6; poor, 3-4; very poor, 1-2. A MacBeth standard light source (6800°K) was used for evaluation of the color.

Amino acids

The modified ninhydrin method, as described by Spackman et al. (1958) and Luh and Kanujoso (1967) adapted for the autoanalyzer was used. An apple sauce sample made with sucrose as sweetener and another with 75% sucrose and 25% corn syrup solids (62 D.E.) were stored at 68° and 86°F respectively for 12 months prior to analysis. 100g of sample was mixed with 100 ml of water. The mixture was shaken for 30 min and then centrifuged for 30 min. The supernatant liquid was filtered. 50 ml of the filtrate was passed through an ion-exchange column, packed with Dowex 50W-X4 ion exchange resin in hydrogen form (30 ml volume).

The column was washed with about 150 ml of water and then eluted with 200 ml of 2N NH_4OH. The eluate was evaporated almost to dryness in a flash evaporator. The residue was dissolved with acidified water, pH 1.4. The solution was transferred to a 50-ml volumetric flask and diluted to volume with acidified water. The solution was filtered

through a Hercules sterilizing filter sheet, type ST, size DIE 36. A 0.5-ml aliquot of the filtrate was used for amino acid determination in the autoanalyzer.

The area under the peak for each amino acid was calculated from the recording strip chart by the height × width method of Spackman et al. (1958).

Another recording strip chart (standard) was produced by using a mixture of known amino acids of known concentration (2.5 μm of amino acid/ml). The height and width were also calculated for each amino acid. The calculations were made on an IBM model 7044 computer.

RESULTS

Soluble solids and pH value

The soluble solids content of the apple sauce ranged from 20.8-21.0° Brix at 68°F, and the pH values 3.18-3.38 (Table 1). The samples with corn syrup replacements were slightly lower in pH value. When the product was stored at 68°F for 12 months, a decrease in pH value of 0.1 unit was observed. Storage of the canned products at 86°F for 12 months resulted in a decrease in pH value of 0.13 units. The titratable acidity of Sample A (sweetened with sucrose only) was 0.53% as malic acid at the beginning of the storage period, that of Sample B (25% corn syrup solids replacement for sucrose) was 0.54%, and that of Sample C (50% corn syrup solids replacement for sucrose) was 0.56%. The titratable acidity of the products stored at 86°F increased gradually in 12 months by 0.015-0.020% which may result from slow chemical degradation of the apple constituents.

Gardner color difference meter readings

Table 2 shows the Gardner color difference meter readings of the apple sauces stored at 68° and 86°F respectively. The Rd value decreased more rapidly at 86°F than at 68°F. Samples of higher Rd values were lighter in color. The samples with corn syrup replacement for sucrose were higher in Rd values. The Gardner "a" value increased gradually as the storage time at 86°F lengthened. It changed progressively from −5.8 to +0.4 in Sample A (sucrose as sweetener) stored at 86°F. Similar changes were observed in Samples B and C.

Table 1—Soluble solids and pH value of canned apple sauce

Sample	Sweetener type	Storage time, months	Stored at 68°F: Soluble solids, °Brix at 68°F	Stored at 68°F: pH	Stored at 86°F: Soluble solids, °Brix at 68°F	Stored at 86°F: pH
A	Sucrose only	0	21.0	3.38	21.0	3.38
		4	20.8	3.30	21.0	3.30
		8	21.0	3.30	21.0	3.22
		12	21.0	3.28	21.0	3.25
B	75% Sucrose	0	21.0	3.35	21.0	3.35
	25% Corn	4	20.9	3.29	21.0	3.28
	syrup solids	8	21.0	3.28	21.0	3.20
		12	21.0	3.25	21.0	3.22
C	50% Sucrose	0	21.0	3.31	21.0	3.31
	50% Corn	4	21.0	3.28	21.0	3.25
	syrup solids	8	21.0	3.25	21.0	3.20
		12	21.0	3.22	21.0	3.18

Table 2—Effect of storage temperature on Gardner color difference meter readings of canned apple sauce

Sample	Sweetener	Storage time, months	68°F Rd	68°F a	68°F b	86°F Rd	86°F a	86°F b
A	Sucrose only	0	22.7	−5.8	+14.0	22.7	−5.8	+14.0
		4	22.3	−6.2	+13.1	20.0	−4.2	+13.1
		8	21.2	−4.9	+13.4	16.1	+0.4	+13.3
B	75% Sucrose	0	27.5	−5.4	+14.4	27.5	−5.4	+14.4
	25% Corn	4	24.1	−6.1	+13.7	20.3	−4.2	+12.5
	syrup solids	8	22.1	−4.9	+13.0	16.7	+0.6	+13.1
C	50% Sucrose	0	30.0	−5.4	+15.0	30.0	−5.4	+15.0
	50% Corn	4	25.4	−6.1	+13.8	21.3	−4.5	+12.7
	syrup solids	8	22.6	−5.1	+12.5	17.3	+0.7	+13.0

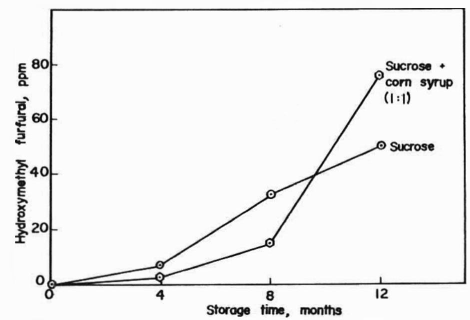

Fig. 1—Formation of hydroxymethyl furfural in canned apple sauces stored at 86°F.

Hydroxymethyl furfural (HMF)

HMF was not present in the samples stored at 68°F for 12 months. Storage of the product at 86°F resulted in gradual increase in HMF content (Fig. 1). After storage at 86°F for 12 months, the sample with corn syrup solids replacement (1:1) was higher in HMF content than the sample sweetened with sucrose alone. Partial replacement of sucrose by corn syrup did not result in HMF formation in the canned apple sauce when stored at 68°F.

Consistency

A Bostwick consistometer was used to evaluate the change in consistency during post-canning storage of apple sauce. Figure 2 shows the importance of storage temperature on consistency. Poor consistency was shown by a higher Bostwick reading. The samples stored at 68°F were heavier in consistency than those stored at 86°F. Since consistency is a quality factor in grading apple sauce, it is advisable to store the product at 68°F or lower.

Serum color

The serum color test is a useful criterion for storage changes in canned foods. When the canned product receives excessive heat treatment during processing or is stored at a high temperature for an extended period, the serum of the product darkens. Figure 3 shows the effect of storage at 68 and 86°F on serum color of apple sauce. There was practically no difference in serum color between the samples stored at 68°F. The type of sweetener appears to have no significant effect on serum color darkening. Storage temperature appears to be an important factor influencing the stability of the canned product. Darkening of the serum color occurred as the storage duration at 86°F was lengthened.

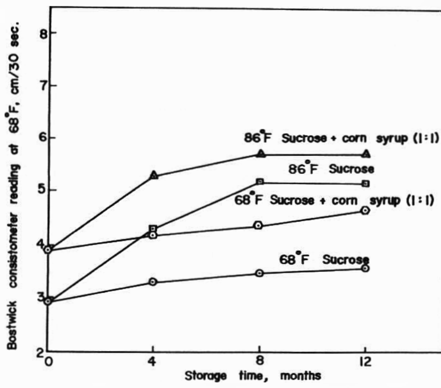

Fig. 2—Effect of storage on consistency of canned apple sauces varying in sweetener types.

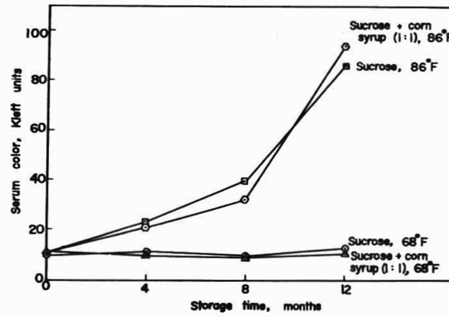

Fig. 3—Effect of storage on serum color of canned apple sauces varying in sweetener types.

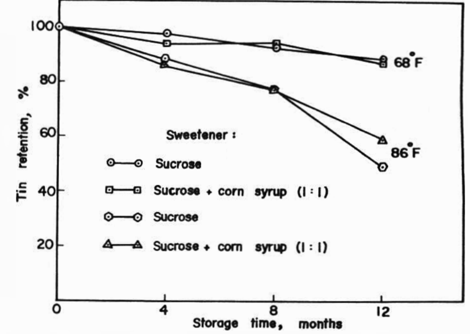

Fig. 4—Effect of storage on corrosiveness of canned apple sauces.

Can corrosion

Figure 4 shows the effect of storage temperature on tin retention as measured by the electrolytic tin-stripping method. Faster detinning was observed in the cans stored at 86°F than at 68°F.

In the present investigation, there was no significant difference in percent tin retention between cans differing in sweetener types. The shelf life of canned apple sauce can be shortened if the post-canning storage temperature is raised. It is advisable to store canned apple sauce at 68°F or lower for better storage stability.

Organoleptic evaluation

The effect of partial replacement of sucrose by 62 D.E. corn syrup on the organoleptic quality of canned apple sauce is shown in Table 3.

The visual color score of the canned product was affected by storage temperature. Storage at 86°F resulted in a gradual lowering of the visual color score. The products stored at 68°F were more stable in color. Samples B and C, which were sweetened with a mixture of sucrose and corn syrup, were slightly ligher in color as compared with that sweetened with sucrose alone (Sample A).

The flavor score of the canned product decreased when stored at 86°F. Both the color and flavor scores of canned apple sauce were influenced more by storage temperature and duration than by the type of sweetener used.

Amino acids

The effect of storage at 68 and 86°F for 12 months on free amino acids in canned apple sauces is shown in Table 4. Generally speaking, with the exception of aspartic acid, some decrease in quantity of free amino acids occurred when the product was stored at 86°F as compared with that at 68°F.

DISCUSSION

ORDINARILY, the soluble solids content of canned apple sauce varies from 19–21%. To evaluate the effect of sweetener types on the flavor acceptance, it is necessary to have the samples at comparable solids level. The three samples used in this investigation were reasonably uniform in soluble solids readings. Thus the flavor acceptance data obtained may be attributed to the difference in quality of the sweeteners.

The titratable acidity of the apple sauce stored at 86°F increased gradually in 12 months by 0.015–0.020%. The increase in acidity resulting from storage at 86°F was small as compared with that resulting from the differences in ripeness level and varietal characteristics of the apple. Sometimes it is desirable to combine different varieties of apples for sauce manufacture so that the resulting product is well balanced in acidity and flavor quality.

The Gardner color difference meter is useful to determine color changes in canned apple sauce. When browning reaction occurs in the product, the Rd value decreases, and the "a" value increases. The values correlated well with visual color score.

It is generally known that furfural will be formed when hexoses are heated in an acid solution. When canned apple sauce is stored at higher temperatures, HMF is formed from hexoses and amino-carbonyl reactions. The HMF test may be used as a criterion for storage changes in canned apple sauce. There was no HMF formation in the sample stored at 68°F for 12 months. The changes in flavor and color score of the

Table 3—Effect of partial corn syrup solids replacement for sucrose on organoleptic score of canned apple sauces stored at 68 and 86°F

Sample	Sweetener	Storage time, months	Average visual color score 68°F	Average visual color score 86°F	Average flavor score 68°F	Average flavor score 86°F
A	Sucrose only	0	7.5	7.5	8.8	8.8
		4	7.4	4.4	8.0	6.3
		8	8.0	3.5	8.2	5.0
		12	7.8	3.3	8.7	4.8
B	75% Sucrose	0	8.3	8.3	8.5	8.5
	25% Corn	4	8.3	5.3	8.0	6.1
	syrup solids	8	8.6	3.9	7.6	4.7
		12	7.7	3.4	7.7	4.7
C	50% Sucrose	0	9.1	9.1	8.0	8.0
	50% Corn	4	9.1	5.6	7.6	5.9
	syrup solids	8	8.9	4.1	7.2	4.7
		12	7.7	2.9	8.0	4.6

L.S.D. at 95% probability level:

	0	4 mo	8 mo	12 mo
Color	0.08	1.04	0.98	1.42
Flavor	0.67	1.63	1.38	1.36

Table 4—Amino acids in canned apple sauce

Amino acids[a]	Retention time, min.	(A) Sucrose as sweetener		(B) 25% Sucrose + 75% 62 D.E. corn syrup as sweetener	
		Stored at 68°F	Stored at 86°F	Stored at 68°F	Stored at 86°F
		μmole/100g	μmole/100g	μmole/100g	μmole/100g
Aspartic acid	180	51.60	67.60	36.40	48.80
Threonine	206	65.60	38.40	43.20	26.40
Serine	213	22.80	16.40	16.00	14.80
Glutamic acid	247	29.60	9.60	31.20	11.20
Glycine	342	4.40	3.20	2.80	3.20
Alanine	368	21.20	18.00	23.20	6.00
Valine	476	2.40	Trace	1.60	Trace
Ethanolamine	834	10.00	9.60	8.40	7.20
Ornithine	963	1.60	4.40	2.40	1.60
Lysine	1004	0.80	1.60	0.40	Trace
Tryptophane	1026	20.00	17.60	19.60	18.80

[a]There were trace amounts of: methionine sulfoxide (retention time 162 min.), methionine (572 min.), isoleucine (632 min.), leucine (655 min.), tyrosine (713 min.), phenylalanine (738 min.), histidine (1053 min.), and arginine (1248 min.) present in the samples.

product stored at 86°F appear to be in parallel with formation of HMF from hexoses and amino-carbonyl reactions. Although the effect of HMF on the flavor acceptance of apple sauce is not known, the presence of this compound in the product may be used as an indicator for deteriorative changes.

The decrease in consistency of apple sauce stored at 86°F might be attributed to the conversion of protopectin in the cell walls to water-soluble pectin. This reaction results from acid catalysis or thermal degradation. Storage of the product at 68°F or lower would inhibit or retard such changes. The results obtained here confirm those reported by Luh and Kamber (1963) who observed that consistency of canned apple sauce was lowered when stored at 86°F and 98°F but not at 68°F.

The serum color test appears to be useful for evaluating post-canning storage changes caused by high storage temperature. It may be used concurrently with the Gardner color difference meter and the HMF test to give objective criteria for quality changes. Formation of water-soluble pigments in the product is an indication of improper storage or processing. The effect of storage temperature on internal can corrosion has been reported by Luh and Sioud (1966). They reported that higher storage temperature caused faster corrosion of the tin coating and the formation of hydrogen gas in the head space of canned pear puree. The results obtained here on apple sauce showed a similar phenomenon as that in pear puree. For better storage stability, the product should be stored at 68°F or lower. The humidity in the warehouse should also be controlled so that external corrosion of the cans would also be eliminated. Dissolution of tin and iron into the product during post-canning storage would cause undesirable metallic off flavor. It may also cause darkening of the product due to the reaction of iron with tannins. This phenomenon would be more evident when the can is opened.

Free amino acids appear to be involved in quality degradation in canned apple sauce. Storage at 86°F or higher temperatures results in gradual decrease in free amino acids accompanied by quality deterioration.

Apple sauce is one of the important dessert items. It has been popular in the United States and European countries because of its attractive flavor and aroma. Results obtained here indicated that corn syrup of 62 dextrose equivalent can be used to replace 25% of the sucrose in making apple sauce. When 50% of the sucrose was replaced by 62 D.E. corn syrup solids, there appears to have been some lowering of flavor score. An important factor influencing the color and flavor of the canned product was the storage temperature. Storage at 68°F or lower appears to be advisable for better quality retention.

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J. B. MICKLE, WANDA SMITH, J. M. TIETZ, T. C. TITUS and MARTHA JOHNSTON
Dept. of Animal Sciences & Industry, Oklahoma State University, Stillwater, Okla.

INFLUENCE OF EMULSIFIER TYPE AND SOLUBILITY ON THE STABILITY OF MILK FAT-WATER EMULSIONS

SUMMARY—The purpose was to measure the amount of change in emulsion stability caused by different chemical types of emulsifiers in relation to the amount of change caused by emulsifier HLB. Seven emulsifiers used as 12 different binary mixtures were evaluated in model systems containing 10, 25 and 40% fat in water. Each emulsifier mixture was used at HLB numbers of 7, 10 and 13. The effect of chemical type on emulsion stability was minor in relation to the large changes caused by the fat percentage in the model system and the HLB of the emulsifier. A method was developed, using gas–liquid chromatography, to more accurately measure the HLB numbers of the emulsifiers used in this work. With these measurements it was learned that none of the differences in emulsion stability could be traced to the chemical type of emulsifier.

INTRODUCTION

USE OF emulsifiers to improve the physical characteristics of many food products is well known. The literature (as reviewed by Becher, 1965) indicated that one consideration when selecting an emulsifier was its relative fat-water solubility. More recently Titus et al. (1968) showed that maximum emulsion stability resulted when the fat-water solubility of the emulsifier was matched with the fat-to-water ratio of the food emulsion in which the emulsifier was to be used. These authors also presented data to be used in determining the proper fat-water solubility and the amount of emulsifier needed for foods containing various percentages of fat and water.

One method of expressing fat-water solubility is termed hydrophile-lipophile balance (HLB). Griffin (1949) defined this number as the ration of the weight of the water soluble (hydrophilic) portion of the emulsifier molecule to the weight of the fat soluble (lipophilic) portion. Becher (1965) reviewed the techniques of measuring HLB and the most precise of these involves gas-liquid chromatography (GLC) using the emulsifier whose HLB is to be measured as the liquid phase of the GLC column (Becher and Birkmeier, 1964).

According to Becher (1965) HLB numbers are algebraically additive. This involves the assumption of a "straight-line relationship" between HLB numbers of any two emulsifiers and all combinations thereof. However, it is not known whether this assumption is valid; since deviations from linearity have been observed in some cases. Becher (1965) also summarized the data of Griffin showing that certain pairs of emulsifiers (with different chemical compositions) result in more stable emulsions than do other pairs of emulsifiers—at the same HLB numbers.

It seems logical to assume that the chemical composition of an emulsifier would determine the relative water-fat solubility of the compound; i.e. it's HLB. If this were true, any differences in emulsion stability attributed to differences in the chemical composition of the emulsifier, must in fact be caused by inaccurate measurements of HLB numbers. Since HLB numbers were thought to be similar in the work of Griffin (1949), the different stabilities must have been due to nonlinearity of HLB numbers as suggested by Becher (1965) or an inaccuracy in measuring these numbers. However, the possibility exists that the chemical composition of an emulsifier does influence the effectiveness of that compound through some attribute whose effect is not usually measured; e.g. the size or shape of the molecule. At present, there is insufficient quantitative data on the effects of different chemical types of emulsifiers so that one is unable to determine whether chemical composition as such affects emulsion stability in addition to HLB values.

The purpose of this study was to measure the effectiveness of different chemical types of emulsifiers under conditions where HLB numbers were precisely measured and controlled to relate the magnitude of changes in emulsion stability associated with chemical types to the size of the changes caused by HLB number.

EXPERIMENTAL

SEVEN EMULSIFIERS from various commercial sources were chosen to represent different types of nonionic emulsifying agents. The HLB numbers of these compounds were approximated using the water-solubility method of Griffin (1949) as summarized by Atlas Chemical Industries (1962). Four of the emulsifiers had HLB numbers below 7 and three had numbers above 13. These seven compounds were used in 12 combinations of two, with each combination containing an emulsifier with a low HLB number and one with a high HLB. These emulsifiers were combined in ratios such that each of the 12 combinations resulted in three emulsifier mixtures with HLB numbers of 7, 10, and 13, or a total of 36 binary emulsifier mixtures as shown in Table 1. These 36 emulsifiers were added to the three different water-fat systems at the rate of 0.25%. The anhydrous milk fat used in this work was obtained from a single source to ensure a uniform supply. (Anhydrous butterfat, 99.9% fat, with a titration of < 0.1 meq/ml acidity calculated as lactic was obtained from Odell Concession Specialties Co., Caldwell, Idaho 83605.) This fat plus one of the 12 emulsifier mixtures and distilled water were weighed and combined to make fat-water systems containing 10, 25 and 40% fat.

The stability of these emulsified water-fat systems was evaluated using a technique previously described by Titus et al. (1968). This involved homogenizing the emulsified fat-water systems, then sampling the emulsion immediately. After quiescent incubation in test tubes at 37°C for 6 hr, a second sample representing the bottom half of the emulsion was obtained. The fat was extracted with ether and weighed, then the two fat percentage measurements were used to calculate a "stability index." This "index" was calculated by dividing the % fat in the 6-hr sample by the % fat in the initial sample and represented the relative change in stability of the initial emulsion during incubation. A stability index of 50 indicated that 50% of the emulsion had separated during incubation.

The variability of data from the above trials, as indicated by the size of the error terms in the statistical analysis, showed that a more precise method of measuring HLB numbers was desirable. For this, the GLC procedure similar to that described by Becher and Birkmeier (1964) was modified for use in this work as follows: A Varian Aerograph model 600 GLC instrument with a hydrogen flame detector was used with column temperature set up at 90°C ± 0.5°C. The recorder chart speed was set at 1 in./min so retention times could be obtained by direct measurement of distances on the chart paper. Prior to running each sample the carrier gas (nitrogen) flow rate was adjusted so ethyl ether was eluted exactly 1.4 min. after injection. The 6-ft columns used in this work 1/8 in. O.D. copper tubing. The support was Chromosorb G (60-70 mesh) and this was coated with 5% by weight of the emulsifier being measured. After injecting a variety of compounds, alcohols were chosen as the class of compounds most useful as test substances. Isoamyl alcohol was selected because its retention time was long enough to adequately measure small differences in emulsifier HLB.

RESULTS & DISCUSSION

STABILITY INDICES reported in Table 1 indicate that the fat-water sys-

Table 1—Stability indices of milk fat-water emulsions[a] with different fat levels and different types of emulsifiers[b] at selected HLB numbers

	10% Fat			25% Fat			40% Fat		
	HLB			HLB			HLB		
Emulsifier	7	10	13	7	10	13	7	10	13
1. Span 60[c]-Tween 60[d]	35	42	52	49	59	81	80	77	93
2. Span 60-L44[e]	45	51	57	66	69	80	89	91	93
3. Span 60-F68[f]	33	37	47	56	62	73	79	73	89
4. Marvic acid[g]-Tween 60	18	33	48	17	66	73	61	72	92
5. Marvic acid-L44	32	41	55	29	79	85	54	89	96
6. Marvic acid-F68	23	33	47	17	61	69	49	83	91
7. Myverol[h]-Tween 60	38	43	57	54	66	71	69	74	95
8. Myverol-L44	51	52	54	64	71	79	79	86	91
9. Myverol-F68	47	45	53	59	62	77	81	81	91
10. Drewlate[i] 10-Tween 60	33	35	57	28	65	72	72	78	94
11. Drewlate 10-L44	13	47	56	6	77	77	61	88	94
12. Drewlate 10-F68	15	36	48	12	63	71	14	83	92

[a]Averages of duplicate data.
[b]Emulsifiers added at the rate of 0.25%.
[c]Span 60: Sorbitan monostearate, Atlas Chemical Co.
[d]Tween 60: Polyoxyethylene sorbitan monostearate, Atlas Chemical Co.
[e]Pluronic L44: Mol. wt. 2200, 40% polyoxyethylene groups, Wyandotte Chemical Co.
[f]Pluronic F68: Mol. wt. 8350, 80% polyoxyethylene groups, Wyandotte Chemical Co.
[g]Marvic acid: Lactylic stearate, Durkees Famous Foods.
[h]Myverol 18-00: Glycerol monostearate, Distillation Products Inc.
[i]Drewlate 10: Propolene glycol monostearate, Drew Chemical Co.

Table 2—Analyses of variance of emulsion stability indices

Source	Mean squares HLB 10 & 13 only
Principal variables	
Fat	19,465[a]
HLB	4,139[a]
Emulsifier type	189[a]
Within emulsifiers	
1, 2, 3 vs others[c]	3
4, 5, 6 vs others	46
7, 8, 9 vs others	56
10, 11, 12 vs others	1
1, 4, 7, 10 vs others	226[b]
2, 5, 8, 11 vs others	1,755[a]
3, 6, 9, 12 vs others	722[a]
Interactions	
Fat × HLB	25
Fat × Emul.	30
HLB × Emul.	44
Fat × HLB × Emul.	25
Duplicates	34

[a]$P < 0.01$.
[b]$P < 0.05$.
[c]The numbers 1, 2, 3 etc. refer to the number of the emulsifier mixtures listed in Table 1.

tems emulsified with HLB 7 emulsifiers were very unstable. The water and fat phases of these systems often separated immediately after homogenization and before the initial sample could be obtained. Thus, it often was impossible to obtain representative samples of the system and the data obtained from such samples were of questionable value. Representative samples could be obtained from the other systems which contained emulsifiers with HLB numbers of 10 or 13 and the data from these appeared to be valid.

Statistical analysis of the data from the HLB 10 and 13 systems (Table 2) showed that the principal variables (fat, HLB and emulsifier) all were associated with statistically significant differences ($P < 0.01$) in emulsion stability. The relative size of the mean square values for these variables, indicated that influence of fat content in emulsion stability was almost five times greater than the influence of emulsifier HLB, and about 22 times greater than the effect of emulsifier type.

When these emulsifiers were grouped by individual compounds, none of the four lipophilic emulsifiers (HLB numbers of 3.7–4.4) had an effect on emulsion stability that was statistically different ($P < 0.05$) from the effects of the other three compounds (See Table 2: 1, 2, 3, vs others, etc.). However, the influence of the different hydrophilic emulsifiers (HLB 13.6–14.7) on emulsion stability was statistically significant ($P < 0.05$). The relative size of the mean square values for these comparisons indicated that these differences were largely caused by the emulsifier mixtures containing Pluronic L-44 (numbers 2, 5, 8 and 11) which were much more stable than the mixtures containing the other two hydrophilic emulsifiers. These differences can be seen in Figure 1 where the stability indices are graphed for the systems containing the HLB 10 emulsifier mixtures.

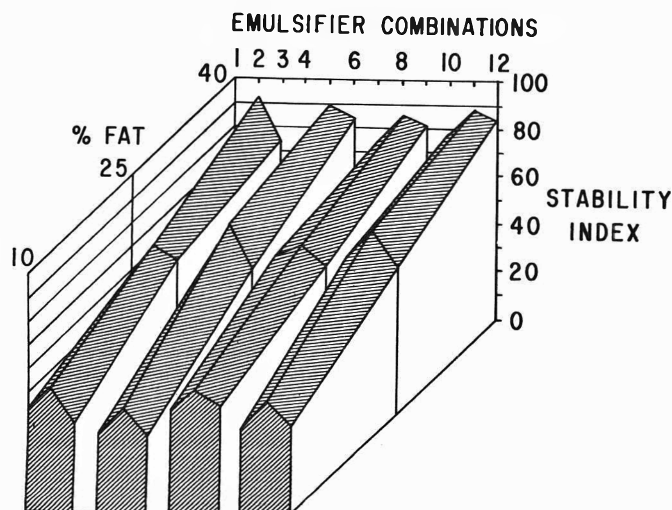

Fig. 1—Stability indices of milk fat-water systems containing 10, 25 and 40% fat with 0.25% of 12 different emulsifier mixtures at HLB 10.

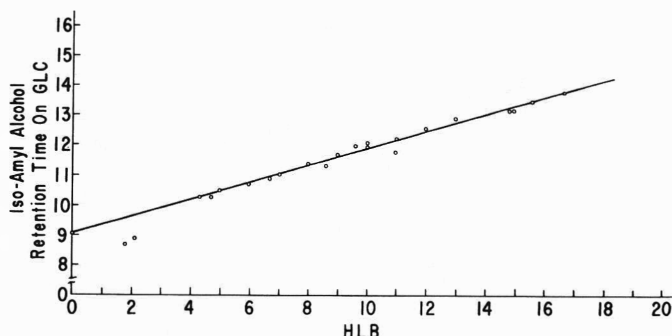

Fig. 2—HLB values of single emulsifiers (from Table 3) vs GLC retention times of isoamyl alcohol.

Table 3—Retention times of isoamyl alcohol on GLC columns coated with selected emulsifiers

Single emulsifiers of known HLB[a]	HLB (mfg)	Retention time[b] (min)
Span 85	1.8	8.7
Span 65	2.1	8.9
Span 80	4.3	10.3
Span 60	4.7	10.3
Span 40	6.7	10.9
Span 20	8.6	11.3
Tween 61	9.6	12.0
Tween 81	10.0	12.1
Tween 85	11.0	11.8
Tween 60	14.9	13.2
Tween 80	15.0	13.2
Tween 40	15.6	13.5
Tween 20	16.7	13.8
Mixtures of Span 60 and Tween 60		
	5.0	10.5
	6.0	10.7
	7.0	11.0
	8.0	11.4
	9.0	11.7
	10.0	12.0
	11.0	12.2
	12.0	12.6
	13.0	12.9
	14.0	13.2

[a]HLB values for single emulsifiers were those furnished by the manufacturer. For mixtures, HLB values were calculated algebraically assuming a straight-line relationship between HLB numbers and emulsifier weights (Atlas Chemical Industries, 1962).
[b]GLC columns were 6 ft copper tubing of 1/8" O.D., packed with 60–70 mesh Chromosorb G which was coated with 5% of the emulsifier to be measured. Retention times of isoamyl alcohol were measured at a chart speed of 1 in./min and a column temperature of 90°C ±0.5°.

This experiment shown in Table 1 gave no indication of why one emulsifier should cause greater stability in a fat-water system than did the other emulsifier. One possible explanation was that the water solubility technique used to measure HLB numbers for the single emulsifiers, was not precise enough and thus the HLB numbers of the mixtures used in the experiment were not accurate. To check this, the HLB numbers of all emulsifiers and mixtures used in this experiment were measured with the GLC technique described previously. To begin, retention times of emulsifiers with known HLB numbers were measured (Table 3). When plotted against HLB values, these retention times formed a straight line (Fig. 2). Using this as a "standard curve," the HLB numbers of the emulsifiers used in this work were determined (Table 4). These data show that the HLB numbers determined by GLC for Drewlate, Marvic acid, Myverol and Span 60 were lower than those obtained by the water solubility technique. The GLC value for Pluronic L-44 was higher than the corresponding water solubility value. However, the GLC value for Pluronic F-68 was lower and the value obtained for Tween 60 was about the same.

Table 4—Emulsifier HLB measurements by water-solubility and GLC techniques

Emulsifier	HLB H_2O	HLB GLC
Span 60	4.7[a]	4.2
Drewlate	5.0	4.0
Marvic acid	5.2	3.7
Myverol 1800	5.2	4.4
Pluronic L-44	13.2	13.6
Pluronic F-68	14.5	13.6
Tween 60	14.9[a]	14.7

[a]HLB numbers of Span 60 and Tween 60 were provided by the manufacturer, and these corresponded to measurements made by the water solubility technique. HLB numbers of the other emulsifiers were measured with the water solubility technique only.

If one assumes the GLC measurements were correct, then the HLB numbers of binary mixtures were not 10 or 13 as had been calculated on the bases of the water solubility measurements of single emulsifiers. Instead, all the mixtures with a calculated HLB value of 10 were 0.1–1.1 units lower than expected. The mixtures numbered 1, 3, 4, 6, 7, 9, 10 and 12 were 0.3–0.8 HLB units less than the calculated value of 13 and the mixtures numbered 2, 5, 8 and 11 were 0.2 more than the expected HLB value of 13 and 0.5–1.0 units higher than the value of the mixtures with which they were being compared (Table 5).

This work and that of Titas et al. (1968) have shown that HLB differences of 1.0 or more will cause significant differences in emulsion stability. Thus, the fact that HLB values for mixtures 2, 5, 8 and 11 were often as much as 1.0 HLB unit higher than the HLB numbers of the mixtures with which they were being compared probably accounts for most, if not all, of the differences attributed to the chemical type of emulsifier in this work.

The stability indices used in this work are only relative values and give only an abstract indication of an emulsifier's performance. One should remember that all of these values could have been increased, or decreased, by changing the length of the incubation time in the test procedure. However, these stability values do give a relative indication of which emulsifier system will give maximum performance under commercial conditions. This has been demonstrated by Titus et al. (1968) when similar data were used to pick the best emulsifier system for cakes. After baking, the cake volumes showed that these abstract stability indices were suitable to use in predicting which emulsifier system would give maximum performance under practical conditions.

Table 5—Calculated HLB values of emulsifier mixtures using GLC and water-solubility measurements of single emulsifiers[a]

Emulsifier mixture[b]	Calculated HLB H_2O	GLC	H_2O	GLC
1. Span 60-Tween 60	13	12.7	10	9.7
2. Span 60-L44	13	13.2	10	9.9
3. Span 60-F68	13	12.4	10	9.4
4. Marvic acid-Tween 60	13	12.5	10	9.1
5. Marvic acid-L44	13	13.2	10	9.5
6. Marvic acid-F68	13	12.2	10	8.9
7. Myverol-Tween 60	13	12.7	10	9.5
8. Myverol-L44	13	13.2	10	9.8
9. Myverol-F68	13	12.3	10	9.3
10. Drewlate-Tween 60	13	12.6	10	9.4
11. Drewlate-L44	13	13.2	10	9.7
12. Drewlate-F68	13	12.2	10	9.1

[a]HLB measurements from Table 3.
[b]Emulsifier mixtures as described in Table 1.

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G. S. MORRISON,[1] N. B. WEBB, T. N. BLUMER, F. J. IVEY and A. HAQ
Department of Food Science, North Carolina State University, Raleigh, N.C. 27607

RELATIONSHIP BETWEEN COMPOSITION AND STABILITY OF SAUSAGE-TYPE EMULSIONS

SUMMARY—A laboratory technique for the preparation of small batches of sausage emulsion was developed for a comparative study of the effectiveness of fresh and frozen beef in achieving emulsion stability. The technique consisted of three basic steps: (1) low speed chopping and blending of ingredients at −2° to +2°C; (2) low speed blending with the gradual addition of soybean oil at 2°–8°C; and (3) high speed chopping to a temperature of 15°–16°C. The preparation and evaluation of emulsions with widely varying compositions revealed that the lean and fat percentages could be varied over wide ranges without significantly affecting emulsion stability, but the range for percentage of water was narrow and critical to stability. The relative stability for fresh and frozen lean was influenced by the amount of added water. For example, at 30% fat level, there was a sharp drop in stability as the added water was reduced below 16% for fresh beef; whereas, an equivalent drop in stability was found as the added water was reduced below 21% for frozen beef. The theory is proposed that emulsion instability is highly dependent upon the level of added water based on results of this investigation.

INTRODUCTION

A MAJOR PROBLEM in comminuted sausage production is the tendency of fat droplets to coalesce during thermal processing. The problem is greater when frozen meat is substituted for fresh meat. However, recognition of the fact that this commercial product is essentially an emulsion has resulted in the application of emulsion principles in explaining the problem of fat separation. Photomicrographs (Hansen, 1960) have revealed that the colloidal-sized fat globules are surrounded by a protein film. The role of protein as the emulsifying agent has been confirmed recently by electron microscopy (Borchert et al., 1967). In recognition of the important role of protein in emulsion formation, much work has been done to evaluate the effectiveness of various proteins and protein-containing products (Swift et al., 1961; Hegarty et al., 1963; Saffle and Galbreath, 1964; Trautman, 1964; Borton et al., 1968; Maurer et al., 1969; Ivey et al., 1971).

Saffle and Galbreath (1964) found that pre-rigor beef contains a much higher fraction of salt-soluble protein than 48 hr post-mortem beef. Recently, Acton and Saffle (1969) demonstrated that the use of pre-rigor meat produces stable emulsions at a much higher fat level than 48 hr post-rigor meat. These results appear to support the theory that salt-soluble protein is largely responsible for emulsion stability. However, previous work in this laboratory (Ivey et al., 1971) suggested that water levels may play a much more important role in emulsion stability than has heretofore been recognized.

[1]Current address: Department of Chemistry, Northern Arizona University, Flagstaff, Ariz. 86001.

The purpose of this investigation was to develop a method of preparing small amounts of sausage-type emulsions with laboratory equipment to determine the effect of water level on the stability of sausage-type emulsions prepared with fresh and frozen meats.

EXPERIMENTAL

Sampling procedure

Samples of beef muscle (semimembranosus) were taken from utility grade cows 3 days post-mortem, trimmed free of surface fat and connective tissue; ground once through a 3/8-in. plate and thoroughly mixed. Treatment lots (400g) were placed in moisture proof plastic bags and the samples stored at 2°C (fresh storage) for use within 3 days or at −27°C (frozen storage) for 12–26 days. The frozen samples were thawed at 2°C for 48 hr immediately before use. On the day of use, fresh or frozen and thawed treatment lots were reground three times, using a chilled (2°C) grinder with a 3/16-in. plate, with thorough mixing and incorporation of any drip accumulation. An electrometric pH determination was made on a slurry and moisture, fat and protein were determined for each sample as described in the AOAC (1965).

Formula calculations

In order to maintain constant temperature and grinding conditions, all emulsions were made to a total weight of 160g, plus the weight of the NaCl, since it was not a part of the compositional calculations. The desired weights of lean, oil and added water were determined as follows:

$$\text{Weight (g) added water} = 160 - (\text{wt lean added} + \%\ \text{fat desired} \times 1.6) \quad (1)$$

If the weight of added water was less than 40g, it was added in the form of ice which had been stored at −20°C. If the weight of added water was 40g or more, the first 40g was added as ice at 0°C with the remainder added in the form of liquid water at 0°C. NaCl was added at a level of 3% of the weight of lean and water, but was not included in the compositional calculation.

In order to compensate for differing moisture levels in the meat samples, the amount of lean was expressed as muscle tissue (MT), which was defined as 4 × the dry weight of fat-free lean. This definition was necessary since lean does not have a specific composition. The value of four was chosen as a mechanical means of maintaining a constant, based upon the present industry practice. Thus, percentage muscle tissue (% MT) in the emulsion was expressed as:

$$\%\ \text{MT} = \frac{\text{wt lean} \times 4\,[100 - (\%\ \text{moisture} + \%\ \text{fat}]}{\text{total wt of emulsion}} \quad (2)$$

In order to compensate for differing fat levels in the meat samples, the total amount of fat present was expressed as total lipid (TL), which was defined as the sum of the weight of added soybean oil and the weight of fat occurring naturally in the meat. Thus, percentage of total lipid (% TL) in the emulsion was expressed as:

$$\%\ \text{TL} = \left[\frac{(\text{wt soybean oil}) + (\text{wt lean} \times \%\ \text{fat})}{\text{total wt of emulsion}}\right] \times 100 \quad (3)$$

The term added water (AW) was defined as water in the emulsion exclusive of MT fraction. Since the emulsion was calculated exclusive of added salt, it was considered as a three-component system and percentage added water (% AW) was expressed as:

$$\%\ \text{AW} = 100 - (\%\ \text{MT} + \%\ \text{TL}) \quad (4)$$

When a high-fat emulsion (% TL desired) was to be prepared, a low-fat (30% TL low-fat) emulsion was prepared first and a calculated weight removed and replaced by an equal weight of chilled soybean oil. The amount of low-fat emulsion to be removed was determined by the following equation:

$$\text{Wt removed} = \left(\frac{\%\ \text{TL}_{desired} - \%\ \text{TL}_{low\text{-}fat}}{100 - \%\ \text{TL}_{low\text{-}fat}}\right) 160\text{g} \quad (5)$$

The resulting lower percentage of muscle tissue (% MT_{final}) and of added water (% AW_{final}) were calculated as follows:

$$\%\ \text{MT}_{final} = \%\ \text{MT}_{low\ fat}\left(1 - \frac{\text{g removed}}{160\text{g}}\right) \quad (6)$$

$$\%\ \text{AW}_{final} = \%\ \text{AW}_{low\ fat}\left(1 - \frac{\text{g removed}}{160\text{g}}\right) \quad (7)$$

Emulsion preparation

Preparation of emulsions was accomplished in three steps.

Step 1. The specified amount of lean tissue was added to a chilled (1°C) 200 ml stainless steel Omni-mixer cup, the calculated amounts of ice, water and salt added, and the ingredients blended with a counter rotating mixer (Brookfield) modified by sharpening the leading edge of the blades. The mixer was started slowly by using a rheostat to prevent spattering. The operation was performed by maintaining the cup in an ice bath. In order to bring all of the sample in contact with the blades, the cup was repeatedly raised and lowered so that the blades could engage the entire mass, the sample being freed from the sides of the cup with the stem of a metal thermometer. Speed and duration of the stirring were limited by temperature which was not allowed to exceed 2°C. Step 1 was terminated as soon as a smooth blend was obtained.

Step 2. The procedures were similar to Step 1, except that higher stirrer speeds and temperatures were permitted. The rate of chopping was controlled by not allowing the temperature to exceed 8°C. The desired weight of chilled soybean oil was added in 15g portions, temperature control being maintained by stirring with a metal thermometer until recooled to 4°C before each addition of oil.

In the case of high-fat emulsions (>35% TL), after the completion of the low-fat emulsion preparation, the calculated weight of emulsion (Eq 5) was removed and an equal weight of chilled oil (1°C) was added in the same stepwise sequence. The same temperature, speed and quantity of oil were maintained.

Step 3. In order to simulate a commercial sausage emulsitator, the emulsions were subjected to further comminution using the Omni-mixer with the rotor-knife blade assembly designed for the 200 ml cup and suspended in an ice bath. Through preliminary experimentation, the degree of cooling and time of chopping was selected that would most effectively give the desired final temperature (15–16°C) within the desired time interval (50–70 sec). Using maximum speed on the Omni-mixer, intermittent chopping was used so that the temperature would gradually and uniformly increase. A maximum of five chopping intervals was used and, in the case of thick emulsions, the emulsion was repacked in the cup to maintain mechanical contact with the blades. It was found that the thicker the emulsion, the more rapid was the temperature rise. Cooling was not done between stirring intervals so that the temperature rise could be kept uniform.

Electrical conductivity

Electrical conductivity of the emulsions was determined in a specially designed conductivity cell as illustrated in Figure 1. The cell was prepared by cutting the bottom end from a clear, thin-wall plastic centrifuge tube, resulting in a cell 25 mm (diameter) and 50 mm (length). Each end was fitted with a No. 5 rubber stopper drilled to accommodate a size D, dry cell battery carbon rod and placed so as to be the specified distance apart. The carbon electrodes were projected 12 mm into the cell. Copper lead wires were attached to the outer ends of the electrodes and attached to a standard conductivity bridge. One of the rubber stoppers was fitted with a plastic overflow tube so that entrapped air could be eliminated. The cell constant was determined to be 0.64 using a KCl solution of known conductivity.

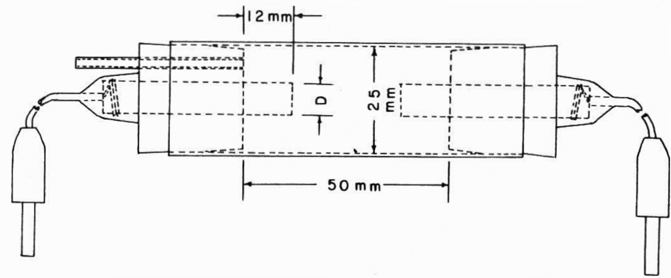

Fig. 1—Schematic diagram of the conductivity cell used to measure electrical conductivity of sausage-type emulsions.

Resistance reading in ohms, R, was taken when the temperature of the emulsion was 18 ± 1°C. Conductivity in millimhos/cm (σ) was calculated:

$$\sigma = (1000/R) \times 0.64$$

Table 1—Physical properties rating system for sausage emulsions heated by the Townsend cook stability test

Factors	Rating score
Resilience	
Good	8
Fair	6
Poor	4
Very poor	2
None	0
Firmness	
Firm	8
Slightly firm	6
Soft	4
Very soft	2
Mushy	0
Binding	
Good	8
Fair	6
Poor	4
Very poor	2
None	0

Cooked stability

The stability of the emulsions was determined by the method of Townsend et al. (1968), modified by using duplicate 28 × 102 mm centrifuge tubes with 30.0g of emulsion. The tubes were tightly covered and heated for 30 min in a water bath at 70°C. Any resulting liquid was decanted into a graduated cylinder and the water and oil volumes recorded separately. The remaining solid was weighed and cook stability (CS) recorded as the percentage of the emulsion remaining in the solid state.

Physical properties

A trained panel, using subjective analyses, rated the physical properties of the emulsions as follows: (1) resilience, the ability to return quickly to original shape after pressing; (2) firmness, resistance to pressing; and (3) binding, resistance to tearing. These properties were rated on a descriptive scale using numerical values of zero to eight as shown in Table 1.

The scores for the three properties were added to obtain a total possible rating of 24. A rating of 19 or higher was considered to be a satisfactory emulsion.

RESULTS & DISCUSSION

Effects of sample variation

Proximate analyses of semimembranosus samples from four animals are shown in Table 2.

Table 2—Proximate composition, pH, and storage time of beef muscles (semimembranosus) used for sausage emulsion preparation

Beef muscle sample no.	Cooler storage time (post-mortem age), days	Freezer storage time, days[a]	pH	Composition, %		
				Moisture	Protein	Fat
1	7-9	18-20	5.51	73.5	21.64	3.70
2	3-5	14-26	5.52	71.9	22.28	7.24
3	3-5	12-14	5.50	75.5	22.55	1.02
4	2-5	—	5.50	73.2	25.08	1.09

[a]The freezer storage time is in addition to the post-mortem age and a 48-hr thaw time at 2°C. Freezer storage studies were not conducted on sample number 4.

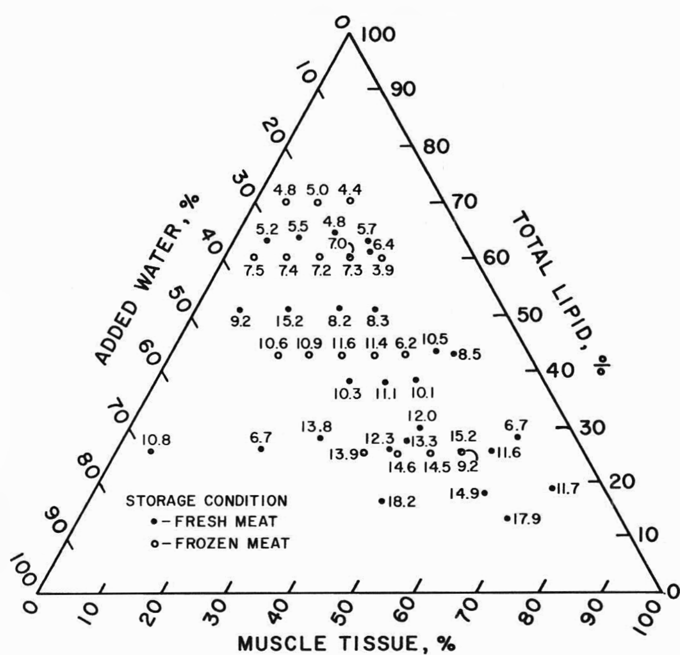

Fig. 2—Electrical conductivity (mmhos/cm) at 1000 Hz. of emulsions having various compositions when prepared from fresh and frozen meat.

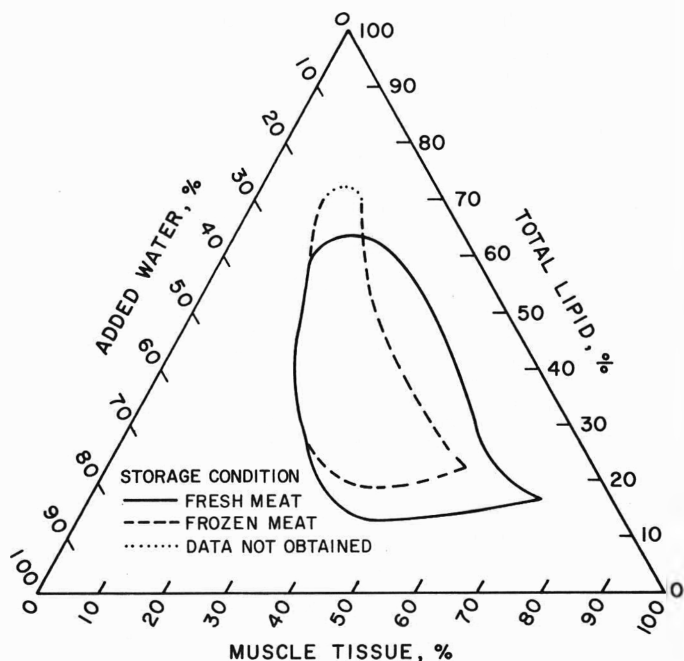

Fig. 3—Limiting boundary (score 19) for satisfactory physical properties of emulsions having various compositions when prepared from fresh and frozen meat.

Although significant differences were present in individual fat and moisture percentages of the four samples, effects of these differences were virtually eliminated by correcting emulsion compositions, as previously described. The variations in storage time of the samples within either the fresh or frozen lots did not significantly influence the results of subsequent treatments. The pH of the beef muscle did not differ substantially from the pH range (5.4–5.85) for 48 hr post-mortem beef as reported by Saffle and Galbreath (1964). The range difference in pH among the four samples was only 0.02 unit. Therefore, any differences in emulsion properties could not be attributed to pH. Since there was no reason to expect significant differences in treatment results among the individual samples, all results for individual samples were pooled and treated statistically as one group.

Emulsion composition

The points in Figure 2 represent the compositions of all emulsions prepared. Percent total lipid (TL) is represented by the perpendicular distance from the base of the triangle to the intersecting point; percent muscle tissue (MT) by the perpendicular distance from the left leg of the triangle to the intersecting point; and the percent added water (AW) by the perpendicular distance from the right leg of the triangle to the intersecting point. In this manner, the sum of % TL, % MT, and % AW is always 100%.

Conductivity as a criterion of emulsion stability

The number located at each point in Figure 2 represents conductivity of the emulsion in millimhos per centimeter. There are two noteworthy features of these data. First, as one proceeds up the composition diagram toward higher total lipid, conductance decreases. This trend is due to an increase in the virtually nonconducting fat phase, thereby reducing the effective cross-sectional area of the aqueous phase, upon which conductivity depends. The second and more significant feature involves points of the composition region of low water which are relatively close to the right leg of the triangle. Cook stability data revealed in this region four fresh compositions (reading down, conductivities of 8.5, 6.7, 11.6, and 11.7) and three frozen compositions (reading down, conductivities of 3.9, 6.2, and 9.2) which had broken as shown by significant oil pouroffs. Figure 2 shows that these emulsions had significantly lower conductivities than the corresponding emulsion immediately to the left. This marked decrease in conductivity upon inversion confirms previous work by Webb et al. (1970) in this laboratory, which suggested the potential value of electrical conductivity as a guide to emulsion stability.

Physical properties and emulsion composition

Figure 3 shows the limiting boundaries for fresh meat (solid curve) and frozen meat (broken curve) inside of which lie all scores of 19 or higher for the physical property evaluations. In comparing the emulsion compositions illustrated in Figure 2 with scores obtained in physical property evaluation (Fig. 3), it was observed that satisfactory scores (>19) were restricted to a relatively narrow region of the composition diagram. It is evident that the minimum percentage of added water (% AW) that can be tolerated in a satisfactory emulsion is represented by that portion of the curve running approximately parallel to the right leg of the triangle. It may be seen that the minimum % AW lies between 11% and 16% for fresh meat, and between 16% and 21% for frozen meat. These values were 16% and 21% for fresh and frozen meat, respectively, at 30% total lipid level. These results indicate that the use of frozen meat in sausage emulsions increases the minimum water requirement for satisfactory physical properties. Although the above evaluations were made on cooked emulsions, a limited number were observed to be markedly different in physical properties from the majority. The emulsions of this limited number had, in common, abnormally low viscosities, abnormally low conductivities and appreciable oil accumulation for the Townsend test. It

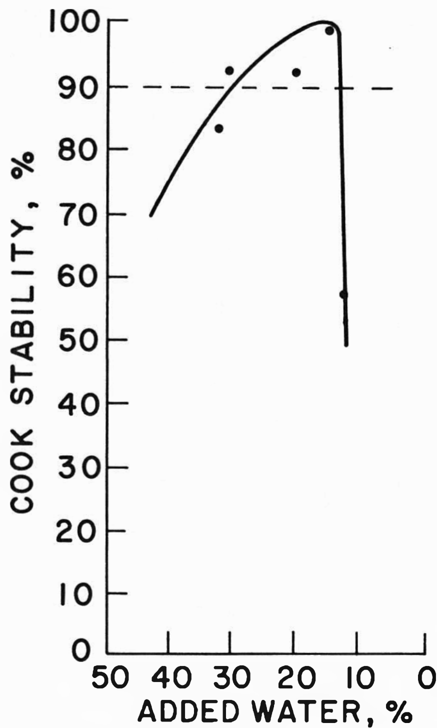

Fig. 4—Effect of added water on cook stability. Muscle tissue level constant at 43%.

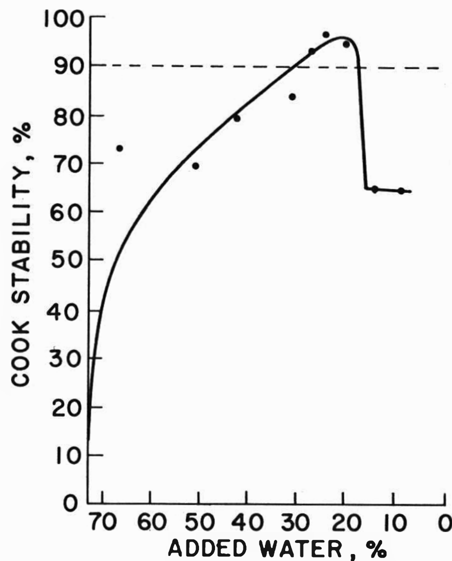

Fig. 5—Effect of added water on cook stability. Total lipid constant at 27%.

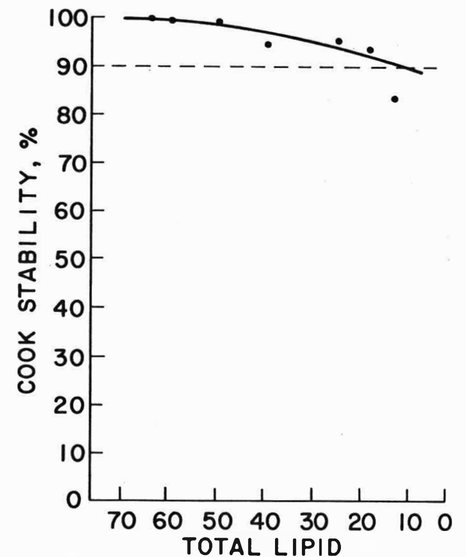

Fig. 6—Effect of total lipid on cook stability. Added water constant at 19%.

was evident that all of these emulsions had broken at varying degrees of intensity. Emulsions within this group were those occurring close to the right leg of the triangle and the same as those described as having a low conductivity.

Cook stability and emulsion composition

Of the three evaluations performed on the emulsions, the Townsend cook stability test appeared to give the most definitive results over a wide range of compositions. For this reason, the relationship between cook stability and composition was examined in more detail by keeping one component constant and plotting cook stability against one of the other components. This was done by selecting five or six each of constant MT, TL, and AW lines, which pass through or close to a number of fresh or frozen meat emulsion composition points, as illustrated in Figure 2. Cook stabilities along each of the constant MT lines or constant TL lines were plotted against % AW; while the cook stabilities along each of the constant AW lines were plotted against % TL. In this way, 17 constant component curves were constructed from each of the fresh and frozen meat data at compositions shown in Figure 2. In cases where the emulsion composition was slightly off the constant component line, cook stability was corrected by multiplying the distance from the line to the point (i.e., the difference in percentage) by the slope of another nearby constant component line. These corrections were small and ranged from 0.5%-2.0%.

Constant component curves are presented as examples for fresh meat emulsions at constant MT, TL, and AW in Figures 4, 5 and 6, respectively. These three curves are presented because they were the closest to commercial sausage composition.

Most of the curves rise to cross the 90% cook stability line, but subsequently fall again below the 90% cook stability line. Thus, most curves yielded two 90% intercepts, with the distance between them representing the composition range over which stable emulsions can be produced.

The 90% intercepts from all fresh and frozen constant component curves are plotted on one triangular graph, as shown in Figure 7. It may be clearly seen by comparing the dashed curve

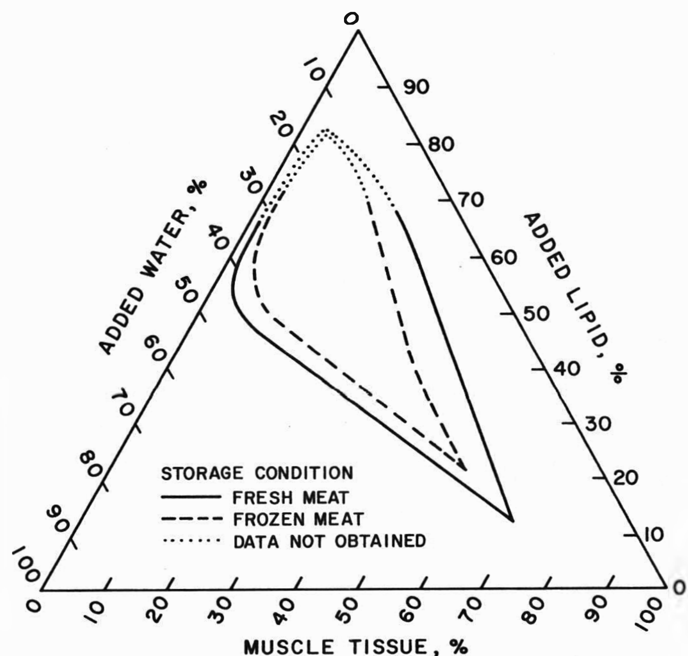

Fig. 7—Limiting boundary for cook stability (90%) of emulsions having various compositions when prepared from fresh and frozen meat.

with the solid curve that the area of 90% or greater cooked stability is greatly reduced by frozen storage. It may also be seen from the slopes of the curves in Figures 4 and 5 that the region of maximum stability is very close to the right hand boundaries of the points in Figure 7. Since both of these boundaries change strikingly less with respect to % AW than with respect to either % MT or % TL, the significant effect of frozen storage is an increase in moisture requirement of about 5% AW for the maintenance of emulsion stability.

Role of water in emulsion stability

It is obvious that one component in the emulsion cannot be varied without affecting at least one of the other two. For example, if one has a fresh meat emulsion containing 40% MT, 40% TL, and 20% AW, in order to increase MT to 50% one can either reduce TL to 30%, or reduce AW to 10%. On the basis of this investigation, reducing %TL to 30% can be done without introducing instability, but reducing % AW will cause the emulsion to break. It is evident that % MT and % TL can be varied over wide ranges as long as an increase in one is compensated by a decrease in the other. Since % AW cannot be similarly varied over a wide range, it was concluded by the investigators that of the three components in an emulsion, the added water level is by far the most critical in maintaining emulsion stability. This view is substantiated by the rather sharp maxima obtained for the curves in Figures 4 and 5 where water was a variable; as opposed to the lack of a maximum in Figure 6, where water was held constant.

While it may be true that in commercial operations, the stability of an emulsion is improved by increasing the salt-soluble protein level, it is possible that this benefit is not entirely explained by the addition of protein per sec. According to the results of this investigation, the addition of protein could be deleterious to emulsion stability. These results are supported by a basic theory relative to the denaturation in some systems. (Mahler and Cordes, 1966). This theory indicates that the denaturation of the protein, under selected situations, allows sufficient unfolding of the molecular chain to increase the stability of the system. Thus, in a relatively dilute system, such as those sausage-type emulsions having higher levels of added water, greater stability is obtained. In the more dilute system, protein-to-protein intermolecular reaction would be minimized but in a more concentrated system it would be increased, thereby reducing the effectiveness of the protein molecule to react with the oil droplets to stabilize the system. Certainly, the model system evaluated in this investigation is less complex than that found in commercial sausage emulsions. The use of one type of protein source and soybean oil for this study, rather than a variety of protein sources and animal fats, presents a different situation than presently used by the industry. However, the authors believe that this study is indicative of the potential for utilizing selected protein fractions more effectively in the stabilization of sausage emulsions. The selection of certain types of meat could have an enhancing effect by either using more efficient protein, or of actually increasing the amount of water which would be inherent in the selected meat. In any case, the work herein described leads to the conclusion that the fundamental solution to emulsion instability, at commercial fat levels, lies either in removing some of the protein from the oil-water interface, or in substituting a more efficient protein for a less efficient protein. If this theory proves to be correct for commercial operations, the practice of merely adding more protein to the interface to obtain greater stability could be eliminated. It is evident that further research will be needed to develop this theory into practice.

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GEORGE A. SCHULER[1] and E. O. Essary
Department of Food Science and Technology, Virginia Polytechnic Institute and State University, Blacksburg, Va. 24061

FATTY ACID COMPOSITION OF LIPIDS FROM BROILERS FED SATURATED AND UNSATURATED FATS

SUMMARY—The effect of feeding saturated fat (tallow) and unsaturated fat (safflower oil) to broilers on the change in fatty acid composition of lipids deposited in broiler tissues at 4, 6, 8, and 10 wk of age was determined. Fatty acids from raw and cooked skin, excluding that on the neck and third wing joint, breast meat, thigh meat, and abdominal fat were identified by using gas liquid chromatography. Fatty acids from water in which carcasses were cooked were also identified. The degree of unsaturation of fatty acids in these tissues was influenced by the degree of unsaturated fatty acids in the diet and tended to assume the fatty acid composition of the diet. In some cases, however, the higher levels of certain fatty acids in depot fat was not present in broilers fed the higher levels in the diet. Fatty acids in the larger amounts in all broiler tissues were palmitic, stearic, oleic, and linoleic but varied in amount among the different age broilers fed the same ration as well as different rations. In most cases there tended to be an inverse relationship between oleic and linoleic acids in the tissues. Lipids from cooked tissues contained a larger amount of 18-carbon unsaturated fatty acids than the other fatty acids combined. Fatty acids collected from cooking water were similar to those in cooked tissues. The presence of 13- and 25-carbon chain fatty acids noted in tissues of 4 wk-old broilers suggests a difference in the metabolism of fat in different age birds. Futher research is needed to substantiate this finding.

INTRODUCTION

AN INCREASING percentage of turkeys and broilers in the United States is being marketed in the form of further processed convenience foods. With the advent of mechanical deboning machines, this trend in marketing shows evidence of continued expansion and emphasizes the importance of studying factors which might influence carcass composition and other tissue characteristics.

Most of the research during the past three decades concerned with the storage of fat in poultry carcasses was conducted using laying hens or broiler chickens of one age with little attention directed towards deposition of fat in broilers of different ages.

65% of the fatty acids present in the body fat of hens were reported to be unsaturated (Hilditch et al., 1934). Marion and Woodroof (1963) isolated 20 carbon chain fatty acids containing five double bonds from skin and adipose fat and 24 carbon chain acids containing six double bonds from thighs and breast meat.

The addition of 5% corn oil in the diet of mature birds resulted in higher levels of linolenic acid and lower levels of palmitoleic and oleic acids (Marion and Edwards, 1963).

Since carcass fat influences the acceptability and shelf life of poultry meat products, it is important to know the amount of fat deposited in the carcass and how its composition might change as broilers approach marketable age.

The purpose of this study was to determine the influence of feeding saturated fat (tallow) and unsaturated fat (safflower oil) in rations on the possible change in fatty acids composition of lipids deposited in broilers at bi-weekly intervals at 4, 6, 8, and 10 wk of age. The amount of fatty acids left in the tissues after broilers were simmered as well as the amount of fatty acids removed from the tissues and left in the water in which broilers were cooked were also investigated.

Table 1—Ingredients and fatty acid composition of experimental rations

Ingredients	Control (lbs)	Beef Tallow (lbs)	Safflower Oil (lbs)
Ground yellow corn	55.99	39.99	39.99
Soybean oil meal, 45% crude protein	22.00	30.00	30.00
Bleached beef tallow	0.00	8.00	0.00
Safflower oil	0.00	0.00	8.00
Other ingredients[a]	22.01	22.01	22.01
Total	100.00	100.00	100.00

[a]Other ingredients and amounts (in lb) fed in each ration are as follows: wheat flour middlings 5.0; corn gluten meal 2.0; menhaden fish meal 5.0; meat scraps (52% crude protein) 2.0; alfalfa meal (17% crude protein) 3.0; dried whey 1.5; ground limestone 1.3; deflourinated rock phosphate 0.8; iodized salt 0.4; trace mineral concentrate (8% manganese, 3% zinc, 24% min calcium) 0.5; vitamin premix #7 (1.5 mg vitamin B_{12} and 2g chloretetracycline per lb) 0.5; and antioxidant (25% butylated hydroxy toluene) 0.01.

EXPERIMENTAL

300 DAY-OLD male broiler-type chicks were fed three diets from day-old to 10 wk of age. One ration contained 8% beef tallow, another 8% safflower oil, and the third diet, the Control, no added fat, Table 1.

The fatty acid composition of the tallow and safflower oil used in the rations is shown in Table 2. Safflower oil contained 89% unsaturated fatty acids of which 76% contained two or more double bonds. Beef tallow contained 51% unsaturated fatty acids, with less than 4% containing two or more double bonds.

Chicks were raised in battery brooders, housed in a temperature controlled brooder house and supplied feed and water ad libitum. Ten broilers from each treatment were selected at random and processed at biweekly intervals from 4-10 wk age.

Uncooked skin, excluding that on the neck and third wing joint, muscle tissue from the breasts and thighs, and the abdominal fat from five of the carcasses were removed, ground separately, freeze dried, and the fatty acids extracted. The other five carcasses in each treatment were labeled and cooked at 180°F for 1 hr in 5 gal of water in a steam jacket kettle. The fat in the cooked tissues was removed, ground, freeze-dried, and extracted as done with the raw tissues. Fat in the cooking water was separated by physical means in a separatory flask and prepared for analysis by gas liquid chromatography.

Lipid samples were esterified and analyzed by gas chromatography by the methods of Metcalfe et al. (1966) and Wijngaarden (1967). The following steps were used in esterification:

1. Approximately 150 mg of fatty material was placed in a 50 ml volumetric flask.

Table 2—Fatty acid composition of dietary fats

Chain length and number of double bonds	Fatty acid	Beef tallow	Safflower oil
8:0	Caprylic	0.2	0.0
10:0	Capric	0.1	0.0
12:0	Lauric	0.1	0.0
14:0	Myristic	2.3	0.1
14:1	Myristoleic	0.7	0.0
16:0	Palmitic	27.4	8.2
16:1	Palmitoleic	3.4	0.1
18:0	Stearic	17.9	2.7
18:1	Oleic	43.0	12.5
18:2	Linoleic	2.7	74.7
18:3	Linolenic	0.6	1.4
Miscellaneous		1.6	0.3

[1]Present address: Extension Food Science Department, Cooperative Extension Service, University of Georgia, Athens, Ga. 30601.

2. 4 ml of 0.5N methanolic NaOH was added to the mixture.
3. The mixture was heated for 2–5 min on a steam bath until the fat globules went into solution.
4. 5 ml of BF_3 methanol was added to the flask and the mixture boiled for 2 min.
5. 5 ml of distilled hexane was added to the flask and the mixture boiled for 1 min.
6. Enough saturated sodium chloride solution was added to bring the liquid level into the neck of the flask.
7. The upper layer (hexane) was pipetted into a glass-stoppered centrifuge tube.
8. The volume was adjusted to 0.5 ml by the addition of hexane or the evaporation of excess hexane under a stream of nitrogen gas.
9. A pinch of anhydrous sodium sulfate was added to bind traces of water.
10. Between 0.1–0.6 μl of this solution was injected directly into the gas chromatographic column.

The fatty acids found in the tissue samples were identified by using known concentrations of known fatty acids obtained from Hormel Institute (Austin, Minn.) and Supleco Inc. (Bellefonte, Pa.) These fatty acids were also identified by a commercial laboratory which further lends credibility to the correct detection of these acids. The retention time for each fatty acid was compared with a known standard since it is known that under the same parameters fatty acids have different retention times but each fatty acid has its own repeatable retention time. Relative percentages of the fatty acids in the tissue were calculated by using integrator counts adjusted to a standard attenuation.

A total of 660 chromatographs were made by fractioning the fatty acid methylates with an F and M model 400 gas liquid chromatograph equipped with a hydrogen flame detector. Aluminum columns (9-1/2 ft × 1/4 in) packed with 15% by weight of diethylene glycol succinate (DEGS) on 60-80 mesh chromsorb W were used at an isothermal oven temperature of 222°C for the first two months of analysis. It was found, however, that the DEGS column broke down rapidly at this temperature. Therefore, in subsequent analysis a glass column (5-1/2 ft × 1/4-in) packed with 15% DEGS on 60-80 mesh chromsorb W was used at an isothermal oven temperature of 190°C.

Other parameters used in the GLC analysis were prepurified nitrogen gas as a carrier gas at 50 ml/min, hydrogen and breathing air at 40 ml/min to maintain the hydrogen flame, and a flash heater at 200°C for quick vaporization of the injected mixture.

The methyl ester peaks were also identified by programmed temperature analysis and by the use of column packings to change the order of peak appearance (3% CC Grade SE-30 on 80-100 chromsorb WH which reverses the appearance of unsaturated members of a carbon series).

RESULTS & DISCUSSION

THE FATTY acid composition of lipids from raw and cooked skin, breast and thigh tissues from birds fed the Control ration, and the rations containing tallow and safflower oil are presented in Table 3. The fatty acids eluted from the diethylene glycol succinate column in the larger quantities and in carbon chains 13 and 25 are listed in the order of their emergence from the column.

Fatty acids of 13- and 25-carbon chains from raw skin tissue of 4 wk-old broilers were detected in our laboratory as well as in a commercial laboratory. To the authors' knowledge, this is the first study to show the presence of these fatty acids in the skin of 4 wk-old birds. Since carbon 13 fatty acid was prevelant only in 4 wk-old broilers and work in literature concerned with fatty acids in broilers has been conducted primarily with 8 wk-old birds, there was little

Table 3—Fatty acid composition of lipids from the skin, breast muscles and thigh muscles from broilers fed tallow (T), safflower oil (S.O.) and the Control (C) diet (Avg 5 broilers)

	Raw												Cooked					
	Age 4 wk			Age 6 wk			Age 8 wk			Age 10 wk			Age 8 wk			Age 10 wk		
Fatty Acid[a]	C %	T %	S.O. %	C %	T %	S.O. %	C %	T %	S.O. %	C %	T %	S.O. %	C %	T %	S.O. %	C %	T %	S.O. %
Skin																		
13:0	0.1[b]	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
16:0	26.6	25.0	16.6	29.0	15.0	20.5	26.3	17.1	17.0	28.3	18.5	14.7	21.0	17.9	18.9	24.5	20.5	24.3
16:1	2.9	4.1	3.4	6.8	5.7	5.3	7.3	5.0	4.9	4.1	5.3	3.3	6.6	8.1	1.4	7.4	5.5	0.0
18:0	4.6	4.8	5.4	7.0	7.5	11.1	6.3	8.8	8.9	4.2	7.8	5.8	6.3	10.4	6.9	7.6	8.5	3.5
18:1	35.2	48.2	22.7	33.9	31.9	24.6	36.0	30.4	27.4	36.4	37.4	24.3	31.2	29.8	26.3	37.2	41.0	38.4
18:2	20.8	14.1	25.1	23.5	34.2	29.8	18.6	28.8	34.8	19.2	20.3	48.9	20.7	22.1	36.1	20.1	20.0	18.5
18:3	2.9	0.0	0.2	0.1	1.1	0.8	0.4	0.9	0.4	0.2	0.0	0.0	4.9	0.0	2.3	0.1	0.0	1.1
25:0	0.4	0.0	1.5	0.0	0.0	0.2	0.0	0.0	0.0	0.2	0.0	0.0	0.1	0.1	0.0	0.0	0.0	1.1
Breast Muscles																		
13:0	0.0	0.1	1.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
16:0	26.1	23.4	16.4	24.3	20.1	18.4	27.3	22.0	16.1	20.8	20.8	18.3	21.3	16.8	18.6	36.5	19.3	18.6
16:1	3.0	5.4	4.2	6.6	2.1	4.9	6.7	6.9	3.1	9.1	5.8	2.8	7.6	7.5	5.4	5.5	5.5	7.5
18:0	8.8	9.8	8.0	8.1	8.0	9.1	5.0	10.4	7.3	6.5	9.2	7.5	8.9	10.8	8.9	5.4	10.4	6.3
18:1	37.7	42.9	22.9	31.6	31.5	30.2	39.0	32.4	26.0	32.9	41.7	27.3	34.4	30.2	32.4	39.8	34.3	36.1
18:2	20.1	15.6	39.8	23.7	26.9	28.7	19.2	17.4	44.0	18.5	18.3	40.7	18.6	24.6	26.6	18.4	24.5	19.2
18:3	0.1	0.0	0.5	0.1	0.0	0.2	0.2	0.7	0.3	0.0	0.0	0.5	1.1	0.0	0.1	0.0	0.0	0.9
25:0	0.0	0.5	0.6	0.5	0.1	0.4	0.0	0.0	0.0	0.5	0.0	0.0	0.0	0.1	0.1	0.0	0.0	0.3
Thigh Muscles																		
13:0	0.1	0.2	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
16:0	24.1	20.1	21.9	23.5	18.1	17.6	24.7	19.0	18.2	23.6	20.3	13.6	23.1	16.6	17.5	26.1	20.9	17.0
16:1	7.5	7.6	1.8	5.9	5.6	5.3	8.1	8.9	3.1	8.5	5.8	2.6	8.1	8.3	3.4	7.0	5.1	3.1
18:0	7.4	6.9	5.3	7.8	8.5	9.1	6.4	9.2	8.2	4.1	8.6	6.0	6.7	11.0	8.2	6.2	9.8	9.2
18:1	32.9	38.9	22.8	34.2	31.0	33.5	40.1	31.8	29.7	37.0	39.7	22.5	32.8	30.5	28.1	40.0	40.9	21.8
18:2	19.6	16.5	24.6	23.1	23.9	27.3	17.9	20.8	34.0	18.5	20.1	43.8	19.2	21.8	32.6	18.0	19.7	44.4
18:3	0.0	0.1	0.0	0.1	0.5	0.3	0.6	0.0	0.1	0.0	0.0	0.2	3.7	0.0	0.0	0.0	0.0	0.0
25:0	0.6	0.4	0.4	0.4	0.2	0.0	0.0	0.2	0.0	0.4	0.0	0.0	0.1	0.1	0.3	0.0	0.0	0.0

[a] Carbon chain length: number of double bonds.

[b] Values rounded off to one decimal place. Standard evaluations were not calculated unless retention time of the fatty acid in four or more samples matched the retention time of the standard. Carbons 12–26 were isolated and identified but most of them in small amounts are not included.

Table 4—Fatty acid composition of lipids from abdominal fat and fat in water after cooking (Avg 5 broilers)

	Raw											
	Age 4 wk			Age 6 wk			Age 8 wk			Age 10 wk		
Fatty Acid[a]	C[b] %	T[b] %	S.O.[b] %	C %	T %	S.O. %	C %	T %	S.O. %	C %	T %	S.O. %
13:0	0.2	0.2	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
16:0	25.2	26.4	19.3	24.1	19.1	19.8	23.3	16.4	18.9	25.5	21.7	15.0
16:1	4.8	0.0	0.0	7.5	3.3	3.3	8.0	3.1	1.1	8.1	4.3	4.7
16:2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.5	0.0	0.0	2.3
18:0	5.4	3.8	2.7	6.8	5.2	8.5	5.7	5.8	7.0	6.3	6.5	18.6
18:1	36.8	39.2	25.1	33.9	33.2	27.9	37.7	43.6	28.9	33.8	41.5	11.9
18:2	17.1	15.5	38.5	22.4	31.3	28.2	22.1	19.8	35.5	16.8	18.4	41.0
18:3	2.5	0.0	0.0	0.1	0.7	1.0	0.8	0.0	0.4	0.0	0.0	0.0
25:0	0.2	1.8	0.1	0.3	0.3	0.0	0.0	0.0	0.0	0.2	0.0	0.1
Fat from Cooking Water												
16:0	Not obtained from 4 and 6 wk-old broilers.						32.8	25.6	18.9	26.0	14.3	26.7
16:1							0.0	0.0	0.0	7.7	2.3	7.1
16:2							0.0	0.0	0.0	0.0	0.0	0.0
18:0							3.4	4.9	5.2	6.5	6.0	0.4
18:1							38.6	39.2	29.3	36.9	19.8	10.0
18:2							19.2	21.3	34.4	19.4	47.8	49.8
18:3							0.0	0.0	0.4	0.1	2.9	0.0
25:0							0.7	0.4	0.6	0.0	0.0	0.0

[a]Carbon chain length: number of double bonds.
[b]C = control; T = tallow; S.O. = safflower oil.
[c]See footnote b Table 3.

chance of detecting these acids by previous research workers.

Even though the source of 13-carbon fatty acid is not known there are several plausible explanations for its origin. Tridecanoic acid may be an intermediate in the fatty acid metabolism of young birds or it might be the beta oxidation product of the 15-carbon fatty acid petadecanoic acid.

Pentacosanoic acid, 25:0-carbon, was found in larger amounts in broiler skins than tridecanoic acid but was present more often in tissues in older broilers. Both pentacosanoic and tridecanoic acids may have been synthesized in the tissues of broilers or they may have had their origin in the egg. There is also the possibility that minute amounts of these fatty acids were present in the fishery products added to rations.

The fate of these two acids could be determined by feeding radioactive labeled carbon in fats added in rations and subsequently determining their presence in fatty acids of depot fat. It is possible that the peaks interpreted as 13- and 15-carbon fatty acids could have been caused by lipid compounds not recognized or by the fractionation of longer chain fatty acids. Further study is needed to substantiate the source of these compounds.

Lipids from raw skin of broilers fed the different diets contained the same fatty acids but the amounts varied slightly among weeks. The safflower oil fed birds contained the lowest amount of 18 carbon fatty acids (approx. 53%) at 4 wk of age but the highest (approx. 79%) at 10 wk. The total percentage of the 18-carbon fatty acids in the other two diets were similar for the different periods. Linolenic acid was higher at 4 wk in broilers fed the Control diet as compared to broilers fed safflower oil and tallow. Palmitic acid (16:0) was highest each week in the skin of Control fed birds. The reason for this phenomenon is difficult to explain since the amount of this acid was much higher in the diet containing tallow, Table 2, and would be expected to be deposited in smaller amounts in the Control diet which contained no added fat. Apparently the broilers were able to synthesize this acid as needed but the unanswered question is why birds fed lower amounts of this acid would deposit higher amounts of it in the skin than those on feed containing fat with the higher of this fatty acid. The level of palmitoleic acid (16:1) in skin lipids varied in amounts among weeks and treatments.

In lipids from breast muscles the three acids in the largest amounts were palmitic, oleic and linoleic. Palmitic acid was highest in birds fed the Control diet. Birds fed safflower oil contained the lowest amount of palmitic acid which was consistent in amounts among weeks. The total amount of oleic and linoleic acid in the Control fed birds was similar among weeks but fluctuated in broilers fed tallow and safflower oil. The reason for these differences is not understood since the beef tallow ration contained much higher amounts of both acids than the other two rations. Linolenic acid was noted in small amounts in the different age broilers. Also noted was small amounts of 13- and 25-carbon chain fatty acids.

Lipids in thigh muscle contained about the same amounts of palmitic, oleic and linolenic acids as those in breast muscles. Palmitoleic acid (16:1) was deposited in smaller amounts in thighs from broilers fed safflower oil than in the other two diets. The amount of oleic acid in thighs of birds fed the same ration varied among weeks. Since the amount of this acid deposited in lipids did not consistently increase or decrease with the age of the bird, it is difficult to make a logical explanation as to the reason for the bi-weekly variations noted.

The cooked tissues often contained smaller quantities of the different fatty acids than was noted in the raw tissues. This did not hold true, however, with linolenic acid which was in larger quantities in the cooked tissue than in raw tissue. Chang and Watts (1952) also reported a substantial increase in the linolenic acid content of cooked chicken meat. This phenomenon has not been noted in ground pork, bacon, ham, beef roasts, leg of lamb, or lamb chops but was destroyed in these meats by cooking. These workers reported that cooking caused an increase in the 18:3 carbon fatty acid in chickens. It was also shown that linolenic acid varied from 20–30% in chicken and turkey fat but only 1–2% in beef and lamb fat. The higher levels

of linolenic acid in cooked tissues may be explained on the basis that the oxidation of unsaturated fatty acids may cause an additional double bond to appear in the acids during alkali isomerization.

The fatty acid composition of abdominal fat from broilers fed the three diets is presented in Table 4. It may be noted that the 13-carbon chain fatty acid was present in abdominal fat of 4 wk-old broilers. Carbon 25-chain fatty acid was found in varying amounts in fat from different age broilers. Palmitic acid levels varied each week in fat from birds fed all three rations and was higher in broilers fed the Control ration. The tallow diet contained the highest level of palmitic acid and yet palmitic acid was the lowest in the abdominal fat of birds fed this diet in two of the five periods. The reason for this result is not known.

There appeared to be an inverse relationship between the amount of oleic and linoleic acids in the abdominal fat. Broilers fed safflower oil contained a higher level of linoleic acid in the abdominal fat than broilers fed the other two rations. Birds fed the Control diet and the diet containing tallow showed higher levels of oleic acid than did broilers fed safflower oil. This would be expected in broilers fed tallow because of the level of this acid in the ration. The amount of 18:3 fatty acid in the abdominal fat was relatively small and varied among weeks and treatments.

Fatty acids in lipids recovered from cooking water at 8 and 10 wk were similar to those in the tissues cooked in water.

The data presented in this study further demonstrate a marked similarity among the fatty acids in lipids from poultry tissues of different age birds. These results are in agreement with those of Cruickshank (1934) who showed a great similarity in fatty acids among different poultry tissues. However, Marion and Woodroof (1963) reported a difference in fatty acid composition of poultry tissues. These workers stated that appreciable quantities of 22- and 24-carbon fatty acids were detected in breast tissues (6.9%) in the Control group, while smaller quantities (6.7%) were generally found in thigh tissues of the Control group.

The amount of a particular fatty acid included in the ration was not necessarily deposited in the same relative amounts in different areas in broilers. In many cases, the fatty acid found in the largest amounts in lipids from different tissues of broilers was not present in the largest amount in the ration. Lipids from broilers fed the Control ration had a higher level of palmitic acid than did lipids from birds fed tallow or safflower oil. A logical reason for this observation is not known.

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P. E. BOUTON, P. V. HARRIS and W. R. SHORTHOSE
CSIRO Division of Food Preservation—Meat Research Laboratory
P.O. Box 12, Cannon Hill, Brisbane, Queensland, Australia 4170

EFFECT OF ULTIMATE pH UPON THE WATER-HOLDING CAPACITY AND TENDERNESS OF MUTTON

SUMMARY—Ultimate pH values in the musculature of sheep ranging from 5.6–7.0 have been obtained by using pre-slaughter injections of epinephrine. A high speed centrifugal method was used to measure the water-holding capacity of raw M. semitendinosus (ST), semimembranosus (SM) and biceps femoris (BF). Results showed a high correlation with ultimate pH. Cooking losses and the amounts of centrifugally expressed juice were determined for the SM and BF cooked for 1 hr at either 65°C or 90°C. Cooking losses at 65°C decreased linearly with increasing pH while the losses at 90°C showed little change up to a raw meat pH of ca. 5.9, then decreased linearly with increasing pH. The amount of juice centrifugally expressed from the cooked meat, which has a high positive correlations with organoleptic juiciness, increased linearly with pH. Tenderness of the cooked SM and BF muscles was measured using a Warner-Bratzler shearing device and an Instron Universal Testing Machine: both gave high objective-subjective correlations. Instron measurements have high negative linear correlations with ultimate pH for both the 65°C and 90°C cooked samples. Hardness of these muscles, cooked at 65°C or 90°C, decreased approximately three-fold as ultimate pH increased from 5.6–6.9. Results obtained using the Warner-Bratzler device showed linear regressions with a significant quadratic component for one muscle at both 65°C and 90°C.

INTRODUCTION

WATER-HOLDING capacity (WHC) of raw and cooked meat has been related to such important organoleptic properties as juiciness and tenderness (Hamm, 1960). Change in WHC of muscle homogenates has been shown (Hamm, 1958a, b; 1959) to be closely related to pH, and to be a sensitive indication of variations in the charges and structure of muscle proteins. The pH of these homogenates was adjusted by the addition of acid or alkali (Grau et al., 1953). It has often been assumed that these results with meat homogenates were directly applicable to intact meat samples.

Winkler (1939) obtained a range of pH values in whole meat samples by injecting lactic acid or ammonia and produced changes in tenderness although these solutions were probably not evenly distributed through the meat.

Pre-slaughter stress or drug administration has been used (Bouton et al., 1957) to vary ultimate pH. Other workers have relied on normal variation in pH and investigated the relationship between pH and meat properties.

Lewis et al., (1967a, b) stressed cattle before slaughter to produce an increase in ultimate pH but their results were equivocal. Penny et al. (1963) and de Fremery (1963) induced high ultimate pH by pre-slaughter injection of epinephrine and/or iodoacetate and found that meats with a high pH were more tender and juicy than those with a low pH.

There have been few studies of the variation in the properties of meats over a wide range of ultimate pH. Although Mackey et al. (1952) used pork with a pH range of 5.57–6.39, they found no relationship between pH and tenderness, probably because only five animals were used. Bouton et al. (1957) reported a curvilinear relationship between the ultimate pH of beef (range 5.55–6.48 but with very few readings above 5.8) and sensory toughness; peak toughness occurred in meat with a pH of about 6.0. From the results available, it has been difficult to determine the precise relationships between pH and meat properties. Often only a limited pH range had been studied or samples were unevenly distributed within the range. Frequently, only contrasts were made between meats of high and low pH.

In this present investigation drugs were administered to sheep before slaughter to produce muscles with a wide range of ultimate pH values (5.6–7.0) in an attempt to investigate the relationship between pH and WHC of the raw meat. The effects of pH on moisture losses and on the tenderness of samples cooked at 65°C and 90°C were also determined. Tenderness was assessed using two methods, viz, the Warner-Bratzler shear and a penetrometer method involving an Instron Universal Testing Machine (Instron Ltd., Bucks., England).

MATERIALS & METHODS

Animals and pre-slaughter treatments

The 16 sheep used were Merino X wethers, 3-4-1/2 years old, mean liveweight 45.2 ± 0.7 Kg. They were penned individually and fed alfalfa hay and a commercial sheep concentrate, 15% protein, for 8 wk prior to slaughter.

To produce muscles with a high ultimate pH, subcutaneous injections of 1:1000 or 1:500 (w/v) adrenaline chloride were used, usually administered in two doses, and in some cases supplemented by an intravenous injection shortly before slaughter. The total amount of adrenaline given subcutaneously in the two injections varied from 6–30 mg. The first dose was given 23-1/2–29 hr before slaughter. The interval between the second dose and slaughter varied from 52–375 min.

To produce muscles with a low-normal pH "Myanesin" (B.D.H. Ltd., London) [3-(2-methylphenoxy)propane-1:2-diol] was administered either intravenously [50 or 65 ml of a 2% suspension, in 0.9% (w/v) NaCl] as one dose immediately prior to stunning or orally as two doses of 1.5 and 2.0 g, given 28 hr and 4 hr prior to stunning the animal in its own pen.

Animals were bled immediately after stunning with a captive-bolt pistol. In the case of animals stunned in their pens the time between stunning and bleeding varied from 1-1-1/2 min. The animals were dressed and then chilled at 1°C for 72 hr.

Measurement of pH

pH of whole or comminuted, raw or cooked meats was measured directly on the meat using an IL175 meter (Instrumentation Laboratory Inc., Boston, Mass.) with a probe-type combined electrode. Ten pH readings were made on each sample and the mean of these used. Meat temperature was approximately 20°C when pH determinations were made. The pH measured for the raw meat was the ultimate or minimum pH, taken after the carcases had been held for 3 days at 1-2°C.

Measurement of WHC

Many methods for measuring WHC have been described in the literature. Möhler and Kiermeier (1953) used sedimentation while others, including Marsh (1952), Wierbicki and Deatherage (1958), Hamm (1958b), Penny et al. (1963) and Miller et al. (1968), have used centrifugation. The most commonly used methods seem to be modifications of the press method of Grau et al. (1953) and Grau and Hamm (1953, 1957). All methods attempted to take meat samples, generally homogenized, through arbitrarily defined treatments and measure relative changes between the various samples.

The method used here was a modification of the high speed centrifugation method of Akroyd (private communication), in which either whole or minced meat samples weighing 3-4g were centrifuged at 120,000–190,000 × G for 30-60 min. Samples were placed in polypropylene tubes and were spun without added water. These samples were weighed before centrifuging and again after decanting

the centrifugally expressed juice. This method was considered to have distinct advantages over all previously reported methods since, (a) it could be used for both raw and cooked samples; (b) it could give juice suitable for further analysis; (c) it gave coefficients of variation of better than 5%; and (d) although the conditions were arbitrarily set, they were easily reproducible.

In the present study accurately weighed meat samples 1-1/2–2-1/2g were centrifuged in a Beckman Spinco Preparative Centrifuge (Spinco Division of Beckman Instruments Inc., Palo Alto, Calif.) Model L at 100,000 × G (36,000 rpm) for 60 min. Cellulose nitrate tubes were used. These tubes had either to be filled with meat completely or topped with distilled water to prevent collapse. It was not possible to determine accurately the solids content of expressed juice (EJ) when water had been added.

After centrifuging, the raw or cooked meat samples were removed from the tubes with forceps, carefully dried with tissues and then reweighed to determine liquid loss. Four to six replicates were centrifuged for each sample and with or without additions of water, the coefficient of variation was approximately 5%.

Total moisture content of the raw samples was determined by measuring the weight loss after heating in an oven at 100°C for 18–24 hr. Fat content was determined by the AOAC (1960) method. Expressed juice from the raw or cooked meat samples was defined as the loss in weight after centrifuging expressed as a percentage of the initial weight of the uncooked sample. Total juice loss (TJL), expressed as a percentage, was defined as the sum of this expressed juice and the percentage cooking or drip loss. Water-holding capacity was defined as the fraction of the total moisture content (TW) remaining after deducting the total juice loss, viz,

$$WHC = (TW - TJL)/TW = 1 - (TJL/TW)$$

For raw meat where there had been no drip or cooking loss this expression simplified to give:

$$WHC = 1 - (EJ/TW)$$

A correction for fat content was made so that the total moisture content and total juice loss were expressed on a fat-free basis. No corrections were made for the actual solids contents of the centrifugally expressed juice or of the "cooking" juice. Measurements have indicated that the former was about 10% while the latter was about 4%. It was assumed that these values would not vary greatly over the pH range studied and that the absence of such corrections would not alter any trends with pH.

Objective assessment of tenderness

The tenderness of the meat samples was determined by using a modification of the Warner-Bratzler type shearing device (Bratzler, 1932), and a table model TM-M Instron Universal Testing machine.

Cooked meat samples used in the Warner-Bratzler device had a rectangular cross-section 0.66 cm × 1.5 cm. They were cut so that the knife blade of the device cut across the fibers at right angles. At least six determinations were made on each sample and the mean of the peak shear forces used as an estimate of tenderness.

With the Instron machine, a 0.63 cm diameter flat-ended plunger was driven vertically 80% of the way through a 1.30 ± 0.01 cm thick sample of the cooked meat. The meat samples were cut and presented so that the fibers were perpendicular to the direction of plunger penetration. The plunger was driven into the meat twice at each location and the work and force penetration curve for each cycle recorded. Six to ten sites were used within each sample. The parameters determined were similar to those defined by Friedman et al. (1963), and involved "hardness" or the force required to achieve the first penetration and "cohesiveness" or the ratio of the work done during the second penetration to that performed on the first. A secondary parameter, "chewiness", was defined as the product of "hardness" and "cohesiveness". The coefficients of variation for the three parameters, hardness, cohesiveness and chewiness, were about 8, 8 and 14% respectively. The latter was approximately equivalent to that obtained using the Warner-Bratzler device.

Sample preparation and cooking methods

Before use, carcases were chilled at 1°C for 3 days after death. Samples weighing about 130g were cut from the middle section of both the SM and BF, weighed and placed in polyethylene bags. These polyethylene bags were tightened around the samples using clips and the samples were then completely immersed in water baths at 65 ± .5°C or 90 ± .5°C for 1 hr. As the samples were of approximately equal weight the time taken for the internal temperature of any individual sample to reach the temperature of the bath was considered to be sensibly constant. After cooking, they were removed and cooled in cold running water still in their exuded juice. The cooked samples were then carefully dried with tissues to remove excess surface moisture and re-weighed to determine cooking losses. Cooking losses were determined on whole samples of the SM and BF at 65°C and 90°C. The pH of the cooked muscles was determined on these cooked samples. The ST was used only in the raw state.

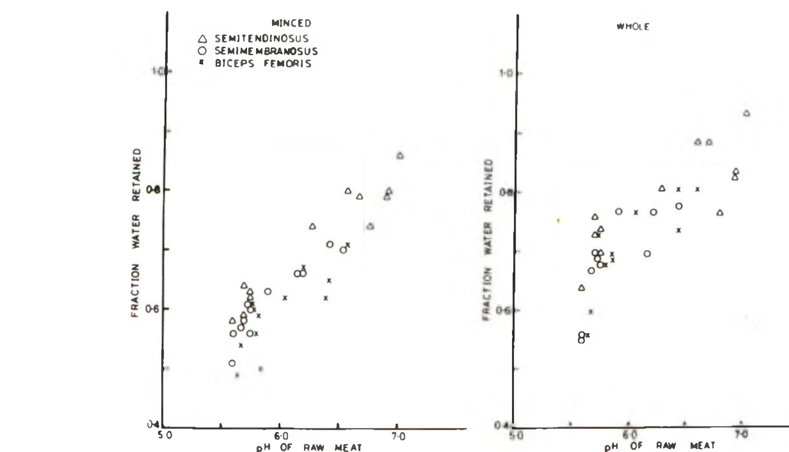

Fig. 1—The water-holding capacity of several ovine muscles expressed as a function of the ultimate pH of the raw meat.

RESULTS & DISCUSSION

Ultimate pH

The ranges of ultimate pH values produced in the three muscles were 5.60–7.0 for the ST; 5.60–6.55 for the SM; and 5.65–6.60 for the BF. Myanesin treatments did not result in muscles having ultimate pH values of less than 5.6.

Moisture measurements

Raw meat. The WHC of whole or minced raw samples from the 3 muscles was determined and plotted as a function of pH (Fig. 1). When the results for all three muscles were combined the WHC was significantly correlated ($P < 0.001$) with pH for whole ($r = 0.80$) and minced samples ($r = 0.88$). The minced samples retained significantly less moisture than the intact or whole samples ($P < 0.001$). The ST had a significantly greater WHC ($P < 0.01$) than the other two muscles

The difference in WHC between intact and minced samples was expected since considerable structural damage occurs during the mincing process. However the reason for the difference in WHC between the muscles over very similar pH ranges was less obvious. Hamm (1960) reported differences in pH-hydration curves for different muscle homogenates when their pH was adjusted with acid or alkali; however, the present work has shown that there may have been WHC differences which were characteristic of different muscles. Bouton et al. (1957) have shown differences in drip losses which were characteristic of different muscles.

Cooked meat. The moisture losses, measured as a percentage of the raw

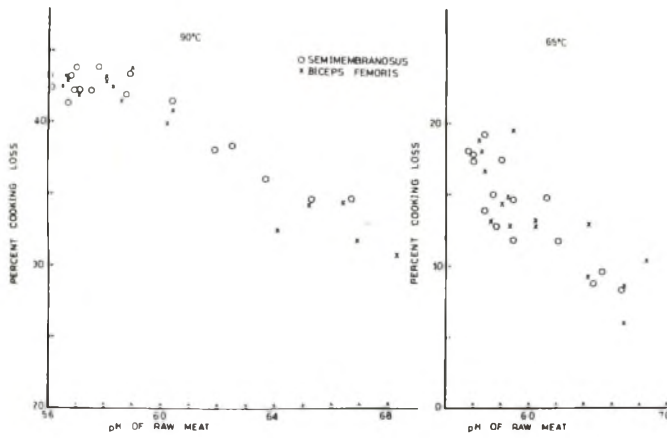

Fig. 2—The cooking losses of the M. biceps femoris (X) and M. semimembranosus (O) after cooking for 1 hr at 90°C and 65°C as a function of the ultimate pH of the raw meat.

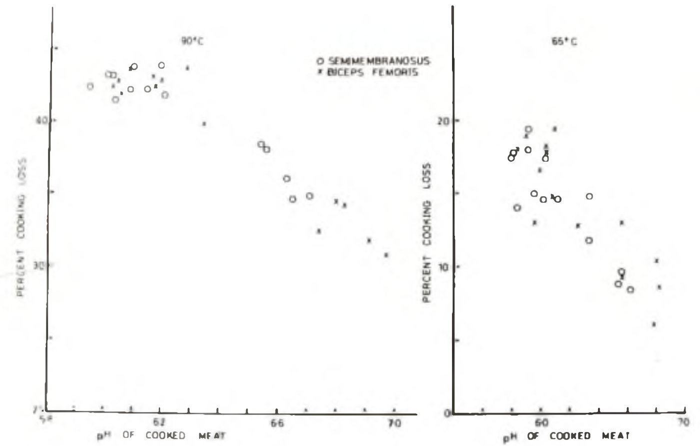

Fig. 3—The cooking losses of the M. biceps femoris (X) and M. semimembranosus (O) after cooking for 1 hr at 90°C and 65°C as a function of the pH of the cooked meat.

weight, were determined for the BF and SM muscles cooked at 65°C and 90°C for 1 hr. The relationship between the pH of the meat both before and after cooking, and these cooking losses were plotted in Figures 2 and 3.

Cooking losses at 90°C were sensibly constant with increasing pH of the raw meat at 42–43% until a pH of about 5.9 was reached when it decreased linearly to approximately 31% at pH 6.8. Cooking losses at 65°C decreased linearly as raw pH increased. The relationships between cooking loss and cooked pH were very similar to those obtained for the cooking loss and raw pH except that the curves were displaced because of the rise in pH produced during the cooking process. The increase in pH with cooking changes from about 0.4 units when the pH of the uncooked meat was 5.6 to nearly zero when the pH of the raw meat was 7.0. This increase apparently represents changes of protein charge (Hamm et al., 1960). The decrease in cooking loss, at 90°C, occurred at a pH, after cooking, of about 6.1-6.2.

These results agreed with WHC results of Hamm and Deatherage (1960). Their results showed a steady linear increase with increasing pH, in the WHC of beef homogenates cooked at 60°C. In the present experiment there was a linear decrease, with increasing pH, in cooking loss at 65°C. Hamm and Deatherage (1960) did not use temperatures above 80°C. When their 80°C results were compared with the 90°C results reported here there were obvious similarities, with a difference in minimal WHC which occurred at pH 6.0–6.1 at 80°C instead of 6.1–6.2, at 90°C. If cooking losses paralleled changes in WHC, than a minimum WHC and maximum cooking loss would be expected at pH of the cooked meat of about 6.1–6.2. The results of Hamm and Deatherage (1960) showed there was very little change in WHC as the pH of the meat after cooking varied from 5.8–6.1 but a relatively sharp change from 6.1–7.0. These results were in agreement with Hamm's results on muscle homogenates.

The total juice loss, cooking loss and expressed juice for SM cooked at 65°C and 90°C have been plotted (Fig. 4) as a function of the cooked meat's pH. Linear regressions were calculated for all three parameters at both temperatures, and all were very highly significant ($P < 0.001$) except for the EJ from meat cooked at 65°C. Cooking and total juice losses decreased linearly with pH while the EJ increased.

In earlier unpublished work by Harris et al. (private communication) using meat of pH 5.5–5.8, it was shown that cooking loss over a range of about 20 to nearly 50% had a highly significant ($P < 0.001$) negative linear correlation ($r = 0.95$) with organoleptic juiciness. A similar correlation (although positive) was obtained for the centrifugally expressed juice. The results shown on Figures 2, 3, and 4 indicate that juiciness should increase with increasing pH. This evidence obviously does not support the suggestion of Bouton et al. (1957) that there was a minimum juiciness at a raw pH of about 6.0. The greater WHC of the higher pH meat was reflected in decreased total juice loss and cooking loss.

Changes in pH due to cooking

Since pH of the cooked meat relates to the WHC of cooked muscle, the changes (△ pH) in pH occurring during cooking at 65°C and 90°C for 1 hr have been determined as a function of ultimate pH of the raw meat. At 65°C a highly significant ($P < 0.001$) correlation

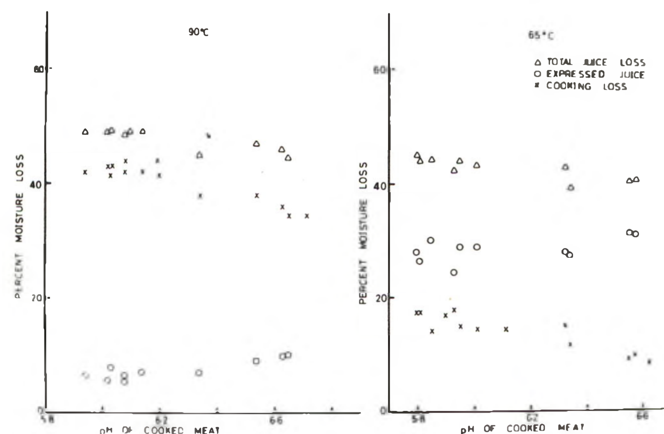

Fig. 4—The total moisture losses from M. semimembranosus cooked at 90°C and 65°C for 1 hr versus the pH of the cooked meat.

(r = −0.81) was obtained and a linear regression equation fitted $\triangle pH = 1.120 - 0.153\ pH_{RAW}$.

This gave an estimated △pH of 0.26 at pH 5.60 which decreased to 0.04 at pH 7.03. Similarly for the 90°C samples a correlation coefficient of −0.87 ($P < 0.001$) was obtained giving a △pH of 0.41 at pH 5.60 and 0.08 at pH 7.03, $\triangle pH = 1.684 - 0.227\ pH_{RAW}$. A raw meat pH of 5.40 would give a cooked meat pH of about 5.65 at 65°C and of 5.86 at 90°C assuming that the linear regressions hold in that region.

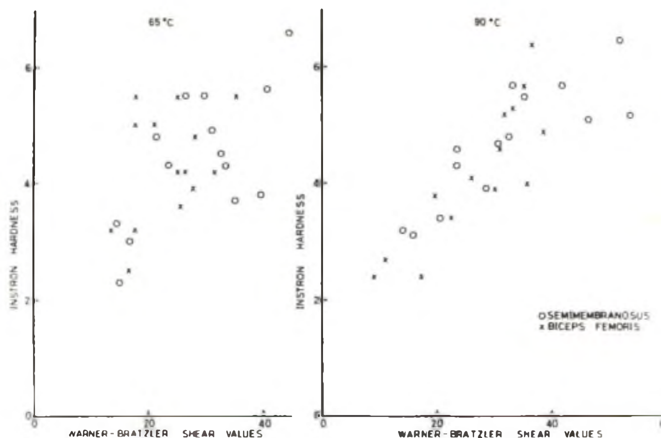

Fig. 5—A comparison of the results obtained from the Warner-Bratzler shear and Instron measurements for M. biceps femoris (X) and M. semimembranosus (O) cooked at 65°C and 90°C for 1 hr.

Objective measurements of tenderness

Objective measurements of tenderness or toughness taken with the Instron have previously been correlated, Harris et al. (private communication) with results from taste panels. In these comparisons a much simplified version of the texture profile method of Brandt et al. (1963) was used to assess the meat texture. The panel members were asked to score their initial and residual impression of texture using a linear or non-structured scaling system in which only the upper and lower limits of tenderness or juiciness were defined. Instron "hardness" was highly correlated r = 0.88 ($P < 0.001$) with initial impression while "chewiness" correlated well r = 0.90 ($P < 0.001$) with residual impression. Correlations of 0.8–0.9 have also been obtained using a more conventional structured scaling system.

In this laboratory both instruments have been correlated with a taste panel using a hedonic scale giving highly significant ($P < 0.001$) linear correlations of 0.80–0.82 with the Warner-Bratzler shear values and 0.86 with the Instron. In the present investigations results from the two instruments were correlated, see Figure 5. The correlation at 65°C for both muscles was less than 0.5 but 0.86 for the 90°C samples. The poor correlation at 65°C was probably due to some difficulty in cutting these comparatively wet and easily deformed samples to the precise dimensions.

The results for the Warner-Bratzler shear and Instron methods have been plotted in Figures 6 and 7 as a function of ultimate pH of the raw meat. Both "hardness" and "chewiness" gave very similar results and only the "hardness" results have been shown. The samples cooked at 65°C gave highly significant ($P < 0.001$) linear negative regressions for both muscles for the Instron method but the Warner-Bratzler method gave a significant ($P < 0.05$) quadratic relationship with a peak at about pH 6.0 for the SM and a significant ($P < 0.01$) linear regression for the BF. When both muscles were grouped together, only linear regressions were significant.

The samples cooked at 90°C gave similar results; highly significant ($P < 0.001$) linear regressions for both muscles on the Instron and, for the SM only, on the Warner-Bratzler. Results from the BF showed a significant ($P < 0.05$) quadratic relationship between pH and shear force with a peak toughness at about pH = 6.0. Again when results from the muscles cooked at 90°C were grouped together, only linear regression was significant ($P < 0.001$).

There was strong evidence for increasing tenderness as the pH increased from 6 to 7. There was some evidence, from Warner-Bratzler shear force values only, of a peak toughness at

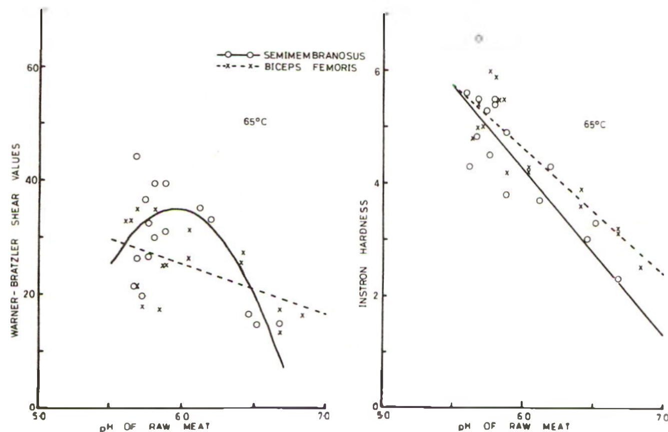

Fig. 6—Objective measurements for M. semimembranosus and M. biceps femoris cooked at 65°C for 1 hr plotted against the ultimate pH of the raw meat.

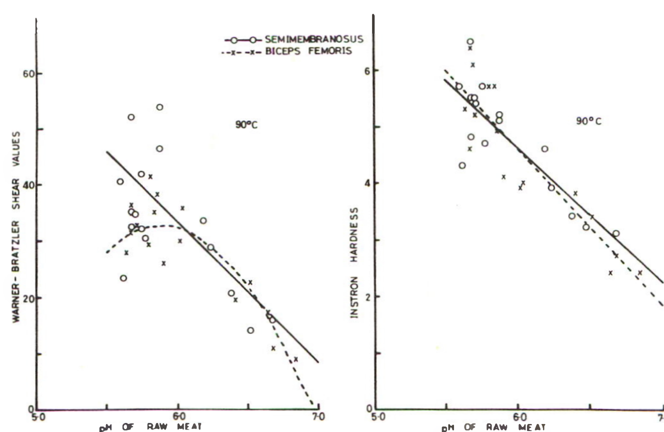

Fig. 7—Objective measurements for M. semimembranosus and M. biceps femoris cooked at 90°C for 1 hr plotted against the values of the ultimate pH of the raw meat.

about pH 6. This evidence was equivocal as it occurred in different muscles at different cooking temperatures.

In a recent unpublished experiment with beef, we found that "hardness" and Warner-Bratzler shear force varied curvilinearly with pH, in the range pH 5.6 to 6.5, and that the beef was toughest at about pH 6. There is other evidence that a peak in sensory toughness occurs at a pH of 6 in beef (Bouton et al., 1957), and mutton (Bouton and Shorthose, 1969). Winkler (1939) altered the pH of meats with injections of lactic acid or ammonia, and found a maximum toughness at pH 5.0–6.0. Mackey et al. (1952) used five pigs, pH range 5.57–6.39, and found no apparent relationship between pH and shearing strength.

Kelley et al. (1966), and Kelly (1969), working with fish have found a very significant positive, linear, relationship between ultimate pH and tenderness which is in agreement with the results of the present work on mutton. The ultimate pH of fish in their experiments varied from 6.3–7.1, with a few exceptional readings near 6.1. Many workers including Penny et al. (1963, 1964); Lewis et al (1967a, b); Judge et al. (1960); and Kauffman et al. (1964) have shown tenderness differences when comparing high with low ultimate pH meats. Penny et al. (1963, 1964) found that meat with a high pH was tender and juicy.

CONCLUSIONS

THESE results showed that pH values of 5.6–7.0 could be obtained in sheep muscles by the use of pre-slaughter injections. Cooking losses at 65°C decreased linearly with increasing raw or cooked pH over the whole pH range while the cooking losses at 90°C showed little change until at a pH of about 5.9 (uncooked meat) or about 6.2 (cooked meat) they decreased linearly with increasing pH. These results paralleled the changes in WHC for cooked muscle homogenates as shown by Hamm and Deatherage (1960). Juiciness, assessed by the measurement of cooking loss and by centrifugally expressed juice, generally increased with increasing pH.

Objective measurements of tenderness using two dissimilar methods have shown that a three-fold increase in tenderness was associated with increases in ultimate pH from about 5.9–7.0. Evidence for a peak toughness for meat with a raw pH of 5.9 was not clearly demonstrated. This could have been due to the failure to obtain any meat having a pH of less than 5.6. The remarkable correspondence of tenderness changes with cooking losses and WHC indicated that large differences in tenderness below pH 5.9 would be unlikely. A pH of 5.4 for the raw meat would give a pH for the cooked meat of about 5.8.The decrease in the WHC of cooked muscle homogenates (Hamm and Deatherage, 1960) from 5.8 to the minimum at about 6.2 was much less pronounced than the increase from 6.2–7.0. Animal to animal variation could well have proved to be too great to permit the toughness peak at a raw pH of 5.9 to be unequivocally established.

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HELEN L. NORRIS, DOROTHY L. HARRISON, LOIS L. ANDERSON, BETTINA von WELCK and HAROLD J. TUMA
Departments of Foods & Nutrition and Animal Science & Industry, Kansas State University, Manhattan, Kansas 66502

EFFECTS OF PHYSIOLOGICAL MATURITY OF BEEF AND MARBLING OF RIB STEAKS ON EATING QUALITY

SUMMARY—Selected characteristics of beef rib steaks representing three physiological maturity levels and two marbling levels were investigated. Paired steaks at the 11th thoracic vertebra were used to obtain cooking time and losses, palatability scores, Warner-Bratzler shear values, and selected histological data. In addition, steaks from the left 7th and right 8th thoracic vertebrae positions were used to measure cooking time and losses, Warner-Bratzler shear values, waterholding capacity, pH, color-difference, and total moisture. Ether extract was obtained for raw longissimus dorsi at the 12th thoracic vertebra. Maturity and marbling affected total moisture ($P < 0.001$), quantity of fat in raw muscle (histological measurement, $P < 0.01$), ether extract ($P < 0.001$), and dripping losses (7th and 8th thoracic vertebrae $P < 0.001$). Other factors were not affected significantly. Maturity and marbling, at levels represented, had little effect on palatability of the steaks, although generally, measurements for fat content were higher and those for moisture lower at the higher marbling level. Most correlation coefficients for overall acceptability and histological measurements were low. Tenderness had more influence on the overall acceptability of the meat than flavor or juiciness. Moderate to high relationships occurred between cooking time and both initial weight of steaks and cooking losses. Generally, waterholding capacity was not related to pH, total moisture, or cooking losses. Correlation coefficients for pH vs. shear value and pH vs. color-difference were low.

INTRODUCTION

TRADITIONALLY, the beef grading system of the U.S. Department of Agriculture (USDA) has emphasized maturity and marbling of beef carcasses. However, changes in feeding management and animal breeding, increasingly important to characteristics related to the quality of beef, may point to overemphasis of maturity and marbling (Lawrie, 1966). Research with conflicting results on the effect of maturity and marbling on the quality of beef (Blumer, 1963; Gilpin et al., 1965) were in agreement with revised specifications for a reduced number of marbling levels and less emphasis on maturity in the Prime, Choice, Good, and Standard Grades (USDA, 1965).

Most studies found in the literature based maturity on chronological age of the animal. Information is needed on the effect of maturity and marbling when maturity is based on physiological age, i.e. size, shape, and ossification of the bones and cartilages and color and texture of the lean, as specified in the USDA standards (USDA, 1965).

This study, part of a larger investigation at Kansas State University (KSU), used steaks from two positions in beef ribs to measure the effects of three levels of physiological maturity and two levels of marbling at each maturity level on properties related to the cooking and eating quality of the steaks.

EXPERIMENTAL METHODS

2-IN. RIB STEAKS from 60 beef carcasses (241–438 kg) representing three levels of physiological maturity (youthful, A− A°; intermediate, A+ B−; approaching maximum maturity permitted for U.S. Good, B° B+), corresponding to the approximate chronological ages of 12–18, 18–30, and 30–38 mo were used. Two marbling levels (small, moderate) were represented equally within each maturity group (USDA, 1965). Wholesale rib cuts were selected by two meat scientists and were taken to the Meat Processing Laboratory at KSU, where paired steaks at the 11th thoracic vertebra (11T) and steaks at the left 7th thoracic vertebra and right 8th thoracic vertebra (7–8T) were cut, wrapped in laminated freezer paper, frozen at −20°F and stored at −20°F until used. Between 3–5 mo after processing, steaks from the 11T position were cooked; those from the 7–8T position were cooked between 9–12 mo after processing.

Cooking time and losses and Warner-Bratzler shear values were obtained for steaks from both the 7–8T and 11T positions; palatability and histological characteristics of longissimus dorsi (LD) muscle were measured at the 11T position; and total moisture, waterholding capacity (WHC), pH, and Gardner color-difference values were determined for LD at the 7–8T position. Data for steaks from each position were analyzed separately. Ether extract was measured on raw LD at the 12th thoracic vertebra.

Prior to each cooking period four steaks were defrosted 4 hr at room temperature (78°F) and then 20 hr in a refrigerator (40°F). Steaks were cooked at 400°F by a modified broiling method (Hay et al., 1953) to an end point temperature of 70°C. The design for cooking and evaluation consisted of 30 periods for steaks from each of the two positions in the ribs; four steaks, randomized by pairs, were cooked and evaluated at each period.

Cooking time and losses

Total cooking time, in min and in min/lb, was determined. Percentage total, volatile, and dripping cooking losses were calculated.

Palatability scores

1/2-in cubes of cooked LD muscle were selected at random by a laboratory panel of 10–14 members, who evaluated the muscle on a 7-point scale for flavor, juiciness, tenderness, and overall acceptability. Flavor and overall acceptability were scored on a desirability scale and tenderness and juiciness on an intensity scale: a score of seven represented extremely desirable or intense characteristics.

Warner-Bratzler shear values

Warner-Bratzler shear values (25-lb dynamometer) were measured on three cores (1/2-in dia from the lateral position in the LD of all steaks and on three cores from the medial position in the LD of each steak from the 7–8T position. Two shears were made on each core.

Histological values

For each maturity-marbling level, samples of LD muscle (5 raw, 5 cooked; each approx 5 × 3.75 × 1.25 cm) were wrapped in plastic bags, frozen (−20°F) and stored (−20°F) for 8–12 wk. Specimens (approx 1.5 × 1.0 × 0.5 cm) cut from each sample were sectioned 8–10μ thick with a cryostat microtome; five sections from each specimen were mounted on slides and stained with Sudan IV and Harris hematoxylin. Each member of a three-member panel used an ocular micrometer and a magnification of 430× to measure the width of three fibers, selected at random, from each section. This procedure provided for the measurement of 75 raw and 75 cooked fibers per panel member and 225 raw and 225 cooked fibers for each of the six maturity-marbling levels. Panel members also used a 7-point scale to estimate the quantity and distribution of fat at a magnification of 100×.

One person used a Bausch and Lomb microprojector to obtain an objective measurement of the quantity and distribution of fat (microprojector fat value) in raw and cooked LD. Each section was projected on graph paper with 20 × 20 squares to the inch. Each square represented 0.1 mm on the section as measured by focusing a Lovins Micro-Slide Field Finder on the graph paper. A distance between the slide and the graph paper of 23-3/8 in gave a magnification of 16.1×. The area of fat projected in 3,000 squares on the graph paper was colored on the paper. The colored squares were counted to obtain the area of fat per 300 sq mm of the section.

Total moisture

Percentage total moisture in cooked LD was determined with the C. W. Brabender semi-automatic moisture tester. Duplicate 10g samples of ground muscle were subjected to a temperature of 121°C for 60 min.

Waterholding capacity

WHC of the cooked muscle was determined as reported by Miller and Harrison (1965). The ratio of the area of pressed muscle to the area of expressed liquid on filter paper on which the sample was pressed was designated the expressible-liquid index. Values for WHC were obtained by subtracting the expressible liquid index from 1.0, which arbitrarily was chosen as the maximum expressible-liquid index. For each steak at the 7-8T position, three WHC values were obtained on samples taken at random from medial and lateral positions in the LD muscle.

pH

Homogenates of 5g of cooked, ground LD from each steak and 50 ml of distilled water were used to measure pH. Two measurements were made on each homogenate.

Color-difference

Duplicate measurements of Rd (reflectance), a+ (redness), and b+ (yellowness) were measured with a Gardner Color Difference Meter on 25g of ground LD packed in a Gardner glass cell. The instrument was standardized using a satin finish ceramic tile with calculated values of: Rd, 15.53; a+, 9.33; b+, 13.10.

Table 1—Means, standard deviations[a] and F-values for initial weight, cooking time, and cooking losses of beef rib steaks as influenced by maturity and marbling

		Maturity and marbling levels						
		Youthful		Intermediate		Approaching maturity		
Measurement	Position[b]	Small	Moderate	Small	Moderate	Small	Moderate	F-value
Initial weight, (g)	11T	680 (84.9)	731 (104.4)	714 (118.4)	725 (51.5)	746 (92.0)	738 (100.7)	1.23 ns
	7-8T	538.7 (100.4)	512.6 (52.0)	573.4 (111.9)	548.0 (106.3)	586.4 (67.9)	584.7 (95.5)	2.04 ns
Cooking time, total min	11T	61.2 (8.3)	61.9 (8.0)	61.8 (6.4)	64.5 (5.2)	64.9 (7.8)	63.6 (7.6)	0.90 ns
	7-8T	51.7 (9.1)	51.4 (5.9)	51.0 (7.0)	54.2 (8.2)	56.1 (8.6)	55.4 (8.2)	1.60 ns
Min/lb	11T	41.1 (5.9)	38.9 (3.1)	40.2 (6.6)	40.4 (3.1)	39.9 (4.5)	39.5 (4.4)	0.48 ns
	7-8T	44.5 (4.9)	45.6 (4.4)	41.5 (5.9)	46.1 (8.0)	42.5 (6.0)	43.5 (5.5)	1.83 ns
Cooking losses, total, %	11T	23.7 (5.3)	24.6 (2.5)	23.2 (4.3)	24.8 (2.1)	25.4 (3.0)	25.1 (2.8)	1.16 ns
	7-8T	23.4 (2.0)	24.5 (3.4)	23.1 (2.0)	23.6 (2.5)	23.5 (2.8)	24.3 (2.7)	0.95 ns
Volatile, %	11T	15.3 (4.9)	15.5 (2.3)	14.6 (3.6)	16.1 (1.8)	17.0 (1.6)	15.4 (1.9)	1.52 ns
	7-8T	18.1 (1.9)	17.4 (2.2)	18.2 (1.8)	17.5 (2.4)	18.1 (2.0)	17.3 (2.1)	0.70 ns
Dripping, %	11T	8.3 (1.5)	9.1 (2.1)	8.6 (1.8)	8.7 (2.1)	8.4 (2.2)	9.7 (2.0)	1.30 ns
	7-8T	5.2 (1.4)	6.9 (1.7)	4.6 (1.2)	5.8 (1.8)	5.1 (1.6)	6.8 (1.9)	7.04***

[a]Values in parentheses are standard deviations from the mean.
[b]7-8T, steaks at 7th and 8th thoracic vertebrae; 11T, steaks at 11th thoracic vertebra.
ns, not significant.
***, significant at the 0.1% level.

RESULTS & DISCUSSION

THERE WERE NO significant differences for any of the measurements that were attributable to side of the carcass. Therefore, data for left and right sides of the carcass were pooled and analyzed as for a completely randomized design. Also, Covington et al. (1970) found that shear force and chemical and histological characteristics of LD at the 9th and 12th thoracic vertebrae in the same 60 ribs used in this study did not differ between sides of the carcass.

Other similarities and differences between the work of Covington et al. (1970) and the study reported here are given throughout the discussion. Data in these two papers point out some variations in results that may be obtained for similar work done in two or more laboratories and variations among positions relatively near to each other within the same carcass.

Initial weight

The initial weight of steaks assigned to the maturity-marbling levels did not vary significantly (Table 1).

Cooking time and losses

Cooking time, both total min and min/lb, and total and volatile cooking losses were not affected significantly by maturity and marbling level. However, at the 7-8T position, percentage dripping losses were affected ($P < 0.001$) by maturity and marbling levels (Table 1).

Comparison of dripping and volatile losses for steaks at the 7-8T and 11T positions indicated these differences (Table 1): dripping losses were consistently higher ($P < 0.001$) for steaks from the region of the 11th thoracic vertebra; volatile losses were consistently higher ($P < 0.001$) for steaks from the region of the 7th and 8th thoracic vertebrae. Since dripping losses are primarily lipid and volatile losses primarily moisture, the data may indicate that the marbling, or lipid content, was different at the two locations in the LD.

Palatability scores and Warner-Bratzler shear values

Panel scores for tenderness, juiciness, flavor, and overall acceptability and Warner-Bratzler shear values were not affected significantly by level of maturity or marbling (Table 2). However, for flavor, orthogonal comparisons indicated a significant ($F = 4.43$, $P < 0.05$) interaction between maturity and marbling. At both levels of marbling, steaks from carcasses in the youthful group scored slightly higher in flavor than steaks with a moderate amount of marbling in the intermediate group and those with a small amount of marbling in the group approaching maturity. The differences (0.2-0.3 point) between mean flavor scores for those groups of steaks are too small to be of practical importance.

Covington et al. (1970) found that shear force of cooked LD at the 9th thoracic vertebra was not affected significantly by maturity, but was lower ($P < 0.05$) for the moderate than for the small level of marbling. Other workers (Wellington and Stouffer, 1959; Walter et al., 1965; Goll et al., 1965) found that shear force of beef LD was not affected by marbling. Field et al. (1966) reported that when marbling was held constant, maturity (chronological age) had no effect on shear force.

Shear values were consistently higher ($P < 0.001$) at the 7-8T lateral position than at the 11T lateral position. For the 7-8T position, at any one level of maturity and marbling the difference in mean shear force between medial and lateral positions was never greater than 0.3 lb. When all levels of maturity and marbling were considered, the greatest difference was 0.9 lb (Table 2). Covington et al. (1970) reported that at the 9th thoracic vertebra lateral shear cores were less tender ($P < 0.05$) than central or medial cores.

Table 2—Means, standard deviations[a] and F-values for palatability scores and shear values of beef longissimus dorsi as influenced by maturity and marbling

	Maturity and marbling levels						
	Youthful		Intermediate		Approaching maturity		
Measurement	Small	Moderate	Small	Moderate	Small	Moderate	F-value
Palatability, scores 7-1, (11T)[b]							
Tenderness	5.4 (0.9)	5.9 (0.4)	5.8 (0.4)	5.6 (0.4)	5.7 (0.3)	5.8 (0.5)	1.14 ns
Juiciness	6.0 (0.3)	5.9 (0.4)	6.0 (0.5)	5.6 (1.4)	5.8 (0.4)	5.9 (0.6)	0.96 ns
Flavor	5.8 (0.3)	5.8 (0.3)	5.8 (0.3)	5.5 (1.3)	5.6 (0.3)	5.8 (0.4)	1.19 ns
Over-all acceptability	5.6 (0.6)	5.7 (0.3)	5.8 (0.4)	5.4 (1.3)	5.4 (0.3)	5.8 (0.5)	0.78 ns
Shear value, lb/½-in core							
lateral (11T)	5.6 (1.6)	5.2 (0.8)	5.7 (1.4)	5.3 (0.9)	5.3 (0.9)	5.5 (1.2)	0.57 ns
(7-8T)[b]	7.7 (1.8)	7.0 (1.0)	7.7 (1.5)	7.1 (1.4)	7.3 (0.9)	7.0 (1.4)	1.17 ns
medial (7-8T)	7.9 (1.4)	6.9 (1.0)	7.9 (1.6)	6.9 (1.6)	7.3 (0.9)	7.3 (1.5)	2.29 ns

[a]Values in parentheses are standard deviations from the mean.
[b]7-8T, steaks at the 7th and 8th thoracic vertebrae; 11T, steaks at the 11th thoracic vertebra.

Table 3—Means, standard deviations[a] and F-values for histological measurements and ether extract of beef longissimus dorsi as influenced by maturity and marbling

	Maturity and marbling levels						
	Youthful		Intermediate		Approaching maturity		
Measurement	Small	Moderate	Small	Moderate	Small	Moderate	F-value
Fiber width, μ							
Raw	38.4 (6.1)	39.9 (3.5)	43.2 (3.3)	39.2 (5.1)	38.9 (4.4)	41.8 (3.4)	1.83 ns
Cooked	35.5 —	38.8 —	36.9 —	38.6 —	38.1 —	38.1 —	0.71 ns
Fat quantity, (7-1 = large to none)							
Raw	3.1 (0.7)	4.3 (0.7)	3.2 (0.5)	3.9 (0.8)	3.7 (0.8)	3.9 (0.7)	3.67**
Cooked	4.8 (1.1)	4.8 (1.2)	4.5 (1.1)	4.8 (0.8)	4.7 (1.1)	5.2 (0.6)	0.53 ns
Fat distribution, (7-1 = large droplets to cloudy aggregate)							
Raw	4.8 (0.8)	5.5 (0.7)	5.1 (0.8)	5.6 (0.6)	5.2 (0.9)	5.5 (0.6)	1.36 ns
Cooked	4.6 (0.9)	4.9 (1.0)	4.6 (0.9)	4.6 (0.6)	4.9 (1.1)	4.8 (1.0)	0.21 ns
Microprojector fat value[b]							
Raw	51.0 (41.0)	73.3 (37.8)	35.6 (18.6)	125.1 (165.3)	70.1 (44.4)	83.3 (48.7)	1.61 ns
Cooked	72.7 (54.4)	122.6 (124.7)	64.8 (34.8)	99.8 (59.3)	147.4 (143.6)	122.7 (56.1)	1.30 ns
Ether extract, %	16.5 (2.8)	22.2 (4.2)	17.5 (1.7)	21.7 (3.3)	17.3 (2.7)	24.2 (4.8)	17.34***

[a]Values in parentheses are standard deviations from the mean.
[b]Area of fat per 300 sq mm of muscle section (16.1X).
ns, not significant.
**, significant at the 1% level.
***, significant at the 0.1% level.

Histological and ether extract values

Analysis of variance indicated that LD fiber width at the 11T position was not affected significantly by maturity and marbling (Table 3). However, orthogonal comparisons indicated that small and moderate levels of marbling exhibited different trends. With a small degree of marbling, tissue from the intermediate maturity level had wider fibers than that from the young or approaching maturity levels. With moderate marbling, tissue from the intermediate maturity level had narrower fibers than tissue from either the youthful or approaching maturity levels.

Covington et al. (1970) reported increased ($P < 0.05$) fiber "diameter" with increased maturity for samples at the 9th thoracic vertebra, but no significant differences in fiber "diameter" between the two marbling levels. They separated the fibers in a Waring Blendor with the blades reversed, then measured fiber "diameter" at a magnification of 100×. In the study reported here fiber "width" was measured on fibers in longitudinal sections of muscle tissue. Differences between the two studies relative to the effect of maturity on fiber "diameter" or "width" may be accounted for partly by method of measurement.

Generally, analysis of variance indicated that both panel estimates and microprojector fat values for quantity and distribution of fat did not vary significantly among maturity and marbling levels. The exception was for quantity of fat in raw tissue (Table 3). Moreover, orthogonal comparisons of panel estimates for quantity of fat in raw tissue also indicated a significant ($F = 12.60$, $P < 0.001$) effect of maturity and marbling and a significant ($F = 4.40$, $P < 0.05$) interaction between maturity and marbling. Raw tissue from youthful and intermediate maturity groups contained more fat at the moderate than at the small level of marbling, whereas for the group approaching maturity there was little difference in the quantity of fat in raw tissue representing the two marbling levels. Moreover, orthogonal comparisons indicated that panel scores for fat distribution in raw tissue were affected significantly ($F = 5.33$, $P < 0.05$) by marbling level; tissue representing the moderate level contained the larger fat droplets. Similarly orthogonal comparisons pointed out a significant ($F = 4.44$, $P < 0.05$) effect of marbling on microprojector fat values. Moderately marbled tissue had more fat per 300 sq mm than tissue with a small degree of marbling.

Both analysis of variance (Table 3) and orthogonal comparisons ($F = 80.05$, $P < 0.001$) indicated that ether extract

differed significantly among maturity and marbling levels and that the differences were attributed to the two levels of marbling. Tissue in the moderate marbling level had slightly more ether extract than that in the small marbling level (Table 3). Covington et al. (1970) suggested that as maturity increased the proportion of ether extractable material was not visibly increased.

Comparison of histological data for raw and cooked tissue indicated that raw tissue tended to have wider fibers, less fat, and slightly larger droplets of fat than cooked tissue.

Total moisture

Total moisture was affected ($P < 0.001$) by level of maturity and marbling. As would be expected, total moisture decreased ($P < 0.05$) with increased marbling. In moderately marbled muscle, total moisture tended to decrease with increased maturity, and the difference between youthful and approaching maturity was significant ($P < 0.05$). In muscle with a small amount of marbling, total moisture was consistent for all three levels of maturity (Table 4). Trends reported by Covington et al. (1970) for the moisture content of LD at the 12th thoracic vertebra were similar to those reported in this paper.

WHC, pH, color-difference

WHC, pH, and all color-difference factors (reflectance, redness, yellowness) were not affected significantly by level of maturity and marbling. However, the trend was toward slightly decreased reflectance (darker color) with increased maturity. Moreover, a+ values decreased (less red color) slightly with increased marbling (Table 5.).

Relationships between selected measurements

Correlation coefficients for selected paired variates within each of the six treatment combinations are given in Table 5. A wide range of r-values was noted for most of the relationships reported.

Apparently, tenderness generally had more influence on the overall acceptability of the meat than flavor or juiciness. Correlation coefficients were high ($r = 0.80$ or above) for tenderness vs. overall acceptability of samples with a small degree of marbling at youthful maturity, and with moderate marbling for the intermediate and mature groups. For both flavor and juiciness vs. overall acceptability, coefficients were high only for moderate marbling in the mature group. However, there were moderate (r between 0.40–0.79) relationships between those paired variates for all treatments except for moderate marbling in the youthful group (both flavor and juiciness) and for small marbling in the mature group (juiciness).

Moderate negative relationships were indicated between tenderness scores and Warner-Bratzler shear values for three maturity-marbling groups, and between tenderness scores and fiber width of cooked muscle for one group. Also, for cooked muscle, moderate to high relationships were noted between quantity and distribution of fat for all except two

Table 4—Means, standard deviations[a] and F-values for total moisture, waterholding capacity, pH, and color-difference of beef longissimus dorsi as influenced by maturity and marbling

	Maturity and marbling levels						
	Youthful		Intermediate		Approaching maturity		
Measurement	Small	Moderate	Small	Moderate	Small	Moderate	F-value
Total moisture, %	62.6 (1.7)	61.1 (2.1)	62.5 (2.0)	60.0 (2.1)	62.6 (2.0)	59.6 (2.1)	9.45***
WHC[b]	0.62 (0.07)	0.60 (0.07)	0.55 (0.09)	0.60 (0.07)	0.61 (0.07)	0.59 (0.06)	2.13 ns
pH	5.83 (0.09)	5.77 (0.16)	5.79 (1.11)	5.84 (0.08)	5.80 (0.17)	5.82 (0.08)	1.04 ns
Color-difference							
Rd (reflectance)	20.9 (1.6)	20.5 (1.9)	20.0 (2.0)	19.6 (1.7)	19.8 (1.2)	19.8 (1.3)	1.84 ns
a+ (redness)	11.1 (1.7)	10.0 (2.6)	10.4 (1.8)	9.8 (1.7)	11.4 (2.3)	10.5 (2.3)	1.80 ns
b+ (yellowness)	11.0 (0.5)	11.0 (0.7)	11.1 (0.7)	10.7 (0.7)	11.1 (0.4)	11.0 (0.5)	1.36 ns

[a]Values in parentheses are standard deviations from the mean.
[b]WHC, waterholding capacity (1.0 − expressible liquid index).
ns, not significant.
***, significant at the 0.1% level.

Table 5—Correlation coefficients for selected paired variates on the basis of maturity and marbling

	Maturity and marbling levels					
	Youthful		Intermediate		Approaching maturity	
Paired variates	Small	Moderate	Small	Moderate	Small	Moderate
Overall acceptability vs.[a]						
Flavor	0.73*	0.05	0.76**	0.51	0.58	0.83**
Juiciness	0.66*	−0.07	0.41	0.62	0.34	0.80**
Tenderness	0.97***	0.25	0.54	0.88***	0.62	0.80**
Tenderness score vs.[a]						
Warner-Bratzler shear value	−0.65*	0.18	0.17	−0.41	−0.04	−0.77**
Fiber width, cooked muscle	−0.73*	−0.01	−0.25	−0.19	0.00	0.31
Fat quantity, cooked muscle vs.[a]						
Fat distribution, cooked muscle	0.48	0.86**	0.37	0.64*	0.75*	0.18
Microprojector fat, cooked muscle	0.76*	0.77**	0.61	0.83**	0.70*	0.25
Ether extract, raw muscle vs.[a]						
Fat quantity, raw muscle	0.02	−0.74*	−0.05	0.01	0.62	0.59
Fat quantity, cooked muscle	0.84**	0.81**	0.19	0.28	0.28	0.69*
Initial weight vs. cooking time						
11th thoracic vertebra[a]	0.63	0.92***	0.52	0.17	0.60	0.74*
7th and 8th thoracic vertebrae[b]	0.82**	0.65**	0.74**	0.64**	0.70*	0.82**

[a]D/F = 8; r-value required for a significant relationship: $P < 0.05$, 0.632; $P < 0.01$, 0.765; $P < 0.001$, 0.872.
[b]D/F = 18; r-value required for a significant relationship: $P < 0.05$, 0.444; $P < 0.01$, 0.562.
*, P = 0.05.
**, P = 0.01.
***, P = 0.001.

groups, and between quantity of fat and microproejctor fat values for all but one group. Better relationships were observed between ether extract of raw muscle and quantity of fat in cooked than in raw muscle. At the 7th and 8th thoracic vertebrae, relationships between initial weight of the steaks and total cooking time were moderate to high for all maturity-marbling groups, and for all but one group at the 11th thoracic vertebra.

Relationships among measurements for waterholding capacity, total moisture, total cooking losses, and pH are not reported in Table 5, but, in general, they were low (r between 0.0–0.39). Also, relationships between pH and shear value or color-difference factors were poor.

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Contribution No. 238, Department of Foods and Nutrition, and No. 386, Department of Animal Science and Industry, Kansas Agricultural Experiment Station, Manhattan, Kansas 66502.

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G. C. SMITH, T. C. ARANGO[1] and Z. L. CARPENTER
Department of Animal Science, Texas A&M University, College Station, Texas 77843

EFFECTS OF PHYSICAL AND MECHANICAL TREATMENTS ON THE TENDERNESS OF THE BEEF LONGISSIMUS

SUMMARY—280 steaks from 56 beef carcasses were evaluated to determine the effects of different mechanical and physical methods for increasing the tenderness of the longissimus muscle. Changes in method of suspension, carcass integrity or chilling procedures resulted in increases in tenderness of approximately 47.5% for shear force and 53.6% for taste panel ratings. Experiment 2 (suspension from the achilles tendon, severance between vertebrae in 5 locations, severance of the ligamentum nuchae and the attachment of 68 kg weights) resulted in a 15% increase in tenderness as evaluated by both shear force values and the taste panel ratings. Experiment 3 (suspension via the obturator foramen, severance of the vertebrae in 5 different locations, severance of the ligamentum nuchae and the attachment of 68 kg weights to the neck) gave the greatest positive response in taste panel tenderness ratings (53.6%). Experiments 3, 4, 5 and 6 had the disadvantage of resulting in irregularly shaped carcasses, which could present problems in fabrication. The assumption that increases in carcass length are necessary to achieve a positive response in the tenderness of the longissimus appears unjustified, since post-mortem chilling at elevated temperatures (16°C) increased tenderness 47% with no appreciable change in carcass length. It was concluded from this study that the degree of post-mortem muscle contraction which occurs during the development of rigor mortis is a primary factor influencing the ultimate tenderness of beef. The degree of such contraction is related to the temperature at which the carcass is stored during the initial period of cooling. Chilling the carcass in a 16°C cooler during the first 16 hr post-mortem resulted in a 40.2% increase ($P < .05$) in ratings of longissimus tenderness and a 47.5% decrease ($P < .01$) in shear force requirement, yet involved no additional labor expense nor any irregularity in carcass form. Of the methods studied, the procedure involved in experiments 8 and 9 (chilling the carcasses for the first 16–20 hr in a cooler at 16°C) appears to be the most practical for industrial utilization.

INTRODUCTION

DURING the development of rigor mortis, muscles change physically from the highly extensible, elastic state characteristic of the freshly killed animal to that of the inextensible or rigid condition characteristic of full rigor. Locker (1960) observed that a gradual shortening of sarcomeres accompanied the change in extensability which occurs during onset of rigor.

The contraction or shortening of unrestrained muscles during the onset of rigor mortis is a well established phenomenon that is highly dependent upon the temperature during the onset of the death-stiffening process (Locker and Hagyard, 1963; Marsh and Leet, 1966; Marsh et al., 1968). Excision, restraint, tension or stretching and their effects on the contractile state of muscle fibers are highly associated with meat tenderness (Locker, 1960; Herring et al., 1965a,b; Marsh and Leet, 1966; Howard and Judge, 1968; Ramsbottom and Strandine, 1949; Partmann, 1963; Buck and Black, 1967; Weideman et al., 1967; Herring et al., 1967).

Gillis and Henrickson (1967) and Reddy and Henrickson (1967) reported that as excised muscles shortened there was a decrease in sarcomere length and an increase in fiber diameter. When tension is maintained during the development of rigor mortis, rigor kinks or contracture nodes are often found in localized areas along some muscle fibers (Gillis and Henrickson, 1967). Tuma et al. (1962) and Herring et al. (1965b) reported decreases in beef tenderness as muscle fiber diameter increased.

Locker (1959) observed that various bovine muscles enter rigor mortis in differing states of contraction which are undoubtedly related to the tension present in the muscles of the suspended carcass. The extent of rigor-induced shortening of muscles in a carcass depends on the physical restrictions imposed by their attachments to the skeleton (Hostetler et al., 1970). Changes in carcass position during the onset of rigor mortis have been observed to produce pronounced effects on sarcomere length, muscle fiber diameter and tenderness (Eisenhut et al., 1965; Herring et al., 1965b; Hostetler et al., 1970). Herring et al. (1967) concluded that, from the standpoint of ultimate tenderness, it was more important to prevent post-mortem shortening of muscles than to ensure maximum stretching.

The present study was designed to investigate physical or mechanical methods for increasing the tenderness of the beef longissimus muscle. Changes in method of suspension, carcass or skeletal integrity and temperature during chilling were studied in relation to their effects in the prevention of muscle shortening and/or on the subsequent tenderness of the beef longissimus.

EXPERIMENTAL

NINE TRIALS (Table 1) were conducted using 280 muscle samples from 56 carcasses. A description of the animals in each individual treatment is presented in Table 2. Left and right sides were allotted to treatments at random and each carcass was stored after treatment in a 2°C cooler until fabrication 7 days post-mortem.

Body length measurements

Measures of body length were taken from the anterior border of the symphysis pubis to the anterior border of the first rib for each side in experiments 1, 2, 3, 4, 5, 7, 8 and 9. For experiment 6, measures of carcass length were taken from the caudal edge of the dorsal spinous process of the 6th lumbar vertebra to the cranial edge of the superior spinous process of the 1st thoracic vertebra. These measures were taken immediately post-slaughter, after the treatments were initiated and at the end of the trial (immediately before the carcasses were processed).

Severance treatments

In experiments 1, 2, 3 and 5, individual cuts were made between certain vertebrae which severed the intervertebral fibro-cartilages and the ventral and dorsal longitudinal ligaments. These cuts were made between the 6th and 7th cervical; 5th and 6th, 9th and 10th, 12th and 13th thoracic; and the 4th and 5th lumbar vertebrae. In experiments 1, 2 and 3, the funicular part of the ligamentum nuchae was severed at the anterior end, posterior to the dorsal spinous process of the first cervical vertebra, and the lamellar portions were freed from their attachments to the cervical spines.

Weighted load treatments

In experiments 2, 3 and 5, weight loads of 68 kg were attached to the neck (3rd to 6th cervical vertebrae) for 48 hr in an attempt to maintain carcass length during periods of subsequent storage in the holding cooler (2°C).

Body curvature treatments

One side of each carcass in experiment 6 was bent by use of two small hooks, one of which was attached to the achilles tendon and the other attached to either the anterior border of the sternum or the 4th cervical vertebra. Tension was achieved by connecting the two hooks with a wire to effect lateral curvature along the vertebral column.

[1] Present address: Veterinary College, Universidad Nacional, Bogota, Colombia.

Table 1—Description of the treatments employed

Exp	Number of carcasses	Side	Method of suspension	Severance treatments[a]	Attachment of weights to the neck	Body curvature	Holding conditions Temp °C	Time hr
1	18	control	achilles tendon	none	none	none	2	168
		treated	achilles tendon	A B	none	none	2	168
2	3	control	achilles tendon	none	none	none	2	168
		treated	achilles tendon	A B	68 kg	none	2	168
3	8	control	achilles tendon	none	none	none	2	168
		treated	obturator foramen	A B	68 kg	none	2	168
4	4	control	achilles tendon	none	none	none	2	168
		treated	b	none	none	none	2	168
5	4	control[c]	obturator foramen	none	none	none	2	168
		treated	obturator foramen	A	68 kg	none	2	168
6	4	control	achilles tendon	none	none	none	2	168
		treated	achilles tendon	none	none	d	2	168
7	5	control	achilles tendon	none	none	none	2	168
		treated	achilles tendon	none	none	none	16	8[e]
8	5	control	achilles tendon	none	none	none	2	168
		treated	achilles tendon	none	none	none	16	16[e]
9	5	control	achilles tendon	none	none	none	2	168
		treated	achilles tendon	none	none	none	16	20[e]

[a]A = intervertebral severance between the 6th to 7th cervical; 5th to 6th, 9th to 10th, 12th to 13th thoracic; and 4th to 5th lumbar vertebrae. B = severance of the funicular and lamellar portions of the ligamentum nuchae.
[b]During the first 24 hr the carcass was suspended via the obturator foramen. The carcass was resuspended via the achilles tendon for the remainder of the 168 hr storage period.
[c]Both sides of each carcass in experiment 5 were suspended via the obturator foramen and thus the paired side would not be considered a control in the untreated sense.
[d]Body curvature was achieved by inducing tension from the achilles tendon to either the anterior border of the sternum or to the fourth cervical vertebra.
[e]Individual sides were stored in a 16°C cooler for 8, 16 or 20 hr and subsequently stored in the 2°C cooler for 160, 152 or 148 hr, respectively.

Table 2—Allocation of animals to the individual experiments

Experiment	n	Age at slaughter	Sex condition
1	18	10–14 mo	steers
2	3	10–14 mo	steers
3	8	30–42 mo	steers
4	4	12–18 mo	2 steers
		Unknown[a]	1 cow
		12–18 mo	1 heifer
5	4	12–18 mo	steers
6	4	Unknown[b]	steers
7	5	12–18 mo	steers
8	5	12–18 mo	steers
9	5	12–18 mo	steers

[a]Subjective estimates of the degree of bone ossification suggested that this animal was 5 yr of age or older at time of slaughter.
[b]Slaughter steers obtained from a livestock show which were subjectively evaluated as being less than 18 mo of age at time of slaughter.

Suspension treatments

Two methods were compared in experiments 3 and 4; suspension from the achilles tendon (normal method) vs. support via the obturator foramen (in the manner previously described by Hostetler et al., 1970). Both sides of each carcass in experiment 5 were suspended via the obturator foramen.

Temperature treatment

In experiments 7, 8 and 9, one side of each carcass was placed in a 2°C cooler and the opposite side of each carcass was placed in a cooler maintained at 16°C. Following 8, 16 or 20 hr of storage (post-mortem) in the 16°C cooler these sides were placed in the 2°C cooler for the remainder of the aging period (168 hr).

Tenderness determinations

In experiments 1, 2 and 3, individual steaks (3.8 cm thick) from the neck (6th cervical), chuck (5th thoracic), rib (12th thoracic) and loin (4th lumbar) regions were removed, packaged, frozen at −18°C and stored for a period of 10–25 days. For experiments 4 through 9, a 3.8 cm steak was removed from the rib (12th thoracic) area, packaged, frozen at −18°C and stored for a period of 10–25 days. The steaks were removed from frozen storage, thawed at room temperature (23°C) and broiled at 177°C to an internal temperature of 75°C. Individual samples (1.27 cm × 2.54 cm) of each steak were presented to a trained four-member panel for tenderness evaluations. Samples were evaluated by use of a 9-point hedonic scale ranging from 9 (very tender) to 1 (very tough). Core samples (1.27 cm) for shear force determination were removed parallel to the orientation of the muscle fibers after the steaks had reached room temperature (23°C).

Sarcomere length determinations

Unfixed samples of longissimus were blended for 40 sec in cold (4°C) 0.25M sucrose solution using a Virtis homogenizer. The suspension of muscle fibers was examined directly in a phase contrast microscope (10X ocular, 100X objective). Sarcomere length was determined by use of a filar micrometer and values are reported as the average length of 250 sarcomeres from each muscle.

Statistical treatment

Means, standard deviations and coefficients of variation were computed for each of the variables studied. The paired t distribution analysis was employed to test for significance of differences between mean values (Li, 1957).

RESULTS & DISCUSSION

MEANS, standard deviations and coefficients of variation for shear force values, tenderness ratings and sarcomere lengths for the 18 steer carcasses in experiment 1 are presented in Table 3. Sides which were treated by severance of the vertebral column and ligamentum nuchae provided steaks that were significantly

lower in shear force requirements for longissimus samples from the neck (P < .01), chuck (P < .05) and loin (P < .01) areas. Tenderness ratings were significantly higher (P < .05) for chuck steaks from the treated sides. These findings are in general agreement with those of Herring et al. (1965a, b), Tuma et al. (1962), Gillis and Henrickson (1967), Howard and Judge (1968) and Hostetler et al. (1970).

Severance of the vertebrae and ligamentum nuchae in experiment 1 resulted in increased carcass lengths (Table 4) immediately after the treatment and this increase was partially maintained during subsequent storage. While an increase (P < .01) in sarcomere length was observed at the 12th thoracic area (Table 3), the effect was not manifested via increases in the tenderness of corresponding rib steaks. Marsh and Leet (1966) postulated that cold shortening occurs in muscles which remain attached to the skeleton. The carcasses in experiment 1 were subjected to cold shock upon exposure to cooler temperatures of 2°C prior to and during the onset of rigor mortis. Tenderness increases may have occurred in specific carcass locations (e.g. chuck, Table 3) because steaks from that region were obtained adjacent to areas stretched extensively by the severance procedure; while cold shortening may have occurred in those areas between the severed vertebrae. The lack of uniformity in the effect of shortening along the long axis of the longissimus would result in relatively minor changes in carcass length.

Means, standard deviations and coefficients of variation for shear force values, tenderness ratings and sarcomere lengths for experiment 2 are presented in Table 5. Weights (68 kg) were attached to the neck in an attempt to maintain the increase in body length which resulted from vertebral severance treatments, and to prevent decreases in carcass length resulting from cold shortening when the carcasses were exposed to low temperatures (2°C) post-slaughter. The attachment of 68 kg weights to the neck (prior to rigor mortis) effectively maintained the increased body lengths gained via severance (Table 4), during the period of cooler storage.

Nonsignificant decreases in shear force requirement for steaks from the neck, chuck and rib regions and increases in tenderness ratings for steaks from the neck and rib were observed. The lack of significant differences for all values except the tenderness rating of the chuck steak (P < .05) is attributed in part to the size of the sample observed (n = 3).

Results of experiment 3 are presented in Table 6. Significant reductions in shear force requirement were observed

Table 3—Means, standard deviations (S.D.) and coefficients of variation (C.V.) for shear force values, panel tenderness ratings and sarcomere lengths at different locations from the Longissimus muscle in Experiment 1

Trait	Location[a]	Mean		S.D.		C.V.	
		C[b]	T[b]	C	T	C	T
Shear force (kg)	neck	4.96[e]	4.07[e]	1.00	0.82	20.15	20.08
	chuck	4.76[d]	4.10[d]	1.36	1.21	28.55	29.59
	rib	4.58	4.38	2.06	1.84	44.97	42.13
	loin	4.51[d]	3.75[d]	1.33	0.89	29.57	23.77
Taste panel scores[c]	neck	4.52	4.18	0.97	1.46	21.43	34.88
	chuck	6.01[e]	7.19[e]	1.72	1.13	28.61	15.70
	rib	5.49	5.96	1.57	1.60	28.67	26.82
	loin	6.13	6.14	1.64	1.75	26.72	28.50
Sarcomere length (μ)	rib	1.83[e]	2.09[e]	0.17	0.15	9.19	7.24

[a]Neck steaks were removed from the 6th cervical region; chuck steaks from the 5th thoracic region; rib steaks from the 12th thoracic region and loin steaks from the 4th lumbar region.
[b]C = control or untreated side; T = treated side. The treatment consisted of severance of the vertebrae at five locations and severance of the funicular and lamellar portions of the ligamentum nuchae.
[c]Hedonic ratings based on a 9-point scale (9 = very tender).
[d]Values bearing the same superscript differ significantly (P < .05).
[e]Values bearing the same superscript differ significantly (P < .01).

Table 4—Mean values for body length resulting from the treatment imposed upon the carcasses in each experiment

Experiment	Average carcass length (m)[a]				
	Warm carcasses		Chilled carcasses		
	Before treatment	After treatment	After storage	Difference (m)	Change (%)
1	1.15	1.17	1.16	0.01	0.86
2	1.17	1.23	1.23	0.06	5.12
3	1.16	1.30	1.29	0.13	11.20
4	1.14	1.23	1.23	0.09	7.89
5	1.15	1.22	1.22	0.07	6.08
6[b]	1.03	1.14	1.13	0.10	9.70
7	1.21	1.21	1.19	−0.02	−1.65
8	1.10	1.09	1.09	−0.01	−0.91
9	1.19	1.20	1.20	0.01	0.83

[a]Measures of carcass length (with the exception of those in experiment 6) were taken from the anterior border of the symphysis pubis to the anterior border of the first rib.
[b]Measures of carcass length were taken from the caudal edge of the dorsal spinous process of the 6th lumbar vertebra to the cranial edge of the superior spinous process of the 1st thoracic vertebra.

Table 5—Means, standard deviations (S.D.) and coefficients of variation (C.V.) for shear force values, tenderness ratings and sarcomere lengths at different locations in the longissimus muscle for Experiment 2

Trait	Location[a]	Mean		S.D.		C.V.	
		C[b]	T[b]	C	T	C	T
Shear force (kg)	neck	4.84	3.25	2.06	0.76	42.68	23.53
	chuck	6.00	4.05	2.07	0.15	34.47	3.60
	rib	3.70	3.13	1.07	0.76	28.93	24.38
	loin	3.70	3.72	0.54	1.68	14.61	45.15
Taste panel scores[c]	neck	4.42	5.71	0.80	1.74	18.20	30.47
	chuck	5.17[d]	7.08[d]	1.01	0.38	19.56	5.39
	rib	6.92	8.00	1.61	0.50	23.24	6.25
	loin	6.33	6.08	1.38	1.04	21.74	17.11
Sarcomere length (μ)	rib	1.86	1.96	0.10	0.15	5.23	7.65

[a]Neck steaks were removed from the 6th to 7th cervical region; chuck steaks from the 5th to 6th thoracic region; rib steaks from the 12th to 13th thoracic region and loin steaks from the 4th to 5th lumbar region.
[b]C = control or untreated side; T = treated side. The treatment consisted of severance between vertebrae in 5 locations, severance of the ligamentum nuchae and the attachment of 68 kg weights to the neck.
[c]Hedonic ratings based on a 9-point scale (9 = very tender).
[d]Values bearing the same superscript differ significantly (P < .05).

for steaks from the treated side in the neck (P < .01), chuck (P < .01) and loin (P < .01) regions. Taste panel ratings for tenderness were significantly higher for steaks from the treated side in the rib (P < .01) and loin (P < .01) regions. Carcasses treated in this manner exhibited the highest percent increase in body length (Table 4) observed in this study and significant (P < .01) differences were observed in sarcomere length. The 11.2% increase in carcass length appears to be sufficient to significantly affect sarcomere lengths. The treated sides exhibited some degree of longissimus elongation and as a result produced steaks with low shear force values and high tenderness ratings. These results are in agreement with the previous reports of Herring et al. (1965a, b), Tuma et al. (1962), Gillis and Henrickson (1967) and Hostetler et al. (1970).

In experiment 4, one side from each of the four beef carcasses was suspended via the obturator foramen in the manner previously described by Hostetler et al. (1970). Carcasses were suspended in this manner for 24 hr post-slaughter, whereupon they were resuspended by the achilles tendon for the remainder of the 168 hr aging period. This procedure was followed to determine its effectiveness in increasing the tenderness of the longissimus when suspension time via the obturator foramen was reduced. It was of further interest to ascertain the extent to which carcasses would return to that shape or form characteristic of carcasses suspended by the achilles tendon. No significant differences were observed for shear force values, tenderness ratings or sarcomere lengths (Table 7). After an aging period of 7 days, carcasses supported during the onset of rigor mortis by the obturator foramen and subsequently resuspended by the achilles tendon did not regain the shape characteristic of those carcasses which were supported in the normal manner for the entire storage period.

Experiment 5 was conducted using carcasses from four steers, 12-18 months of age at slaughter. This experiment was designed to determine the relationship between tenderness and changes in the manner of carcass suspension (Hostetler et al., 1970) and to determine whether further increases in carcass length and/or tenderness could accrue from severance between vertebrae plus weighted loads attached to the neck (Table 4). No significant differences in shear force values, tenderness ratings or sarcomere lengths were observed (Table 7).

Experiment 6 was conducted using carcasses from four steers, 12-18 months of age at slaughter. Body curvature was achieved by the use of tension applied upon a wire connecting the fore and rear portions of the side to effect lateral deformation of the vertebral column. No significant differences were observed for any of the traits studied (Table 7). Body curvature treatments may elongate fibers in some areas of the longissimus, while other areas either shorten or do not stretch enough to significantly affect tenderness in that location. The carcasses treated in this manner were irregular in form, undesirably shaped and would not be acceptable commercially.

In experiments 7, 8 and 9, three groups of 5 steers each, 12-18 months of age, were used to study the effects of cold shortening (Marsh et al., 1968) on changes in beef tenderness. One side from each carcass was stored in a 16°C cooler for 8, 16 or 20 hr and at 2°C for the remainder of the 168 hr aging period. The untreated sides were held in a 2°C cooler for the entire storage period. No significant differences were found in experiment 7 (Table 8) while carcasses stored 16 hr at 16°C (experiment 8) had significantly lower shear force values (P < .01) and higher tenderness ratings (P < .05). Shear force requirements were significantly lower (P < .01) for the treated sides in experiment 9. The standard deviations and coefficients of variation were quite high in experiment 9 for shear force and tenderness ratings, which may explain partially the lack of a significant difference between control and treated sides for taste panel tenderness.

Interestingly, total body length was not increased by storing the treated carcasses at elevated cooler temperatures in either experiment 7 or 8. Significant differences in tenderness were not associated with increases in body length, thus changes in body length may not be a desirable index of the contractile state of the sarcomeres. Locker and Hagyard (1963) and Cassens and Newbold (1967) have demonstrated that exposure to low

Table 6—Means, standard deviations (S.D.) and coefficients of variation (C.V.) for shear force values, tenderness ratings and sarcomere lengths at different locations in the longissimus muscle for Experiment 3

Trait	Location[a]	Mean		S.D.		C.V.	
		C[b]	T[b]	C	T	C	T
Shear force (kg)	neck	7.31[d]	5.12[d]	0.93	0.51	12.67	9.87
	chuck	6.23[d]	3.60[d]	1.02	0.46	16.38	12.90
	rib	5.60	5.06	1.80	1.42	32.10	28.04
	loin	5.70[d]	4.33[d]	0.64	0.66	11.15	15.37
Taste panel scores[c]	neck	2.45	2.50	0.56	0.64	22.75	25.63
	chuck	5.66	5.97	1.18	0.90	20.86	15.09
	rib	4.16[d]	6.39[d]	0.91	0.55	21.79	8.58
	loin	4.56[d]	6.57[d]	1.16	0.73	25.49	11.09
Sarcomere length (μ)	rib	1.82[d]	2.19[d]	0.22	0.15	12.03	6.75

[a]Neck steaks were removed from the 6th to 7th cervical region; chuck steaks from the 5th to 6th thoracic region; rib steaks from the 12th to 13th thoracic region and loin steaks from the 4th to 5th lumbar region.
[b]C = control or untreated side; T = treated side. The treatment consisted of hanging the carcass by the obturator foramen, severance between vertebrae at 5 locations, severance of the ligamentum nuchae and the attachment of 68 kg weights to the neck.
[c]Hedonic ratings based on a 9-point scale (9 = very tender).
[d]Values bearing the same superscript differ significantly (P < .01).

Table 7—Means, standard deviations (S.D.) and coefficients of variation (C.V.) for shear force values, tenderness ratings and sarcomere lengths for the longissimus muscle from Experiments 4, 5 and 6

Trait	Experiment	Location[a]	Mean		S.D.		C.V.	
			C[b]	T[b]	C	T	C	T
Shear force (kg)	4	rib	3.76	3.22	0.94	0.53	25.08	16.34
	5	rib	3.91	3.77	1.25	0.83	32.07	21.97
	6	rib	3.73	3.11	0.46	0.80	12.41	25.63
Taste panel scores[c]	4	rib	5.69	6.63	1.36	1.38	23.91	20.78
	5	rib	5.94	6.38	1.34	0.66	22.64	10.38
	6	rib	5.25	6.31	0.94	1.03	17.82	16.29
Sarcomere length (μ)	4	rib	2.01	2.05	0.17	0.11	8.50	5.50
	5	rib	2.36	2.34	0.36	0.55	15.45	23.76
	6	rib	1.78	2.03	0.29	0.09	16.50	4.53

[a]Rib steaks derived from the 12th to 13th thoracic vertebrae region.
[b]C = control or untreated side; T = treated side.
[c]Hedonic ratings based on a 9-point scale (9 = very tender).

Table 8—Means, standard deviations (S.D.) and coefficients of variation (C.V.) for shear force values, tenderness ratings and sarcomere lengths for the longissimus muscle from Experiments 7, 8 and 9

Trait	Experiment	Location[a]	Mean		S.D.		C.V.	
			C[b]	T[b]	C	T	C	T
Shear	7	rib	5.63	5.38	1.30	1.83	23.14	34.14
force	8	rib	6.23[e]	3.27[e]	1.09	0.93	17.53	28.62
(kg)	9	rib	3.73[d]	2.54[d]	1.86	0.70	49.94	27.61
Taste	7	rib	5.95	5.80	1.35	1.16	22.70	20.00
panel	8	rib	4.10[d]	5.75[d]	0.52	0.73	12.64	12.68
scores[c]	9	rib	4.73	6.06	2.53	1.80	53.48	29.72
Sarcomere	7	rib	1.87	1.84	0.35	0.12	1.87	6.54
length	8	rib	1.96	1.89	0.56	0.13	28.67	7.06
(μ)	9	rib	1.83	1.93	0.09	0.19	4.75	9.85

[a]Rib steaks derived from the 12th thoracic vertebra region.
[b]C = control or untreated side; T = treated side. The treated carcasses were chilled for the first 8, 16 or 20 hr at a temperature of 16°C and at 2°C for the remainder of the 168 hr aging period.
[c]Hedonic ratings based on a 9-point scale (9 = very tender).
[d]Values bearing the same superscript differ significantly (P < .05).
[e]Values bearing the same superscript differ significantly (P < .01).

Table 9—A summary of the effects of each treatment on changes in shear force values and tenderness ratings of rib steaks

Exp	Difference (control minus treated) shear force, kg	Decrease in shear force value (%)[a]	Level of probability	Difference (treated minus control) tenderness rating[b]	Increase in tenderness rating (%)[c]	Level of probability
1	0.20	4.4	n.s.[d]	0.47	8.6	P < .15
2	0.57	15.4	P < .10	1.08	15.6	P < .15
3	0.54	9.6	P < .25	2.23	53.6	P < .01
4	0.54	14.4	P < .10	0.94	16.5	P < .25
5	0.14	3.6	n.s.	0.44	7.4	P < .25
6	0.62	16.6	P < .10	1.06	20.2	P < .10
7	0.25	4.4	n.s.	−0.15	−2.5	n.s.
8	2.96	47.5	P < .01	1.65	40.2	P < .05
9	1.19	31.9	P < .05	1.33	28.1	P < .10

[a]Percent decrease computed as control minus treated divided by control.
[b]Hedonic ratings based on a 9-point scale (9 = very tender).
[c]Percent increase computed as treated minus control divided by control.
[d]Values were not significant at P < .25.

temperatures (0-16°C) is necessary for cold shortening. The discontinuity in the enzymatic properties of myosin ATPase that occurs at 16°C may reflect a change in the shape of the active site on the myosin molecule at this temperature (Szent-Gyorgyi, 1951) thereby resulting in the formation of fewer bonds between actin and myosin filaments during rigor. Unless muscles are either subjected to blast freezer temperatures while on the carcass or are excised and exposed to 0° -2°C temperatures, cold shortening is not likely to occur in the longissimus. Restraint of the longissimus is removed during the suspension of the carcass by the achilles tendon. Also, perhaps some factor such as proteolysis may be playing a role in tenderization during the time the carcass is held at 16°C for 16-20 hr and the cathepsins may be activated at the lower pH and higher temperature. It is apparent from these results that increases in carcass length do not necessarily result in elevated tenderness levels; nor is increased carcass length necessary for attaining significant improvement in longissimus tenderness.

A summary of the effects of each treatment on changes in shear force values and tenderness ratings of rib steaks is presented in Table 9. Chilling carcasses at 16°C for the initial 16 or 20 hr post-mortem yielded the greatest response in reducing shear force requirements (experiments 8 and 9, P < .01 and P < .05, respectively). Suspension via the obturator foramen, severance of the vertebrae in five locations, severance of the ligamentum nuchae and the attachment of 68 kg to the neck (experiment 3) gave the greatest positive response (P < .01) in tenderness ratings. However, chilling at elevated temperatures for 16 hr post-slaughter (experiment 8) significantly enhanced tenderness (P < .05) yet required no change in carcass integrity or manner of suspension.

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T. A. 8734, Texas Agricultural Experiment Station.

R. J. BOTHAST, PAUL P. GRAHAM and R. F. KELLY
Department of Food Science and Technology
Virginia Polytechnic Institute and State University, Blacksburg, Va. 24061

INFLUENCE OF CONTROLLED MICROBIAL CURING ON PORCINE MUSCLE

***SUMMARY**—A previously developed technique was adapted to study the influence of certain microbiological populations and their effects on processed meat. The technique consisted of an initial reduction of surface bacteria on conventionally handled muscle tissue via a hot water dip, followed by processing at 28°C in a sterile plastic isolator where Pediococcus cerevisiae was introduced into the curing solution. This treatment was compared to reduced initial count and conventional samples. Identification of the bacteria in the curing solution of each treatment indicated that a Lactobacillus spp. was predominant in the reduced initial count treatment. The inoculated Pediococcus cerevisiae was predominant in the reduced initial count inoculated treatment while Staphylococcus epidermidis and Flavobacterium diffusum were predominant in the conventional treatment depending upon the trial. Tenderness and bacterial load were significantly ($P < .01$) increased by treatment while pH was significantly ($P < .01$) decreased. Oxidation and muscle composition were not affected by treatment. Samples from all treatments were acceptable organoleptically.*

INTRODUCTION

CONSIDERABLE RESEARCH concerning the influence of microorganisms on meat products has involved primarily mixed cultures and their spoilage of the product. Little work has been done using bacteria in the development of new, safe and appealing meat products which have acceptable keeping qualities. Use of bacteria in foods as starter cultures provides a possible means of controlling certain types of spoilage as well as food poisoning hazards (Niven, 1952). In addition, the usefulness of microorganisms as flavor enhancers greatly overshadows their economic importance as spoilage bacteria. Therefore, if the meat scientist is going to control and use bacteria, the effects of microorganisms on foods must be studied. In this connection, Ockerman et al. (1964) and Bothast et al. (1968) have developed surgical and dip techniques, respectively, for obtaining undenatured muscle tissue with a low bacterial count. These techniques furnish the meat scientist with a new tool to study the influence of bacteria (pure or mixed cultures) on meat products. Consequently, this research was designed to further develop a technique to study the influence of bacteria on meat.

Pediococcus cerevisiae (reduced initial count inoculated treatment), an accepted starter culture (Deibel et al., 1961) was used in the pickle curing of pork loins. In addition, the predominant microorganisms of conventional and reduced initial count treatments were studied to determine their influence on the product.

EXPERIMENTAL

PORCINE longissimus dorsi was selected as the muscle tissue for this experiment. Two 1-yr-old sows were slaughtered in the Meat Laboratory, V.P.I., according to the procedure outlined by Cole (1951). A third sow—similar size and age—was slaughtered by a commercial establishment. The loins from the first carcass were removed according to the methods outlined at the Fourth Reciprocal Meat Conference (Cole, 1951), and the longissimus dorsi muscles were cut to yield three 20-cm sections for Trial I. Each section was then randomly assigned to one of the three treatments—reduced initial count (RIC), reduced initial count inoculated (RICI), and conventional (C). Trials II and III were conducted similarly, using loins from carcass #2 in Trial II and loins from carcass #3 in Trial III.

The RICI treatment was designed to serve as an indicator of the success of the dip technique being developed, i.e., does the technique allow an inoculated organism to predominate and its influence on a meat product to be studied? In this particular research, *Pediococcus cerevisiae* was inoculated into the curing solution to take advantage of any beneficial properties it might impart to the product. The RIC treatment was designed to produce low bacterial loads on meat so that a direct comparison could be made between this treatment and the RICI treatment. The conventional treatment functioned as the control. This treatment was "conventional" in the sense that loins were cured by a process (temperature, time, technique, etc.) similar to the one used by meat processors today.

The sections assigned to the RIC treatment were processed in a plastic isolator according to the procedures described by Bothast et al. (1968) and Graner et al. (1969), with slight modifications. Once the loin sections were in the isolator, they were put into a large beaker and 2.2 l of curing solution were added. The curing solution contained 4% sodium chloride, 5% dextrose, and 0.3% of a 12/1 (dry weight) nitrate to nitrite mixture. The meat was allowed to cure in the isolator for 7 days at 28°C. Filtered air circulated in the isolator at all times over the solution covered samples.

The RICI treatment was essentially the same as the RIC treatment except that 1.12g of *Pediococcus cerevisiae* (ACCEL starter culture produced by Merck and Co., Inc.) were included per 2.2 l of curing solution.

The conventional treatment consisted of a 7-day cure at 4°C without inoculation or sanitary treatment. The loin sections assigned to this treatment were submerged in a large beaker containing 2.2 l of brine. This solution consisted of the same ingredients as the other two treatments but was mixed manually and not autoclaved.

When the curing was completed for the three treatments, samples were evaluated for bacteria; pH; oxidation of fat; taste differences; tenderness; composition; and color. Uniform 10 ml curing solution samples were obtained aseptically from each treatment. A standard plate count was made, according to the procedure recommended by the American Public Health Association (1966), on 1 ml aliquots of these 10 ml samples, using tryptone glucose extract (TGE) agar (Difco) incubated at 30°C for 48 hr. The viable bacterial load was determined. Three representative colonies for each treatment were picked from TGE plates that were countable (between 30 and 300 colonies) and inoculated on brain heart infusion agar (Difco) and APT broth (Difco). Identification procedures according to Breed et al. (1957) were then used to identify the respective organisms. The pH was determined on uniform 25-ml curing solution samples from each treatment.

The loin sections were removed from their respective solutions and approximately 200g samples were obtained from the end of each section. These samples were wrapped in aluminum foil and refrigerated over night. The next day 10g subcutaneous fat samples were obtained from the 200g samples and oxidation was measured by TBA (2-thiobarbituric acid) values, according to the procedure of Tarladgis et al. (1960). The remainder of the 200g samples were rewrapped and frozen. When all three trials were completed, these samples were thawed at room temperature and analyzed for moisture, fat and protein according to the procedures described by A.O.A.C. (1965).

The remaining loin sections (those left after removal of a 200g sample) were subjectively evaluated for quality of color and then cooked in a 177°C oven to an internal temperature of 73°C. Sections from each treatment were sliced (3.1 mm thickness) and 25 mm diameter discs were made. These discs were then administered in a triangle taste test (Amerine et al., 1965) to a panel of five members. The taste panel members were trained by several practice sessions prior to the actual test. The meat was warm when served. The panel was offered all possible triangle comparisons—with the three treatments—in duplicate at each testing ses-

Table 1—Standard plate count per millilter of curing solution[a]

Trial	Treatment: reduced initial count	Treatment: reduced initial count inoculated	Treatment: conventional
I	7.68	7.36	3.20
II	7.72	7.48	4.08
III	8.08	6.64	3.56
	—	—	—
X	7.83	7.16	3.61**

[a]Counts are expressed in logarithms.
**Means significantly different ($P < .01$).

sion. In addition, 25 cm diameter cores were obtained from the cooked sections and evaluated for tenderness by the Warner-Bratzler shear.

Statistical analysis of all data was conducted according to Li (1964).

RESULTS & DISCUSSION

Viable bacteria

Table 1 shows the results of standard plate counts on each curing solution. These data agree with counts determined by the Petroff-Hauser counting chamber. The curing solution of the conventional treatment was significantly ($P < .01$) lower in bacteria than solutions of the other two treatments. This lower level of the conventional treatment can be attributed to the 4°C curing temperature. At 4°C the metabolic processes of most microorganisms are restricted and in 7 days very little microbial growth will occur. No significant difference existed in the bacterial level of the RIC and RICI solutions. The physical and chemical environment of the RIC and RICI treatments appeared to be excellent for certain microbial growth and development.

Isolation and identification of the predominant bacteria

Table 2 shows the predominant microorganisms found in the curing solutions and the morphological and physiological properties upon which identification is based. Representative colonies from all three trials for the RIC treatment yielded *Lactobacillus* spp. No differentiation of the species in the genus *Lactobacillus* was made because of the inadequacy of comparative information.

Several investigators (Niven, 1951, 1952; Jensen, 1954; Deibel and Niven, 1958, 1959; Frazier, 1958) have reported that lactobacilli are the most common bacterial contaminants in fermented foods and commercial curing solutions. The importance of these organisms has been stressed in the development of a pleasant "tangy" flavor, establishment of reducing conditions for proper color formation, and in green discoloration. Leistner (1960) studied the microbiology of ham curing and found *Lactobacillus* in 70% of the pumping brines and 95% of the cover brines examined. Therefore, it was not surprising that *Lactobacillus* spp. predominated in the RIC brine. Certain species of this genus are heat tolerant; thus, this property may account for their survival of the hot water dip and subsequent growth in the curing solution.

Pediococcus cerevisiae was the predominant organism isolated from representative colonies of RICI solutions for all trials, as would be expected since it was inoculated as a starter culture (ACCEL) in the curing solution.

In addition to *Pediococcus cerevisiae* being found in RICI brine, a species of *Lactobacillus* was also isolated from the RICI brine in Trial I, and only in Trial I. This organism appeared to be the same as the one found in the RIC brine for all three trials.

Isolates from the representative colonies of the conventional treatment for Trials I and II yielded *Staphylococcus epidermidis* while *Flavobacterium diffusum* was isolated from the representative colonies of Trial III. Leistner (1960) found *Staphylococcus* in 5% of ham pumping brines and 10% of ham cover brines. Graham (1970) found coagulase negative *Staphylococcus aureus* prevalent in dry-cured hams. Breed et al. (1957) reported that *Staphylococcus epidermidis* are very salt tolerant, growing vigorously in media containing 10% sodium chloride and are aerobic to facultatively anaerobic. They are normally found on the skin and mucous membranes of man and other animals and in a variety of food products. Therefore, since the ingredients were mixed by hand, it was not unusual to find *Staphylococcus epidermidis* in the conventional solution. Leistner (1960) found *Flavobacterium* in 10% of ham pumping brines and in 15% of ham curing brines. Breed et al. (1957) stated that this organism was aerobic to facultatively anaerobic and normally found in soil, fresh and sea water. Thus, again it was not surprising to find this organism in the conventional solution. However, it was unusual that *Flavobacterium diffusum* did not show up in the first two

Table 2—Isolation and identification of the predominant bacteria found in the curing solution

Treatment	Trials		Growth at 30° on TGE	Growth at 37°	APT broth	Brain Heart Infusion	Gram stain	Morphology	Pigment	Motility	Catalase	Growth on TPEY	Dextrose (acid)	Lactose (acid)	Mannitol (acid)	Gelatin hydrolysis	Litmus milk (acid)	Nitrate reduction	Indole	Urease	Coagulase
			Morphological and Physiological Properties																		
Reduced initial count	(I, II, III)	*Lactobacillus* spp.	E	+	+	(+)	+	Rc	–	–	–		+	+	+	–	+	–			
Reduced initial count inoculated	(I), (II, III)	*Pediococcus cerevisiae*	E	+	+	(+)	+	C	–	–	–		+	+	–	–	–	–			
Conventional	(I, II)	*Staphylococcus epidermidis*	S	+	+	+	+	Cc	–	–	+	+	+		–		+				–
	(III)	*Flavobacterium diffusum*	S, Sp	–	+	+	–	R	V	+	+	–	(+)	–	–	–	–	+	–	–	

E = embedded colonies (+) = restricted growth C = cocci in pairs, tetrads and single S = surface colonies R = rods Cc = cocci in clusters Sp = spreading growth Rc = rods in chains V = variable

Table 3—Final pH of the curing solutions

Trial	Treatment: reduced initial count	reduced initial count inoculated	conventional
I	4.6	4.6	5.8
II	4.9	4.7	5.6
III	4.9	4.9	5.7
X	4.8	4.7	5.7**

**Means significantly different (P < .01).

trials. Slaughtering was conducted at a different location in Trial III and this may account for the different predominating flora.

Final pH of the curing solutions

Acidity of the curing brine was affected by treatment as expected and shown in Table 3. The pH of the conventional solution was significantly higher (P < .01) than the pH of either RIC or RICI solutions. However, no difference in pH existed between the RIC or RICI solutions. These results are generally correlated with the counts given in Table 1, i.e., as the number of organisms increases so does acid production; consequently, the pH was lower for both the RIC and RICI treatments. Also, the predominant organisms (*Lactobacillus* and *Pediococcus*) in these treatments are extremely active acid formers. From previous work (Niven, 1952), it is assumed that the major acid formed was lactic but no determination of acid products was made. Nevertheless, the organism of the RIC and RICI treatments were at a similar stage of acid production. The pH range (4.6-4.9) attained in the RIC and RICI solutions agreed with the values of 4.5-4.8 reported by Everson et al. (1969) and Mills and Wilson (1958). The pH range of the conventional solutions (5.6-5.8) appeared to be similar to the ultimate pH reported by Lawrie (1966). Bacteria did not appear to influence, measurably, the pH of the conventional solution due to both the level and type of organism present.

Oxidation of fat

Oxidation of fat was not significantly affected by any of the experimental treatments as measured by 2-thiobarbituric acid (TBA) values. The peracetic acid (used to sterilize the isolator), the 28°C curing temperature, and the hot water dip did not appear to increase oxidation. This is contrary to earlier work reported by Bothast et al. (1968) where the peracetic acid and hot water dip increased oxidation. Perhaps the lower fat oxidation observed in this research was the result of the loin sections being submerged throughout the curing process and by the short duration (7 days) of the process. It appears that the microorganisms involved in this study were not increasing oxidation, but on the contrary may be establishing reducing conditions (Leistner, 1960) and thereby inhibiting oxidation.

Composition of longissimus dorsi

The composition of porcine longissimus dorsi muscle was not significantly different between treatments.

Tenderness

When Warner-Bratzler shear values were combined over trials (Table 4), a significant difference (P < .01) existed between cores within treatments and between the treatments. RIC and RICI treated samples required significantly less force to shear a 2.5 cm diameter core than conventional samples. A significant difference (P < .05) was found between trials. Trial I was less tender than either Trial II or III. This difference may be explained by animal variation.

An explanation of why the RIC and RICI samples were more tender than

Table 4—Warner-Bratzler shear values combined over trials[a]

Trial[b]	reduced initial count Core 1	reduced initial count Core 2	reduced initial count inoculated Core 1	reduced initial count inoculated Core 2	conventional Core 1	conventional Core 2	$\bar{X}_R$
I	6.6	10.6	12.2	9.0	10.2	13.8	10.4*
II	6.4	4.5	5.8	4.7	10.1	9.9	6.9
III	5.2	8.0	4.9	5.0	9.4	12.5	7.5
$\bar{X}_c$	6.1**	7.7	7.6	6.2**	9.9**	12.1	
$\bar{X}_t$	6.9		6.9		11.0**		

[a]Values are expressed as pounds necessary to shear a 2.5 cm diameter core.
[b]$\bar{X}_c$—Core mean; $\bar{X}_R$—Trial mean; $\bar{X}_t$—Treatment mean.
*Mean significantly different (P < .05).
**Means significantly different (P < .01).

Table 5—Treatment difference and preference as determined by triangle tests

Reduced initial count samples vs reduced initial count inoculated samples

	No. of judgments	No. of correct judgments	% accuracy
Trial I	20	13	65
Trial II	20	12	60
Trial III	20	6	30
Total	60	31	52

Preference of correct judgments[a] R = 18, I = 9, O[b] = 4

Conventional samples vs reduced initial count samples

	No. of judgments	No. of correct judgments	% accuracy
Trial I	20	8	40
Trial II	20	16	80
Trial III	20	13	65
Total	60	37	62

Preference of correct judgments[a] R = 19, C = 10, O[b] = 8

Reduced initial count inoculated samples vs conventional samples

	No. of judgments	No. of correct judgments	% accuracy
Trial I	20	14	70
Trial II	20	12	60
Trial III	20	12	60
Total	60	38	63

Preference of correct judgments[a] I = 14, C = 18, O[b] = 6

[a]R, I, and C represent the three treatments—reduced initial count, reduced initial count inoculated and conventional, respectively.
[b]Indicates no preference.

conventional samples probably lies in the activity of proteolytic enzymes within the muscle. Sharp (1963) has shown that these enzymes are more active at higher temperatures and lower pH's. Since these treatments were conducted at 28°C and a pH of 4.7 was attained, it is reasonable to suspect meat proteolytic enzymes as the cause of this increased tenderness. However, bacterial effects cannot be ruled out completely. Certain genera of bacteria (*Proteus, Clostridium, Pseudomonas,* etc.) manufacture and excrete proteolytic enzymes that might also tenderize muscle. Consequently, tenderization may result from combined bacterial-proteolytic enzyme action. Nevertheless, the predominant organisms identified in these treatments are not known for their proteolytic activity. Also, the samples did not have rank odors which are associated with proteolysis.

Triangle taste test for acceptability

Triangle taste test data showed extreme variation between and within individuals from trial to trial and within trials. Therefore, no statistical analysis was made on these data but some overall comparisons were made as shown in Table 5. Generally, it can be concluded that the taste panel results agree with the tenderness and acidity results previously presented. The RIC and RICI samples are similar, while the conventional samples differ. Preferences of the panel on correct judgments showed that RIC samples were favored over RICI and conventional samples, and conventional samples were slightly preferred over RICI samples. Nevertheless, sizeable preference for, or dissatisfaction with, any of the treatments was not shown.

Cured color

A subjective evaluation for color development of all loin sections showed the cured red color to be more uniform, deeper, brighter and more desirable after 7 days in the RIC and RICI loins than in the conventional loins. Perhaps the higher curing temperature of the first two treatments accounted for this, but it is also likely that bacteria, via establishing reducing conditions (Greenwood et al., 1940; and Lawrie, 1966) are responsible for this enhanced color.

This work demonstrates that meat can be processed at room temperature with the production of a wholesome product. Although, the technique described may be too involved for present commercial use, further application may alter processing as we know it today. Additionally, this research introduces alternatives for studying the microbial influences (beneficial or harmful) on processed meats.

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C. R. REY and A. A. KRAFT
Department of Food Technology, Iowa State University, Ames, Iowa 50010

EFFECT OF FREEZING AND PACKAGING METHODS ON SURVIVAL AND BIOCHEMICAL ACTIVITY OF SPOILAGE ORGANISMS ON CHICKEN

SUMMARY—A study was conducted to determine effects of freezing, thawing, and subsequent holding at about 5°C on survival or growth of aerobic bacteria on chicken packaged with various materials. Production of fluorescent pigment, and extracellular proteinase and lipase activities were used as indices of the ability of the organisms to produce spoilage. Growth of bacteria was determined by colony counts. Assays for proteolysis were made by means of a dye binding method; lipolysis of chicken fat was determined by titration of free fatty acids. Fluorescent pigment formation was evaluated by means of a photofluorometer. The influence of availability of oxygen on proteolytic and lipolytic spoilage of poultry was studied after chicken was packaged with materials having high or low oxygen permeability and by vacuum packaging. Fluorescent pigment production, proteolytic and lipolytic spoilage of chicken stored at 5°C was directly related to the availability of oxygen provided by the packaging procedure. Bacterial numbers paralleled increases in biochemical indices of deterioration. Freezing of chicken at −29°C for 35 days followed by defrosting and refrigerated storage increased the proportion of biochemically active psychrophiles on the surface of the meat. Vacuum packaging generally limited amount of spoilage as measured by the criteria specified. When samples were analyzed after alternate freezing and thawing, total aerobic bacterial counts were only slightly different from those on chicken frozen continuously for 22–25 days.

INTRODUCTION

FREEZING of poultry and the use of this preservation method in connection with potential centralized packaging in the U.S.A. has stimulated much interest in effects of freezing and packaging methods on shelf life. However, at low temperatures, psychrophilic bacteria may survive and eventually grow sufficiently to cause spoilage. Of the psychrophiles, *Pseudomonas* species are the most important spoilage organisms of poultry (Ayres et al., 1950; Walker and Ayres, 1956; Barnes and Shrimpton, 1959; Nagel et al., 1960; and Ayres, 1960). These bacteria are usually strongly lipolytic and/or proteolytic (Witter, 1961) and these deteriorative actions may contribute to alteration of flavor. A water soluble fluorescent pigment, pyoverdine, is also produced by some species of *Pseudomonas* (Turfreijer, 1941), and may indicate spoilage of the poultry. High bacterial counts are associated with off-odor and slime formation on poultry. However, as Witter (1961) emphasized, even low levels of contamination with biochemically active psychrophiles may result in poor keeping quality of foods stored at low temperatures.

The influence of packaging materials on bacterial populations and shelf life of poultry was reviewed by Ayres (1966). Since typical spoilage organisms are highly aerobic, the permeability of materials to oxygen and the effect of exclusion of air on growth and biochemical activity of the bacteria were factors considered in the present study. In addition, the effects of freezing and subsequent refrigerated storage after thawing were investigated in relation to growth and certain metabolic functions of bacteria associated with spoilage of poultry.

MATERIALS & METHODS

CHICKEN WINGS were purchased at a retail store on the day the poultry was delivered to the store, and packaged and stored under conditions shown in Table 1.

Lipolytic and proteolytic activity, and fluorescent pigment production were properties selected as indexes of metabolic activity related to spoilage.

A "wash and rinse" method was used to extract extracellular proteolytic and lipolytic enzymes, and the fluorescent pigment, pyoverdine, liberated on the surface of the wings by the organisms producing spoilage. Wings were placed individually in polyethylene bags containing 50 ml of 0.01M phosphate buffer at pH 7, and washed by manually shaking the bags, in accordance with procedures suggested by the American Public Health Association (1966). The liquid drained from the bags, the "wash water," was centrifuged for 30 min at 9,000 rpm (10,300 R.C.F. × G) at 5°C to aid in separation of insoluble material. Low temperature facilitated solidification of lipid material and also retarded activity of extracted enzymes. The supernatant liquid was filtered twice through glass wool to separate low density insoluble material in the upper layer.

The fluorescence intensity of the supernatant was measured with a Coleman Model C photofluorometer (Coleman Instruments, Inc., Maywood, Ill.) equipped with B_1 and PC_2 filters. 6 ppm of fluorescein was used as standard. Procedures were similar to those described by Kraft and Ayres (1964).

The wash water was dialyzed in 0.01M phosphate buffer at pH 7.0 for stabilizing the pH of the extract during partial purification

Table 1—Packaging, freezing, and storage conditions for chicken

Packaging material	Oxygen permeability cc/100 sq in./ 24 hr at 1 atm	Packaging conditions	Storage conditions
195-LSAD cellophane[a]	100 (80–90% R.H.) 25°C	Atmospheric	Frozen at −29°C for 35 days, thawed at 5°C for 5 days, or stored at 5°C without freezing
Maraflex 7F[b] (polyester/Al foil/ polyethylene)	0–0.03 (90% R.H.) 38°C	Atmospheric or vacuum (28.5 in. Hg)	
Polyethylene[c] (0.0025 in.)	approx. 350 (0% R.H.) 25°C	Atmospheric	Alternate freezing at −29°C and thawing, or continuously frozen for 22–25 days

[a]E. I. DuPont de Nemours & Co., Wilmington, Del.
[b]American Can Co., Neenah, Wisc.
[c]Freez-Pak, Milwaukee, Wisc. Oxygen permeability calculated from data given by Landrock and Proctor, 1952.

at 2°C. Tests for proteolytic and lipolytic activities were then performed.

Proteolysis was estimated spectrophotometrically by the dye-binding method described by Hammond et al. (1966). 2 ml of sample and 1 ml of sterile skim milk were incubated for 6 hr at 35°C. The optimum time for reaction of enzyme and substrate was determined to be 6 hr, as measured by plotting proteolysis indexes against time. According to this method, the proteolysis index, modified for our experimental procedures, was calculated as As/(0.883 × A dye), where As = absorbancy of sample, A dye = absorbancy of dye before incubation, and 0.883 is the correction for dilution (15/18). An increase in the index indicates increased proteolytic activity.

Lipolysis was evaluated by titration of free fatty acids released from chicken fat. For this test, the dialyzed wash water was analyzed for enzyme activity with chicken fat as substrate, in accordance with the method of Rey et al. (1969). 4 ml of sample were employed for each test. Samples were shaken on a gyratory shaker at about 250 rpm during the 12 hr incubation period. This period was previously shown to give optimal results for enzyme-substrate reaction, and so it was used in subsequent analyses.

Bacteriological analyses of the chicken were performed using media and methods listed in Table 2.

In order to test the reliability of the methods used, 15 chicken wings were analyzed prior to the freezing and storage tests described. The chicken was packaged in aluminum foil and stored at −29°C for 21 days. The wings were then thawed at 5°C, packaged in polyethylene bags and stored in a display case at 5°C. Samples were analyzed at intervals for 6 days, and statistical analysis of variability of the assays was performed.

For the study of effects of alternate freezing and thawing, wings were packaged in polyethylene bags, frozen at −29°C and thawed for 24 hr at 5°C once, twice, or three times after 11, 16 and 22-25 days of storage respectively. After thawing at the time intervals specified, all companion samples were replaced into the freezer for subsequent analyses as indicated. Control samples were kept frozen continuously for 22-25 days. Tests on these samples included only total aerobic bacterial counts and counts of fluorescing bacteria.

Table 3—Test measurements and variability from chicken after freezing, thawing and refrigerated storage

Observations	Days stored at 5°C	Mean	Standard deviation	Standard error	Coefficient of variation
Numbers of bacteria per cm^2 × 1000					
Total aerobes	0	4.3	7.5	2.4	1.7
	3	170,000	60,000	19,100	0.35
	6	1,000,000	530,000	170,000	0.52
Proteolytic bacteria	0	1.1	2.1	0,7	1.9
	3	51,000	29,000	9,000	0.56
	6	530,000	380,000	12,000	0.72
Lipolytic bacteria	0	0.4	0.2	0.1	0.50
	3	72,000	39,000	12,000	0.55
	6	790,000	450,000	140,000	0.57
Fluorescent bacteria	0	0.2	0.3	0.1	1.2
	3	33,000	17,000	5,500	0.52
	6	310,000	260,000	83,000	0.83
Proteolysis indexes	0	—	—	—	—
	3	0.04	0.02	0.01	0.54
	6	0.24	0.07	0.02	0.32
Ml of 0.01 N acid released by enzymatic hydrolysis of fat	0	0.07	0.10	0.03	1.39
	3	0.97	0.43	0.14	0.45
	6	4.12	2.56	0.81	0.62
Photofluorometer readings of wash water	0	3.90	0.90	0.30	0.23
	3	10.30	2.00	0.60	0.20
	6	34.10	17.30	5.50	0.50

All determinations were made with triplicate samples.

RESULTS & DISCUSSION

BACTERIAL COUNTS, fluorescent pigment production, and measurements of proteolytic and lipolytic activity from the chicken examined for evaluation of test methods are presented in Table 3, along with analyses of variability. Considerable variation existed among samples analyzed during the storage period. The greatest variability occurred prior to storage of the chicken in the refrigerated display case. These differences, at least partly, reflected differences in initial loads of bacteria after freezing and thawing. However, it may be noted that all measurements of spoilage increased during holding. Information on the limits of variability of bacterial counts and biochemical activity of spoilage organisms was desirable for estimating reliability of the procedures. Such information is also of value for other studies of a similar nature. Deteriorative changes were pronounced after bacterial populations reached high levels on the poultry meat. Biochemical criteria of spoilage paralleled increases in total aerobes, lipolytic, proteolytic and fluorescent bacteria. It should be recognized that lipolytic and proteolytic activity as measurements of spoilage are relative. No comparisons were made between these chemical determinations and flavor evaluation, nor was any attempt made to assign values for these measurements to bacterial counts or onset of off-odor and slime. This approach would be an interesting study and might be worthy of further investigation.

Table 4 and Figures 1 and 2 compare the effect of frozen storage and subsequent refrigerated holding after thawing with that of continuous refrigeration at 5°C for 5 days. Chicken was packaged in accordance with methods described previously. Packaging materials and method of packaging (atmospheric vs. vacuum) produced distinct differences in numbers and types of bacteria recovered from the chicken, even when the variability previously determined was taken into account. When LSAD

Table 2—Media and methods for enumeration of bacteria

Organism types	Medium	Method, incubation
Total aerobic bacteria	Trypticase soy agar (BBL)	Swab of 10 cm^2 of surface,[a] pour plate, 15°C, 5 days
Fluorescing bacteria	Medium B (King et al., 1954)	Swab-surface plating, 15°C, 6 days
Lipolytic bacteria	Nile blue sulfate medium (Goldman and Rayman, 1952) with chicken fat substrate	Swab-plating as described by Alford and Steinle (1966) 15°C, 21 days
Proteolytic bacteria	Medium of Smith and Goodner (1958)	Swab-surface plating, 15°C, 4 days

[a]Swab technique described by Ayres et al., 1956.

Table 4—Effect of packaging materials and methods on spoilage of fresh and previously frozen and thawed chicken stored for 5 days at 5°C

		Packaging procedure		
		Atmospheric pressure		Vacuum-pack
Observations	Storage method	LSAD cellophane	Maraflex	Maraflex
No. of bacteria per cm^2 × 10,000				
Total aerobes	F.R.[a]	110,000	100,000	11,000
	F.D.[b]	372,000	31,500	24,700
Proteolytic bacteria	F.R.	63,000	54,000	7,000
	F.D.	238,000	24,000	10,000
Lipolytic bacteria	F.R.	54,000	43,000	7,600
	F.D.	232,000	26,000	7,700
Fluorescent bacteria	F.R.	6,000	11,000	400
	F.D.	58,000	5,000	5,000

[a]Freshly refrigerated (freshly cut chicken wings packaged and stored for 5 days at 5°C).
[b]Frozen-defrosted (freshly cut chicken wings packaged, frozen for 35 days at −29°C, defrosted and stored for 5 days at 5°C).

cellophane was used as the packaging material, values for all criteria of spoilage were markedly increased by freezing and frozen storage before holding at low temperature above freezing. Although total aerobic counts did not differ greatly, frozen-defrosted poultry meat stored at 5°C showed higher proportions as well as actual numbers, of proteolytic, lipolytic, and fluorescent bacteria than those observed when the chicken was not previously frozen. Earlier work (Rey et al., 1969) demonstrated that survival of psychrophiles in pure culture stored in the frozen state was proportional to the metabolic activity of the culture. On frozen poultry, the most metabolically active psychrophiles would be expected to survive better than less active organisms. After thawing of the meat, the surviving active bacteria would be able to grow at refrigeration temperatures with fewer competing organisms than those present on fresh chicken that had not been frozen. The permeability of LSAD cellophane ensured sufficient oxygen for the active survivors to grow to high numbers and to perform their metabolic functions. By all criteria given in Table 4 and Figures 1 and 2, with the environmental conditions provided by the highly permeable cellophane film, spoilage was more pronounced with frozen-defrosted poultry than with the fresh product on refrigerated storage.

Contrary to the results obtained for chicken packaged with LSAD cellophane, when Maraflex film was used at atmospheric pressure, bacterial growth was more extensive on fresh chicken after refrigeration than on frozen defrosted poultry. However, increased percentages of proteolytic, lipolytic and fluorescent bacteria present on the meat previously held in the frozen condition

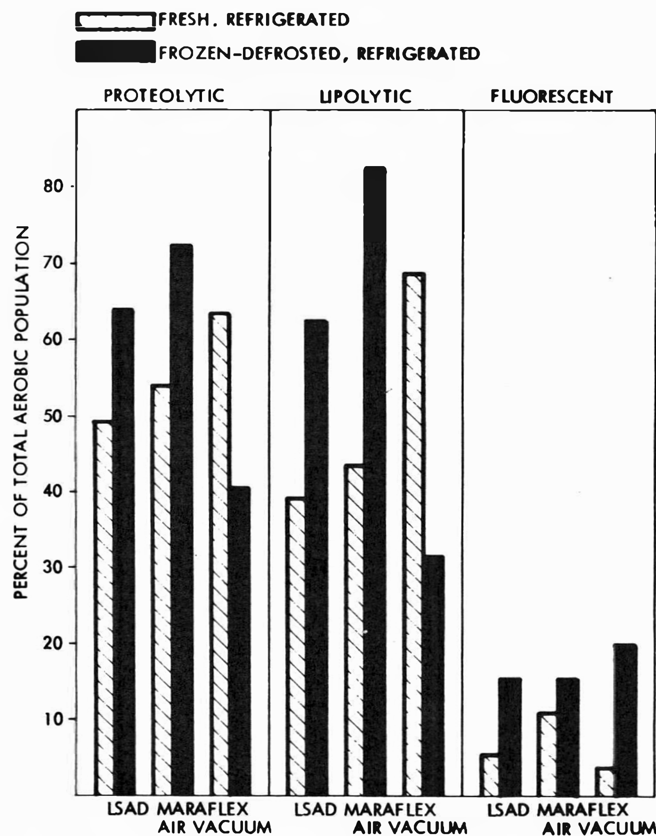

Fig. 1—Effect of freezing, packaging materials and methods on percent of spoilage organisms recovered from chicken stored for 5 days at 5°C.

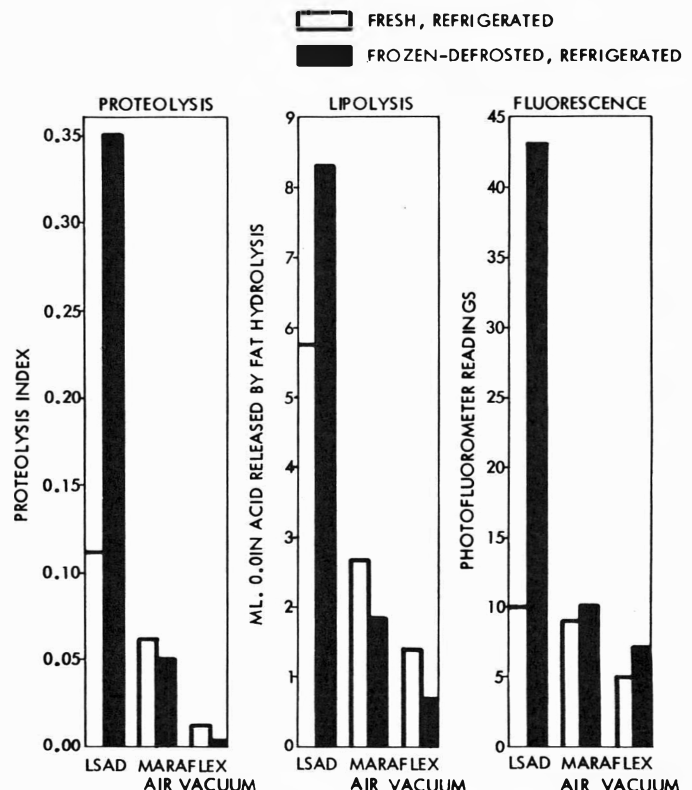

Fig. 2—Effect of freezing, packaging materials and methods on biochemical indexes of spoilage of chicken stored for 5 days at 5°C.

indicated that freezing caused the same type of modification of the bacterial flora as it did when LSAD cellophane was used (Fig. 1). The most metabolically active organisms survived to a greater extent after freezing than did less active bacteria. The oxygen available in the Maraflex packages was probably depleted at a more rapid rate with the frozen-defrosted chicken during subsequent refrigerated storage than it was with freshly refrigerated chicken, due to the higher proportion of biochemically active psychrophiles. More rapid exhaustion of oxygen possibly limited growth on the frozen-defrosted meat. Conversely, the microflora of the fresh samples, being composed of a greater percentage of less active organisms, was able to multiply to greater numbers while consuming the oxygen available in the package at a slower rate. Reduction of oxygen tension in the packages of previously frozen chicken apparently affected enzyme activity of lipolytic bacteria as well as growth of these organisms, but did not greatly influence proteolytic activity. Morihara (1963) observed that low oxidation-reduction potentials did not influence activity of the protease of *Pseudomonas* that he studied. A similar situation may have existed during growth of psychrophiles on samples packaged with the low oxygen-permeable Maraflex laminate in the present work.

Bacterial growth was only slightly greater on frozen-defrosted than on freshly refrigerated poultry when oxygen was excluded from the environment by packaging with Maraflex film under a vacuum (Table 4). Except for fluorescent bacteria, differences were not great. The percentages of different types of bacteria were also modified by frozen holding, but the trends were opposite to those described for the previous packaging methods. The combined effects of freezing, thawing, and exclusion of oxygen resulted in lowering the proportions of proteolytic and lipolytic bacteria of the total aerobic microflora. These organisms may have had an increase in nutritional requirements. The morphology of colonies on agar plates inoculated with organisms recovered from the chicken is worthy of mention as an effect of lack of oxygen in the packages. Although the plates were incubated in air, samples packaged in Maraflex under vacuum yielded only minute punctiform colonies, suggestive of nonlethal injury. Also, the flora was apparently composed of a smaller proportion of aerobic spoilage organisms with high biochemical activity than observed when chicken was packaged with either material at atmospheric pressure. Upadhyay and Stokes (1961) observed that elimination of oxygen decreased both rate and extent of growth of facultative psychrophiles, indicating nonlethal metabolic injury. Straka and Stokes (1959) reported that nonlethal metabolic injury caused an increase in nutritional requirements of psychrophilic bacteria. However, for vacuum-packaged samples, the observation that the actual counts on frozen-defrosted poultry were at least as great, and sometimes slightly higher, than on freshly refrigerated chicken may be explained by the action described by Mossel and Ingram (1955). They stated that free fluids rich in nutrients are withdrawn from tissues by freezing, favoring microbial development on foods. Nonproteolytic and nonlipolytic bacteria injured by lack of oxygen may have been provided with readily available nutrients on frozen defrosted meat. These organisms were apparently able to multiply to form a greater proportion of the total aerobic flora, since the more biochemically active psychrophiles suffered from impairment of enzyme production or activity.

Greater populations of fluorescent bacteria, both in numbers and percentage of total aerobes, developed on frozen-defrosted samples than on meat that was not frozen before refrigerated storage when Maraflex was used with vacuum packaging (Table 4 and Fig. 1). Photofluorometer readings, although differing only slightly, indicated the same trend (Fig. 2). Again, availability of nutrients after freezing and thawing may have reduced the need for pyoverdine as an accessory respiratory pigment. The pigment that was not utilized in respiration of the cells was secreted onto the skin surfaces of the chicken. Under reduced oxygen pressure, with comparatively little withdrawal of chicken tissue fluids on unfrozen meat, the organisms used pyoverdine in their metabolism to a greater extent. The fluorescent pigment, therefore, was secreted only sparingly on the chicken.

Regardless of whether the poultry was frozen before refrigerated storage or not, vacuum packaging with the highly impermeable Maraflex film resulted in reduction in numbers and activity of spoilage organisms when compared with other methods.

Sulzbacher (1952) used pure culture inocula with ground meat stuffed in test tubes and determined growth of the organisms in the refrigerated or frozen defrosted meat. He concluded that frozen meat was no more perishable after thawing than was fresh meat. Results of his work with meat in test tubes were similar to the present findings for counts of total aerobes on chicken packaged with Maraflex film in an atmosphere of air. Sulzbacher made no determinations of proteolytic or lipolytic organisms. However, in an earlier investigation Sulzbacher (1950) observed an increase in lipase-producing bacteria on pork after frozen storage for 12 wk. Kitchell and Ingram (1956) attempted to repeat Sulzbacher's work with a pure culture of *Pseudomonas multistriata* inoculated on fresh horse meat and pig muscle and on similar meat subsequently frozen. The inocula were added "in numbers sufficiently large to make negligible the contribution of the miscellaneous natural flora to the total counts." No differences in the storage life of the meats were observed. As stated by Kitchell and Ingram "none of these effects of freezing (generation time, duration of lag phase) on the growth of *P. multistriata* were at all comparable to those observed by Sulzbacher in his apparently similar tests."

It should be emphasized that, unlike the present study, the natural flora of the meat was overcome by a pure culture inoculum in the work of Kitchell and Ingram (1956). Also, in contrast to the present work, no investigation was made of proteolytic or lipolytic spoilage organisms, or of such activity as determined by chemical analyses.

Consideration must be given to earlier observations made in this laboratory (Rey et al., 1969) that psychrophilic *Pseudomonas* may differ considerably in their metabolic spoilage activity; this may partly explain the discrepancy reported by Kitchell and Ingram (1956) between some of their results and those of Sulzbacher (1952). When selected pure cultures are used as inocula at levels which form a major portion of the microflora, differences may be expected when compared with spoilage caused by naturally occurring contaminants present in their usual populations. As shown in our study, total counts alone are not as indicative of spoilage as are numbers and proportions of proteolytic and lipolytic bacteria or lipolysis and proteolysis caused by such bacteria. As mentioned earlier, the most metabolically active spoilage organisms may be expected to survive freezing to a greater extent than less active psychrophiles. The survivors subsequently could cause deterioration of poultry meat after thawing; such activity might be enhanced because fewer competing bacteria would be present than on unfrozen chicken (Rey et al., 1969).

In other work in our laboratory, the effects of alternate freezing and thawing were compared with continuous frozen storage and a single thaw. Counts of total aerobes were within the limits of variation observed and did not differ greatly during frozen holding when the poultry was thawed once, twice, or three times as compared with prolonged frozen storage for the entire interval. Total aerobes were only slightly greater after the

chicken was frozen and thawed two or three times than after a single freezing and thawing period for either 11 or 22-25 days. These data suggest that psychrophilic populations are not stimulated in growth to any appreciable degree by practices permitting thawing to occur with subsequent re-freezing. Fluorescent bacteria did not change appreciably in numbers by alternate freezing and thawing of the chicken.

From these studies, it may be stated that there is no simple relation between packaging procedures, frozen storage and spoilage of packaged chicken. However, if centralized packaging of frozen poultry is to be successful, consideration must be given to the oxygen permeability of the packaging material; films that allow free exchange of oxygen should be avoided when poultry is held under frozen conditions prior to refrigeration during normal marketing periods.

CONCLUSIONS

THE FOLLOWING conclusions were derived: (1) frozen storage causes a change in the microflora of poultry meat which results in an increase in proportion of biochemically active psychrophiles; (2) freezing of poultry meat prior to refrigerated storage enhances development of spoilage if packaging films highly permeable to oxygen are used; (3) a packaging film impermeable to oxygen reduces growth and proteolytic and lipolytic activities of psychrophilic bacteria on poultry; this effect is increased by vacuum packaging; and (4) uncooked poultry may be frozen and thawed several times without an appreciable increase in aerobic bacteria over that resulting from continuous frozen storage.

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BASIC SCIENCE

NELL I. MONDY, CONNIE METCALF and R. L. PLAISTED
Graduate School of Nutrition, Department of Human Nutrition and Food
New York State College of Human Ecology, Cornell University, Ithaca, N.Y.

POTATO FLAVOR AS RELATED TO CHEMICAL COMPOSITION
1. Polyphenols and Ascorbic Acid

SUMMARY—A study was made on the relationship of flavor of potatoes to their phenolic and ascorbic acid content. Material for the study was from two main sources: (1) clones that are hybrids between varieties of Solanum tuberosum subsp. tuberosum grown in New York and varieties of Solanum tuberosum subsp. andigenum grown in Peru; (2) established commercial American varieties such as Ontario, Pontiac, and Katahdin. Bitterness and astringency were selected as the character notes most related to phenolic content. A significant correlation was found between phenolic content and bitterness and a highly significant correlation between phenolic content and astringency. No significant correlation was found between ascorbic acid content and flavor.

INTRODUCTION

POTATOES are produced in every state and are harvested every month of the year in the United States. With a yield per acre greater than most crops its sustaining power depends primarily on its acceptability. A variety of high quality should have acceptable qualities of flavor, texture and color when cooked. In this paper we deal largely with the flavor aspect of potato quality.

The word "flavor" has been reserved to mean the overall sensation resulting from the impact of food on the chemical sense receptors in the nose, mouth and throat. Taste is perceived in the mouth by taste buds and odor is detected by olfactory epithelium in the nose and is called odor when the volatile molecules producing the sensation are inhaled directly through the nose. When the odorous molecules pass from the mouth to the nose, via the inner passages, during the eating process then the complex sensation of taste and odor is called flavor.

Attempts to relate flavor of substances to their chemical composition are not new, but the subject is a complex one for the palate is very sensitive to what seems to the chemist to be only minor variations in structure. The generlizations that inorganic salts are salty, sugars sweet, acids sour, and alkaloids bitter are not always reliable and help very little in predicting the taste of the multitude of compounds that do not fall into one of these categories. By focusing on a series of closely related substances it is possible to perceive relations between their taste and the presence or absence of certain structural features (Horwitz, 1964).

As early as 1926 Salaman (1926) suggested that the strength of potato flavor was a varietal characteristic, whereas Rathsack (1935) believed that the strength of the potato flavor was related to the mineral content of the soil, especially the ratio of potassium to nitrogen. Kroner (1944) suggested that an inferior taste resulted when excess sugar accumulated in the badly-stored tubers, and Hilton (1951) attributed bitterness to solanine. Kroner and Wegner (1942) analyzed an ether extract of steam-volatile constituents of potatoes and detected an oil-containing pentanol, esters, fatty acids and a high-boiling sulfur compound. Wegner (1949) suggested that a phenolic compound was also present in the oil extracted from potatoes in this way.

Self et al. (1963a, b) and Gumbmann and Burr (1964) identified some of the sulfur compounds in the volatiles of cooked potato and found that methanethiol and dimethyl disulfide together constituted 90% of the total sulfide. These workers studied four different varieties of potatoes during cooking and concluded that the varieties differed noticeably in the content of volatiles.

It is also possible that nonvolatile compounds such as phenols also contribute to flavor. Phenolic compounds are important constituents related to taste and enzymatic darkening in several different foods (Swain, 1962). Differences in relative astringency of fruits such as peaches and persimmons have been shown to be directly related to differences in tannin or phenolic content (Guadagni and Nimmo, 1953). Blake and Davidson (1941) classified a number of different peach varieties according to tannin content and found some of these so high in tannin that they were regarded as relatively unpalatable. The phenolic content of potatoes is relatively high, particularly in the periderm and cortex areas (Mondy et al., 1959).

Since potato varieties are known to differ widely in their phenolic content and also in their flavor, work was undertaken in our laboratory to determine the relationship of phenolic content to certain flavor characteristics.

MATERIALS & METHOD

Source of material

Potatoes for the study were selected from two main sources: (1) clones that are hybrids between varieties of *Solanum tuberosum* subsp. *tuberosum* grown in New York and varieties of *Solanum tuberosum* subsp. *andigenum* grown in Peru; and (2) established commercial American varieties such as Ontario, Pontiac, and Katahdin. Clones were chosen since it was assumed they would have considerable genetic diversity and offer opportunity for selection of a range of expression of phenols and flavor. The varieties selected have been used in our laboratory for several years becuuse of their diversity of phenolic content and their different tendencies toward enzymatic darkening.

During the first year of the study, 50 clones were analyzed for total phenolic content and grouped into "high" and "low" categories according to their relative phenolic content. Four clones from each of these groups were selected to be grown and used for the second year of the study. Those relatively

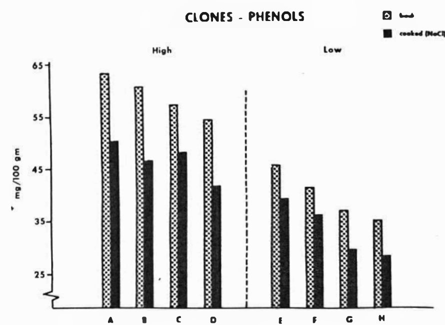

Fig. 1—Phenolic content of cortex tissue of fresh and cooked clones.

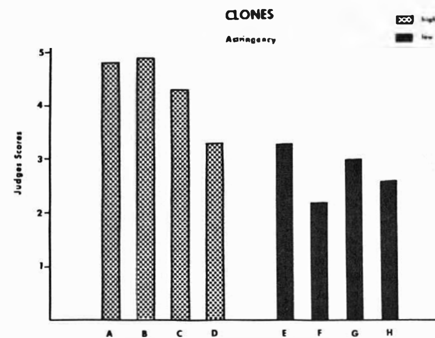

Fig. 2—Flavor scores for astringency. Clones are arranged in order of their decreasing phenolic content. Scale for astringency: 1—extremely weak to 7—extremely pronounced.

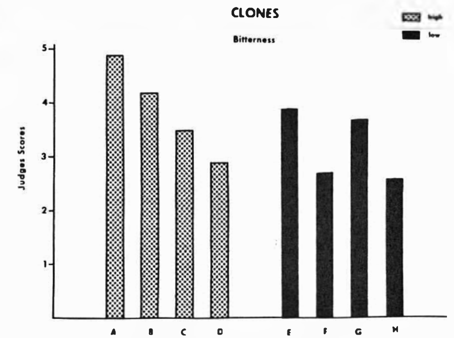

Fig. 3—Flavor scores for bitterness. Clones are arranged in order of their decreasing phenolic content. Scale for bitterness: 1—extremely weak to 7—extremely pronounced.

high in phenolic content were labeled A–D and those lower designated by the letters E–H.

The clones were grown by the Department of Plant Breeding in experimental plots near the Ithaca area. The three varieties were grown at the Cornell Research Farm in Riverhead, Long Island. The varieties, as well as the clones, were harvested 20 wk after planting and stored at 40°C until analyzed for phenolic content and evaluated for flavor.

Phenolic content was determined using the method of Rosenblatt and Peluso (1941) which uses tannic acid as the standard. Four tubers of each group were selected at random for each extract and duplicate extracts were made for each group used in the phenol analyses. Cortex tissue and the peridem were sampled from bud to stem end of the tuber. A 50g sample was blended with 150 ml of 95% ethanol for 5 min. The total extract was measured, filtered, and 35 ml of the filtrate used for phenol determinations. Since this method is not specific for phenols but also includes ascorbic acid, corrections were made for ascorbic acid. Potato tissue was extracted with meta-phosphoric acid and analyzed for ascorbic acid by the indophenol dye method (Nelson and Somers, 1945) simultaneously with the analyses for phenols. Corrections were made for ascorbic acid.

Flavor evaluation

Whole, unpeeled tubers selected at random from each group were boiled for 40 min in a 3.5% NaCl solution. The cooked potatoes were drained, cooled to room temperature, the peels removed and the cortex area separated from the pith, mashed, and served to a panel of trained judges.

Preliminary studies were made to determine if phenolic content affected flavor. Flavor was first evaluated using the Flavor Profile method which attempts to analyze and define flavor. During the preliminary testing, agreement was reached that the character notes, "bitterness" and "astringency", were the ones most closely related to phenolic content. Astringency is generally associated with the puckering sensation caused by tasting unripe fruits and bitterness is associated with the sensation observed on the back of the tongue and throat. These character notes were then selected as the ones to be evaluated in the study. The intensity of each character note was rated by a panel of six trained judges using a seven-point scale. The scale ranged from 1 (extremely weak) to 7 (extremely pronounced) in intensity. The judges were selected from a group of graduate students, faculty, and technicians in the Department of Food and Nutrition and were selected on the basis of their sensitivity to the character notes to be studied.

Preparation of thin-layer chromatograms

The alcoholic extract prepared for total phenolic analyses were used for the thin-layer chromatograms. Activated Eastman chromatogram plates were used. The standards, chlorogenic acid and caffeic acid, as well as the potato extracts were spotted using a 10 λ micro-pipette, and the chromatograms were developed for 6 hr and then viewed under ultraviolet light. The developing solvent used was a mixture 10:2:8 of n-butanol:glacial acetic acid:distilled water.

RESULTS & DISCUSSION

Studies with clones

The clones were very irregular in shape, often had unusual bluish or purple markings, deep eyes, and frequently a large and highly irregular cortex.

Phenols. The eight clones studied during the second year of the experiment were analyzed in the fresh state for phenolic content and arranged in the order of their decreasing phenolic content (Fig. 1). Each clone was assigned a letter A through H. There was no significant variation in the phenolic content between replicate samples of the clones.

Cooking of the clones resulted in a decreased phenolic content as shown in Figure 1. The greatest decrease occurred in those clones of highest phenolic content, and with the exception of clone C, the cooked clones remained in the same sequence with respect to phenolic content as the fresh clones. The "high" clones showed an average loss of 21% in phenolic content during cooking, whereas, the "low" clones exhibited only an 11% loss, but the latter continued to have a much lower phenolic content than the "high" group.

Flavor. The cooked clones were submitted to a panel of trained judges who scored them for the character notes of astringency and bitterness (Fig. 2, 3). Phenolic content was positively correlated with both bitterness and astringency. The correlation coefficient for bitterness was 0.72 and was significant; that for astringency was 0.82 and was highly significant. The judges frequently described a lingering, burning sensation in the throat following the sampling of certain potatoes. The specific phenols involved in flavor have not been elucidated, but we are presently attempting to study these. Chlorogenic acid is one of the chief phenols found in potatoes and upon hydrolysis yields caffeic and quinic acids both of which are insoluble in cold water but more readily soluble in hot water. Chlorogenic acid is located largely in the periderm and cortex areas and is hydrolyzed during the cooking of the tuber (Fig. 4).

Factors related to bitterness in citrus fruits have been studied extensively (Horowitz, 1964). Some of the basic chemical structures have been elucidated, and the aglycones of all the bitter compounds were found to be nonplanar, whereas, in each of the tasteless flavonoids which one might expect to be bitter, the aglycone portion of the molecule was highly conjugated and planar in shape. Studies of this nature need to be done with potatoes.

Ascorbic acid. The clones that were higher in phenolic content also tended to be higher in ascorbic acid (Fig. 5). The same trends were observed following cooking, but there was great variation within samples and the differences were not significant. The judges were unable to distinguish differences in flavor due to differences in ascorbic acid content.

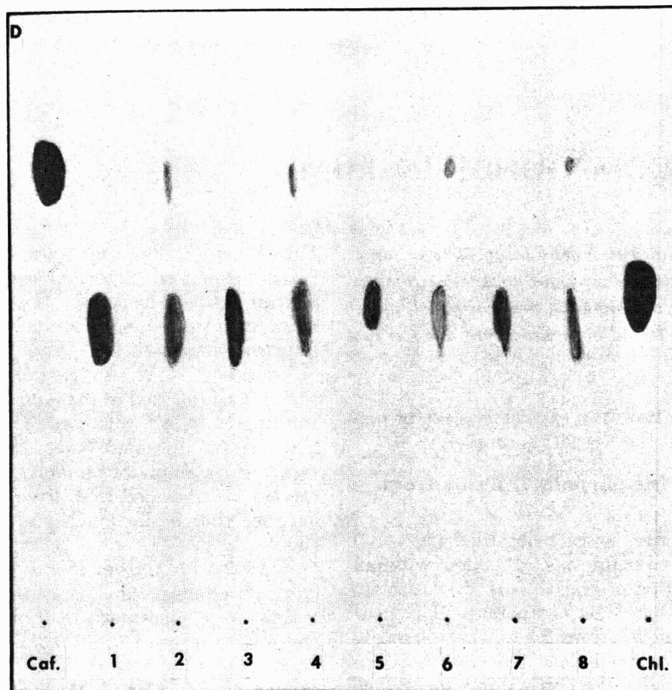

Fig. 4—Chromatogram of cortex tissue of fresh and cooked clones. Caf-caffeic acid; 1-fresh; 2-clone A cooked; 3-clone B fresh; 4-clone B cooked; 5-clone G fresh; 6-clone G cooked; 7-clone H fresh; 8-clone H cooked; Chl-chlorogenic acid.

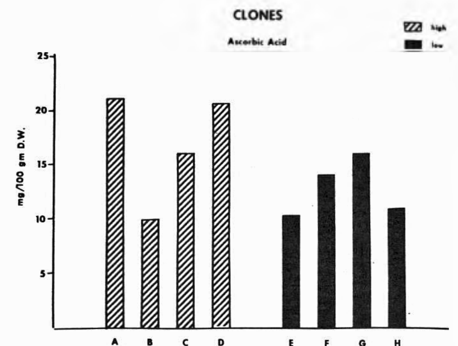

Fig. 5—Ascorbic acid content of cortex tissue of clones.

Studies with varieties

Results of studies on the three varieties are given in Figure 6. The Ontario variety was the highest in phenolic content, Pontiac the lowest, and Katahdin was intermediate. These differences were highly significant. The same trends were observed in the varieties following cooking, although the phenolic content in each was markedly lowered by cooking.

Earlier work in our laboratory had shown that the Ontario variety was most susceptible to darkening or "black spot", whereas Pontiac was the least and Katahdin was intermediate. Darkening was positively correlated with phenolic content.

Flavor scores for astringency and bitterness showed a significant positive correlation with phenolic content.

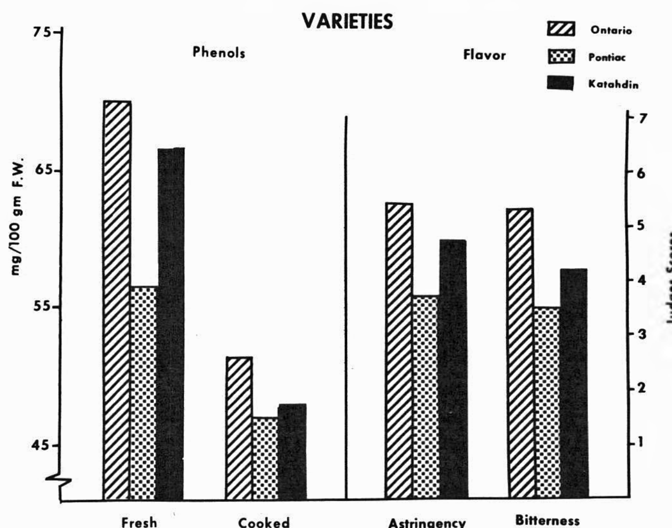

Fig. 6—Phenolic content and flavor scores for three varieties of potatoes.

Comparison of clones with varieties

Comparison of the studies with these particular varieties to those with clones showed that generally the clones were lower in phenolic content and scored lower in both astringency and bitterness than the varieties.

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R. TIMMER, R. ter HEIDE, H. J. WOBBEN and P. J. de VALOIS
N.V. Chemische Fabriek "Naarden," Naarden, The Netherlands

PHENOLIC COMPOUNDS IN RUM

SUMMARY—A study was made of the phenolic fraction of a Jamaica rum containing 75% ethanol. Identification of the phenols was performed by coupled gas chromatography and mass spectrometry and further by infrared spectrometry and thin layer chromatography. Phenol, p-ethylphenol, o-cresol, p-cresol, guaiacol, p-ethylguaiacol and dihydroeugenol were found in Jamaica rum for the first time; the presence of p-methylguaiacol and eugenol was confirmed.

INTRODUCTION

DATA about the composition of the phenolic fraction of rum have scarcely been reported in the literature. Raghunatha Rao (1943) isolated salicylic acid from the fusel oil of cane molasses. Maarse and ten Noever de Brauw (1966) examined Jamaica rum and identified the phenols eugenol and 4-methylguaiacol. Other investigators identified phenolic acids and aldehydes in cognac and whiskey stored in oak barrels for a number of years. It is assumed these are formed by alcoholysis of the lignin in the wood (Otsuka et al., 1965). Therefore it is very likely these compounds also occur in rum which is stored the same way.

This paper deals with the analysis of the volatile phenolic substances isolated from Jamaica rum.

EXPERIMENTAL

Isolation of the phenolic fraction from rum

2 liter of rum was brought to pH 13, with a 4% sodiumhydroxide solution and the volume of the mixture was reduced to approximately 100 ml in a rotary vacuum evaporator on a water bath at about 50°C. The residue was extracted with methylene chloride (5 × 15 ml) to remove the nonacid (phenolic) substances; the emulsions were broken down by centrifugation. The aqueous layer was brought to pH 10 by means of carbon dioxide. At this pH, the phenolic acids are not yet in the free state.

The liberated phenolic compounds were then extracted with methylene chloride (5 × 15 ml). After drying over anhydrous Na_2SO_4, the extract was concentrated by distillation under atmospheric pressure (using a Vigreux column) until a residue of about 1 ml remained.

In order to obtain more material for identification based on infrared spectrometry and thin layer chromatography, the phenolic fraction was also isolated from rum extract.

Preparation of rum extract

100 liter of diluted rum (15% alcohol) was extracted with pentane/ether (2:1) for 24 hr in a modified Kutscher-Steudel extraction apparatus. The rum was diluted in order to obtain a minimum amount of alcohol in the extract. After drying over Na_2SO_4, the solvent was removed from the extract by careful distillation through a Vigreux column.

Isolation of the phenolic fraction from rum extract

20 ml of rum extract obtained from 100 liter of diluted rum was extracted with an aqueous solution of NaOH (pH 13) to isolate the acid and phenolic compounds. The neutrals were removed from the aqueous layer by extraction with methylene chloride (5 × 15 ml) and finally the procedure mentioned before was applied.

Separation and identification

Optimal conditions for gas chromatographic separation of the phenolic fraction from rum were determined on a Becker instrument (Delft, The Netherlands) equipped with two flame ionization detectors. Two columns were employed of 1/8 in. o.d. stainless steel and 2m in length. The first was packed with Carbowax 20M (5% by wt) on silanized Embacel (60-80) mesh). The second column was packed with Apiezon-L (20% by wt) on the same support. The other operating parameters were as follows:

Injection port temperature	230°C
Column temperature	150°C
Carrier gas flow (N_2)	9 ml/min
Hydrogen flow	30 ml/min
Air flow	300 ml/min

Using the above mentioned operating conditions (except the type and amount of carrier gas), a sample of the phenolic fraction isolated from rum was analyzed on a Varian Aerograph Model 1220 gas chromatograph. The effluent of the column was admitted via a Watson-Biemann molecular separator to the inlet of a single focusing 90° magnetic field mass spectrometer (Varian-MAT, CH 5, Bremen, Germany).

In place of N_2 as carrier gas, helium, which is not ionized in the ionization chamber working at 20 eV, was used. The total ionization current obtained from this chamber is recorded as a gas chromatogram. The mass spectra are obtained from the material entering the other ionization chamber working at 70 eV.

Samples for further identification by thin layer chromatography and infrared spectrometry were obtained by preparative gas chromatography of the phenolic fraction isolated from rum extract. The separation was carried out on a F&M Model 1520 gas chromatograph equipped with a thermal conductivity detector using a 1/4 in. o.d. stainless steel column, 2m long, filled with diethyleneglycolsuccinate (20% by wt) on Embacel support (60-80 mesh). The carrier gas (H_2) flow was 75 ml/min and the oven temperature was programmed from 120-200°C at a rate of 2° C/min. The emerging compounds were collected.

Thin layer chromatography was carried out according to the method described by Klouwen and ter Heide (1962). The collected phenols were splotted on Silica Gel G plates (Merck) using chloroform, hexane-acetic acid (95:5 v/v) and hexane-pyridine (95:5 v/v) as developing solvents. The TLC-chamber was lined with filter paper and allowed to equilibrate with the developing solvents for 30 min to achieve complete saturation.

RESULTS AND DISCUSSION

FIGURE 1 shows a gas chromatogram of the phenolic fraction isolated from

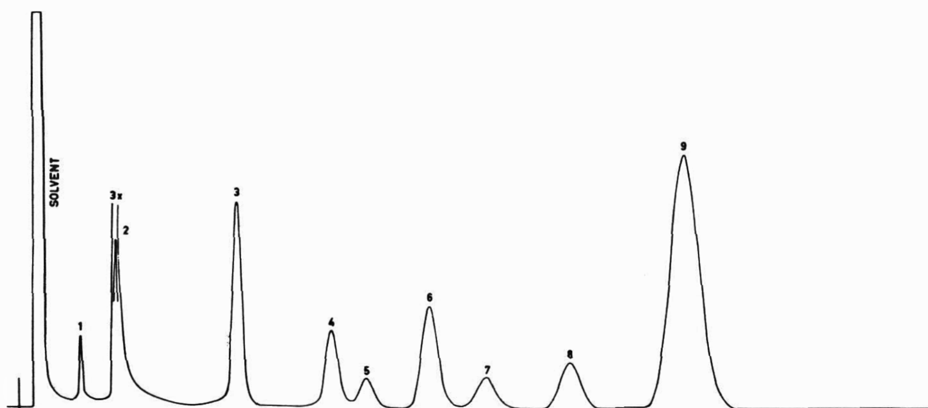

Fig. 1—Gas chromatogram of the phenolic fraction of rum. Column, 2m × 1/8 in. o.d., packed with 5% Carbowax 20M on silanized Embacel (60-80 mesh); temperature 150°C.

Table 1—Phenolic compounds identified in rum

Peak no.	Compound	Relative retention time[a]	Method of identification[b]				Ref.[c]
			GLC	MS	TLC	IR	
3	Guaiacol	0.52	+	+	−	+	−
4	p-Methylguaiacol	0.75	+	+	+	+	+
5	Phenol	0.84	+	+	+	+	−
	o-Cresol	0.84	+	+	−	−	−
6	p-Ethylguaiacol	1.00	+	+	+	+	−
7	p-Cresol	1.13	+	+	+	+	−
8	Dihydroeugenol	1.34	+	+	−	+	−
9	Eugenol	1.64	+	+	+	+	+
	p-Ethylphenol	1.64	+	+	−	+	−

[a]p-Ethylguaiacol = 1.00; gas chromatographic conditions are those mentioned in Figure 1.
[b]Identified by means of GLC, MS, TLC and IR (+ = yes, − = no).
[c]Previously mentioned in the literature (+ = yes, − = no).

Jamaica rum and recorded on the Becker gas chromatograph using Carbowax 20M as stationary phase. Subsequent analysis by GLC on Apiezon-L showed that peaks 5 and 9 consist of two components. Peak 1 had a mass spectrum identical with that of 2-methyl-5-acetylfuran, but no agreement with GLC retention time could be obtained. Work is in progress to identify this component. Peak 2 was identified by means of GLC and MS as dimethylsulfoxide, a polar compound, which was evidently not removed from the aqueous phase by extraction with methylene chloride.

All the other compounds present appeared to be phenolic. They were identified by comparison of GLC retention times and mass spectra with those of reference samples. In most cases the identification was further confirmed by infrared spectrometry and thin layer chromatography. Table 1 summarizes the phenolic compounds found, their relative retention times and the techniques used for their identification. Seven phenolic compounds have not been reported previously as occurring in rum or fusel oils.

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H. J. WOBBEN, R. TIMMER, R. ter HEIDE and P. J. de VALOIS
N.V. Chemische Fabriek "Naarden," Naarden, The Netherlands

NITROGEN COMPOUNDS IN RUM AND WHISKEY

SUMMARY—The nitrogen compounds from a Jamaica rum and a Scotch blended whiskey were isolated and identified by gas chromatography and mass spectrometry. In both spirits were found: pyridine, α-picoline, β-picoline, thiazole, methylpyrazine, 2,5-dimethylpyrazine, 2,6-dimethylpyrazine, 2,3-dimethylpyrazine, 2-methyl-6-ethylpyrazine, 2-methyl-5-ethylpyrazine, trimethylpyrazine and 2,5-dimethyl-3-ethylpyrazine. 2-Methyl-6-vinylpyrazine was detected only in rum. Nine compounds in whiskey and eleven in rum have not previously been reported as constituents of these drinks or fusel oils.

INTRODUCTION

ONLY A FEW data on basic compounds of whiskey and rum have been reported in the literature. Chapman and Hatch (1929) analysed the nitrogen compounds of a fusel oil from molasses and identified trimethylpyrazine, tetramethylpyrazine and possibly a diethylpyrazine. The last-mentioned pyrazine, probably 2,5-substituted, and pyridine were also isolated from the fusel oil of beet molasses by Taira (1936). A review of approximately 400 compounds identified in whiskey and some other beverages produced by yeast fermentation was given by Kahn (1969); concerning the nitrogen compounds in whiskey, pyridine, trimethylamine, 2,5-dimethylpiperazine, 2,5-dimethylpyrazine, tetramethylpyrazine and trimethylpyrazine were mentioned.

This paper presents the results of an investigation into the basic fractions isolated from a Scotch whiskey and Jamaica rum.

EXPERIMENTAL

Isolation of the basic fractions

To 3 liters of rum and 6 liters of whiskey 4N hydrochloric acid was added to pH 1. The volume of the acidified mixtures was reduced to approximately 100 ml in a rotary vacuum evaporator on a water bath at about 50°C. The residues were extracted with methylene chloride (5 x 15 ml) to remove the nonbasic material; the emulsions were broken down by centrifugation.

The aqueous layers were made alkaline with NaOH, and the liberated nitrogen compounds were subsequently extracted with methylene chloride (5 x 15 ml). After drying over anhydrous Na_2SO_4 the solutions were concentrated by careful distillation through a Vigreux column until a residue of about 1 ml remained.

Identification

Gas chromatographic analyses of the basic fractions were carried out using a Becker instrument (Delft, The Netherlands) equipped with a hydrogen flame detector and fitted with a 1/8 in. o.d. stainless steel column 2m in length and packed with Carbowax 20M (20% by wt) on Embacel support (60-80 mesh). The other operating conditions were as follows:

Injection port temperature	230°C
Column temperature	100°C
Carrier gas flow (N_2)	9 ml/min
Hydrogen flow	30 ml/min
Air flow	300 ml/min

In order to obtain comparable gas chromatograms, the same operating parameters with respect to dimensions, temperature and packing of the column were applied to a Varian Aerograph Model 1220 gas chromatograph. Instead of nitrogen as carrier gas, helium was used because the effluent from the column was admitted via a Watson-Biemann molecular spearator to the inlet of a single focusing 90° magnetic sector field mass spectrometer (Varian-MAT, CH 5, Bremen, Germany). The total ionization current obtained from the ionization chamber working at 20 eV (He is not ionized) is recorded as a gas chromatogram. The mass spectra are obtained from the material entering the other ionization chamber operating at 70 eV. Sufficient amounts of pure material could not be trapped from the basic extracts for IR- or NMR analyses because of the very low concentrations (ppm-ppb range) of the nitrogen compounds in these spirits.

RESULTS & DISCUSSION

FIGURES 1 and 2 show chromatograms of the basic fractions of rum and whiskey, respectively, recorded on the Becker gas chromatograph. The mass spectra of some components (peaks 1, 2, 7 and 12 in rum and 1, 7, 12 and 18 in whiskey) were too weak or insufficiently separated to allow interpretation. Some other components in rum and whiskey (peaks 17, 22 and 23) appeared to be nonbasic material i.e. furfural, dimethylsulphoxide and 5-methylfurfural, respectively. Water-solubility accounts for the presence of these compounds in the basic fractions.

The same applies to the peaks 21 and 24 in rum and whiskey. However, the structures of these nonbasic compounds could not be elucidated due to a lack of

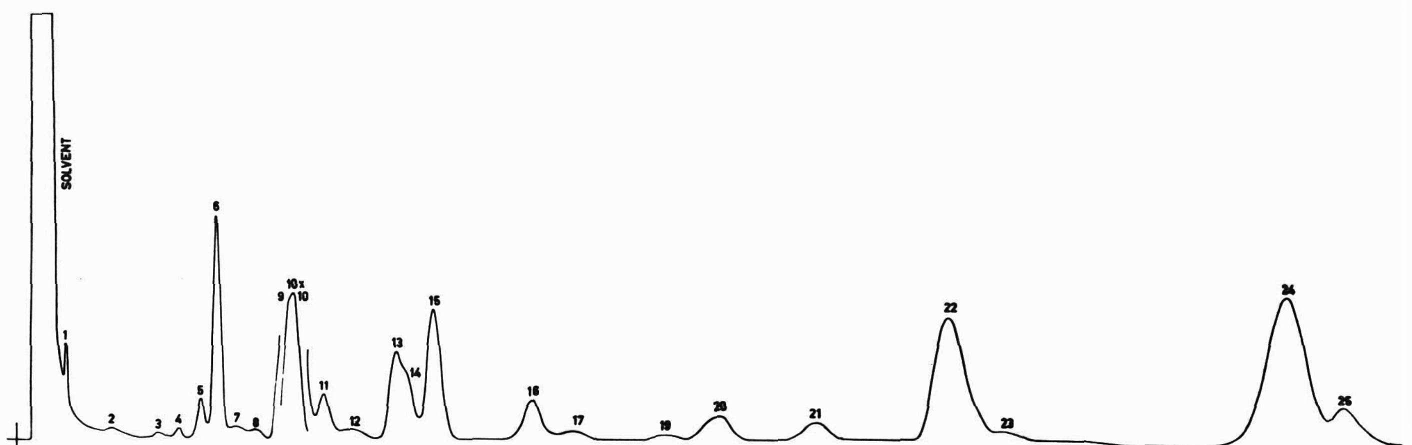

Fig. 1—Gas chromatogram of the basic fraction of rum. Column, 2m × 1/8 in. o.d., packed with 20% Carbowax 20M on Embacel (60-80 mesh); temperature 100°C.

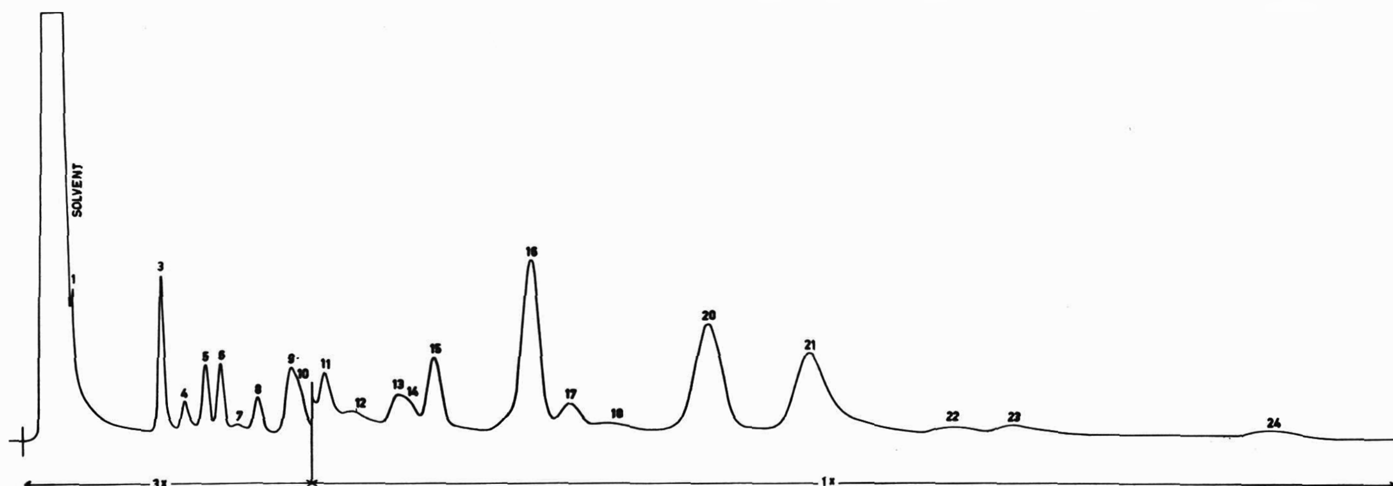

Fig. 2—Gas chromatogram of the basic fraction of whiskey. Operating conditions cf. Figure 1.

Table 1—Nitrogen compounds identified in rum and whiskey

Peak no.	Compound	Relative retention time[a]	Rum: Method of identification[b] GLC	Rum: Method of identification[b] MS	Rum: Ref.[c]	Whiskey: Method of identification[b] GLC	Whiskey: Method of identification[b] MS	Whiskey: Ref.[c]
3	Pyridine	0.51	+	−	+	+	+	+
4	α-Picoline	0.57	+	−	−	+	+	−
5	Thiazole	0.68	+	+	−	+	+	−
6	Methylpyrazine	0.73	+	+	−	+	+	−
8	β-Picoline	0.87	+	−	−	+	+	−
9	2,5-Dimethylpyrazine	1.00	+	+	−	+	+	+
10	2,6-Dimethylpyrazine	1.02	+	+	−	+	+	−
11	2,3-Dimethylpyrazine	1.14	+	+	−	+	+	−
13	2-Methyl-6-ethylpyrazine	1.40	+	+	−	+	−	−
14	2-Methyl-5-ethylpyrazine	1.43	+	+	−	+	−	−
15	Trimethylpyrazine	1.53	+	+	+	+	+	+
16	2,5-Dimethyl-3-ethylpyrazine	1.90	+	+	−	+	+	−
19	2-Methyl-6-vinylpyrazine	2.39	+	+	−			

[a]2,5-dimethylpyrazine = 1.00; gas chromatographic conditions are those mentioned in Figure 1.
[b]Identified by means of GLC and MS (+ = yes; − = no).
[c]Previously mentioned in the literature (+ = yes; − = no).

reference mass spectra. Peak 20 in both spirits appeared to be an amylpyrazine; the substitution pattern could not be determined.

The components identified by gas chromatography and mass spectrometry are listed in Table 1. Although identification by GLC alone usually is not considered strict proof, we feel that in this case the occurrence of pyridine, α- and β-picoline in rum and of 2-methyl-6-ethylpyrazine and 2-methyl-5-ethylpyrazine in whiskey is highly probable, supported by the similarity of the gas chromatograms of the basic fractions of both spirits.

So far it has not been possible to elucidate the structure of peak 25 in rum (Fig. 1). Nine components in whiskey and eleven components in rum have not been previously reported as constitutents of the spirits or of fusel oils.

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JEANA GROSS, MICHAELA GABAI and A. LIFSHITZ
Department of Biochemistry, Tel-Aviv University, Tel-Aviv, Israel

CAROTENOIDS IN JUICE OF SHAMOUTI ORANGE

SUMMARY—More than 50 carotenoids were isolated from the juice of Shamouti orange (*Citrus sinensis* [L] Osb.) using a combination of column and thin layer chromatography. In addition to carotenoids usually found in citrus, others not previously detected in common orange varieties were identified. These include γ-carotene, rubixanthin, the new ketones sinthaxanthin and its OH-derivative, as well as lutein 5,6-monoepoxide. The diol-polyol fraction was most predominant yielding about 70% of the total carotenoids. It was established that low pigmentation of Shamouti orange juice is due to its low total carotenoid content rather than the absence of colored carotenoids.

INTRODUCTION

CAROTENOIDS of citrus fruit (pulp and peel) have been the subject of many investigations (Khan and Mackinney, 1953; Curl and Bailey, 1957 a, c; Curl, 1962a, b, 1965; Yokoyama and Vandercook, 1967; Yokoyama and White, 1967). Some varieties have been studied and characterized in detail, including Valencia *(Citrus sinensis)* and Washington Navel by Curl and Bailey (1954, 1955, 1956, 1957b, 1961); and Sinton citrangequat by Yokoyama and White (1965, 1966 a, b, c) and Yokoyama *et al.* (1965).

Shamouti orange (*Citrus sinensis* [L] Osb.); the principal variety cultivated in Israel, has not previously been studied systematically. Only the peel was investigated recently (Eilati *et al.*, 1969; Gross and Lifshitz, 1970). The total carotenoid content of Shamouti juice is only 6–10 ppm versus 15–20 ppm of other more pigmented orange varieties.

The aim of this work was to investigate the nature of carotenoids present in Shamouti juice and to determine whether its light pigmentation is due to a low concentration of total carotenoids or to qualitative differences among the carotenoids per se.

EXPERIMENTAL

Juice

The juice was obtained during the mid and late season (January–March), when the fruit is in its highly pigmented state. Juice was obtained either by hand pressing of commercially available oranges or collected from the pressing machines of citrus processing factories. All operations were carried out in subdued light. The juice was examined immediately or stored at −18°C until used.

Extraction

The juice was homogenized for 1 min in an Ultra-Turrax homogenizer (Typ. TP 18/2N Janke & Kunkel with 1 vol of petroleum-ether (PE) and 3 vol of isopropanol in the presence of a small amount of ascorbic acid. The mixture was then diluted with an equal volume of diethyl ether, and sufficient saturated NaCl solution was added to form two layers. The upper PE-diethyl ether layer contained all the carotenoids. This upper layer was washed with distilled water until free of alcohol (Higby, 1961).

Saponification of extracts and removal of sterols

Saponification was carried out in the cold. The ether layer was combined with an equal volume of 10% KOH-ethanol, covered with nitrogen, and kept overnight at room temperature. The mixture was then washed free of alkali, dried by adding absolute ethanol and evaporated in vacuo. The nonsaponifiable matter was dissolved in a minimum volume of hot methanol and kept at −4°C over night. The precipitated sterols were removed by filtration. After evaporation of the methanol under reduced pressure, the residue was dissolved in some drops of acetone to which a minimum volume of petroleum ether was then added.

Chromatography

The chromatographic separation of the pigments was carried out using column chromatography followed by thin layer chromatography (TLC). The column chromatography resulted in a preliminary fractionation into hydrocarbons, monols, diols and polyols. Each fraction was then further analyzed by TLC. This method enabled us to separate a great many different compounds and to detect those compounds present in only trace amounts.

Column chromatography. A mixture of MgO–Hyflo Super Cel (1:1 by weight) was used for column chromatography (Seasorb 43-Magnesia FMC West Vaco and Hyflo Super Cel Johns Manville). The column was developed stepwise with increasing amounts of acetone in PE.

The first fraction, of carotenes or hydrocarbons, was eluted with PE and 2–5% acetone in PE. The elution of the fluorescent fraction was followed by illuminating for short periods with a UV lamp. The oxygenated carotenes were eluted with 5–10% acetone in PE.

The second fraction, of monols, was eluted with 10% acetone in PE and divided into two subfractions. The less polar yellow zone contained OH-α-carotene as the main pigment, and the more polar orange zone, cryptoxanthin as the main pigment.

The third fraction, of diols and polyols, was eluted with ethanol–PE (1:1). Owing to the complexity of this fraction a further rechromatography on the same adsorbent was performed. This time the column was developed with a mixture of 10% acetone in PE and ethanol (97:3). Three to four subfractions were thus obtained, the first by elution and the others by mechanically cutting the column and dissolving each zone in diethyl ether-ethanol.

TLC. All adsorbents were activated before use. The pigment extract was applied as a line and since no R_f values were determined, the chromatography was continued until the best separation was obtained.

For the separation of the first fraction, calcium hydroxide–silica gel G (6:1) was used as the adsorbent and the solvent system consisted of light petroleum–benzene (98:2) (Bolliger, 1965). For the separation of a β-carotene isomer of this fraction the best adsorbent was a mixture of $Ca(OH)_2$–MgO (1:1) developed with PE ($Ca(OH)_2$ for chromatography Riedel-DeHaën AG). The oxidized carotenes were analyzed on aluminium oxide G (acc. to Stahl, for TLC, E. Merck) with 3% acetone in PE as the solvent system.

This same adsorbent was used for the separation of the second fraction (monols); the solvent system was 5% acetone in PE.

Silica gel G (acc. to Stahl, E. Merck) was used as the adsorbent for the chromatography of the third fraction. The solvent systems were, according to the polarity of the pigments: 20% acetone in PE; PE–ethyl acetate–isopropyl alcohol 95:10:5; methylene chloride–ethyl acetate (80:20) (Bolliger, 1965); and acetone 40% in PE (Bond, 1967). Compounds were identified on thin layer chromatograms by co-chromatography with known samples, where possible.

Identification of pigments

Identification of the pigments and colorless polyenes was made on the basis of their chromatographic and spectrophotometric properties. For some functional groups, known chemical tests were used.

The absorption spectrum of each chromatographically pure pigment was recorded in a Beckman DB Spectrophotometer from 220–550 nm and compared with values from the literature (Davies, 1965).

Chemical Tests

Carbonyl-reduction test. The reduction test for the carbonyl group was carried out at room temperature by adding a few crystals of sodium borohydride to the carotenoid pigment dissolved in 95% ethanol. The solution was kept under N_2 and shaken occasionally. After 30 min, it was extracted with ether and washed several times with water. A hypsochromic shift in the absorption maxima is indicative for the presence of a carbonyl group (Critchley et al., 1958).

Epoxide test. Isomerization of the 5,6-epoxide into the 5,8-epoxide was studied by observing the magnitude of the spectral shift after addition of hydrochloric acid. The spectrum was recorded before, and 3 min after the addition of 3 drops of 0.1N HCl to the caro-

tenoid solution in a 1 cm cell. A shift of about 20 nm to the shorter wavelengths indicates the presence of a monoepoxy group, while a shift of approximately 40 nm is indicative of a diepoxy (Davies, 1965). The color of the reaction mixture, being also characteristic, was noted. A rapid epoxide test was to expose the chromatogram to HCl gas for a few minutes. The epoxide zones became green, greenish blue while those of furanoid oxides turned ink blue.

Quantitative Determination

The method described by Davies (1965) was used for the quantitative determination of the carotenoids. The optical density of a known volume of carotenoid solution was read in the spectrophotometer at 450 nm using the extinction coefficient of 2500 of β-carotene. The results reported are expressed as β-carotene. Phytoene, phytofluene and ζ-carotene were read at the wavelength of maximal absorption and their reported extinction coefficients used in the calculations. Pigments present in very small quantities were not determined separately.

RESULTS & DISCUSSION

TABLE 1 represents the qualitative analyses of the carotenoids in Shamouti juice presented in order of increasing adsorption affinity on the several adsorbents used for column and thin-layer chromatography.

Fraction I—hydrocarbons

Phytoene was detected in the first petroleum ether eluates from the column chromatograms. Phytofluene moved down the column along with a blue fluorescent zone that might be a terpene derivative from the essential oils. However, these two could be separated by eluting the column with 2% acetone in PE. With this solvent, the green fluorescent phytofluene band is eluted, while the blue band remains on the column close to the α-carotene zone. Phytofluene could not be separated into two isomers as reported by Koe and Zechmeister (1953) in tomatoes, although purification on TLC according to Williams et al., (1967) was attempted. The first colored carotenoid, α-carotene, had identical spectral and chromatographic properties as α-carotene isolated from carrots. β-Carotene was identical with the synthetic pigment obtained from Hoffmann La Roche. One of its cis-isomers, neo-β-carotene, could be separated from ζ-carotene both by column and TLC chromatography. This compound appears above ζ-carotene on the column. ζ-Carotene was easy to identify owing to its light yellow color and its very characteristic spectrum with a pronounced fine structure. Besides this main pigment another more polar ζ-carotene-like pigment could be separated both by column chromatography and by TLC on $Ca(OH)_2$-Silica gel G (6:1) developed with light petroleum-benzene (98:2). This pigment has exactly the same absorption maxima as ζ-carotene but is similar chromatographically to the recently discovered unsymmetrical pigment 7,8,11,12-tetrahydrolycopene which was isolated from the bacterium *Rhodospirillum rubrum* by Davies (1970). This new pigment has its main peak at 397.5 nm.

γ-Carotene was identical with γ-carotene isolated from carrots. Its presence has not previously been noted in other common orange varieties, nor in lemons, but it is mentioned as being a constituent of Sinton citrangequat (Yokoyama and White, 1966c).

Between the hydrocarbon and the monol fractions two oxygenated carotenes, minor components, could be identified.

Mutatochrome (β-carotene, 5,8-epoxide) was detected on TLC using alumina developed with 5% acetone in PE. It appears as a very faint lemon yellow zone and yields an intense blue color with the epoxide test. It was the only epoxide derivative of β-carotene that could be detected. It has been mentioned as a constituent of lemons but has not been detected in other orange varieties. The second oxygenated carotenoid of the intermediate fraction is an apoderivative, β-apo-10′-carotenal. Upon co-chromatography it could not be separated from the synthetic sample (Hoffmann La Roche) and could be reduced with sodium borohydride. After reduction, fine structure appeared in the spectrum but its maxima were at somewhat longer wavelengths than those of the synthetic pigment.

Fraction II—monols

This fraction contains two principal monols; hydroxy-α-carotene that appeared as a single pigment and cryptoxanthin that could be separated into a set of isomers.

In the hydroxy-α-carotene subfraction, the major pigment was preceded by traces of the monoepoxide. OH-α-carotene was identical to that isolated from Navel oranges (Curl, 1956). In the same fraction a poly-cis derivative of cryptoxanthin was present that was also found by Curl (1956) in Valencia orange. Its cis peak is very flat as mentioned by Zechmeister (1962). This pigment has a pronounced yellow-orange color while the OH-α-carotene is pure yellow.

The second monol subfraction was dominated by cryptoxanthin. It was compared and found identical with cryptoxanthin isolated from alfalfa. Cryptoxanthin could be resolved into an isomeric set some of which had a pronounced cis-peak. In this subfraction an uncommon monol, rubixanthin, (3-OH-γ-carotene) was detected. This pigment has an orange-red tint. Like γ-carotene it did not undergo reduction and its epoxide test was negative. Although known to occur in fruits of *Rosa rubiginosa* (Arpin and Liaaen-Jensen, 1969), it has not previously been reported in oranges, only in lemons (Curl, 1962b).

Fraction III—diols and polyols

Due to the complexity of this last fraction is was rechromatographed on MgO-Hyflo Super Cel and subdivided into three main fractions, as described in the experimental section.

The first subfraction had lutein as the major pigment. The least polar pigment of this subfraction is cryptoflavin, the furanoid oxide of cryptoxanthin, easily recognizable by its lemon-yellow color and blue epoxide test. It was followed by cryptoxanthin-5,6,5′,8′-diepoxide, which on HCl treatment gave both reactions for the two epoxide groups i.e., a 20 nm hypsochromic shift for the epoxy-group and a blue color for the furanoid oxide. In some samples it was followed by a pigment with exactly the same spectrum but which did not react in the HCl test. The main pigment of this subfraction, lutein, was separated by TLC from a pigment which was lutein-like chemically but which had clearly different R_f and absorption spectrum. This lutein-like pigment was not however, characterized further. The lutein which was isolated in this manner proved identical with lutein extracted from alfalfa. In addition to the normal trans-lutein, another compound cis-lutein was isolated, which showed the characteristic "cis" peak at 330 nm. This was followed by isolutein (lutein 5,6-epoxide), which gave the characteristic monoepoxy shift of about 20 nm in HCl. It has not been mentioned previously as a constituent of citrus, probably because of the difficulty in separating it from lutein.

The next pigment that followed was violaxanthin, the 5,6,5′6′-diepoxide of zeaxanthin. It has the same spectrum as violaxanthin from grapefruit (Yokoyama and White, 1967), but the maxima are 3 nm shorter than those reported by Karrer and Jucker (1950). It could not be separated from violaxanthin isolated from spinach. Its epoxide test is characteristic for two-epoxy groups, that is, a shift of about 37–40 nm and a greenish-blue color. Sometimes its cis-isomer appeared in the zeaxanthin fraction, following antheraxanthin.

According to its polarity, violaxanthin should follow zeaxanthin chromatographically; however, an inversion in its adsorption affinity was observed. A similar inversion of violaxanthins' adsorption affinity on a MgO-Hyflo Super Cel column is reported by Jungalwala and Cama (1962), who studied the carotenoids in the *Delonix regia* flower. They

Table 1—Characterization of carotenoids from Shamouti orange juice

Identification	Abs max[a] (nm)	Eluent from MgO-Hyflo Super Cel	TLC: Adsorbent	TLC: Solvent system	Epoxide test (HCl treatment): Color	Epoxide test (HCl treatment): Hypsochromic shift (nm)	Carbonyl test: Abs max after reduction with $NaBH_4$
Fraction I—hydrocarbons							
Phytoene	272, 283, 298	PE			—	—	—
Phytofluene	327, 348, 367	PE			—	—	—
α-Carotene	420, 443, 470	Ac. 2–5% in PE	$Ca(OH)_2$-Silica gel G (6:1)	PE-Benzene (98:2)	—	—	—
β-Carotene	425, 450, 475	Ac. 2–5% in PE	$Ca(OH)_2$-Silica gel G (6:1)	PE-Benzene (98:2)	—	—	—
ζ-Carotene	378, 400, 425	Ac. 2–5% in PE	$Ca(OH)_2$-Silica gel G (6:1)	PE-Benzene (98:2)	—	—	—
neo-β-Carotene	422, 444, 470	Ac. 2–5% in PE	$Ca(OH)_2$-MgO (1:1)	PE	—	—	—
ζ-Carotene-like	378, 400, 424	Ac. 5–10% in PE	Aluminum oxide G	PE	—	—	—
γ-Carotene	428, 456, 485	Ac. 5–10% in PE	Aluminum oxide G	PE	—	—	—
Intermediate							
Mutatochrome	404, 425, 450	Ac. 5–10% in PE	Aluminum oxide G	Ac. 2% in PE	Blue	—	—
β-Apo-10′-carotenal	435, (465)	Ac. 5–10% in PE	Aluminum oxide G	Ac. 2% in PE	—	—	390, 410, 432
Fraction IIA—monols							
OH-α-Carotene 5,6-epoxide	418, 440, 468	Ac. 10% in PE	Aluminum oxide G	Ac. 5% in PE	Green	+	—
OH-α-Carotene	418, 444, 473	Ac. 10% in PE	Aluminum oxide G	Ac. 5% in PE	—	—	—
poly-cis-Cryptoxanthin a	418, 443, 472	Ac. 10% in PE	Aluminum oxide G	Ac. 5% in PE	—	—	—
poly-cis-Cryptoxanthin b	419, 442, 472	Ac. 10% in PE	Aluminum oxide G	Ac. 5% in PE	—	—	—
Fraction IIB—monols							
Rubixanthin	435, 460, 488	Ac. 10% in PE	Aluminum oxide G	Ac. 5% in PE	—	—	—
OH-α-Carotene 5,8-epoxide	420, 443, 472	Ac. 10% in PE	Aluminum oxide G	Ac. 5% in PE	Blue	+	—
Sintaxanthin	(428), 447, (473)	Ac. 10% in PE	Aluminum oxide G	Ac. 5% in PE	—	—	395, 421, 450
cis-Cryptoxanthin	333, 420, 444, 472	Ac. 10% in PE	Aluminum oxide G	Ac. 5% in PE	—	—	—
Cryptoxanthin a	425, 450, 475	Ac. 10% in PE	Aluminum oxide G	Ac. 5% in PE	—	—	—
Cryptoxanthin b	424, 450, 475	Ac. 10% in PE	Aluminum oxide G	Ac. 5% in PE	—	—	—
Cryptoxanthin-like a	424, 448, 475	Ac. 10% in PE	Aluminum oxide G	Ac. 5% in PE	—	—	—
Cryptoxanthin-like b	423, 448, 473	Ac. 10% in PE	Aluminum oxide G	Ac. 5% in PE	—	—	—
Cis-cryptoxanthin-like	335, 425, 448, 475	Ac. 10% in PE	Aluminum oxide G	Ac. 5% in PE	—	—	—
Fraction IIIA—diols							
Cryptoflavin	401, 425, 448	Ac. 10% in PE + EtOH (97:3)	Silica gel G	PE-Ethyl acetate-Isopropyl alcohol (95:10:5) and Ac. 20% in PE	Blue	+	—
Cryptoxanthin 5,6,5′,8′-diepoxide	397, 418, 443	Ac. 10% in PE + EtOH (97:3)	Silica gel G	PE-Ethyl acetate-Isopropyl alcohol (95:10:5) Ac. 20% in PE	Blue	20	—
Unknown 418	397, 418, 445	Ac. 10% in PE + EtOH (97:3)	Silica gel G	PE-Ethyl acetate-Isopropyl alcohol (95:10:5) and Ac. 20% in PE	—	—	—
Lutein-like	418, 442, 470	Ac. 10% in PE + EtOH (97:3)	Silica gel G	PE-Ethyl acetate-Isopropyl alcohol (95:10:5) and Ac. 20% in PE	—	—	—

[a]Phytoene and phytofluene determined in hexane; carotenoids from Fractions I and II in petroleum ether; from Fraction III in ethanol.

Table 1—Characterization of carotenoids from Shamouti orange juice (Continued)

Identification	Abs max[a] (nm)	Eluent from MgO-Hyflo Super Cel	TLC		Epoxide test (HCl treatment)		Carbonyl test
			Adsorbent	Solvent system	Color	Hypsochromic shift (nm)	Abs max after reduction with $NaBH_4$
Lutein	422, 445, 473	Ac. 10% in PE + EtOH (97:3)	Silica gel G	PE-Ethyl acetate-Isopropyl alcohol (95:10:5) and Ac. 20% in PE	—	—	—
cis-Lutein	330, 420, 443, 472	Ac. 10% in PE + EtOH (97:3)	Silica gel G	PE-Ethyl acetate-Isopropyl alcohol (95:10:5) and Ac. 20% in PE	—	—	—
Isolutein	418, 442, 470	Ac. 10% in PE + EtOH (97:3)	Silica gel G	PE-Ethyl acetate-Isopropyl alcohol (95:10:5) and Ac. 20% in PE	Green-blue	18	—
cis-Isolutein	332, 416, 440, 468	Ac. 10% in PE + EtOH (97:3)	Silica gel G	PE-Ethyl acetate-Isopropyl alcohol (95:10:5) and Ac. 20% in PE	Green-blue	15	—
Violaxanthin	416, 438, 467	Ac. 10% in PE + EtOH (97:3)	Silica gel G	PE-Ethyl acetate-Isopropyl alcohol (95:10:5) and Ac. 20% in PE	Green-blue	38	—
Fraction IIIB-diols							
Unknown 455	430, 455, 490	Ac. 10% in PE + EtOH (97:3)	Silica gel G	PE-Ethyl Acetate-Isopropyl alcohol (95:10:5) and CH_2Cl_2-Ethyl acetate (80:20)	Greenish	—	—
Chrysanthemaxanthin	400, 423, 450	Ac. 10% in PE + EtOH (97:3)	Silica gel G	PE-Ethyl Acetate-Isopropyl alcohol (95:10:5) and CH_2Cl_2-Ethyl acetate (80:20)	Blue	+	—
Unknown 420	395, 420, 444	Ac. 10% in PE + EtOH (97:3)	Silica gel G	PE-Ethyl Acetate-Isopropyl alcohol (95:10:5) and CH_2Cl_2-Ethyl acetate (80:20)	Green	15	—
Citraurin	450	Ac. 10% in PE + EtOH (97:3)	Silica gel G	PE-Ethyl Acetate-Isopropyl alcohol (95:10:5) and CH_2Cl_2-Ethyl acetate (80:20)	—	—	402, 425, 449

[a]Phytoene and phytofluene determined in hexane; carotenoids from Fractions I and II in petroleum ether; from Fraction III in ethanol.

Table 1—Characterization of carotenoids from Shamouti orange juice (Continued)

Identification	Abs max[a] (nm)	Eluent from MgO-Hyflo Super Cel	TLC		Epoxide test (HCl treatment)		Carbonyl test
			Adsorbent	Solvent system	Color	Hypsochromic shift (nm)	Abs max after reduction with $NaBH_4$
OH-Sintaxanthin a	(426), 447, (475)	Ac. 10% in PE + EtOH (97:3)	Silica gel G	PE-Ethyl Acetate-Isopropyl alcohol (95:10:5) and CH_2Cl_2-Ethyl acetate (80:20)	—	—	400, 423, 446
OH-Sintaxanthin b	(423), 445, (475)	Ac. 10% in PE + EtOH (97:3)	Silica gel G	PE-Ethyl Acetate-Isopropyl alcohol (95:10:5) and CH_2Cl_2-Ethyl acetate (80:20)	—	—	400, 420, 445
Zeaxanthin	423, 451, 478	Ac. 10% in PE + EtOH (97:3)	Silica gel G	PE-Ethyl Acetate-Isopropyl alcohol (95:10:5) and CH_2Cl_2-Ethyl acetate (80:20)	—	—	—
Antheraxanthin	418, 445, 470	Ac. 10% in PE + EtOH (97:3)	Silica gel G	PE-Ethyl Acetate-Isopropyl alcohol (95:10:5) and CH_2Cl_2-Ethyl acetate (80:20)	Green	16	—
cis-Antheraxanthin	330, 418, 442, 470	Ac. 10% in PE + EtOH (97:3)	Silica gel G	PE-Ethyl Acetate-Isopropyl alcohol (95:10:5) and CH_2Cl_2-Ethyl acetate (80:20)	Green	20	—
Luteoxanthin a	400, 424, 448	Ac. 10% in PE + EtOH (97:3)	Silica gel G	PE-Ethyl Acetate-Isopropyl alcohol (95:10:5) and CH_2Cl_2-Ethyl acetate (80:20)	Blue	22	—
Luteoxanthin b	393, 420, 443	Ac. 10% in PE + EtOH (97:3)	Silica gel G	PE-Ethyl Acetate-Isopropyl alcohol (95:10:5) and CH_2Cl_2-Ethyl acetate (80:20)	Blue	20	—
Fraction IIIC—polyols							
Unknown 408	408	Ac. 10% in PE + EtOH (97:3)	Silica gel G	PE-Ethyl acetate-Isopropyl alcohol (95:10:5) and Ac. 40% in PE	—	—	337, 354, 374
Unknown 426	400, 426, 448	Ac. 10% in PE + EtOH (97:3)	Silica gel G	PE-Ethyl acetate-Isopropyl alcohol (95:10:5) and Ac. 40% in PE	—	—	—

[a]Phytoene and phytofluene determined in hexane; carotenoids from Fractions I and II in petroleum ether; from Fraction III in ethanol.

Table 1—Characterization of carotenoids from Shamouti orange juice (Continued)

Identification	Abs max[a] (nm)	Eluent from MgO-Hyflo Super Cel	TLC Adsorbent	TLC Solvent system	Epoxide test (HCl treatment) Color	Epoxide test (HCl treatment) Hypsochromic shift (nm)	Carbonyl test Abs max after reduction with $NaBH_4$
Carbonyl 422	422	Ac. 10% in PE + EtOH (97:3)	Silica gel G	PE-Ethyl acetate-Isopropyl alcohol (95:10:5) and Ac. 40% in PE	—	—	372, 394, 418
Mutatoxanthin	404, 426, 452	Ac. 10% in PE + EtOH (97:3)	Silica gel G	PE-Ethyl acetate-Isopropyl alcohol (95:10:5) and Ac. 40% in PE	Blue	—	—
cis-Mutatoxanthin	310, 400, 424, 452	Ac. 10% in PE + EtOH (97:3)	Silica gel G	PE-Ethyl acetate-Isopropyl alcohol (95:10:5) and Ac. 40% in PE	Blue	—	—
Luteoxanthin-like	394, 418, 444	Ac. 10% in PE + EtOH (97:3)	Silica gel G	PE-Ethyl acetate-Isopropyl alcohol (95:10:5) and Ac. 40% in PE	Blue	20	—
cis-Luteoxanthin-like	310, 394, 416, 443	Ac. 10% in PE + EtOH (97:3)	Silica gel G	PE-Ethyl acetate-Isopropyl alcohol (95:10:5) and Ac. 40% in PE	Blue	18	—
Auroxanthin	376, 398, 417	Ac. 10% in PE + EtOH (97:3)	Silica gel G	PE-Ethyl acetate-Isopropyl alcohol (95:10:5) and Ac. 40% in PE	Blue	—	—
Trollichrome a	400, 423, 450	Ac. 10% in PE + EtOH (97:3)	Silica gel G	PE-Ethyl acetate-Isopropyl alcohol (95:10:5) and Ac. 40% in PE	Blue	—	—
Trollichrome b	396, 420, 448	Ac. 10% in PE + EtOH (97:3)	Silica gel G	PE-Ethyl acetate-Isopropyl alcohol (95:10:5) and Ac. 40% in PE	Blue	—	—
Trollixanthin	396, 420, 447, 470	Ac. 10% in PE + EtOH (97:3)	Silica gel G	PE-Ethyl acetate-Isopropyl alcohol (95:10:5) and Ac. 40% in PE	Green	—	—
Neoxanthin	416, 437, 464	Ac. 10% in PE + EtOH (97:3)	Silica gel G	PE-Ethyl acetate-Isopropyl alcohol (95:10:5) and Ac. 40% in PE	Green	18	—

[a]Phytoene and phytofluene determined in hexane; carotenoids from Fractions I and II in petroleum ether; from Fraction III in ethanol.

Table 1—Characterization of carotenoids from Shamouti orange juice (Continued)

Identification	Abs max[a] (nm)	Eluent from MgO-Hyflo Super Cel	TLC Adsorbent	TLC Solvent system	Epoxide test (HCl treatment) Color	Epoxide test (HCl treatment) Hypsochromic shift (nm)	Carbonyl test Abs max after reduction with $NaBH_4$
Trollixanthin-like	422, 448, 474	Ac. 10% in PE + EtOH (97:3)	Silica gel G	PE-Ethyl acetate-Isopropyl alcohol (95:10:5) and Ac. 40% in PE	Blue-green	—	—
Neoxanthin-like	412, 438, 463	Ac. 10% in PE + EtOH (97:3)	Silica gel G	PE-Ethyl acetate-Isopropyl alcohol (95:10:5) and Ac. 40% in PE	Green	20	—

[a]Phytoene and phytofluene determined in hexane; carotenoids from Fractions I and II in petroleum ether; from Fraction III in ethanol.

mentioned that on MgO–celite, xanthophylls with epoxy groups are less strongly adsorbed than the corresponding xanthophylls.

The second diol fraction is the zeaxanthin fraction and its epoxides. Two developing solvents for TLC were used for separating the pigments in this subfraction: PE-ethyl acetate–isopropylalcohol (95:10:5) and methylene chloride–ethyl acetate (80:20).

The least polar pigment of this fraction is chrysanthemaxanthin (lutein-5,8-epoxide) which has the same spectrum as given in the literature and the characteristics of a furanoid oxide. It has not been mentioned by other authors as a component of citrus. The next pigment was citraurin, found also in the peels of this variety (Gross and Lifshitz, 1970). Zeaxanthin was preceded by the hydroxyl derivative of the recently discovered sintaxanthin. It could be separated into two isomers, the more polar one having its maxima 2 nm shorter than the less polar. They had the same characteristics as described by Yokoyama and White (1966c). Previous methods used could not separate citraurin from zeaxanthin so that OH-sintaxanthin which is located between these two, could not be detected.

Zeaxanthin was identical with an authentic sample from Hoffmann La Roche. Zeaxanthin was followed by the trans and cis isomers of antheraxanthin, the monoepoxide derivative of zeaxanthin. Antheraxanthin was identical with spinach antheraxanthin, although its spectrum was 2 nm shorter. The last pigment of this fraction is luteoxanthin, the 5,6,5′8′-diepoxide of zeaxanthin which gave the characteristic reactions of the epoxide groups.

The last fraction—the most polar—is the fraction containing the polyols, that is three hydroxy derivatives. Because of the difficulty encountered in separating the components of this fraction, an additional solvent system was used for TLC. This solvent system consisted of 40% acetone in PE.

The first pigment, having a yellow-lemon color had an unusually broad spectrum at a very short wavelength, and could be reduced. Since this compound was present in only trace quantities it was not further investigated. The second pigment, a minor constituent, was very difficult to characterize, being very unstable. It has a violet color that becomes brown rapidly. The reduction test was not clear, the pigment being rapidly destroyed.

The next pigment had the broad spectrum of a carbonyl derivative and could be reduced. The epoxide test gave an intense blue color. Being very similar to Curls' pigment detected in Valencia peels it was tentatively identified as violaxanthal, the apo-derivative of violaxanthin with a terminal aldehydic carbonyl (Curl, 1967).

In this fraction, the monofuranoid and difuranoid of zeaxanthin could be separated. The two isomers of mutatoxanthin, the monofuranoid, as well as the two isomers of auroxanthin, the difuranoid, were isolated. Also present in this fraction, although in much greater quantity, was a luteoxanthin-like pigment. The triol (3,3′,6′-OH) derivatives of α-carotene appeared in inverse order of their polarity. The furanoid, trollichrome, appeared first, followed by the epoxide trollixanthin. The triol derivative of β-carotene isolated was neoxanthin (3,3′,5′-trihydroxy-6′7′-dehydro-5, 6-epoxy-β-carotene). The triol derivatives of α-carotene reacted only slightly with HCl, even when the concentration was increased to 34%, as also noted previously (Eugster and Karrer, 1957). In contrast to the other triols, neoxanthin gave a positive epoxide test; two isomeric forms were identified.

The quantitative composition of the carotenoids is given in Table 2. The total content of carotenoids was about 6–7 μg/ml of juice. Toward the end of the season it reached almost 10 μg/ml. As shown, the approximate composition is about 25% hydrocarbons plus monols, and 75% diols plus polyols and their epoxides.

The uncolored pigments phytoene and phytofluene were found in approximately the same percentages as those reported in Navel orange. In lemon, these two pigments make up about 20% of the total carotenoids. Their amount decreases at the end of the season.

In the hydrocarbon fraction there is about 4 times more β-carotene than α-carotene, but it is exceeded by ζ-carotene, which biogenetically is their precursor. In Navel orange there is about 15 times more ζ-carotene than β-carotene and in Valencia 5 times more.

In the monol fraction, cryptoxanthin is the major pigment, there being about 14 times more cryptoxanthin than OH-α-carotene. At the beginning of the season the amount was only half of that.

In the lutein fraction, lutein predominates. Also present in relatively high amounts is the monoepoxide. Violaxanthin is present in a relatively small quantity in comparison with Navel orange where it represents 45% of the total carotenoids. At the beginning of the season violaxanthin was present in a greater quantity, representing 12% of the total.

In the next diol subfraction zeaxanthin and its epoxide predominate. The two isomers of antheraxanthin were analyzed together. Luteoxanthin is found in about half the quantity of antheraxanthin, and both increase at the end of the season. A very high quantity of

Table 2—Quantitative composition of carotenoids from Shamouti orange juice (Percent of total carotenoids)[a]

Phytoene[b]	5.7
Phytofluene[b]	2.4
Fraction I Total	3.00
α-Carotene	0.36
β-Carotene	1.24
ζ-Carotene[b]	1.40
Intermediate and Fraction IIA Total	1.60
Mutatochrome	0.02
β-Apo-10′-carotenal	0.03
OH-α-Carotene	0.50
poli-cis-Cryptoxanthin	1.05
Fraction IIB Total	15.00
Rubixanthin	0.05
Sintaxanthin	0.07
cis-Cryptoxanthin	2.00
Cryptoxanthin	12.88
Fraction IIIA Total	13.0
Cryptoflavin	0.7
Cryptoxanthin diepoxide	0.1
Lutein	5.2
Isolutein	4.0
Violaxanthin	3.0
Fraction IIIB Total	20.0
Chrysanthemaxanthin	0.07
Citraurin	0.11
OH-Sintaxanthin	2.48
Zeaxanthin	6.2
Antheraxanthin	6.83
Luteoxanthin	3.98
Fraction IIIC Total	39.3
Pigment 426	0.39
Carbonyl 422	0.78
Mutatoxanthin	15.13
Luteoxanthin-like	6.92
Auroxanthin	0.58
Trollichrome	1.46
Trollixanthin	9.64
Neoxanthin	3.97

[a]Expressed as β-carotene.
[b]Phytoene, phytofluene and ζ-carotene were read at the wavelengths of maximal absorption using their reported extinction coefficients.

luteoxanthin, about 30%, was found at the beginning of the season. Perhaps as a result of the higher acidity, violaxanthin is partially isomerized to the furanoid oxide. Present in noticable amounts is the newly detected hydroxy ketone 3-OH-sintaxanthin. Citaurin, that was easily detectable in peels, appeared in lower amounts in juice. In the last fraction, which is very rich in the midseason, mutatoxanthin and the luteoxanthin-like pigment appear in large amounts. A relatively small amount of auroxanthin was also detected. In Navel orange pulp it does not appear. The triols derivatives of α-carotene, especially trollixanthin, were present in much higher amounts than neoxanthin, the β-carotene allenic triol derivative.

With the new methods utilized it has been shown that a large number of colored pigments are present in Shamouti orange juice. It may be concluded, therefore, that the light color of Shamouti juice is due to the low, total concentration of carotenoids, rather than the absence of any specific colored pigments.

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B. C. PATIL, D. K. SALUNKHE and B. SINGH
Department of Food Science, Utah State University, Logan, Utah 84321

METABOLISM OF SOLANINE AND CHLOROPHYLL IN POTATO TUBERS AS AFFECTED BY LIGHT AND SPECIFIC CHEMICALS

SUMMARY—Chlorophyll and solanine syntheses as affected by cultivars, specific gravities, light intensities, and chemicals were studied in potato (Solanum tuberosum, L.) tubers. When exposed to 100 ft-c of white fluorescent light for 5 days, tubers of Bounty, Kennebec, Norchip and Red Lasoda were most sensitive to greening and solanine development. LaChipper and Platte tubers were resistant to the light effects. Chlorophyll synthesis was inversely related to specific gravity of tubers, while specific gravity did not affect solanine synthesis. The chlorophyll content of tubers exposed to 50 ft-c and 200 ft-c was significantly less than that of tubers exposed to 100 ft-c and 150 ft-c light intensities. The solanine synthesis was not influenced by the light intensities studied. Chemicals were applied at a concentration of 10,000 ppm by vacuum injection technique. Ethrel® and Alar® were effective in inhibiting chlorophyll and solanine formation. Ethrel was more effective in retarding the chlorophyll formation and alar was most effective in preventing solanine formation. Maleic hydrazide MH®-30 and Cyocel® were ineffective in controlling both chlorophyll and solanine formation.

INTRODUCTION

The common white potato (*Solanum tuberosum*, L.) turns green when exposed to light. This greening is assocciated with bitterness and off-flavor. The synthesis of chlorophyll is responsible for greening; while the bitter taste is due to the presence of solanine, a toxic alkaloid (Hilton, 1951). In recent years, this problem has become more serious because of the technological developments in handling, transportation, and merchandising of potatoes.

Chlorophyll and solanine develop in the periderm and cortical parenchyma layers of the tuber (Larson, 1949). Often, the same factors, such as light intensity and quality, storage duration, and age of tubers, influence the synthesis of both compounds (Hardenburg, 1964). The processes of chlorophyll and solanine syntheses are, however, independent of each other (Conner, 1937).

These investigations were undertaken to study the variations in the chlorophyll and solanine contents among cultivars, as related to specific gravities and light intensities. The chemical control of the synthesis of these two compounds was attempted.

MATERIALS & METHODS

Plant material

Eleven potato cultivars were planted in sandy loam soil on May 7, 1969, at the Utah State University farm, Farmington, Utah. A total of 177 lb of nitrogen and 147 lb of phosphate per acre were applied and the crop was irrigated when needed. The tubers were harvested on September 30, 1969, and transported to the laboratories at Logan. These were then cleaned, selected for uniformity of size (U.S. No. 1), classified on the basis of specific gravity according to the brine flotation method (Clark et al., 1940; Salunkhe et al., 1953), and stored at 40°F. For other series of experiments, potatoes were procured from local markets.

Light

Light was obtained from two banks of fluorescent tubes 8 ft long (ITTF96T12/cw cool white). The light intensity was measured in foot-candles with Weston illumination meter, Model 603 No. 610. The tubers were exposed to light at 70°F and 80-90% relative humidity.

Chemicals

Chemicals were obtained as follows: Alar® (succinamic acid, 2,2-dimethylhydrazide) from uniRoyal Chemical, Division of uniRoyal Inc., Bethany, Conn.; Cycocel® (2-chloroethyltrimethylammonium chloride) from American Cyanamid Co., Agricultural Division, Princeton, N.J.; Ethrel® (2-chloroethylphosphonic acid) from American Products, Inc., Agricultural Chemical Division, Ambler, Pa.; maleic hydrazide (1,2-dihydropyridazine-3,6-dione) MH®-30 from United States Rubber, Naugatuck Chemical Division, Naugatuck, Conn.; and solanine from K. and K. Laboratories Inc., Plainview, N.Y.

Chemical application

The solutions of the chemicals were prepared in distilled water at concentrations of 10,000 ppm. The tubers were treated by vacuum injection technique. The solutions were placed in desiccators and the tubers dipped in the solutions. Air was drawn off by a suction pump for 4 min. The tubers were then allowed to soak in the solutions for 30 min. Control tubers were similarly treated by using distilled water.

Analytical methods

The AOAC (1965) method for chlorophyll analysis was used with modifications for potato tubers. Potato peels ranging in thickness from 1.5-2.0 mm were uniformly removed by a hand peeler. Chlorophyll extraction was made from 50g of peel with total volume of 350 ml of 85% acetone and 120 ml of ether for separation. The chlorophyll-ether layer was removed, dried over anhydrous sodium sulfate, made to 100 ml volume, and then used for optical density readings at 660 mμ. The method for solanine extraction and determination was that of Gull and Isenberg (1960). A regression curve was constructed with known concentrations of pure solanine. The optical density was measured at 565 mμ. The results for both chlorophyll and solanine were expressed as milligrams per 100g of fresh peel.

Statistical analysis

All experiments were arranged in a completely randomized block design. Three replications were used with each treatment randomized within a replication. Analysis of variance was made and the means were compared according to least significant difference (LSD) procedure (Steel and Torrie, 1960).

RESULTS & DISCUSSION

Cultivars

Table 1 indicates that the cultivars differed significantly in chlorophyll and solanine formation. The results are in conformity with those of Gull and Isenberg (1960) and Akeley et al. (1962), which showed that greening potential is a cultivar characteristic.

Specific gravities

Chlorophyll development in tubers was inversely related to their specific gravity (Fig. 1). Solanine synthesis was,

Table 1—Chlorophyll and solanine contents of 11 cultivars exposed to white fluorescent light at 100 ft-c for a period of 5 days during March-April, 1970

Cultivar		Chlorophyll	Solanine
		(mg/100 g of fresh peel)	
Bounty		2.431	70.30
Cascade		0.966	65.69
Kennebec		2.228	96.40
LaChipper		0.691	44.81
LaRouge		1.175	73.06
Norchip		1.395	79.20
Platte		0.720	55.86
Red Lasoda		1.566	69.99
Russet Burbank		2.083	69.07
Shurchip		1.808	44.50
Sioux		1.247	58.93
LSD	0.01	0.19	10.88
	0.05	0.12	7.13

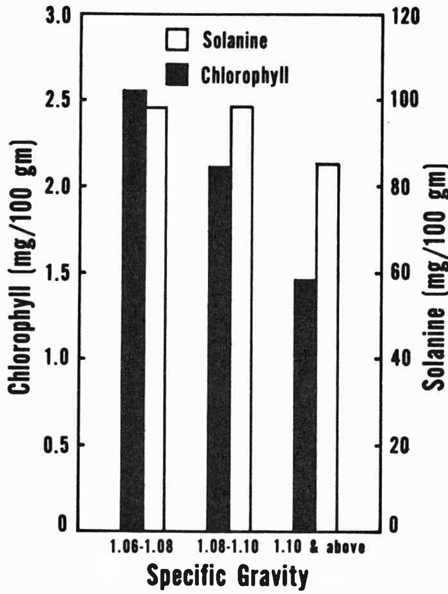

Fig 1—Chlorophyll and solanine contents in relation to specific gravity of Kennebec potato tubers exposed to 100 ft-c of light. (Data expressed on fresh weight basis.)

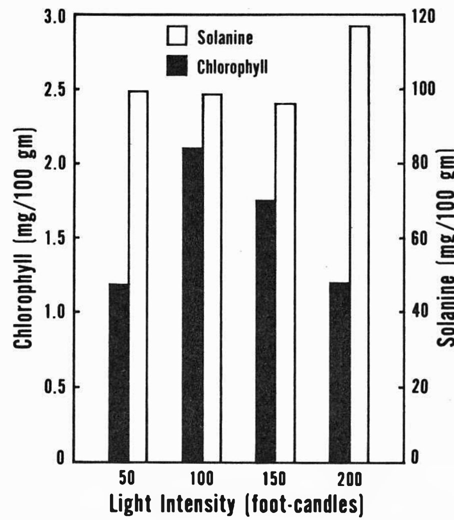

Fig. 2—Chlorophyll and solanine contents of Kennebec potato tubers exposed to four light intensities. (Data expressed on fresh weight basis.)

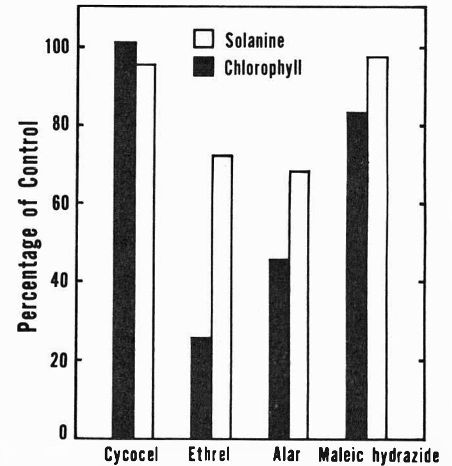

Fig. 3—Chlorophyll and solanine contents of Kennebec potato tubers subjected to chemical treatments (a solution of 10,000 ppm of Cycocel, Ethrel, Alar or maleic hydrazide by vacuum infiltration technique) and exposed to 100 ft-c of white fluorescent light during December, 1969.

however, not dependent on specific gravity of tubers. Data analysis showed that the chlorophyll content of tubers with specific gravities of 1.1 and above was significantly less as compared to tubers of lower (1.06–1.08 and 1.08–1.10) specific gravities. Howard et al. (1957) observed that the apical end of potato tubers appeared greener than the stem end. The apical end of the tuber had a lower specific gravity as compared to the basal end (Thiessen, 1947). The immature potato tubers are often subject to greening (Hardenburg, 1964), and it is known that the maturity of potato tubers had a good relation to specific gravity (Salunkhe et al., 1954; Talburt and Smith, 1959).

Light intensities

The effect of four light intensities on chlorophyll and solanine formation of potato tubers that were stored for nearly 6 months after harvest is illustrated in Figure 2. Maximum greening was observed at 100 ft-c. More chlorophyll formed at 100 and 150 ft-c than at 50 and 200 ft-c. There were no significant differences between 100 and 150 ft-c. Generally, chlorophyll formation increased with increased light intensity up to 100 ft-c, slowly and steadily declined up to 150 ft-c, and sharply declined at 200 ft-c. Possibly, the higher and more intense light may have degraded the chlorophyll already formed.

Gull and Isenberg (1960) subjected Kennebec, Cherokee, and Katahdin potatoes to 25, 50, 75 and 100 ft-c for 5 days. They reported that more greening occured at 50 ft-c than at 25 ft-c, but found no increase in the amount of greening at intensities above 50 ft-c. Yamaguchi et al. (1960) tested light intensities from 10–135 ft-c on White Rose potatoes and found increasing quantities of chlorophyll as the intensity was increased. The efficiency of light in causing greening was lower at higher light intensities, which was attributed to the lower rate of protochlorophyll formation or to a light filtering effect from the chlorophyll already present. Liljemark and Widoff (1960) studied the effect of increasing light intensities on chlorophyll development from 15–360 ft-c. They observed the increase in chlorophyll content with increase in light intensity and further noted that the chlorophyll content appeared to rise in proportion to the logarithm of light intensity values in lux.

The differences in the solanine content after exposure to the four light intensities were not significant (Fig. 2). Gull and Isenberg (1960) and Liljemark and Widoff (1960) also found higher solanine contents in potato tubers stored at lower temperatures for a period of about 5 months. This initial rise in solanine content was attributed to bud activity and sprout growth (Gull and Isenberg, 1960). However, in our experiments, no sprout growth was observed. The results obtained in this experiment which were in conformity with those already reported warrant the conclusion that solanine synthesis occurred in the potato tubers stored at lower temperatures and increased gradually during the storage period.

Chemicals

Table 2 and Fig. 3 show that Ethrel and Alar were significantly effective in inhibiting chlorophyll and solanine synthesis. Ethrel was most effective in controlling the chlorophyll formation, and the least solanine was formed after the Alar treatment. There were, however, no significant differences between these two treatments. Maleic hydrazide and Cycocel were observed to be ineffective to control both chlorophyll and solanine formations. The tubers treated with Cycocel appeared greener as compared to other treatments and control. Schwimmer and Weston (1958) also reported maleic hydrazide to be ineffective in controlling postharvest greening.

Table 2—Chlorophyll and solanine contents of Kennebec potato tubers subjected to chemical treatments and exposed to 100 ft-c of white fluorescent light for a period of 5 days during December, 1969

Chemical		Chlorophyll (mg/100 g of fresh peel)	Solanine
Alar		0.941	58.63
Cycocel		2.077	82.37
Ethrel		0.524	62.52
Maleic hydrazide		1.738	84.22
Control		2.073	86.06
LSD	0.01	0.48	18.99
	0.05	0.31	12.19

According to Conner (1937), the processes of chlorophyll and solanine syntheses are independent. Gull and Isenberg (1960) confirmed the findings of Conner. They noted, however, that greened potatoes contain more solanine than ungreened. Guseva et al. (1960) showed the utilization of acetate and mevalonate for the biosynthesis of solanine in potato sprouts. Studies of Dennis et al. (1965) and Ryugo and Sachs (1969) revealed that Alar depressed the incorporation of mevalonic acid into isoprenoids. Perhaps Alar inhibits the synthesis of solanine by inhibiting conversion of mevalonic acid into isoprenoid moiety of solanine.

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STANLEY D. KOCH[1], ASHER A. HYATT[2] and DOLORES V. LOPIEKES[3]
Monsanto Research Corp., Boston Laboratory, Everett, Mass. 02149

BASIS OF STABILITY OF AMINE SALTS OF LINOLEIC ACID
1. Generality of the Oxidation Protection and Effect of Physical State

SUMMARY—The mechanism and generality of the known stabilization against autoxidation conferred on linoleic acid by certain basic amino acids, such as lysine and arginine, was investigated. Basic amino acids were the only class of compounds found to confer the effect. However, the smallest basic amino acid, 2,3-diaminopropionic acid, was not effective, nor was a β,ω-diamino acid, 3,6-diaminohexanoic acid, although a simple isomer of lysine. The stabilization was observed only in the solid phase. Inclusion of sodium chloride in the solid matrix was deleterious to the effect. A large number of physical and chemical observations were made and correlated but it has not been possible to draw detailed conclusions about the mechanism of stabilization, nor can a detailed structure of the stabilized complex be suggested. The cause of the phenomenon appears to be closely associated with the physical arrangement of the ions in the crystal lattice.

INTRODUCTION

SALTS of basic amino acids and linoleic acid were described by Chang and Moyer (1960) and Chang and Linn (1964). They reported that the salts were far more resistant to becoming rancid by autoxidation than the linoleic acid. No synergism with phenolic additives was involved. At the beginning of this work it was thought that these salts might find important use if meats and other foods containing unsaturated fatty acid derivatives could be protected against autoxidation by means of edible additives.

For convenience throughout these two papers, the 1:1 compound of lysine and linoleic acid is called lysinium linoleate, and referred to as a salt, without the intention of prejudicing the evaluation of data on the structure of the compound or the mechanism of the effect.

The generality of the oxidation protection phenomenon was studied thoroughly with the Warburg apparatus and a variety of natural and synthetic basic amino acids, simple amino acids, monoamines, diamines, and derivatives of lysine with linoleic acid. Other unsaturated acids and derivatives of them were examined in the Warburg apparatus with lysine. This apparatus was also used to study the effect of physical state on the autoxidation of linoleic acid and lysinium linoleate, and the effect on the latter of contamination with trace metals or with sodium chloride.

EXPERIMENTAL

SALT COMPLEXES were prepared by the method of Chang and Moyer (1960). An alcoholic solution of the basic amino acid was treated with an equimolar alcoholic solution of linoleic acid or other fatty acid to give a 95–98% yield of white solid. The solid was washed thoroughly with anhydrous ether and dried in vacuo.

The stabilizing effect of the salts was investigated in comparison to the free fatty acid by oxidation experiments carried out on a "Precision" 20-unit Warburg manometricon using conventional procedures (Umbreit et al., 1949). Oxidations were conducted on samples containing 90 mg of fatty acid or the equivalent amount of salt. The dimensions of the manometers limited the total oxygen uptake to 300 μliters (1.3×10^{-5}mole). Thus, the Warburg reading represents a ratio of moles of oxygen consumed to moles of compound charged of 0.06. A leveling off of oxygen uptake below 300 μliters indicated a stable system.

Infrared studies were carried out on all reaction products, using a Perkin-Elmer Infracord Model 137 Spectrophotometer. Comparison of the spectra of the free bases with those of the salts showed a complete absence of the N-H band at 3 μm in the spectra of the salts. Comparison of the spectra of the fatty acids with those of the salts showed the disappearance of the 5.8-μm carbonyl absorption band from the spectrum of the fatty acid.

Unless specified, all reaction studies were carried out at 25°C. The various amino acids were purchased from Sigma Chemical Co., California Corp. for Biochemical Research, and Nutritional Biochemicals Corp. The linolelaidic acid and derivatives were purchased from the Hormel Institute, while other fatty acids came from Sigma Chemical Co. All materials were of the highest purity commercially available.

RESULTS & DISCUSSION

Generality of the oxidation protection effect

The oxidation of various basic amino acid–linoleic acid complexes is summarized in Table 1. The table lists the time required for an oxygen uptake of 300 μliters, which gives an index of degree of inhibition of autoxidation.

A solution of linoleic acid in aqueous ethanol oxidized in 27 hr or less. Although not shown in the table, the same rapid oxidation took place when solutions of the fatty acid in butanol or Nujol were studied. Furthermore, it was found that 20% and 50% mulls of lysinium linoleate in linoleic acid autoxidized rapidly, as did various mulls of linoleic acid with lysine/2,4-diaminobutyric acid, lysinium oleate, and lysinium stearate. These experiments emphasized that oxidation stability is associated with the dry salts and that the phenomenon does not extend to the protection of greater-than-stoichiometric ratios of linoleic acid to basic amino acids.

The simple analogs of lysine studied were ornithine, 2,4-diaminobutyric acid, and 2,3-diaminopropionic acid. Ornithine and 2,4-diaminobutyric acid were found to behave like lysine and to protect linoleic acid from autoxidation. However, although 2,3-diaminopropionic acid, the smallest basic amino acid, formed a salt when reacted with linoleic acid, it did not retard the autoxidation at all. It was thus clear that the chain length, and presumably stereochemical fit, of the amino acid were important factors affecting oxidation protection ability.

In connection with the possibility that the effect being studied is a solid-state phenomenon, it is of interest that the salts of linoleic acid with basic amino acids were always solids. Full protection against autoxidation was obtained only with basic amino acids. Salts of linoleic acid with other compounds tested were not solid. However, the fact that the salts with 2,3-diaminopropionic and 3,6-diaminohexanoic acids are solids, and yet oxidize, shows that the solid state alone is not the basis for the stability.

One basic amino acid, arginine, was examined for salt formation and oxidation protective ability in both the D- and L-forms. No difference was found.

To ascertain whether free amino groups were necessary for the protective ability of a basic amino acid, N,N,N′,N′-tetramethyllysine was synthesized (Bowman and Stroud, 1950) and reacted with linoleic acid. The product was not a

[1] To whom inquiries should be directed. Present address: Horizons Research, Inc., Cleveland, Ohio 44122.
[2] Present address: Division of Research Grants, National Institutes of Health, Bethesda, Md. 20014.
[3] Present address: Gillette Toiletries Co., Boston, Mass. 02106.

Table 1—Warburg oxidations, basic amino acids. Time required for uptake of 300 μ liters of oxygen; charge equivalent to 90 mg of linoleic acid; temperature 25°C

Name	Uptake time, hr
Lysinium linoleate	1440[a,c]
2,3-Diaminopropionic acid/ linoleic acid	24
2,4-Diaminobutyric acid/ linoleic acid	330[a,c]
Ornithinium linoleate	791[a,c]
L-Argininium linoleate	552[a,c]
D-Argininium linoleate	552[a,c]
3,6-Diaminohexanoic acid/ linoleic acid	456
N_ϵ-Benzoyllysine/linoleic acid	24
N,N,N',N'-Tetramethyllysine/ linoleic acid	120
Linoleic acid + aqueous ethanol	18–<24
Lysinium linoleate + aqueous ethanol	<17–<24
Lysinium linoleate (0.13g) + 30% aqueous ethanol	<20
Lynsinium linoleate (0.13g) + 0.5g water	288
Lysinium linoleate (0.13g) + 1g water	264
Lysinium linoleate (0.13g) + 1g water + 0.001g EDTA	384
50% Mull of lysinium linoleate and linoleic acid	30–<48
20% Mull of lysinium linoleate + linoleic acid	20–124
50% Mull of lysine free base + linoleic acid	598
20% Mull of lysine free base + linoleic acid	100–240
50% Mull of 2,4-diaminobutyric acid linoleate + linoleic acid	264[a,b]
20% Mull of 2,4-diaminobutyric acid linoleate + linoleic acid	168–216
Deuterated lysinium linoleate	528
Lysinium linoleate freeze-dried	960
Lysinium linoleate + copper acetate	1032[a,b]
Linoleic acid/butylamine/ L-norleucine	<72
Linoleic acid/butylamine/ glycine	456

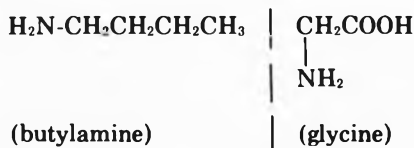
[a]Deliberately terminated.
[b]Slight oxidation.
[c]No oxidation at all.

normal salt and the material oxidized rapidly.

One basic amino acid without an α-amino group was examined for salt formation and oxidation inhibition of linoleic acid: 3,6-diaminohexanoic acid, $NH_2CH_2CH_2CH_2\underset{NH_2}{\underset{|}{CH}}CH_2COOH$. The compound was lysine in which the α-amino group has simply been moved to the β-position. The compound was synthesized (van Tamelen and Smissman, 1953; Sheehan et al., 1952; Pelizzoni and Jommi, 1959) and reacted with linoleic acid to give a solid salt that was normal as judged by the infrared spectrum. It was slightly impure, showing some absorption attributed to $-OH$ or $-NH$. Warburg experiments showed that the salt did not offer total inhibition of autoxidation; the salt gradually oxidized, absorbing 300 μliters of oxygen in 19 days. This acid, which did not protect, was the only β,ω-diamino acid tested.

The rate of autoxidation of deuterated lysinium linoleate was also measured. The samples oxidized, although very slowly. The method of preparation differed from the usual procedure for lysinium linoleate in that freeze drying was involved. This caused a change in physical appearance. Since changes in physical state caused changes in oxidation behavior (described below), a sample of freeze-dried lysinium linoleate was tested as a control. The protonated freeze-dried sample did resist autooxidation. This leads to the conclusion that deuteration had a real effect. In the absence of corroborating experiments, however, we perfer to regard this result as an artifact.

In order to form a lysinium linoleate analog in which the lysine skeleton had been broken, linoleic acid was complexed with butylamine in the presence of glycine to give lysinium linoleate in which the lysine skeleton was broken along the carbon chain:



This combination did not show the protective effect, but was only slowly autoxidized (456 hr for 300 μliter oxygen uptake). On the other hand, linoleic acid complexed with butylamine and norleucine showed no such inhibition.

When amino acids are used as synergists with phenolic antioxidants, the effect is usually ascribed to chelation of prooxidative metal traces (Marcuse, 1962). To evaluate this mechanism a study was made of lysinium linoleate deliberately contaminated with a copper prooxidant. Cupric acetate, 0.2 mg, was dissolved in 1000 ml of methanol. A 100-ml portion of this solution was added to 0.3g of lysinium linoleate dissolved in 10 ml of methanol. The reaction mixture was evaporated to dryness and the recovered solid subjected to oxidation experiments. It was found that the stability of the salt had been retained. The infrared spectrum of the lysinium linoleate was unchanged by the addition of the copper acetate.

Related to these experiments was a Warburg experiment with linoleic acid and ethylenediaminetetraacetic acid (EDTA). This was found not to protect linoleic acid from autoxidation. Salt formation could not occur as EDTA has four carboxyl groups but only two basic nitrogen atoms and so probably exists as a dizwitterion with two free carboxyl groups. However, if trace metals had been responsible for initiating oxidation, the EDTA would effectively have removed them from solution and so protected the linoleic acid. That this did not occur further demonstrated the inapplicability of this trace-metal hypothesis.

In order to study the generality of the autoxidation inhibition effects, salts and mixtures of linoleic acid with a variety of lysine analogs other than basic amino acids were prepared for Warburg oxidation studies. The analogs were derived from lysine by systematically varying the chain length and the presence or absence of each of the functional groups. The data collected showed that compounds that were not basic amino acids did not protect linoleic acid from autoxidation. In some cases, the materials prepared for Warburg experiments were shown to be salts by infrared spectroscopy; in other cases, they were physical mixtures or solutions. Some of the materials were solids, while others were liquids. Physical state, salt formation, and behavior in the Warburg apparatus could not be correlated. The Warburg experiments on these lysine analogs are summarized in Table 2.

Modification of lysine by elimination of the carboxyl group and one amino group leaves a straight chain primary amine. The simple primary amines investigated gave liquid complexes with linoleic acid. Infrared spectra showed that the primary amine stretching frequency at 3 μm was replaced by the RHN_3^+ band and the 5.8 μm carbonyl absorption band replaced by the vibration associated with the carboxylate anion. Since these simple alkylammonium linoleates were all liquids, the experiments did not answer the key question of whether the inhibition of autoxidation was due to the solid physical state or to a chemical effect peculiar to basic amino acids. An attempt was made to find an alkylammonium linoleate that was itself a solid, but none was found. Salts of higher molecular weight amines were viscous liquids. Although they did not fully protect linoleic acid from autoxidation, such amines did decrease the rate of autoxidation; the magnitude of this effect apparently increased with the molecular weight of the amine and the viscosity of the salt.

Ammonium linoleate itself was prepared and subjected to Warburg oxidation and it was found to autoxidize very rapidly.

Table 2—Warburg oxidations, lysine analogs other than basic amino acids. Average time required for uptake of 300 μliters of oxygen; charge equivalent to 90 mg of linoleic acid; temperature 25°C

Name and structure of compound mixed with linoleic acid		Physical state	Salt or mixture	Avg uptake time, hr
ε-Aminocaproic acid	$NH_2(CH_2)_4CH_2COOH$	semi-solid	M	<24
L-Norleucine	$CH_3(CH_2)_3CHCOOH$ NH_2	semi-solid	M	<48
Valeric acid	$CH_3(CH_2)_3COOH$	liquid	M	<48
Adipic acid	$HOOC(CH_2)_4COOH$	semi-solid	M	<36
Glutamic acid	$HOOC(CH_2)_2CHCOOH$ NH_2	semi-solid	S	<24
Heptadecylamine	$C_{17}H_{35}NH_2$	semi-solid	S	<240
Dodecylamine	$C_{12}H_{25}NH_2$	liquid	S	240
Butylamine	$C_4H_9NH_2$	liquid	S	72
Putrescine	$NH_2(CH_2)_4NH_2$	viscous liquid	S	576
Cadaverine	$NH_2(CH_2)_5NH_2$ (1 mole)	viscous liquid	S	792
Cadaverine	$NH_2(CH_2)_5NH_2$ (1/2 mole)	viscous liquid	S	552
1,6-Hexanediamine	$NH_2(CH_2)_6NH_2$	viscous liquid	S	600
1,12-Diaminodecane	$NH_2(CH_2)_{10}NH_2$	viscous liquid	S	360
Piperazine	HN NH (ring)	viscous liquid	S	336
Ammonia	NH_3	viscous liquid	S	28
Ethylenediamine-tetraacetic acid	$HOOCCH_2$ CH_2COOH NCH_2CH_2N $HOOCCH_2$ CH_2COOH	semi-solid	M	<24
Ethylenediamine	$NH_2(CH_2)_2NH_2$	liquid	S	24
Diethylenetriamine	$NH_2(CH_2)_2NH(CH_2)_2NH_2$	liquid	S	144
Lysine hydrochloride	$[NH_2(CH_2)_4CHCOOH$ (NH_2)$]$ HCl	semi-solid	M	24
1,4-Diazabicyclo-[2.2.2]octane	N N (bicyclic structure)	liquid	M	48
3-Azabicyclo-[3.2.2]nonane	NH (bicyclic structure)	liquid	M	48

Modification of lysine by elimination of only the carboxyl group leaves a diamine. The simple straight chain diamines formed normal salts with an equimolar amount of linoleic acid. These salts were liquids of varying viscosities. The infrared spectra of preparations of linoleic acid with the diamines again showed the disappearance of the 5.8 μm carbonyl absorption of linoleic acid and its replacement by the vibration associated with the carboxylate anion. The free $-NH_2$ bands were complemented by $-NH_3^+$ bands showing that salt-formation had occurred. It was not possible to determine whether the products consisted of all monoamine-monosalt or a mixture of disalt and unchaged diamine. Thin layer chromatography was attempted to settle this point in the case of cadaverine, but the results were inconclusive. This variable was obviated in the case of cadaverine by also preparing the salt with two molar equivalents of linoleic acid. It was found that autoxidation proceeded at the same slow rate for the 1:2 salt as for the 1:1 salt. This is of interest because after 1:2 salt-formation has taken place, each NH_3^+ group of the diamine is associated with a linoleate COO^- and there are no functional groups left to interact with the unsaturated region. These salts with diamines did not fully protect linoleic acid from autoxidation, but exhibited the same retardation phenomenon as the simple amines, the effect again being most noticeable with higher molecular weight diamines. The weights of the samples used in the Warburg experiments were chosen to allow for the molecular weight of the amine so that the same amount of linoleic acid was present in each case. Thus, the retardation effect is real and not caused by dilution. However, the effect could be associated with the increase in viscosity of the salt with increase in molecular weight observed with both the monoamines and diamines.

The structural modification made by elininating one or both amino functions gives rise to simple amino acids and carboxylic acids, respectively. Salts were not formed with linoleic acid and no protection at all against autoxidation was found. The infrared spectra in these cases were essentially the sums of the spectra of the components.

All of these experiments suggested that salt formation was essential for protection and that it was the linoleate ion that was protected. That these conditions were necessary but not sufficient was emphasized by the fact that calcium linoleate autoxidized rapidly in the Warburg apparatus.

These premises were confirmed by studying the autoxidation of mixtures of lysine with linoleyl alcohol, methyl linoleate and trilinolein. In each case, autoxidation was very rapid; the lysine clearly did not protect. The infrared spectra of these mixtures showed that salt-formation did not occur.

A mixture of lysine hydrochloride and linoleic acid also autoxidized rapidly. The spectrum of the preparation was the sum of the spectra of the components. Similar observations were found for a mixture of N,-benzoyllysine and linoleic acid. These two experiments confirmed the need for an unionized amino group in the protective agent.

Lysine was also studied with oleic and stearic acids. These acids were found to oxidize at much slower rates than linoleic acid, so the question of whether or not they are stabilized as lysinium salts is less meaingful. Protection of these two acids is of less practical interest than protection of linoleic acid. They were not protected from autoxidation by the lysine.

Oxidation results with all linoleic acid analogs are accumulated in Table 3.

Pure linolelaidic acid, the trans,trans isomer of linoleic acid, was found to oxidize quite rapidly, although not as fast as its stereoisomer, cis,cis-linoleic acid. It formed a normal salt with lysine, and the salt was resistant to autoxidation. Thus, the stereochemical configuration of the carboxylic acid surprisingly does not seem to be a factor in the phenomenon.

Effect of the physical state

As mentioned above, although lysinium linoleate did not oxidize in Nujol mulls, when dissolved in aqueous ethanol there was no inhibition of autoxidation. The inhibitory effect was also considerably reduced when the salt was in aqueous solution. These experiments are summarized in Table 1.

Lysinium linoleate was found to be insoluble in anhydrous ethanol and in polar nonhydroxylic solvents such as dimethyl sulfoxide, dimethylformamide, methylene chloride, the sulfolanes, and glymes: solvents which might have placed the salt in the liquid phase without breaking up the complex. If such a nonhydroxylic solvent had been found, experiments would have been run on the solutions to check the apparent need for close association of the ions for inhibition of oxidation.

The part played in the inhibition of autoxidation by the solid state was investigated by running oxidation studies of linoleic acid at −9 and −12°C, just above and below the freezing point of linoleic acid, and comparing with lysinium linoleate at the same temperatures. The results of the experiments, summarized in Table 4, showed that the solid state alone was not the cause of the inhibition of autoxidation of linoleic acid. The induction period was prolonged the lower the temperature, but once oxi-

Table 4—Warburg oxidations at various temperatures, lysine and lysinium linoleate. Time required for uptake of 300 μliters of oxygen; charge equivalent to 90 mg of linoleic acid

System	Avg uptake time, hr
Linoleic acid at 37°C	24
	24
Lysinium linoleate at 37°C	72[a]
Linoleic acid at 25°C	48
	48
Lysinium linoleate at 25°C	1336[a]
	391[a]
Linoleic acid at −9°C	144
	240
Lysinium linoleate at −9°C	168[a]
	312[a]
Linoleic acid at −12°C	384
	408
Lysinium linoleate at −12°C	384[a]
	480[a]

[a]No oxidation; deliberately terminated.

Table 3—Warburg oxidations of lysine with linoleic acid derivatives. Time required for uptake of 300 μ liters of oxygen; charge equivalent to 90 mg of linoleic acid; temperature 25°C

Name and structure of compound mixed with lysine		Physical state	Salt (S) or mixture (M)	Avg uptake time, hr
Linoleyl alcohol	$C_5H_{11}CH=CHCH_2CH=CH(CH_2)_7CH_2OH$	viscous liquid	M	<72
Methyl linoleate	$C_5H_{11}CH=CHCH_2CH=CH(CH_2)_7COOCH_3$	viscous liquid	M	120
Trilinolein	$CH_2OOC(CH_2)_7CH=CHCH_2CH=CHC_5H_{11}$ / $CHOOC(CH_2)_7CH=CHCH_2CH=CHC_5H_{11}$ / $CH_2OOC(CH_2)_7CH=CHCH_2CH=CHC_5H_{11}$ (1 mole)	viscous liquid	M	52
Trilinolein	$CH_2OOC(CH_2)_7CH=CHCH_2CH=CHC_5H_{11}$ / $CHOOC(CH_2)_7CH=CHCH_2CH=CHC_5H_{11}$ / $CH_2OOC(CH_2)_7CH=CHCH_2CH=CHC_5H_{11}$ (1/3 mole)	viscous liquid	M	48
Oleic acid	$CH_3(CH_2)_7CH=CH(CH_2)_7COOH$	solid	S	791
Stearic acid	$CH_3(CH_2)_{16}COOH$	solid	S	791[a,c]
Oleic acid (no lysine)		liquid	—	792[a,c]
Stearic acid (no lysine)		solid	—	792[a,c]
50% Mull of lysinium oleate + linoleic acid		liquid	—	576[a,b]
20% Mull of lysinium oleate + linoleic acid		liquid	—	120
50% Mull of lysinium stearate + linoleic acid		liquid	—	<68
20% Mull of lysinium stearate + linoleic acid		liquid	—	28
Linolelaidic acid	*trans, trans*-$C_5H_{11}CH=CHCH_2CH=CH(CH_2)_7COOH$	solid	S	1416[a]
Linolelaidic acid (no lysine)		liquid	—	168

[a]Deliberately terminated.
[b]Slight oxidation.
[c]No oxidation at all.

dation began, it continued in the characteristic manner of uninhibited linoleic acid.

The hypothesis that it was the linoleate ion in a specific crystalline matrix whose autoxidation was inhibited was tested by studying the autoxidation of lysinium linoleate prepared in the presence of sodium chloride by several routes. When equimolar amounts of lysinium linoleate and sodium chloride were ground together in the solid state, the product was as completely resistant to oxidation as if the sodium chloride were not present. But, when this mixture was prepared by evaporating a solution containing lysinium linoleate and sodium chloride, the product was no longer completely resistant. Oxidation occurred, although it was very slow. This slow oxidation was also observed when lysinium linoleate was dispersed in potassium bromide and pressed at 20,000 psi into a pellet in the manner used for infrared spectroscopy. (It should be noted that this last experiment did not bear on the validity of the infrared work on lysinium linoleate, for those spectra were taken in Nujol mulls, a state in which the oxidation inhibition phenomenon had been shown to be retained.) The infrared spectrum of lysinium linoleate-sodium chloride ground dry was identical to that of lysinium lineolate in a Nujol mull or potassium bromide pellet. However, the spectrum of the evaporation co-solution, although grossly similar, differed in several details characteristic of a change of state. These results suggested that when the crystal lattice of lysinium linoleate was altered by the inclusion of foreign ions, the autoxidation inhibition phenomenon was damaged.

The effect was even more pronounced when lysinium linoleate-sodium chloride was prepared from equimolar amounts of sodium linoleate and lysine hydrochloride, either ground together or recovered by evaporation of their cosolution. The oxidation rates were then comparatively fast. The infrared spectra differed in several details from that of lysinium linoleate prepared in the usual manner, again suggesting a change of state.

The Warburg oxidation results for this series are shown in Table 5.

Table 5—Warburg oxidations, effect of sodium chloride. Time required for uptake of 300 μliters of oxygen; charge equivalent to 90 mg of linoleic acid; temperature 25°C

Mixture	Physical state	Avg uptake time, hr
Lysinium linoleate and sodium chloride (from solution)	solid	576
Lysinium linoleate and sodium chloride (solid mix)	solid	696[a]
Sodium linoleate and lysine hydrochloride (from solution)	solid	236
Sodium linoleate and lysine hydrochloride (solid mix)	solid	216
KBr pellet of lysinium linoleate	solid	720
(Control) Lysinium linoleate	solid	888[a]

[a]No oxidation; deliberately terminated.

CONCLUSIONS

EXPERIMENTS to delineate the generality of the effect showed that compounds that were not basic amino acids did not protect linoleic acid from autoxidation. Some amines and diamines formed salts with linoleic acid that were found to decrease the rate of autoxidation, but these did not provide the total protection provided by the lysine compound. This result might have been a viscosity effect.

All the salts with basic amino acids were solids. Two were found that did not show the oxidation-inhibition effect as claimed in the patent: 2,3-diaminopropionic acid, the smallest basic amino acid possible; and 3,6-diaminohexanoic acid, the only basic amino acid tested that was not α,ω-diamino. The only basic amino acids available commercially or much studied are the α,ω-diamino examples which apparently give the effect.

It was thus clear that the chain length and stereochemical fit were of some importance, although it it important in this context that lysine was found to protect linolelaidic acid, the trans,trans-isomer of linoleic acid, which has a totally different shape from cis,cis-linoleic acid.

It seemed possible that the effect was specifically associated with the solid state, the rigid crystalline lattice of lysinium linoleate acting as a barrier to the diffusion of oxygen and oxidized intermediates and to the propagation of chain reactions. The fact that simple solid salts such as ammonium linoleate and frozen solid linoleic acid both oxidized showed that the solid state alone was not sufficient to cause inhibition. Thus ionization and solid state are both necessary but neither is sufficient to explain the oxidation inhibition effect.

A series of experiments in which sodium chloride was used to contaminate lysinium linoleate on a mole-to-mole basis showed that the more intimately foreign ions were included in the crystal lattice of lysinium linoleate, the greater was the damage to the autoxidation-inhibition phenomenon.

In summary, the following minimum requirements were found for the inhibition of autoxidation of linoleic acid by a basic amino acid: (a) Salt formation must occur, for it is the linoleate ion that is protected; (b) The salt must be solid and dry; (c) The ions in the solid state must be closely associated; and (d) There must be an undefined stereochemical fit (which cannot be met by 2,3-diaminopropionic or 3,6-diaminohexanoic acids).

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DOLORES V. LOPIEKES[1] *and STANLEY D. KOCH*[2]
Monsanto Research Corp., Boston Laboratory, Everett, Mass. 02149

BASIS OF STABILITY OF AMINE SALTS OF LINOLEIC ACID
2. Structure-Property Correlations on Lysinium Linoleate

SUMMARY—The solid compound formed by treating L-lysine with linoleic acid has been examined by chemical stoichiometry, electrical conductivity, infrared and nuclear magnetic resonance spectroscopy. The compound has been shown to be a true equimolar salt. Evidence from nmr and ir spectroscopy shows that the diene system of the linoleic acid is not changed from that in the free fatty acid. Conductivity measurements show that the salt is a moderately strong electrolyte. Salts of other basic amino acids, ornithine and 2,4-diaminobutyric acid, with linoleic and oleic acids were also examined and showed similar evidence. Interpretation of the data and the significance of the findings to stability of amino acid salts of unsaturated fatty acids are discussed.

INTRODUCTION

SALTS of basic amino acids and linoleic acid are described by Chang and Moyer (1960) and Chang and Linn (1964). The inhibition of autoxidation of the linoleic acid component of the salts was discussed in the first paper of this series (Koch et al., 1971). The present study was undertaken to investigate the interaction of amino acids with fatty acids. It was hoped that the structural studies of lysinium linoleate would provide information on the mechanism of the oxidation protection effect of basic amino acids on unsaturated fatty acids.

EXPERIMENTAL

AMINO ACIDS used were obtained from Sigma Chemical Co. in highest purity available. Linoleic and linolenic acids of 99% purity were purchased from Hormel Institute, and oleic acid, 99% purity, was procured from Nutritional Biochemicals Corp. The salts were prepared by the method of Chang and Moyer (1960).

Nuclear magnetic resonance spectra were determined on a Varian Model A-60 instrument, referred to tetramethylsilane in a concentric tube system. Samples were run as 5–10% solutions in deuterochloroform, deuterium oxide and pyridine, and deuterium oxide and methanol, at a wide sweep width of 500 cps.

Electrical conductivity was determined on an Industrial Instruments Inc. conductivity bridge, model RC-16B2, at 1000 cps.

RESULTS & DISCUSSION

Stoichiometry

The salts were made by the interaction of equimolar quantities of the two ingredients. To confirm the stoichiometry quantitatively, lysinium linoleate was treated with calcium chloride:

lysinium linoleate + $CaCl_2$ →
calcium linoleate + lysine·HCl

To 0.005 mole of lysinium linoleate dissolved in water was added with stirring an equivalent amount of calcium chloride also dissolved in water. The white precipitate was filtered off and dried. Calculated for calcium linoleate: 1.4980g; found: 1.4944g. The infrared spectrum of the calcium linoleate obtained in this manner agreed with the spectrum for calcium linoleate made directly from calcium hydroxide and linoleic acid.

The identity and purity of the precipitated calcium linoleate was confirmed by ashing. Ash, calculated for CaO: 9.3%; found: 9.8%

The filtrate from the reaction mixture was evaporated to dryness and constant weight to give a white solid. Calculated for lysine monohydrochloride: 0.9135g; found: 0.9110g. Melting point after recrystallization 234–235°C; reported (Rice, 1949), 235–236°C. When mixed with an authentic sample of lysine monohydrochloride there was no melting point depression. Infrared spectrum was identical with authentic lysine monohydrochloride. Monopicrate, yellow needles mp 264–266°C; reported (Greenstein and Winitz, 1961), 266°C.

Electrical conductivity

Conductance at infinite dilution of lysinium linoleate. For the strong electrolytes sodium chloride, sodium linoleate, and lysine hydrochloride, the equivalent conductance is seen to be a linear function of the square root of the concentration, Figure 1. Lysinium linoleate also gave a straight-line plot, indicating that it, too, was dissociated in solution and behaved as a strong electrolyte. Extrapolation of these lines to infinite dilution gave accurate values of Λ_0.

The equivalent conductances in 90% methanol of all the salts examined, Figure 1, were lower than for strong electrolytes in water (Glasstone, 1942).

The equivalent conductance of lysinium linoleate was calculated from:

$$\begin{array}{l} \Lambda_0\,(\text{Lys HCl}) + \Lambda_0\,(\text{NaLin}) \\ \quad (60.5) \quad + \quad (63.5) \\ \quad - \Lambda_0\,(\text{NaCl}) = \Lambda^0\,(\text{LysLin}) \\ \quad - \quad (87) \quad = \quad (37) \end{array}$$

The calculated Λ_0 is 37 $ohms^{-1}\ cm^2$; the extrapolated experimental value for lysinium linoleate is 39 $ohms^{-1}\ cm^2$. This is considered very good agreement and confirmation of the normal behavior of lysinium linoleate as a strong electrolyte in solution.

Degree of dissociation of lysinium linoleate. The degree of dissociation of lysinium linoleate in 90% methanol was calculated from the equivalent conductance and found to be 0.897 at 0.001N. Comparison with typical values in water (Glasstone, 1942) and with the experimental values in 90% methanol of 0.851 for sodium chloride, 0.902 for lysine hydrochloride, and 0.859 for sodium linoleate, shows that lysinium linoleate behaves as a uni-univalent electrolyte.

The ionization constant was calculated for lysinium linoleate from the degree of dissociation at several concen-

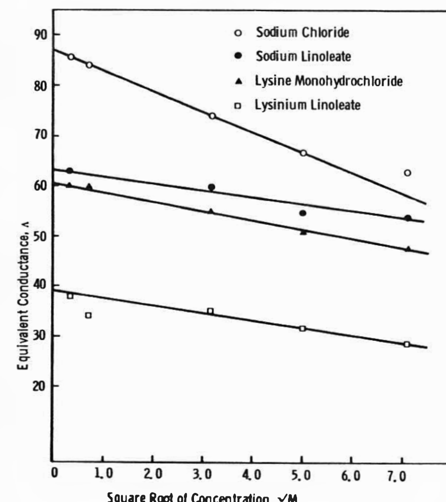

Fig. 1—Equivalent conductance in 90% methanol as a function of the square root of concentration for lysinium linoleate and related compounds.

[1]Present address: Gillette Toiletries Co., Boston, Mass. 02106
[2]To whom inquiries should be directed. Present address: Horizons Research, Inc., 23800 Mercantile Road, Cleveland, Ohio 44122.

trations. The activity coefficient was neglected and the degree of dissociation assumed to be equal to the conductance ratio. The results showed that in 90% methanol lysine hydrochloride, sodium chloride, and sodium linoleate had pK values of about 2, and the value for lysinium linoleate was 2 to 2.5. In water, values of pK of 2–3 are usually considered to show a moderately strong electrolyte, 5 weak, 9 very weak, and 12 extremely weak. Thus, lysinium linoleate was shown to be a moderately strong electrolyte of the uni-univalent type.

Conductivity of lysinium linoleate analogs. Ornithinium linoleate, 3-amino-3-carboxypropylammonium linoleate, lysinium oleate, and ornithinium oleate were made by the same procedure (Chang and Moyer, 1960); Chang and Linn, 1964).

All conducted a current in solution. For the linoleates a straight-line plot was obtained when equivalent conductance was plotted against the square root of concentration, indicative of a strong electrolyte. The oleates, however, gave steep curves typical of a weak electrolyte.

Infrared spectrum of lysinium linoleate

Gross structural features. The decreases in vibration frequencies which result from the formation of a charge-transfer complex involving increase in bond lengths are not observed in the infrared spectrum of Nujol mull preparations of lysinium linoleate. Neither are new, strong infrared bands corresponding to a complex. Thus it appears (Andrews and Keefer, 1964) that if charge transfer plays any role at all it is limited to a very weak interaction with relatively little distortion of the bonds.

Comparison of the N-H stretching vibration region in the spectrum of lysine free base with lysinium linoleate (Fig. 2) showed a complete absence of this band at 3 μm in the latter. It was concluded from these observations that the free amine was no longer present after reaction with linoleic acid.

Table 1—Reconstructed 6.0–6.8 μm region for lysinium linoleate

	Absorption contribution, absorption units			
	1610–1635 cm^{-1}	1560 cm^{-1}	1510 cm^{-1}	1580 cm^{-1}
Linoleate ion	0.017	0.33	0.034	0.34
Lysine monohydrochloride	0.269	0.056	0.35	0.0525
Sum, calculated	0.286	0.386	0.384	0.3925
Lysinium linoleate, observed	0.24	0.31	0.38	0.39

A comparison of the spectrum of linoleic acid with that of lysinium linoleate (Fig. 2) showed the disappearance of the 5.8 μm carbonyl absorption band in the latter. This indicated formation of the carboxylate anion.

The 6.0–6.8 μm region of lysinium linoleate was reconstructed from the absorption intensities of lysine monohydrochloride and calcium linoleate, as indicated in Table 1. This table shows that the spectrum of lysinium linoleate is a composite of that of the lysinium ion and the linoleate ion. Small deviations from perfect additivity were to be expected because of displacement of bands by hydrogen-bonding, physical state, and other masking effects. The correlation is considered satisfactory.

Double-bond region. The infrared spectrum of lysinium linoleate in a KBr pellet or a Nujol mull showed a shoulder at the expected frequency of a double bond stretch at 3020 cm^{-1}. Because of the interfering absorption of the NH_3^+ ion, it was not possible to state definitely that unsaturation was present. The 1660 cm^{-1} vibration associated with C=C was also lost in the C=O vibrations. The out-of-plane CH of the cis-olefin was very weak and thus of little value in detecting unsaturation.

In order to eliminate the interfering NH_3^+ absorption between 3300 and 2600 cm^{-1}, the lysinium linoleate was deuterated, converting NH to ND.

The deuteration was carried out as follows: approximately 100 mg of lysinium linoleate was added to 2 ml of deuterium oxide in a stoppered vial. After gentle heating and shaking for an hour, the salt dissolved in the deuterium oxide. The solution was frozen in liquid air and the deuterium oxide and water were removed by freeze drying for 4 hr. The deuterated lysinium linoleate was obtained as a fluffy white solid.

Successful deuteration caused a shift of the NH stretch from 3300–2630 cm^{-1} to 2500–2000 cm^{-1} for the ND stretch. When most of the interfering NH absorptions were removed, only the CH stretching vibration showed in this region. By running the infrared spectrum of deuterated lysinium linoleate in a Kel-F mull (no CH present), the presence of aliphatic CH as well as olefinic CH at 3020 cm^{-1} was seen. This is summarized in Table 2 which shows that unsaturation was present in deuterated lysinium linoleate.

Sinclair et al. (1952) have shown that in the C-H stretching region the band at 3020 cm^{-1} increases with the number of cis-double bonds present, while the relative intensity of the methylene cis-double bond peak at 2920 cm^{-1} diminishes. If d_A is the optical density at 3020 cm^{-1}, and d_B at 2920 cm^{-1}, then a plot of $d_B/(d_B - d_A)$ against the number of double bonds is approximately linear for oleic, linoleic, and linolenic acids, which contain one, two, and three cis double bonds, respectively. Curves similar to the one for cis-unsaturated acids

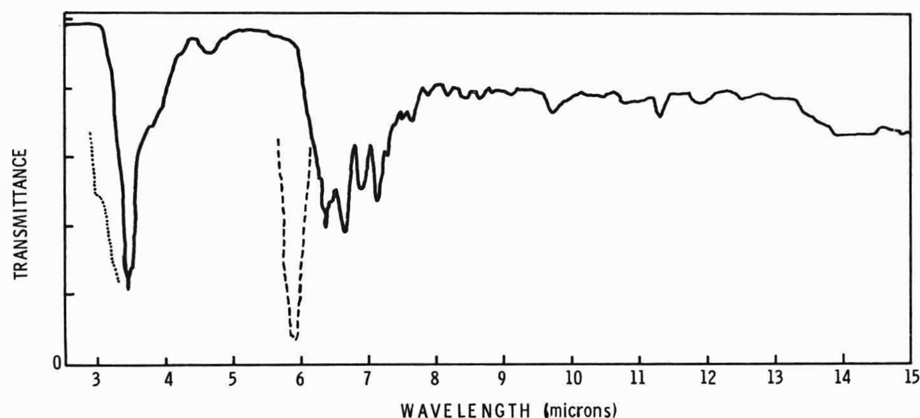

Fig. 2.—Infrared spectra of L-lysinium linoleate (Nujol mull), solid line; L-lysine (Nujol mull), dotted line; and linoleic acid (liquid film), dashed line.

Table 2—Infrared assignments

Lysinium linoleate (KBr)		Deuterated lysinium linoleate (Kel-F mull)	
Frequency, cm^{-1}	Assignment	Frequency, cm^{-1}	Assignment
3300–2630	NH_3^+	2500–2000	ND_3^+
3020	CH=	3020	CH=
2120	NH_3^+	1550[a]	ND_3^+
1563	NH_3^+ def., COO^-	1589 and/or 1560	COO^-
		1170	ND_3^+ def.

[a]Estimated.

Table 3—Infrared absorbance measurements, double bonds

Compound	Absorbance at 3019 cm^{-1} (d_A)	Absorbance at 2919 cm^{-1} (d_B)	$\frac{d_B}{d_B - d_A}$	Number of double bonds
Deuterated lysinium oleate	0.065	0.789	1.09	1
Deuterated lysinium linoleate	0.165	1.111	1.17	2
Deuterated lysinium linolenate	0.124	0.660	1.23	3

Table 4—Assignment of peaks from nuclear magnetic resonance spectrum of linoleic acid, 12% in $CDCl_3$

$CH_3(CH_2)_3CH_2CH=CHCH_2CH=CHCH_2(CH_2)_5CH_2COOH$
f e c a a b a a c e d g

chemical shift, ppm	assignment
11.18	g
5.50–5.25	a
2.9–2.6	b
2.5–1.8	c and d
1.7–1.1	e
0.88	f

Table 5—Assignment of peaks from nuclear magnetic resonance spectrum of L-lysine monohydrochloride, 12% in D_2O

$^-OOCCH(NH_3^+)(CH_2)_3CH_2NH_2 \cdot HCl$
a c b

chemical shift, ppm	assignment
5.30	residual water in D_2O; also all labile protons, i.e., HCl, NH_2, –COOH
4.4–4.1	a
3.7–3.4	b
2.7–1.7	c

Table 6—Assignment of peaks in the diene system from nuclear magnetic resonance spectrum of L-lysinium linoleate, 14.5% in MeOH

$CH_3(CH_2)_3CH_2CH=CHCH_2CH=CHCH_2(CH_2)_5CH_2COOH \cdot NH_2CH_2(CH_2)_3CH(NH_3^+)COO^-$
c a a b a a c d

chemical shift, ppm	assignment
5.5–5.2	a
2.7	b
2.4–1.85	c and d

were established for the methyl esters of these acids, and for their deuterated lysine salts. By this extrapolation, it was shown that there was no anomaly in the number of double bonds in solid lysinium linoleate.

Deuteration of the salts was carried out as described earlier and appeared to be about 80% complete as indicated by the infrared and nuclear magnetic resonance spectra. Contamination by foreign acids (including trans-stereoisomers) was found to be negligible by thin layer chromatography. If there had been appreciable quantities of trans-isomers, a correction would have been necessary because the optical density of the 3020 cm^{-1} band is weaker for trans-olefins than that for cis-olefins. The infrared spectra of deuterated lysine salts were run as hexachloro-1,3-butadiene mulls on the high resolution double grating Perkin-Elmer 421 instrument with a resolution of 0.3 cm^{-1} at 2200 cm^{-1}, sufficient sensitivity for the problem. The absorbance (optical density) measurements were made by the base line technique. The results are summarized in Table 3.

Nuclear magnetic resonance spectroscopy

It was readily possible to obtain nmr spectra of lysinium linoleate in solution. Useful solvents were 5–10% solutions in deuterochloroform, deuterium oxide-pyridine, and deuterium oxide-methanol. Interpretation and assignment of peaks from the nmr spectra of linoleic acid, L-lysine hydrochloride, and L-lysinium linoleate are shown in Tables 4–6.

The most interesting conclusion that may be drawn from these data is that there is no change in the environment of any of the protons of the diene system of linoleic acid observable in solutions of lysinium linoleate. This is shown in the 5.25–5.50 ppm region.

The nmr spectra further substantiate the infrared data since the carboxylic proton of the linoleic acid is no longer observed in lysinium linoleate (11.18 ppm) region.

CONCLUSIONS

CHEMICAL ANALYSES of lysinium linoleate verified that the product is a 1:1 compound from which the components could be recovered unchanged. Conductivity experiments show that lysinium linoleate is a true ionized salt displaying the properties of a uni-univalent strong electrolyte. Infrared and nmr spectra show that the entire diene system of linoleic acid is unchanged after salt formation.

The carboxylic acid group of the fatty acid may form a salt with one of the amine groups of the basic amino acid, leaving the other amino group available to affect the double bond area of the fatty acid. If the latter interaction is a

charge-transfer complex, it is a very weak one involving only partial donation of electrons and little bond distortion.

Attack by oxygen or free radicals on the α-methylene group of the linoleate is prevented by a mechanism which still cannot be explained. Although ionization and solid state are both necessary, neither by itself is sufficient to explain the oxidation inhibition. It would appear that the protective effect is specifically associated with the solid state.

The rigid crystalline lattice of lysinium linoleate could act as barrier to the diffusion of oxygen and oxidizer intermediates, and to the propagation of chain reactions. On the other hand, studies of the oxidation in solution (Koch et al., 1971) suggest that the salt complex also prolongs the induction period and retards the rate of autoxidation. Therefore, one might speculate that initially the solid complex physically prevents oxygen from entering into reaction. After the oxygen succeeds in attacking the organic substrate, the oxidation would be terminated by a mechanism in which the basic amino acid acts as an oxygen scavenger or a free radical chain terminator.

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R. PRESSEY and J. K. AVANTS
USDA Richard B. Russell Agricultural Research Center, ARS, Athens, Georgia 30604

EFFECT OF SUBSTRATE SIZE ON THE ACTIVITY OF TOMATO POLYGALACTURONASE

SUMMARY—Three polygalacturonic acid preparations with widely differing molecular weight distributions were obtained by controlled enzymatic hydrolysis of pectic acid. A study of the action of tomato polygalacturonase on the polygalacturonic acids and pectic acid revealed that the activity is dependent on the molecular size of the substrate. Pectic acid was hydrolyzed optimally at pH 5, with little activity below pH 4. Decreasing the molecular weight of the substrate resulted in a progressive shift of the pH optimum to the acid side. For the smallest substrate, the activity extended to below pH 2. Monovalent cations enhanced the activity at low pH, and this effect was also dependent on the molecular weight of the substrate. Below pH 4, pectic acid inhibited 70% of the hydrolysis of low molecular weight substrates by tomato polygalacturonase. The incomplete inhibition is attributed to the presence of a polygalacturonase isoenzyme which is not inhibited by high molecular weight polygalacturonic acids.

INTRODUCTION

EARLY STUDIES on tomato polygalacturonase indicated that it may be an endo-polygalacturonase with a specificity for large substrate molecules (McColloch and Kertesz, 1948). It caused the disappearance of fractions precipitable as calcium pectate at much lower reducing values than fungal polygalacturonase. Hydrolysis of pectic acid proceeded only to what appeared to be partial degradation, and galacturonic acid could not be detected as a product. With the advent of paper chromatography, other workers (Roelofsen, 1953; McCready et al., 1955) demonstrated that galacturonic acid is formed by the action of tomato polygalacturonase on pectic acid. They found, however, that the rate of monomer formation is much slower than that by fungal polygalacturonase. This has been confirmed by the observation that oligogalacturonides are hydrolyzed very slowly by the tomato enzyme (Luh et al., 1956). Evidence has also been presented which points to polygalacturonase isoenzymes in tomatoes, with possible differences in substrate size specificities. For example, the pH optimum for pectic acid hydrolysis is at 4.5 (McColloch and Kertesz, 1948), but Patel and Phaff (1960a, b) observed an additional peak of activity at pH 2.5 for the hydrolysis of acid-soluble polygalacturonic acid (D.P. 14).

It is clear that the molecular weight of the substrate is an important factor in studies on tomato polygalacturonase. We have conducted studies on the action of this enzyme on substrates with a wide range in molecular weights. The results suggest the existence of polygalacturonase isoenzymes in tomatoes, one of which functions in the degradation of large substrate molecules.

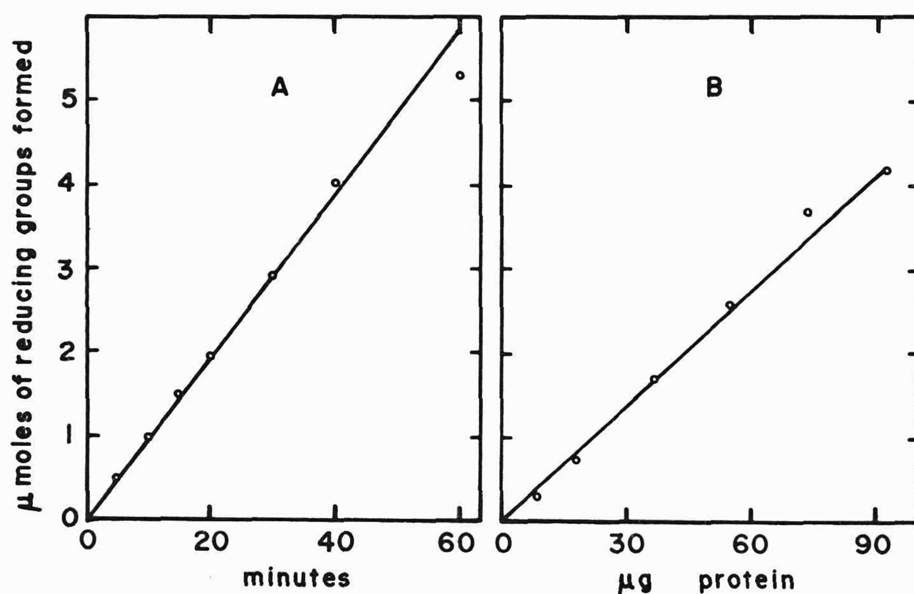

Fig. 1—Linearity of polygalacturonase activity with incubation time (A) and protein concentration (B). Conditions of the experiment were standard using pectic acid as the substrate at pH 4.5.

EXPERIMENTAL

Materials

Sodium polypectate, bovine serum albumin, egg albumin, cytochrome c, and purified fungal pectinase were purchased from Sigma Chemical Company, St. Louis, Missouri. Sephadex G-75 and G-100 and the standard dextrans were obtained from Pharmacia Fine Chemicals, Inc., Piscataway, New Jersey. Bio-Gel A-50m was a product of Bio-Rad Laboratories, Richmond, California.

Methods

Polygalacturonase assay. The standard reaction mixture contained 1 ml of 1% substrate adjusted to the proper pH and 0.95 ml of 0.63M NaCl. After incubation at 37° C for 10 min, 0.05 ml of enzyme solution was added and incubation was continued for 15 min. A heated enzyme blank was run with each sample. The reaction was stopped by placing in a boiling water bath for 2 min. A ml of the solution was then analyzed for reducing groups by the colorimetric method of Nelson (Ashwell, 1957), using galacturonic acid as the standard.

A unit of activity is defined as that amount which catalyzes the liberation of 1 μmole of reducing groups/15 min from the substrate pectic acid at pH 4.5. Specific activity is expressed in units/mg protein. Protein was measured by the method of Lowry et al. (1951), using bovine serum albumin as the standard.

Enzyme activity was linear with respect to both enzyme concentration and incubation time (Fig. 1).

Isolation of tomato polygalacturonase. The enzyme was isolated by a modification of the method of Patel and Phaff (1960a). Firm ripe Rutgers tomatoes were blended with an equal amount of 1M NaCl containing 0.2% sodium bisulfite in a VirTis homogenizer for 5 min at 5°C. The homogenate was centrifuged at 8000Xg for 20 min and the residue was discarded. A portion of the supernatant was dialyzed against 0.2M NaCl and analyzed for protein and polygalacturonase. The total protein content of the crude extract was 171 mg, and the specific activity of polygalacturonase was 19. The remainder of the supernatant was adjusted to pH 5 and solid ammonium sulfate was added to 50% of saturation. The precipitate was removed by centrifugation, and the ammonium sulfate concentration in the supernatant was

increased to 80% of saturation. The small amount of precipitate that formed was collected by centrifugation, dissolved in 32 ml of 0.2M NaCl, and dialyzed against 0.2M NaCl overnight at 3°C. This fraction contained 46 mg of protein and the specific activity of the polygalacturonase was 52. This represents a 2.7-fold purification with 73% recovery.

The enzyme remained soluble and was stable at 3°C for at least a month.

Purification of pectic acid. A 1% solution of sodium polypectate was prepared and adjusted to pH 5. Two volumes of 95% ethanol were added, and the gelatinous precipitate was collected on Miracloth, squeezed dry, and redissolved in the original volume of water. The pectic acid was reprecipitated two more times and then dried by homogenizing with 95% ethanol and finally with acetone. The yield was usually about 45%.

Preparation of polygalacturonic acid I (PGA I). 20g of sodium polypectate were dissolved in 2l of water, and the pH was adjusted to 5. The solution was warmed to 35° C, and 20 mg of fungal pectinase dissolved in 10 ml of 0.2M NaCl was added. Heat was applied immediately to the vigorously stirred sample so that it reached the boiling point in about 15 min. After cooling to 25°C, the solution was filtered and adjusted to pH 2 with HCl. The precipitate was collected by centrifugation, dissolved in 500 ml of water at pH 6, and the precipitation at pH 2 was repeated. The product was collected and dried by homogenizing with ethanol and acetone. The yield was 7g.

Preparation of polygalacturonic acid II (PGA II). 2l of 1% sodium polypectate adjusted to pH 5 and warmed to 35°C was treated with 100 mg of fungal pectinase dissolved in 10 ml of 0.2M NaCl. The solution was heated to boiling in about 15 min. After cooling to 25°C, the pH was adjusted with HCl to 2 and the small amount of precipitate that formed was removed by filtration. The filtrate was stored at 2°C for 48 hr, during which time a fine precipitate formed. This precipitate was collected by centrifugation and dried by washing with acetone. The yield was 1.1g.

Preparation of polygalacturonic acid III (PGA III). The supernatant obtained after the removal of PGA II was treated with 20g $CaCl_2.2H_2O$ and then adjusted to pH 5. The calcium polygalacturonase was collected and dissolved in 400 ml water by lowering the pH to 2. The solution was deionized with Dowex 50 (H^+). 2 vol of ethanol were then added to precipitate the polygalacturonic acids, which were collected by centrifugation and dried by washing with acetone. The yield was about 6g.

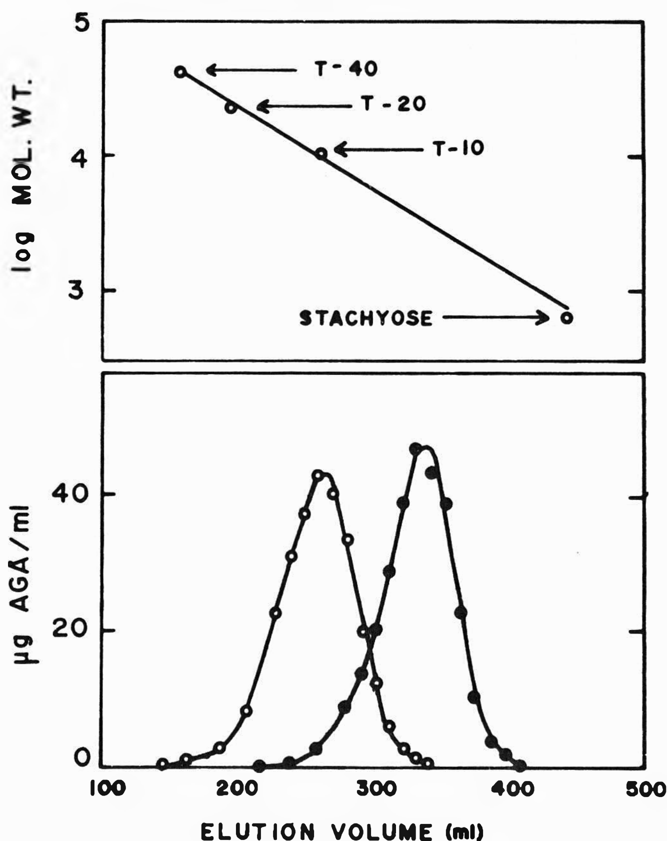

Fig. 2—Determination of the molecular weights of PGA II and PGA III by gel filtration. A 2.5 × 95 cm column of Sephadex G-75 was washed with 0.01M sodium phosphate, pH 7. Each of the polygalacturonic acids (2 mg) was applied in a vol of 2 ml and their elution with 0.01M phosphate, pH 7, was followed by measuring the anhydrogalacturonic acid content of each fraction according to the method of Rouse and Atkins (1955). 10 mg of each standard dissolved in 2 ml of buffer were applied and their elution was followed by the anthrone method (Ashwell, 1957). The fraction size was 5 ml. O———O = PGA II; ●———● = PGA III. T-10, T-20, and T-40 = standard dextrans.

RESULTS

Chromatography of tomato polygalacturonase on Sephadex G-100

When tomato polygalacturonase was filtered through Sephadex G-100, a single peak of activity was observed and it coincided with the only protein peak. This technique therefore was not an effective purification step, but it provided a method for determining the molecular weight of the enzyme which had not been reported previously.

5 ml of purified polygalacturonase was applied to a 2.5 × 95 cm column of Sephadex G-100. The column was equilibrated and eluted with 0.2M sodium acetate, pH 4.5. The elution volumes for the proteins of known molecular weights, cytochrome c (13,000), egg albumin (45,000), and bovine serum albumin (70,000), were 360 ml, 282 ml, and 243 ml, respectively. The elution volume for polygalacturonase was 280 ml which corresponds to a molecular weight of 43,600, calculated according to the method of Whitaker (1963).

Properties of the polygalacturonic acids

A summary of some of the properties of the three polygalacturonic acids and pectic acid is presented in Table I. The molecular weights were determined by gel filtration chromatography at pH 7. Because of the wide range of molecular weights involved, all four samples could not be resolved on any one single gel. Sephadex G-75 was found to be suitable for both PGA II and PGA III (Fig. 2). Using the elution volumes for stachyose, Dextran T-10 ($\overline{M}_w$ = 10,300), Dextran T-20 ($\overline{M}_w$ = 22,300), and Dextran T-40 ($\overline{M}_w$ = 41,800), the elution peak for PGA III corresponds to a molecular weight of 3200. The extremes of molecu-

Table 1—Summary of some of the properties of pectic acid and polygalacturonic acids

	Pectic Acid	PGA I	PGA II	PGA III
Avg mol wt	>1,000,000	110,000	9000	3200
/η/[a]	12.6	1.2	0.18	0.07
Reducing groups, µmoles /g[b]	16	39	150	390
AGA content, %[c]	77	93	93	97

[a]Determined at 30°C with an Ostwald viscometer.
[b]Determined by the arsenomolybdate method of Nelson (Ashwell, 1957)
[c]Anhydrogalacturonic acid content, determined according to the method of Rouse and Atkins (1955).

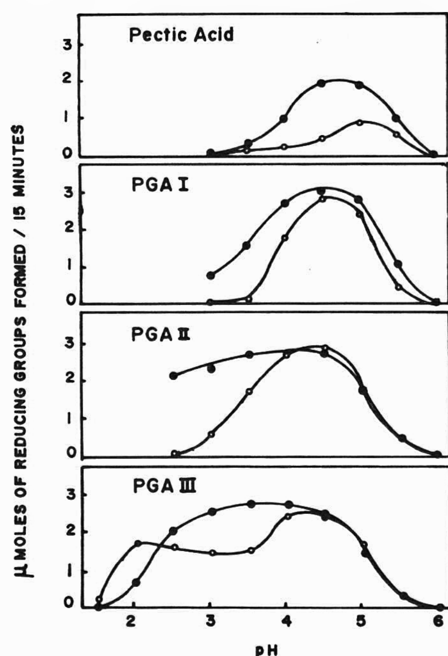

Fig. 3—Effect of substrate molecular weight on the pH optimum for tomato polygalacturonase. ● = standard assay procedure; 0 = standard assay in the absence of NaCl.

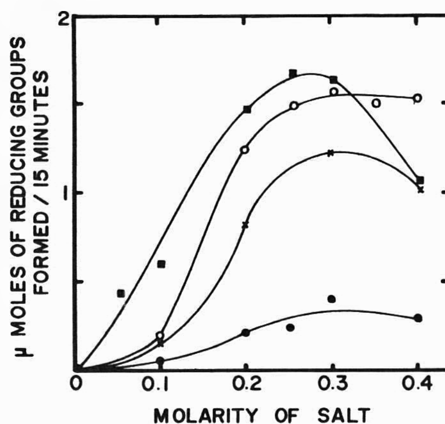

Fig. 4—Effects of salts on the hydrolysis of PGA I by tomato polygalacturonase at pH 3.5. 0 = NaCl; □ = $NaNO_3$; X = CsCl; ● = KCl.

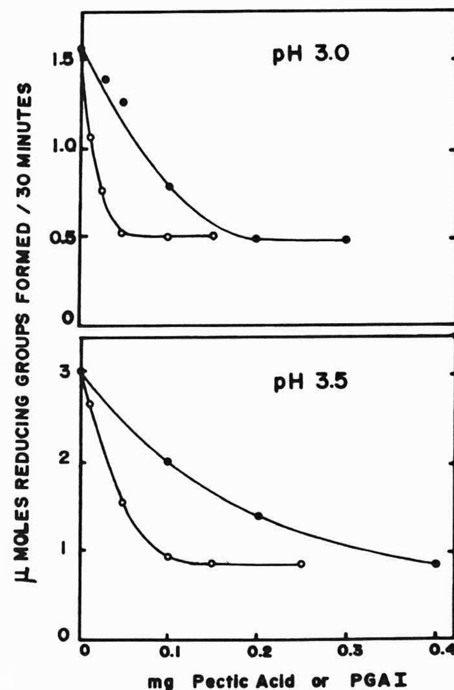

Fig. 5—Effects of pectic acid and PGA I on the hydrolysis of PGA II by tomato polygalacturonase at pH 3.0 and 3.5. The standard assay was used except the incubation period was increased to 30 min and NaCl was left out. 0 = pectic acid; ● = PGA I.

lar weights in PGA III are 1400 and 12,000. The peak for PGA II corresponds to a molecular weight of 9000, with a range from 3400–35,000.

The molecular weights of PGA I and pectic acid were estimated by chromatography on a 2.5 × 80 cm column of Bio-Gel A-50m. By comparison with the elution volumes for Dextrans T-70 ($\overline{M}_w$ = 67,500), T-110 ($\overline{M}_w$ = 100,500), T-150 ($\overline{M}_w$ = 147,000), T-250 ($\overline{M}_w$ = 240,000), and T-500 ($\overline{M}_w$ = 495,000), the peak of the elution pattern for PGA I corresponded to a molecular weight of 110,000. The range in molecular weights in this preparation was from 50,000 to approximately 1,000,000. The elution curve for pectic acid was very broad, with a moderate peak at the upper exclusion limit for the gel. The smallest component in pectic acid had a molecular weight of about 100,000, and the average molecular weight was greater than 1,000.000.

The anhydrogalacturonic acid content of pectic acid was somewhat low but approached the theoretical value for the polygalacturonic acids. The reducing values for PGA II and PGA III were in agreement with the molecular weight results. In contrast, the numbers of reducing groups in PGA I and pectic acid were found to be considerably higher than would be expected on the basis of their molecular weights. Kertesz (1951) has pointed out the difficulty of measuring the reducing power of pectic substances and questioned the reliability of the determinations. The results obtained by different procedures have been highly variable and much higher than would be expected for a linear molecule. It has been suggested that high reducing values are evidence for branching in the pectin molecule (Kertesz, 1951), although physical measurements indicate elongated molecules.

Effect of substrate size on the pH optimum

In the absence of salts, tomato polygalacturonase hydrolyzed pectic acid most rapidly at pH 5, with little activity below pH 4 (Fig. 3). Decreasing the molecular weight of the substrate progressively shifted the activity to the acid side. The rate of hydrolysis at pH 5 was more than two times higher for PGA I than for pectic acid. The hydrolysis of PGA I was maximal at pH 4.5 and the activity decreased sharply to zero at pH 3.5. The activity extended to below pH 3 for the substrate PGA II, with a peak at about pH 4.3. Reducing the molecular weight of the substrate to that of PGA III increased the range of activity to pH 1.5. The activity of the tomato enzyme acting on PGA III was characterized by one peak at about pH 4.2 with a broad shoulder extending to below pH 2.

The addition of NaCl to the reaction mixture activated the hydrolysis of the substrates by tomato polygalacturonase (Fig. 3). This activation was most pronounced at low pH and was dependent on the molecular size of the substrate. Thus, the hydrolysis of pectic acid increased sharply at pH 4.5 in the presence of 0.3M NaCl but the activity was not extended below pH 3.5. The hydrolysis of PGA I shifted to below pH 3, and the hydrolysis of PGA II shifted to below pH 2.5. The solubility of PGA II did not allow studies at lower pH. The hydrolysis of PGA III was activated by NaCl over the pH range 2.5–4, but inhibition occurred below 2.5. The result is a broad peak of activity for this substrate in the presence of NaCl.

The effect of salt concentration on the hydrolysis of PGA I was studied at pH 3.5 (Fig. 4). Activation of polygalacturonase by NaCl was low at concentrations below 0.1M, but it increased sharply to a maximum at about 0.3M. Similar results were obtained for CsCl except that the maximum activation was lower. KCl was much less effective than NaCl and CsCl. The maximum activation obtained with $NaNO_3$ was equal to that with NaCl, but the nitrate was more effective at low concentrations. $CaCl_2$ and $MgCl_2$ did not activate the reaction over the range 0.25–4 mM, and higher concentrations of the divalent cations precipitated the substrate.

Inhibition by high molecular weight substrates

The inability of tomato polygalacturonase to hydrolyze high molecular weight substrates at low pH suggested that these substrates inhibit the enzyme. This was confirmed by studying the hydrolysis of combinations of low and high molecular weight substrates. The effects of pectic acid and PGA I on the rate of hydrolysis of PGA II are shown in Fig. 5. As indicated earlier, PGA II was hydrolyzed by tomato polygalacu-

ronase as low as pH 3. Only 50 μg pectic acid per ml inhibited 70% of the reaction at pH 3. About four times as much PGA I was required to achieve the same inhibition. Higher levels of both inhibitors did not increase the inhibition. Pectic acid and PGA I were less effective at pH 3.5 than at pH 3 and, as at pH 3, the maximum inhibition did not exceed 70%.

Similar results were obtained for the hydrolysis of PGA III at pH 2.5 and 3.0. Pectic acid was about four times as effective as PGA I, and about 30% of the reaction was not inhibited.

DISCUSSION

PECTIC ACID is usually selected as the substrate for measuring polygalacturonase activity because this enzyme appears to be specific for the deesterified polymer (Jansen and McDonnel, 1945). It is prepared by enzymatic or mild alkaline deesterification of pectin so that the colloidal properties are retained. Pectic acid forms highly viscous solutions which tend to gel on acidification and addition of salts or solvents such as acetone and ethanol. This presents problems in purification and handling of the substrate at high concentrations, especially at low pH. Another factor that has been overlooked is the possibility of inhibition of the enzyme by the acidic macromolecular substrate. Pectic acid gels are effective adsorbents for yeast (Phaff and Demain, 1956) and tomato (Patel and Phaff, 1960a) polygalacturonases. Lineweaver and Ballou (1945) have demonstrated that pectic acid markedly inhibits pectinesterase on the acid side of its pH optimum.

Our results indicate that tomato polygalacturonase activity is reduced at low pH by pectic acid and high molecular weight polygalacturonic acids. The lower pH limit for hydrolysis by this enzyme is dependent on the molecular size of the substrate. By decreasing the molecular weight of pectic acid to 3200, the range of activity was extended from pH 4 to below pH 2. Furthermore, this change occurs progressively as the molecular size of the substrate decreases.

There are at least two explanations for the shift in polygalacturonase activity to the acid side with decreasing substrate molecular weight. Either tomato polygalacturonase is inhibited at low pH by large substrates, or it consists of isoenzymes which are substrate size specific. The activation by monovalent cations of hydrolysis at low pH indicates a reversal or prevention of substrate inhibition. Because relatively high levels of salts are required for activation, this effect may be due to weakening of electrostatic interaction between the acidic macromolecular substrates and the ammonium groups of the enzyme. By studying the hydrolysis of low molecular weight substrates in the presence of pectic acid, we have established that the tomato enzyme is inhibited by pectic acid. The inhibition is partial, however, and 30% of the activity at pH 3 and 3.5 is not inhibited even by excesses of pectic acid.

The significant amount of residual activity in the presence of pectic acid suggests that tomato polygalacturonase may consist of at least two isoenzymes. Both enzymes hydrolyze small substrates but one is inhibited by pectic acid at low pH. Because pectic acid is not degraded at low pH indicates that only one enzyme functions on large substrates. This enzyme is inhibited by its substrates at low pH although inhibition can be partially prevented by monovalent cations.

Evidence for polygalacturonase isoenzymes in tomatoes has been presented by other workers. McColloch and Kertesz (1948) found that about 15% of the activity in tomatoes was stable to heating in boiling water. McColloch (1948) separated two enzymes by moving boundary electrophoresis. Patel and Phaff (1960b) postulated the presence of at least two polygalacturonases in tomatoes on the basis of double pH optima for the hydrolysis of oligogalacturonides, but they did not try to separate the different components.

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J. V. POMENTA
Krafts Foods, International Div., Chicago, Ill.
and E. E. BURNS
Dept. of Soil & Crop Sciences, College of Agriculture
Texas A& M University, College Station, Texas 77843

FACTORS AFFECTING CHLOROGENIC, QUINIC AND CAFFEIC ACID LEVELS IN SUNFLOWER KERNELS

SUMMARY—Location of the seed on the sunflower head and storage temperatures were related to chlorogenic, caffeic, and quinic acid levels. Kernels of freshly harvested sunflower seeds located near the center of the head were higher in chlorogenic acid than seeds located near the margin. The opposite was obtained for the caffeic acid content, and quinic acid did not exhibit a positional effect. Chlorogenic acid content decreased during storage at 5°C, 15°C, and 40°C for 120 days; seeds attained the same levels of chlorogenic acid but varied slightly among treatments. Relatively small changes occurred in the caffeic and quinic acids content during storage.

INTRODUCTION

SUNFLOWER SEEDS are an important commercial source of edible oils and animal feeds. The meal resulting from the oil extraction process is a valuable source of proteins, but its use in the human diet is limited by the presence of chlorogenic acid. This phenol darkens upon oxidation and can impair the acceptability of sunflower products.

Although chlorogenic acid is an important factor in the discoloration of the meal, other factors such as particles of hulls, pH, oxidizing agents, and cooking conditions are also concerned with the discoloration.

Chlorogenic acid functions as part of the oxidase system in the plants (Hanson and Zucker, 1963). Removal of chlorogenic acid with organic solvents has been attempted but without practical success (Smith, 1958). The most efficient solvent was 70% ethanol.

Chlorogenic and quinic acids have been isolated from the kernel of sunflower seeds by column chromatography and identified by paper chromatography. The level of both acids in the kernel and hull of sunflower seed, the conversion of chlorogenic acid into quinic acid at temperatures of 100°C and 135°C, and resynthesis of chlorogenic acid during cold storage of the meal have been reported (Milic et al., 1968).

Acid hydrolysis of chlorogenic acid results in caffeic acid. This acid is also present in the sunflower kernel. In the study reported here, the concentration of chlorogenic, quinic and caffeic acid was investigated at different locations on the head and at storage temperatures of 5°C, 15°C and 40°C.

MATERIALS & METHODS

FRESHLY HARVESTED sunflower heads of the Krasnodarets variety grown in College Station, Texas were used. Ten heads were selected for homogeneous appearance and freedom from insect damage. The seeds sampled were located within 2 in. of the center of the head. They were collected and kept separated from subsequent seeds collected from the same heads but located 2 in. from the center to the margin. The two lots of seeds were equally divided for storage treatments at 5°C, 15°C and 40°C for periods of 0, 60, 90 and 120 days.

Sunflowers representative of commercial types grown in various parts of the U.S.A. were chosen to determine the relationship between total sugar content and the levels of chlorogenic acid in sunflower kernels. The sunflowers were grown in College Station as part of the 1968 regional sunflower yield test. The material was fumigated and stored at room temperature prior to analysis. Three replicate plots of each type were used for the determinations.

At the end of each storage treatment, the seeds were dehulled by violent agitation by means of a jet of compressed air in a special container (Fig. 1). A 12-oz Ball refrigerator and freezer jar was covered at the top with two layers of 16 mesh wire screen and a 0.5 cm dia pipe was inserted 1 cm in a small hole at the edge of the wire screen.

50 lb/in.^{2} of air was applied for 30 sec to 1 min depending on the size and variety of the sample being dehulled. 15-20g of each sample were dehulled followed by separation of kernels and hulls in an air stream. This method decorticated the seeds quickly with a minimum damage to the kernels.

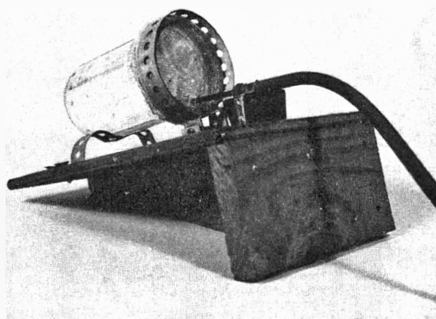

Fig. 1—Apparatus developed for dehulling sunflower seeds.

Kernels were ground in a Waring Blendor for approximately 20 sec to pass a 40 mesh screen. They were oven dried at 75°C for 6 hr and extracted with petroleum ether for 18 hr in a Soxhlet apparatus. 2g of the fat-free residue were extracted for 14 hr with 80% ethanol in a Soxhlet and the extract volume was reduced to 50 ml in vacuo at 40°C.

Chlorogenic acid

100 lambda of the ethanol extract was spotted on Whatman No. 1 chromatography paper 2.5 cm above the bottom and over a length of 4 cm and a maximum width of 1 cm. The chromatogram was developed for 5 hr or until the solvent front moved 14.5 cm. Best results were obtained in small chambers without equilibration periods. The solvent was butanol:acetic acid:water (4:1:5).

Two fluorescent spots appeared: one, with an R_f value of 0.60 and bluish color under UV light, was identified as chlorogenic acid. The spot was removed and quantitatively analyzed by a modification of the Hoepfner reagent (Hoepfner, 1932). The paper was treated with 1 ml of water and 4 ml of a 1:1 mixture of freshly prepared 5% acetic acid and 0.5% sodium nitrite; then it was shaken and thoroughly centrifuged at 2000 rpm for 4 min. Absorption was measured with a Beckman DB spectrophotometer at 520 mμ Absorbancy was compared with a standard curve made of a concentration series and found to be linear over the range of 0.10–1.00 μmol.

Quinic acid

2-Keto-3-deoxy-D-arabo-heptonic acid 7-p synthetase (KDHP) has a similar molecular structure to that of quinic acid. The method for determination of KDHP (Sirinivasan and Sprinson, 1959) was adapted to quinic acid. 1 ml of the ethanol extract was treated with 1 ml of 0.25N HIO_4 in 0.125N H_2SO_4, shaken thoroughly, and let stand for 15 min. The excess periodate was removed with 2 ml of 2% solution of sodium arsenite in 0.5N HCL and shaken until iodine vapors evolved. After 2 min, 2 ml of 0.6% solution of 2-thio-barbituric acid was added. The sample was shaken and placed in a warm bath for 40 min. The pink-colored solution was diluted to 10 ml with acetone and centrifuged for 3 min at 2000 rpm. The absorbancy was measured at 550 mμ and compared with a curve of a concentration series found to be linear over the range of 0.10–0.64 μmol of quinic acid.

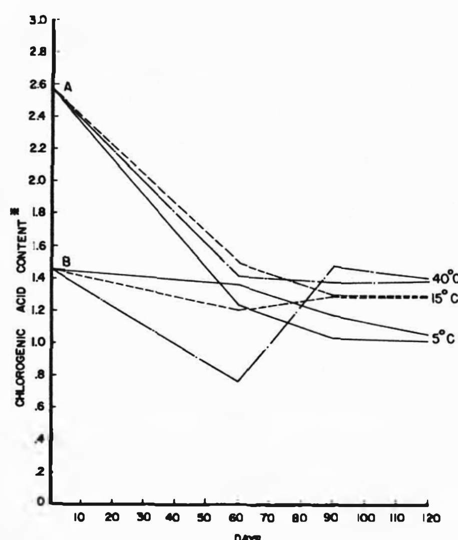

Fig. 2—Effects of temperature and storage on the levels of chlorogenic acid, expressed as per cent dry weight. (A) Seed located near the center of the head; (B) Seed located near the margin of the head.

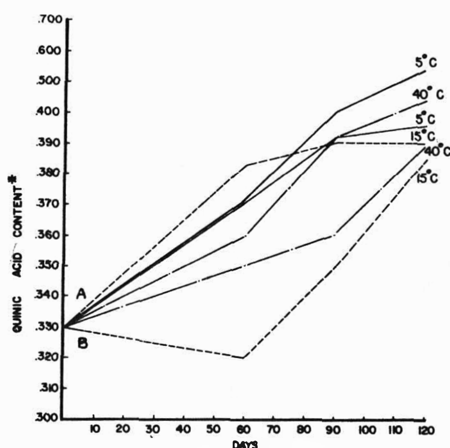

Fig. 3—Effects of temperature and storage on the levels of quinic acid, expressed as per cent dry weight. (A) Seed located near the center of the head; (B) Seed located near the margin of the head.

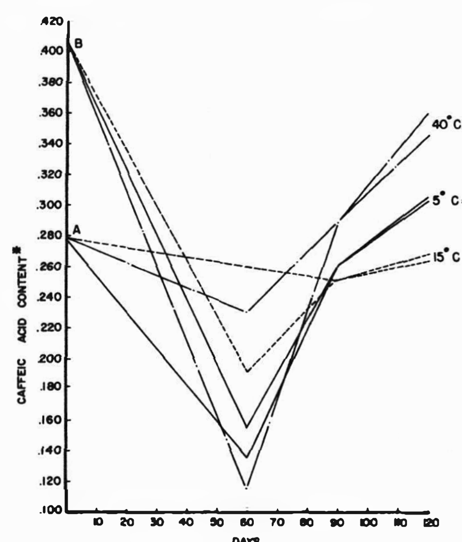

Fig. 4—Effects of temperature and storage on the levels of caffeic acid, expressed as per cent dry weight. (A) Seed located near the center of the head; (B) Seed located near the margin of the head.

Caffeic acid

The second spot on the chlorogenic acid chromatograph was identified as caffeic acid. It had an R_f value of 0.78 and a greenish color under UV light. Caffeic acid was extracted by the same procedure as for chlorogenic acid and the absorbancy measured at 360 mμ. The absorbancy was compared with a concentration series that was found to be linear over the range of 0.10–1.00 μmol.

Total sugars

1 ml of the ethanol extract was diluted to 100 ml with water and total sugars determined by the phenol-sulfuric acid method (Dubois et al., 1956). Glucose standards were used to prepare a standard curve for measuring the total sugar per mg of dry sunflower kernel.

RESULTS & DISCUSSION

INITIAL ANALYSIS of freshly harvested sunflower seed of the Krasnodarets variety revealed that the seed located within 2 in. of the center of the sunflower head contained about twice as much chlorogenic acid as the seeds located 2 in. from the center to the margin of the sunflower head (Fig. 2). A possible explanation may be physiological state of maturity. Sunflower seed located toward the margin of the head reach physiological maturity first (M. L. Kinman, 1969, personal communication). As a consequence, biological activity such as respiration and enzyme activity would be less. Dramatic changes in phenolic composition occur during plant development and some phenolic compounds are synthetized for only a brief period during a particular stage of growth (Zucker et al., 1967). For example, it has been shown that the concentration of chlorogenic acid at the tip of the tobacco leaf was greater than at the base, and this corresponded to the concentration of substances synthesized in situ such as proteins and chlorophyll (Zucker and Ahrens, 1958). This same postulate could apply to sunflower seeds. The initial content of quinic acid was the same for seeds located near the margin and those located toward the center of the head (Fig. 3). Initial content of caffeic acid was higher for seeds located toward the margin than for seeds located toward the center of the sunflower head (Fig. 4).

Storage of the seed at 5°C, 15°C and 40°C for 60 days caused a decrease in chlorogenic acid; this decrease was more pronounced in the seeds located toward the center of the head (Fig. 2). After 120 days of storage, the content of chlorogenic acid was similar for seeds that received the same heat treatment. However, differences were found between storage heat treatments. The highest level was in the seeds treated at 40°C; next was 15°C; and the lowest was for seeds kept below 5°C.

Values for quinic acid (Fig. 3) increased after storage at 5°C, 15°C and 40°C. Leveling off occurred among the seeds that received the same heat treatment after storage for 120 days. The highest concentration was obtained for seeds treated at 5°C; next was 15°C; and the lowest was found at 40°C.

The values for caffeic acid (Fig. 4) decreased after storage at 5°C, 15°C and 40°C for 60 days but increased to the same level among seeds that received the same heat treatment after storage for 120 days. The highest concentration was obtained for seeds treated at 40°C, then 5°C, and the lowest at 15°C. Seeds that received the same heat treatment had similar concentrations of caffeic acid.

Synthesis of chlorogenic acid during cold storage of potatoes has been reported. Glucose, fructose and saccharose accumulate during cold storage of potatoes and are very effective substrates for chlorogenic acid synthesis (Zucker and Levy, 1959). L-Phenylalanine with quinic acid was also found to be an effective substrate for chlorogenic acid synthesis (Hanson and Zucker, 1963).

It was reported (Milic et al., 1968) that a small decrease in chlorogenic acid was associated with a small increase in quinic acid in sunflower kernels which had been heat-treated for 5 hr at 70°C. This was followed by a reversal upon storage at 20°C. This treatment apparently did not damage the mechanism of chlorogenic acid synthesis from the lower degradation complexes of polysaccharides and proteins resulting from the heat treatment.

Analysis of variance of the total sugar and chlorogenic acid levels of the sunflower kernels among the 10 varieties analyzed revealed significant differences at the 99% levels of confidence (Table 1). There were no significant differences among replicate samples. A positive correlation coefficient was obtained between total sugar content and the levels of chlorogenic acid. This may suggest the influence of sugar molecules in the synthesis of chlorogenic acid.

Table 1—Total sugar content and chlorogenic acid in sunflower kernels

Sunflower varieties	Total sugars[a] (% Dry Wt)	Chlorogenic acid[a] (% Dry Wt)
T 56002	4.91	1.68
P-21 ms X HA 60	4.98	1.53
Valley	4.26	1.51
Peredovik	4.99	1.41
VN11MK 8931	5.25	1.58
Krasnodarets	4.41	1.14
Mingren	4.91	1.47
Arrowhead	4.69	1.28
Greystripe	6.28	2.22
Manchurian	5.94	1.62

[a]The correlation coefficient (r) for sugar content and chlorogenic acid was 0.511 and the probability (P) was 0.004.

A possible explanation for the changes occurring in the levels of chlorogenic, caffeic, and quinic acids before and after heat treatments would be the selective induction and repression of enzymes by temperature, time, light, substrate availability, or a combination of these factors in phenolic biosynthesis (Zucker et al., 1967). It can be postulated that immediately after harvesting a breakdown in chlorogenic acid occurred until a steady state was reached. The effect of inducers and repressors such as heat, carbohydrates or enzymes regulated the activity of the phenolic biosynthesis.

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ROBERTO M. de MORAES and EIDIOMAR ANGELUCCI
Instituto de Technologia de Alimentos, Caxia Postal 665, Capinas, São Paulo, Brazil

CHEMICAL COMPOSITION AND AMINO ACID CONTENTS OF BRAZILIAN BEANS (Phaseolus vulgaris)

SUMMARY—The chemical composition of 12 varieties of beans (Phaseolus vulgaris) was determined. Varieties most commonly found on Brazilian markets were chosen for this work. The average moisture content was 11%, ash 3.5%, fat 1%, protein 25%, starch 40%, crude fiber 4% and pentonsans 7%. The content of minerals in mg/100g sample was: phosphate 1000, iron 3.2, calcium 40 and magnesium 210. The content of essential amino acids in mg/g protein, calculated on a dry basis, was: lysine 72–106, threonine 46–61, valine 29–54, methionine 3–18, isoleucine 28–49, phenylalanine 33–118 and tryptophane 103–138. The product was rich in lysine and threonine as compared to the FAO table for essential amino acids require in the human diet. It was poor in methionine, isoleucine, valine, tryptophane and leucine.

INTRODUCTION

BEANS are one of the main components in the everyday dishes in Brazil. Due to the low cost, their use is greatest among the rural population and labor classes. Since protein content is relatively high, beans are one of the main protein sources for these people.

In Latin America, a few studies on the chemical composition of some varieties of beans were carried out in several countries, and the results were summarized by INCAP-ICNND (1961). However, this report did not show the content of the essential amino acids in the samples. Similar work was carried out in Guatemala and Chile with a few local varieties in which the content of the essential amino acids was also considered (Bressani et al. 1961).

The purpose of our work is to report the chemical composition and amino acid content of 12 varieties of beans grown in Brazil. Varieties most commonly found in Brazilian markets were chosen for this work.

MATERIAL & METHODS

BEAN SAMPLES used in this work were furnished by the Instituto Agronômico do Estado de São Paulo, Campinas. Whole beans were initially ground and passed through 20-mesh sieves.

The contents of moisture, ash, phosphate, calcium, iron and magnesium were determined by methods described by AOAC (1965). Fat content was determined by Weibull Stoldt's method; starch by Ewers' method modified by Hadorn and Doevelaar; total sugar content by Munson Walker's method; fiber by the method of Scharrer and Kürschner; pentosans by the method of Peter, Thaler and Täufel. All of these methods, except those of AOAC, were described by Diemair (1963).

The content of the amino acids was determined by use of the Beckman model 120C automatic amino acid analyzer. Ground beans were hydrolysed for 2 hr at 110°C in 6N HCl according to the procedure outlined in the instruction manual which accompanied the amino acid analyzer (Beckman Instruction Manual, 1965). The amino acid contents are reported on a dried protein basis.

RESULTS & DISCUSSION

CHEMICAL composition of the 12 varieties of beans is shown in Table 1. Moisture content was about 11% and ash content about 3.5%. The content of fat was about 1% on the average, but ranged from 0.4% in the variety "roxão" to 2.0% in the variety "roxinho." The highest level of sugar was observed in the variety "pintado" with 8.6% and the lowest in "carioca" with 5.3%. The average was about 6.5%. Starch content averaged 40%, ranging from 34% in "chumbinho opaco" to 44% in "carioca." The amount of pentosans was about 7% in most of the varieties, but "preto G-1" and "chumbinho opaco" had about 13%. Fiber content ranged from 3.5%–5.0%. Protein content was about 25%, ranging from 21%–28%. These results indicate that beans are a relatively good source of protein and carbohydrates; they contain a relatively small amount of fat and a moderate amount of fiber.

Table 2 shows the amount or iron, calcium, magnesium and phosphate in

Table 1—Chemical composition of 12 varieties of beans (P. vulgaris) grown in Brazil

Varieties	Moisture %	Protein %	Ash %	Fat %	Starch %	Total sugars %	Crude fiber %	Pentosans %
Bico de Ouro	10.7	21.9	3.30	0.77	43.6	5.77	4.93	5.85
Jalo	11.2	24.8	3.20	0.86	40.8	5.61	4.52	5.68
Goiano precoce	10.5	28.3	3.46	0.93	34.8	6.57	4.25	6.33
Mulatinho	11.3	23.2	3.58	1.67	40.2	6.06	4.76	7.12
Rico 23	11.3	23.9	3.75	1.72	37.9	5.20	4.81	6.35
Preto G-1	11.7	21.5	3.54	0.48	35.4	6.40	4.32	12.7
Pintado	10.7	22.3	3.23	1.25	37.9	8.67	4.29	7.62
Chumbinho opaco	10.4	24.3	3.79	1.19	34.0	6.32	4.17	12.3
Rosinha G-2	11.1	24.6	3.61	0.82	38.5	6.46	4.78	7.16
Roxão	11.1	23.5	3.39	0.35	39.1	6.83	5.09	7.75
Roxinho	11.4	21.6	4.04	1.99	43.6	5.45	4.08	7.37
Carioca	10.6	22.6	3.52	1.44	44.7	5.34	3.52	7.21

Table 2—Mineral contents (mg/100g) of 12 varieties of beans (Phaseolus vulgaris) grown in Brazil

Varieties	Iron	Phosphate	Calcium	Magnesium
Bico de ouro	2.96	1009	78.1	233.2
Jalo	4.25	1009	41.4	208.8
Goiano precoce	5.76	920	43.0	199.0
Mulatinho	3.51	855	31.6	244.4
Rico 23	3.85	1110	52.3	183.7
Preto G-1	3.51	1009	26.8	237.0
Pintado	3.06	874	50.1	177.5
Chumbinho opaco	3.22	1343	30.3	184.6
Rosinha G-2	2.96	1263	32.2	234.8
Roxão	3.36	1006	35.7	215.4
Roxinho	2.76	1410	50.5	171.5
Carioca	3.44	1183	32.8	219.5

Table 3—Amino acid composition (mg/g protein)[a] of 12 varieties of beans (P. vulgaris) grown in Brazil

Amino acids	Bico de Ouro	Jalo	Goiano Precoce	Mula-tinho	Rico 23	Preto G-1	Pintado	Chumbinho Opaco	Rosinha	Roxão	Roxinho	Carioca
Lysine[b]	83.7	93.3	87.5	106.1	82.0	78.1	78.6	106.2	86.9	87.5	85.4	72.4
Histidine	30.4	31.2	30.0	41.2	37.4	33.6	39.1	45.1	39.6	38.2	42.6	33.6
Ammonia	33.5	38.4	26.6	42.7	36.6	37.1	30.3	47.0	36.6	34.6	36.1	32.8
Arginine	66.5	78.2	59.3	98.1	80.0	65.8	54.8	89.1	79.5	87.9	77.2	78.2
Aspartic acid	126.9	119.4	120.7	133.6	127.7	108.4	120.4	151.5	134.7	133.4	125.7	143.0
Threonine[b]	54.1	59.1	52.1	55.1	52.0	46.2	50.9	61.6	58.1	52.7	51.8	51.7
Serine	67.0	62.6	57.6	60.8	69.8	49.7	64.8	76.4	71.7	66.6	64.8	63.9
Glutamic acid	162.8	151.0	129.2	159.3	173.0	139.9	161.9	195.1	172.0	179.9	166.2	158.8
Proline	45.4	42.3	30.7	39.4	48.0	46.2	45.8	52.0	54.3	51.0	49.9	47.3
Glycine	47.2	48.7	38.9	53.0	46.5	28.8	44.6	52.4	48.1	48.6	51.3	46.2
Alanine	56.8	43.3	40.4	38.1	49.4	34.2	53.5	59.4	56.0	57.8	56.4	54.4
Half-cystine	63.6	36.1	33.2	39.1	43.6	25.7	16.9	44.0	55.5	52.8	46.4	9.8
Valine[b]	51.6	43.1	37.0	47.0	38.0	29.7	43.4	49.0	54.4	51.2	48.8	50.1
Methionine[b]	18.6	8.6	7.5	10.9	9.1	13.9	8.5	8.3	10.5	8.5	9.8	3.5
Isoleucine[b]	44.9	49.2	38.6	49.0	43.9	28.6	42.5	49.8	46.3	47.7	44.0	32.1
Leucine	80.0	77.0	61.4	83.3	77.8	47.4	77.2	88.8	82.0	83.0	76.0	73.5
Tyrosine	35.9	24.7	23.7	30.9	30.0	18.1	26.1	32.1	36.0	37.7	28.6	28.1
Phenylalanine[b]	56.0	46.2	33.4	68.1	52.9	37.8	118.1	65.1	60.8	59.5	61.5	55.9
Tryptophane[b]	138.0	116.3	103.3	119.1	115.4	127.7	135.7	130.2	124.0	110.2	100.1	129.2

[a]Nitrogen × 6.25.
[b]Essential amino acids.

the bean samples which averaged 3.2, 40, 210, and 1000mg/100g, respectively. As a vegetable food, beans are rich in iron, calcium, magnesium and phosphate.

Table 3 shows the amino acid content of beans expressed in mg/g of protein calculated on a dry weight basis. These results indicate that some of the Brazilian beans *(Phaseolus vulgaris)* contain more lysine than rice beans and cowpea (Bressani et al., 1961). Thus they could supplement the lysine deficiency in corn protein when both products are used in the diet. Beans are also rich in threonine, but the content of methionine, isoleucine, valine, tryptophane and leucine is less than the amino acid requirement in foods according to the FAO table for essential amino acids in the diet (FAO, 1968). Methionine is the most deficient amino acid, but it could be supplemented by the use of corn, since corn protein is rich in methionine.

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K. N. PATEL[1] and T. A. NICKERSON
Food Science & Technology Dept., University of California, Davis, Calif. 95616

INFLUENCE OF SUCROSE, GLUCOSE AND LACTOSE ON LOSS OF WATER FROM SOLUTIONS

SUMMARY—Moisture losses from solution of 40, 50, and 60% lactose, sucrose, glucose, and combinations of these sugars were determined at 80°F and 27 in. vacuum. Differences in rate of water loss of 20–50% were observed depending on type of sugar and concentration. Rate of water loss from solution was in ascending order of sucrose, lactose, glucose, and pure water. In mixtures, the rate increased with increased proportion of lactose or glucose, and decreased with increased proportion of sucrose. In all cases, after lactose crystallization occurred, the water loss first increased and then decreased.

INTRODUCTION

THE UNIQUE physical-chemical properties of lactose are being used to advantage in an ever-increasing number of food products. Lactose is present in foods in combinations with sucrose, glucose, and other sugars. Thus, in most confectionery products, the vapor pressure is principally governed by the concentration and nature of the sugar present, and the vapor pressure in the food in relation to that of the surrounding atmosphere determines whether moisture is lost or gained. The moisture level in the food will also determine where the equilibrium shift between α-lactose and β-lactose will occur. It is desirable to know the relative humidity at which sugar or sugar mixtures must be maintained for water content to remain constant, or the point below which the relative humidity must be held to avoid the undesirable effects caused by moisture adsorption.

The studies of Whittier and Gould (1930) were limited to vapor pressures of saturated solutions of sucrose, glucose, galactose and lactose. Those of Money and Born (1951) were limited to sucrose, levulose, invert sugar, glucose, and a mixture of invert sugar and sucrose. There were no data available in the literature on the mixtures of lactose with glucose and sucrose at lower and higher concentrations of these sugars at room temperature (80°F); hence the present study analyzed the influence of sucrose and glucose on the vapor pressure of lactose solutions. Sucrose, lactose, and glucose were selected to represent different chemical types of sugars with varying degree of solubility.

EXPERIMENTAL

RELATIVE vapor pressure can be determined directly with apparatus developed by Grover (1940) in Research Association's Laboratory or by the method of Landrock and Proctor (1951). Alternatively, vapor pressure can be calculated by the formula of Money and Born (1951) or by Grover's (1947) method. However, in this study, all data are expressed in terms of loss of water with time for lactose solution, sucrose solution, glucose solution, mixtures of these sugars in solution at different concentrations, and distilled water under identical conditions.

Water loss from sucrose, lactose, glucose, and mixtures of these solutions were determined with a Mojonnier milk tester, model D, which is operated electrically, has thermostatic control of ovens, and includes accessories for heating, cooling, and vacuum control. A 27 in. vacuum at 80°F was used throughout the experiment.

The U.S.P. regular grade α-lactose hydrate, glucose, and sucrose used were provided by commercial sources. The desired amount of sugars and distilled water were weighed directly into a flask on an analytical balance to make 100g of solution. Thus, concentration of lactose, sucrose, glucose and mixtures of these sugars were prepared as shown in Figures 1 to 4. The solutions were heated in a water bath to 60–75°C, until the sugars dissolved, in a closed flask. Reaching α-lactose and β-lactose equilibrium required long periods of cooling at room temperature. Total solids of the sugar solutions were measured at 68°F using an Abbe refractometer, which was much simpler, but which gave results comparable to polarimetric or drying methods.

Samples from above prepared sugars solutions were weighed into flat-bottomed, aluminum dishes 3 in. dia and 1 in. high. The

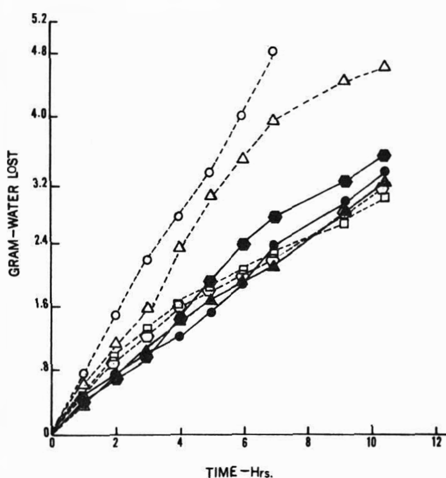

Fig. 1—Vapor pressure of lactose, sucrose, and a mixture of lactose-sucrose having 40% total solids of 80°F and 27 in. vacuum.

Curve	Gram-sucrose	Gram-lactose	Gram-water
—●—●—	10.0050	30.0050	60.0160
—•—•—	30.0010	10.0010	60.0120
—▲—▲—	25.0070	15.0060	60.0102
---○---○---	20.0050	20.0050	60.0000
---□---□---	40.0080	—	60.0090
---△---△---	—	40.0050	60.0020
---○---○---	Distilled Water		

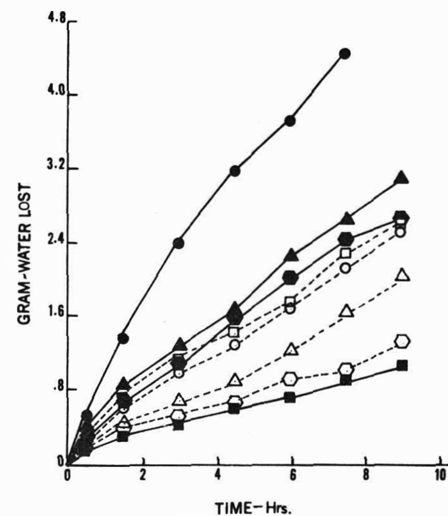

Fig. 2—Rate of water loss from solutions of lactose, sucrose and a mixture of lactose-sucrose having 40, 50 and 60% total solids at 80°F and in 27 in. vacuum.

Curve	Gram-sucrose	Gram-lactose	Gram-water
—▲—▲—	10.0050	30.0050	60.0160
—●—●—	30.0010	10.0010	60.0120
---□---□---	15.0000	25.0090	60.0160
---○---○---	25.0070	15.0060	60.0102
---△---△---	40.0020	10.0010	50.0220
---○---○---	40.0070	20.0040	39.9980
—■—■—	60.0000	—	40.0030
—•—•—	Distilled Water		

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

amount of water per sample was constant; therefore, sample size varied with moisture content. Hence, for example, to have 3g total water it is essential to take 5g of sample having 40% total solids or 7.5g of sample having 60% total solids. All weighed samples were transferred to a vacuum oven at 80°F and 27 in. vacuum; the initial time was noted from this point. The weights of aluminum dishes with samples were taken at regular hourly intervals for at least 10 hr. Any tendency toward crystallization during the process was also observed.

RESULTS & DISCUSSION

FIGURES 1 to 4 show the rate of moisture loss from the various sugars solutions. The amount of water loss in a regular interval is given in terms of the available water originally present in a sample. Figure 1 shows the rate of water loss from lactose, sucrose, and a mixture of lactose-sucrose solutions having 40% total solids at 80°F temperature and 27 in. vacuum, whereas, Figure 2 shows the rate of water loss from lactose, sucrose, and a mixture of lactose-sucrose solutions having 40%, 50%, and 60% total solids under identical condition. Similarly, Figure 3 shows the rate of water loss from lactose, sucrose, glucose, and mixtures of glucose-lactose, and glucose-sucrose solutions having 40% total solids at 80°F temperature and 27 in. vacuum, whereas, Figure 4 shows the rate of water loss from lactose, sucrose, glucose, and mixtures of glucose-sucrose and glucose-lactose solutions having 40% and 60% total solids under identical conditions.

Whittier and Gould (1930) found that the vapor pressure of saturated solutions of sugars at 25°C increased in the order of sucrose, glucose, galactose, and lactose; hence lactose solutions were less likely to adsorb moisture. Money and Born (1951) reported an empirical formula relating the equilibrium humidity value with the molar concentration of sucrose, levulose, invert sugar, glucose and mixtures of sucrose-invert sugar. Grover (1947) observed wide variation in

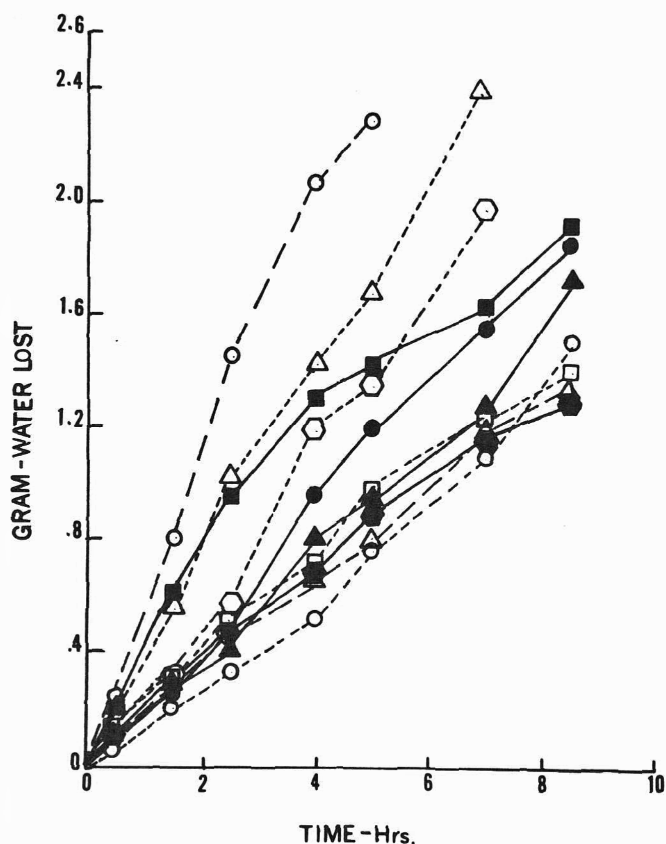

Fig. 3—Rate of water loss from solutions of lactose, sucrose, glucose and mixtures of glucose-lactose and glucose-sucrose having 40% total solids at 80°F and 27 in. vacuum.

Curve	Gram-glucose	Gram-sucrose	Gram-lactose	Gram-water
---△---△---	40.0020	—	—	60.0073
—●—●—	—	40.0000	—	60.0000
---⬡---⬡---	—	—	40.0000	60.0000
---○---○---	20.0035	20.0010	—	60.0134
—▲—▲—	20.0020	—	20.0014	60.0203
—⬢—⬢—	10.0020	30.0000	—	60.0000
---□---□---	10.0010	—	30.0030	60.0170
—△—△—	30.0040	10.0030	—	60.0030
—■—■—	30.0005	—	10.0005	60.0050
—○—○—	Distilled Water			

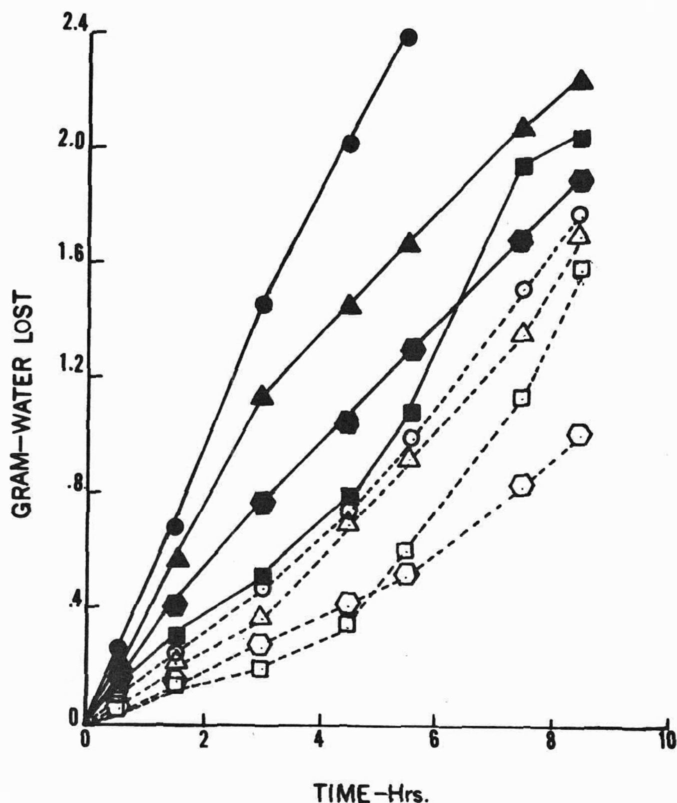

Fig. 4—Rate of water loss from solutions of lactose, sucrose, glucose and mixtures of glucose-sucrose and glucose-lactose having 40% and 60% total solids at 80°F and 27 in. vacuum.

Curve	Gram-glucose	Gram-sucrose	Gram-lactose	Gram-water
—▲—▲—	40.0020	—	—	60.0073
—⬢—⬢—	20.0035	20.0010	—	60.0134
—■—■—	20.0020	—	20.0014	60.0203
---○---○---	30.0005	—	10.0005	60.0050
---⬡---⬡---	60.0000	—	—	40.0120
---□---□---	40.0000	20.0000	—	39.9820
---△---△---	40.0030	—	20.0040	40.0010
—●—●—	Distilled Water			

vapor pressure between different classes of confectioneries. The present study (Fig. 1 to 4) for sucrose, lactose, glucose and mixtures of these sugars in solutions at various concentrations indicates that:

(1) Rate of water loss from individual sugar solutions and pure water was in ascending order of sucrose solution, lactose solution, glucose solution and pure water.

(2) As the percentage of total solids increased, the rate of water loss decreased.

(3) In mixtures of sucrose-lactose, glucose-lactose and glucose-sucrose with the same total solids, the rate of moisture loss varied only slightly.

(4) As the relative proportion of lactose or glucose increased in mixtures of sugars, the rate of water loss increased.

(5) Rate of water loss was higher in mixtures of lactose with other sugars and lower in mixtures of sucrose with other sugar.

(6) After crystallization of lactose, the rate of water loss increased temporarily and then decreased.

Lactose is naturally present in most milk products, while sucrose is added for sweetness or preservation purposes. In some foods, sucrose and lactose are essentially supersaturated, and only their high viscosity prevents crystallization. In this way, once a surface film of moisture has been absorbed, crystallization can proceed and the product is said to have "grained." A consequence of graining is an increase in relative vapor pressure. Similarly, in some foods, a solution of lactose in equilibrium with solid lactose at storage temperature contains α-lactose and β-lactose in the equilibrium ratio with the solution saturated with alpha but unsaturated with respect to beta. If water is evaporated from such a solution, α-lactose will commence to crystallize, and this process will continue until the solution is no longer supersaturated with α-lactose. The separation of α-lactose upsets the alpha-beta equilibrium, and the net change of beta to alpha continues until the normal ratio is attained again. The formation of α-lactose in this process increases the amount of alpha lactose that must crystallize before solubility equilibrium is reached. These factors continue to interplay until both types of equilibria are attained simultaneously (Whittier and Gould, 1930).

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JAMES H. MOY and KING-CHAM CHAN
Department of Food Science and Technology, University of Hawaii, Honolulu, Hawaii 96822
and ALEXANDER M. DOLLAR
Hawaii State Department of Agriculture, Honolulu, Hawaii 96814

BOUND WATER IN FRUIT PRODUCTS BY THE FREEZING METHOD

SUMMARY—An insulated heat-sink containing Freon-12® (N.B.P. – 21.6°F) provides a reproducible system for measurement of thermal properties of fruit products. The difference in time required to remove the latent heat of fusion of the "eutectic" mixture in comparison with distilled water measures the "bound" water. The 34–37% "bound" water in papaya (var. solo) pulp is unaffected by varying pH in the range 3.0–6.0. Less than 10% water is bound in guava, passion fruit and pineapple juice products with up to 35% sucrose added. Thermal conductivity of the solid phase "eutectic" mixture in the 5–50% soluble solids range fits the regression $Y = AB^x$, $Y = (3.69)(0.96)^x$ where x = percent total soluble solids (TSS) and Y = thermal conductivity (cal × $10^3/(cm^2)$ (sec) (°C/cm)). The relationship of freezing point to TSS values as 1.10 + 0.64 (TSS) in the test system is significant ($p < 0.01$) in the range 5–50% TSS. The time for fusion (θ_w) of sucrose/water in the range 5–50% TSS fits the regression: $\log \theta_w = (-0.8325)(-0.0165)(TSS)$ or $\theta_w = (0.984)(0.164)^{TSS}$ sec. Addition of solutes such as sucrose will inhibit gelation without affecting "bound" water values. Pectin gel structures apparently are dependent on secondary bonding and independent of "bound" water per se.

INTRODUCTION

IN PAPAYA, as in many fruits, texture of the intact fruit, referred to as firmness, depends on the water-structural relationships such as the selectivity of cell membranes for solutes and upon intermolecular water binding. Pectins normally present in fruits immobilize water in complex gel structures. Changes in pectins through action of pectinesterase or through changing concentrations of solutes can modify the structural relationships of water and thus the texture of pectin gels.

Behavior of intermolecular water can provide further insight into the structural stability of moisture in whole fruit and in fruit products. A portion of the water which forms intermolecular bonds with pectins and other structural elements behaves as ice over a wide range of temperatures. This water having a close affinity to structural elements and which remains essentially unfrozen until temperatures are below −20°C is defined by Kuprianoff (1960) as "bound" water. The remaining "free" water, which may be relatively mobile within the structure of the gel, behaves as ordinary water acting as a solvent for solutes and freezing with removal of the latent heat of fusion at or near normal temperatures for ice formation. Studies of the responses of fruit gels to various treatments or storage conditions require a simple reproducible technique for estimation of the relative proportions of "bound" and "free" water.

Calorimetric or dilatometric methods measure the "bound" water indirectly by determining the heat lost by a product in cooling to less than 0°F (−18°C). Using these techniques Mannheim et al. (1957) found that water in bread is not completely frozen until below −70°F; Wedemeyer and Dollar (1964) determined the relative importance of "bound" water in free radical reactions induced by exposure of model systems to ionizing radiation using the calorimetric technique.

The objective of the present study was to determine the proportion of "bound" water in fruit products and interactions of natural colloids with added solutes. Measurement of freezing rates and time for removal of heat of fusion under reproducible conditions was chosen as the method for objective measurement of relative proportions of "free" and "bound" water in hydrocolloid systems.

MATERIALS & METHODS

Sample preparation

Fully ripened whole papayas were cut into quarters and seeds and extraneous materials removed. Flesh was removed from the skin, diced into convenient sized pieces and blended for 2 min. All pulp samples were freshly prepared for each series.

The model system was prepared by dissolving 0.8% citrus pectin (a low methoxyl pectin, Matheson, Coleman & Bell), 8% glucose (dextrose, anhydrous, USP, Merck) in 0.15% (w/w) citric acid (anydrous, reagent). pH was adjusted as required by adding either HCl or NaOH. Additives such as gum Tragacanth (USP Type L, Meer Corp.) and synthetic silica (micron-sized, Syloid 224, W. R. Grace) were added to the model system of pectin-glucose-citric acid to study the interaction between various hydrocolloids. All the samples in the model system were also blended for 2 min.

Guava, passion fruit and pineapple were chopped and passed through a pulper fitted with a 0.033 in. screen and a finisher with a 0.020 in. screen. The resultant fruit product was pasteurized (190–200°F, 1 min) and frozen at 0°F in No. 2 (307 × 409) or No. 10 (603 × 700) cans. The juice product was thawed when needed.

Experimental method

Freezing curves for the mixtures were determined by immersing a sample container in liquid Freon-12® (Du Pont dichlorodifluoromethane, N.B.P. −21.6°F) contained in a wide-mouth (7-in. diameter) Dewar flask. This system provides for a reproducible rate of heat removal. A 150g sample in a 6-oz can (202 × 314 size or 5.08 cm × 8.25 cm) was fitted with a copper-constantan thermocouple probe located at geometric center of the liquid volume. The thermocouple was connected to an electronic, automatic cold-junction compensating, 6-point Honeywell recorder, calibrated at −21.6°F with Freon-12 and at 32°F with an ice–water mixture. The can, covered with aluminum foil, was lowered into the Dewar flask until 1/2 in. was exposed above the Freon-12 level. Cooling and freezing was monitored until the sample temperature was less than −10°F.

Calculation of "bound" water

The elapsed time for phase change depends on latent heat of fusion of the "eutectic" mixture adjacent to the thermocouple, thermal conductivity of the concentric frozen portion, the thermal gradient in the system and heat removal from the container surface. The time to complete the phase change is in the range 10–15 min which for any given sample mixture is reproducible to ±0.05°F.

The thermal conductivity of the solid phase of representative fruit–sugar mixtures is determined from the post-freezing rate of temperature change assuming values for specific heat for soluble solids (0.3) and ice (0.49). Thermal conductivity (k) values of frozen mixtures were determined for soluble solids values in the range 5–50% (w/v) on the basis of the equation (Charm, 1963):

$$\text{Thermal conductivity } k = k_{w,s} = \frac{0.398\,\rho\,Cp}{[(1/r^2) + (0.427/h^2)]\,f} = 2.9093\,(\rho\,Cp/f)\ \text{cal}/(\text{cm}^2)(\text{sec})(°\text{C/cm})$$

where

ρ = density g/cm^3
Cp = specific heat = [0.3 (Tss) + 0.49 (100-TSS)] .01
TSS = total soluble solids in g/100 ml
r = radius of container = 2.54 cm
h = half-height of container (coincides with thermocouple junction) = 4.12 cm
f = the time in seconds for $\log [(T_m - T)/(T_m - T_o)]$ to change one log cycle
Tm = temperature of cooling medium – (Freon-12 = −30.1°C)
T = temperature of the solid phase (°C) at time of measurement

T_o = initial temperature (°C) of solid phase

The following expressions summarize the various relationships of observed values to total soluble solid values.

1. $\rho = 0.9901 + 0.00475\ (TSS)$
2. $Cp = [0.3\ (TSS) + 0.49\ (100 - (TSS)]\ .01$
3. $\log f = 2.4577 + 0.0166\ (TSS)$
4. $\log k_{ws} = (-2.4334) + (-0.0167)\ (TSS)$ (or) $k_{ws} = (3.69 \times 10^{-3})\ (0.96)^{TSS}$
5. Freezing point $= 1.10 + 0.164\ (TSS)$ °C
6. Time for fusion (sucrose/water) (θ_w) sec $\log \theta_w = (-0.8325) + (-0.0165)\ (TSS)$

The regression ($Y = AB^x$) of the thermal conductivity fitted by the method of least squares is $k = (3.69)\ (0.96)^{TSS}$ where k = thermal conductivity (cal $\times 10^{-3}$/(cm^2) (sec) (°C/cm)). The correlation coefficient ($r = 0.95$) is highly significant ($p < .01$). Standard error of the estimate is 1.25×10^{-3} cal/(cm^2) (sec) (°C/cm).

"Bound" water was determined for the sample by comparison with values obtained for distilled water using the same system.

The calculation is based on the Nagaoka et al. (1955) modification of the Plank formula. "Bound" water is assumed to exhibit the same thermal conductivity and specific heat as ice. Fusion time (θ) is the elapsed time for phase change as determined by the intercepts of the cooling curves for liquid and solid phases with the portion of the freezing curve representing removal of the heat of fusion of the "eutectic" mixture.

The proportion of "bound" water to total moisture is calculated according to the expression:

$$W\theta_w k_{ws} = (W - B)\, \theta_{eu} k_{eu}$$
$$B = (W\theta_{eu} k_{eu} - W\theta_w k_{ws}) / (\theta_{cu} k_{eu})$$
$$B/W = 1 - (\theta_w k_{ws} / \theta_{eu} k_{eu})$$

Where W = mass of free water; B = mass of "bound" water; θ_w = fusion time of water; θ_{eu} = fusion time for "eutectic"; k_{ws} = thermal conductivity of ice in the temperature range for phase change of the "eutectic" mixture; and k_{eu} = thermal conductivity of "eutectic" mixture as determined from the regression equation.

Table 1—Apparent "bound" water of tropical fruit products

Fruit	Juice base %	Sucrose added %	Soluble solids %	"Eutectic" zone °F	"Bound" water %
Guava	100	—	7	31–26	5
	80	20	25	27–22	5
	60	40	44	22–18	5
Passion fruit	100	—	17	28–23	3
	90	10	26	26–21	4
	65	35	47	15–13	4
Pineapple	100	—	12.5	27–24	7
	85	15	26	26–23	7
	65	35	43	19–16	6

Table 2—Apparent "bound" water fruit products effects of pH

	Model Pectin/Glucose/ 0.8% 8% Citric Acid 0.15%		Papaya Puree
	Percent "Bound" Water CaCl$_2$ added		"Bound" water
pH	None	0.04%	%
3.0	42	58	—
4.0	29	49	34
5.0	18	47	37
6.0	5	9	35

Table 3—Effects of gum tragacanth on apparent "bound" water in fruit products

Added gum* %	"Bound water (Percent) Only gum*		
	Only gum*	Model system**	Papaya puree
0	—	0	34
0.2	17	13	36
0.4	30	17	36
0.6	39	19	39
0.8	48	24	41

*Gum tragacanth.
**Pectin, 0.8%/Glucose, 8%/Citric acid, 0.15%.

RESULTS & DISCUSSION

THE SOLUBLE solids content of fruits and vegetables is normally less than 20%. These products maintain firmness for reasonable post harvest periods at lower temperatures when evaporation is restricted. Cellular integrity is apparently an important factor in this firmness and once injured the fruit undergoes rapid softening. Pulping of papaya results in rapid gelation. This gelation is inhibited by increasing the soluble solids by addition of 13% sucrose (Chang et al. 1965). Reduced pH and addition of divalent ions will enhance gelation in pectin gels.

The present study has demonstrated that water is bound by fruit products and pectin gels in the range 3–48% (Tables 1, 2 and 3). The water bound by fruit nectar and juice products, with the exception of papaya, is less than 10% and unaffected by added sucrose solids (Table 1).

Papaya pulp is unique in that water binding is 34–37% and is not affected significantly by varying pH in the range 3.0–6.0 (Table 2). The water bound by a pectin, sucrose, citric acid model increases as the pH decreases (Table 2). The pectin model does not gel until the pH is 5.0 or less.

While addition of CaCl$_2$ (0.04%) to the pectin model system increases the water binding to that observed for papaya, there is no significant change in the pH effect on gelation. This observation suggests that formation of a gel structure apparently is independent of "bound" water.

Gum tragacanth actively binds water and the binding activity is related to concentration in the range 0.2–0.8% gum without forming a gel (Table 3). Papaya pulp and pectin form gels which bind nearly 50% of the total water in the system. Addition of gums to papaya and pectin gels does not affect the total amount of "bound" water, but weakens or completely inhibits gel formation.

The data indicate that water binding activity of solutes or sols generally do not relate directly to gel formation. Addition of sucrose or hydrocolloids does not affect "bound" water values as measured by the freezing method but competes for space in the "free" water volume. This gel weakening effect of increasing solute concentrations or addition of hydrocolloids suggests that the secondary structure within the "free" water volume is the primary force responsible for increased viscosity gels.

These secondary bonds essential to gel structure presumably forming within the "free" water volume in pectin systems are not measured by the freezing method. Measurement of "bound" water by the freezing method provides an easy, rapid and objective measure of the "free" water volume aiding in the improvement of techniques for controlled formation and analysis of gels.

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ABDUL R. RAHMAN, W. L. HENNING and D. E. WESTCOTT
Food Laboratory, U.S. Army Natick Laboratories, Natick, Mass.

HISTOLOGICAL AND PHYSICAL CHANGES IN CARROTS AS AFFECTED BY BLANCHING, COOKING, FREEZING, FREEZE DRYING AND COMPRESSION

SUMMARY—The effect of processing variables on the cell structure and physical characteristics of carrots were determined. The phloem portion of fresh carrots was subjected to one of the following treatments: blanching; cooking for 10 min; freezing at 0°F, −30°F or −320°F; freeze drying, compressing after freeze drying at approximately 1500 psi. Carrots at each treatment were tested for: (1) texture by means of the Allo-Kramer Shear Press; (2) water holding capacity by centrifuging at 500, 1000, 1500, 2000 and 2500 rpm; (3) histological changes by microscope observation of the tissue structure. Results indicate that among all treatments, freezing temperature is the most critical factor affecting the cell structure of the carrots. Freezing at 0°F or −30°F results in considerable disruption of the cellular structure, whereas it was minimal at −320°F. Carrots frozen at −320°F showed firmer texture as well as higher water holding capacity than the rest. Significant correlation coefficient was established between the shear press values and percent weight loss measured by centrifugation. This suggests that the latter may be used as an objective test for measuring textural changes in processed carrots and perhaps other foods.

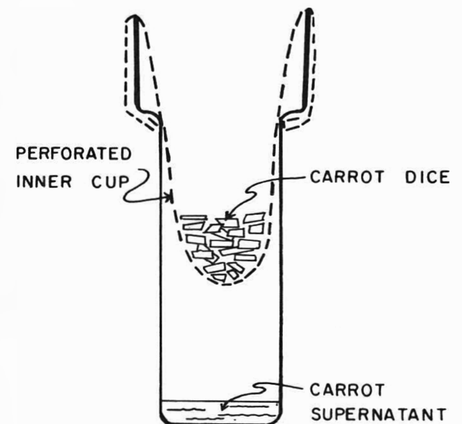

Fig. 1—Diagram of a centrifuge cup fitted with perforated inner cup for determining water holding capacity in carrots by means of centrifugation.

INTRODUCTION

FREEZE-DRIED vegetables, due to their porous structure, rehydrate at a faster rate than the air-dried ones. However, due to blanching and freezing prior to freeze drying, their texture after rehydration as measured by mechanical means is adversely affected. Therefore, rehydrated products possess less firm texture than their fresh counterparts. The physical properties that reflect the turgidity depend largely upon the structural arrangement and chemical composition of the cell walls. Two theories exist regarding the physical effect of cooking on the structure of cell walls in plant tissues. The claim is made that the softening of tissues is due primarily to the easy separation of the cell walls and, secondarily, to the loss of rigidity in the individual cell walls (Sterling, 1955). Since the intercellular cement is generally conceded to be composed in most part of pectic substances (Anderson, 1935; Bonner, 1936; Jolyn and Phaff, 1947), any agent or process which breaks down those substances can obviously bring about cell separation. Kertesz (1951) indicated that heat can cause the breakdown of pectic substances. Sterling and Bettelheim (1955) demonstrated the separation of intact cells in cooked potatoes. This was confirmed by Reeve and Leinbach (1953) in their studies on apples. However, commercially freeze-dried foods are not only heat treated (blanched) but also frozen at temperatures ranging from 0 to −30°F before they are dehydrated. This indicates that further changes to the cells due to freezing may be expected. These changes most often manifest themselves as the loss of characteristic texture, a cracking of the brittle frozen food, and drippage of cell constituents. The source of damage to frozen fruits and other plant tissues is not clear. The rate of freezing is recognized as a critical factor in tissue damage. Under conventional conditions the slow freezing of fruit and other multicellular structures is often harmful (Fennema and Powrie, 1964).

Very little work has been done on the effect of compression on the physical characteristics and cell structure of plant products. Rahman et al. (1969) indicated that freeze-dried spinach, carrots, and green beans compressed under 500–2000 psi show no significant difference from their uncompressed counterpart upon rehydration.

METHODS & MATERIALS

FRESH mature carrots of the Imperator variety were washed and peeled. They were then decorticated to separate the xylem tissue from the phloem portion which was used in these studies. The phloem portion was diced to 3/8 × 3/8 × 1/8 in. The diced carrots were divided into 8 lots. Each lot received only one of the following treatments, and all treatments were replicated:

1. Raw (no treatment)
2. Blanched 3 min in boiling water
3. Cooked 10 min in boiling water
4. Frozen at 0°F
5. Frozen at −30°F (blast freezing)
6. Frozen at −320°F (liquid nitrogen immersion) for comparison
7. Frozen at 0°F, −30°F or −320°F and then freeze dried at platen temperature of 120°F to a final moisture content of less than 2%.
8. Freeze dried as above and then compressed by subjecting to live steam for 5 min, compressing approximately 10(g) of carrots into bars approximately 3 × 1 × 1/2 in and then redried to approximately 2% moisture.

The carrots were prepared for testing as follows: the frozen carrots were thawed and the freeze-dried and freeze-dried compressed carrots were rehydrated by boiling in water for 1 min, soaking for another minute and then draining for 3 min.

Water holding capacity

WHC was measured by placing the carrot dice in a small conical shape screen, that fits snugly inside a centrifuge cup (Fig. 1), and then centrifuging for 10 min at 500, 1000, 1500, 2000 and 2500 rpm respectively. The liquid extracted from the carrots into the centrifuge cup was weighed and the weight loss of the carrot calculated.

Texture

Texture was measured by the Allo-Kramer Shear Press using the 5000-pound ring with 30 sec downstroke.

Histological studies

Carrot dice were fixed in FAA (Formalin-Acetic acid-Alcohol) and then dehydrated with a series of solutions of ethanol and butanol. They were then embedded in paraffin, cut to 10-12 micron ribbons, mounted on slides, and stained with Hematoxylin and Safranin. Several slides for each treatment were prepared. Microscopic examinations were made on these slides and photomicrographs were taken on representative slides.

RESULTS & DISCUSSION

EFFECTS of blanching, cooking, freezing, freeze drying and compression on the water holding capacity of carrots, as

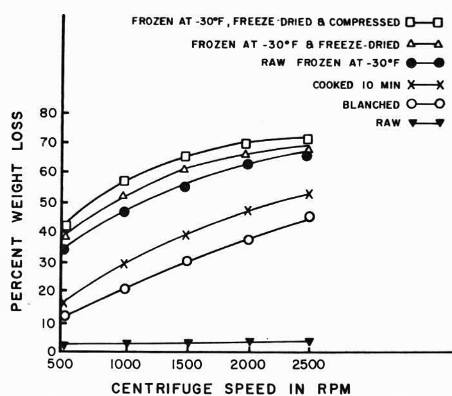

Fig. 2—Weight loss in carrots as affected by processing variables and measured by centrifugation.

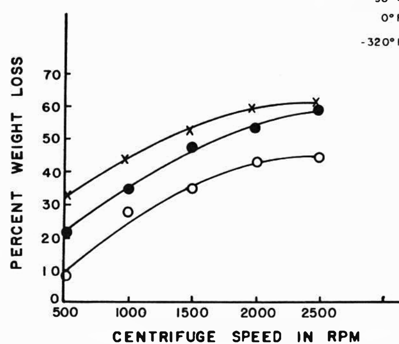

Fig. 3—Weight loss in carrots as affected by freezing temperature and determined by centrifugation.

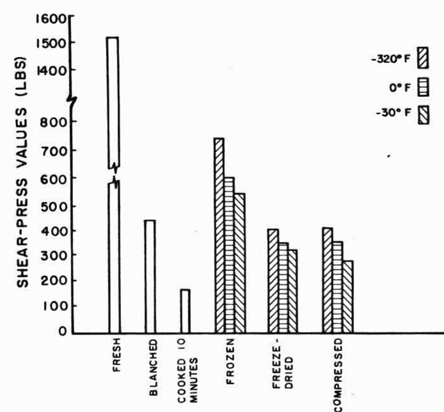

Fig. 4—Texture of carrots as affected by processing variables and measured by the Allo-Kramer shear press.

reflected by the weight loss upon centrifugation, are shown in Figure 2.

Weight loss in carrots at 5 centrifugal speeds ranging from 500–2500 rpm occurred in increasing order upon the following treatments: (1) fresh (no treatment); (2) water blanching; (3) cooking for 10 min; (4) freezing raw carrots at − 30° F and then thawing; (5) freeze drying and then rehydrating; and (6) freeze-drying compression and then rehydrating. This indicates that freezing the raw carrots resulted in a greater weight loss than heating alone. Upon compressing the freeze-dried carrots at 1500 psi and then rehydrating, the weight loss was only slightly higher than the uncompressed freeze-dried carrots.

Weight loss resulting from centrifugation was least on carrots frozen at − 320° F, followed by freezing at 0° F and at − 30° F, respectively (Fig. 3). Therefore, freezing temperature appears to be the major factor affecting the weight loss of the carrots.

The texture of raw carrots softened under all treatments. Degree of softening increased from blanching to cooking, to freezing and thawing, to freeze drying and rehydration. When the freeze-dried carrots were compressed under 1500 psi and then rehydrated, the texture was slightly softer than the uncompressed ones.

Correlation coefficient values of 0.6, 0.7, 0.8 and 0.9 of weight loss upon centrifugation to shear press were established for each respective centrifuge speed upon centrifugation at 500, 1000, 1500, 2000 and 2500 rpm for fresh, blanched, cooked, frozen, freeze dried and compressed carrots. Carrots frozen at − 320° F possessed firmer texture as indicated by higher shear press readings than the ones frozen at higher tempera-

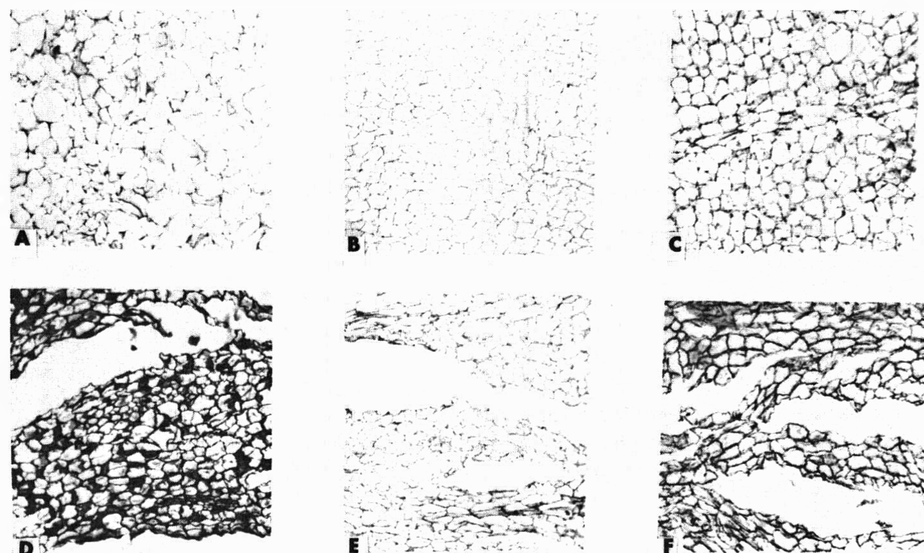

Fig. 5—Cross sections of phloem region of carrots as affected by the following treatments: (A) fresh, no treatment; (B) blanched; (C) cooked for 10 min; (D) frozen at 0° F; (E) freeze dried and rehydrated; (F) freeze dried, compressed and rehydrated. (All figures ×60)

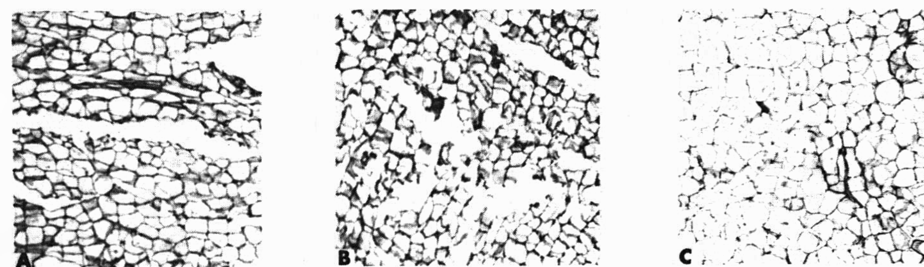

Fig. 6—Cross section of phloem region of carrots as affected by freezing temperature: (A) frozen at 0° F and thawed; (B) frozen at − 30° F and thawed; (C) frozen at − 320° F and thawed. (All figures ×60)

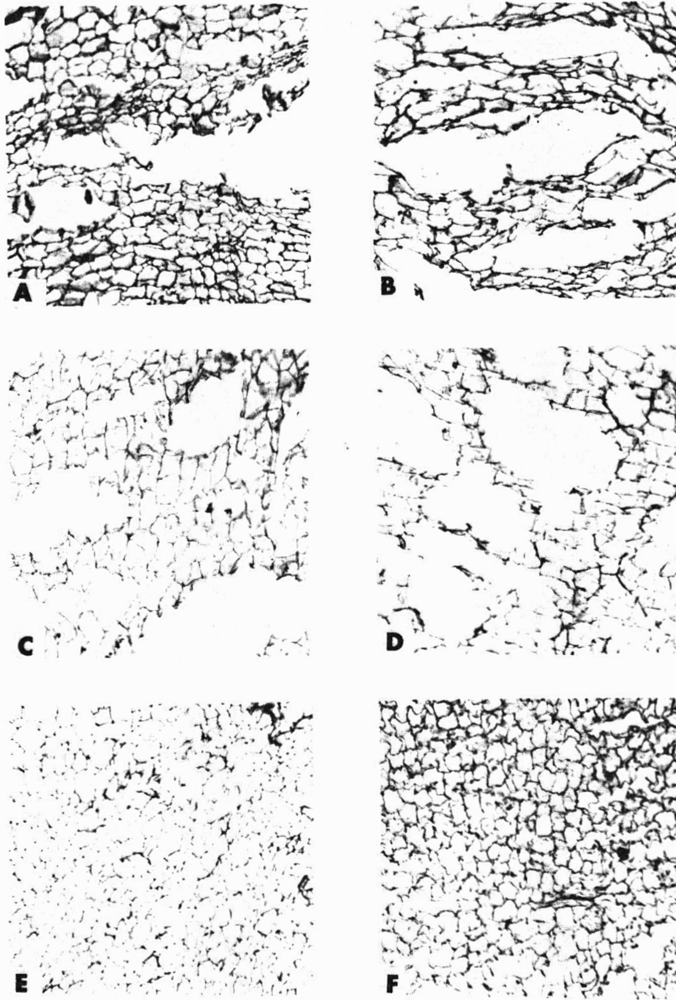

Fig. 7—Cross section of phloem region of carrots as affected by freezing temperature, freeze drying and compression: (A) frozen at 0°F, freeze dried and rehydrated; (B) frozen at 0°F, freeze dried, compressed and rehydrated; (C) frozen at −30°F, freeze dried and rehydrated; (D) frozen at −30° F, freeze dried, compressed and rehydrated; (E) frozen at −320°F, freeze dried and rehydrated; (F) frozen at −320°F, freeze dried, compressed and rehydrated. (All figures ×60)

tures regardless of the treatments (Fig. 4). This softening in texture accompanied by increasing centrifuging weight loss upon blanching, cooking, freezing, freeze drying and compression as compared with raw carrots, suggests definite changes in the cells.

Figure 5 shows physical changes in the tissue as indicated by cell separation and the disruption of cell walls during freezing, freeze drying and compression. However, no tissue disruption is apparent in blanched or cooked carrots. This suggests that physiological and chemical rather than physical changes may have occurred which caused the softness of the tissues and weight loss upon centrifugation. Finkle (1970) suggested that loss in cell viability is caused by a breakdown in selectivity of cellular membranes.

Figure 6 shows that freezing temperature was a major factor in causing cell disruption. Carrots frozen at −320°F showed the least cell disruption followed by 0°F and −30°F.

Figure 7 shows that compressed freeze-dried carrots exhibit a slight increase in cell disruption over the freeze-dried ones regardless of the freezing temperature. Since a significant correlation coefficient was established between the shear press values and percent weight loss measured by centrifugation, the latter may be used as an objective test for measuring textural changes in some processed foods.

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W. J. HARPER, AMANDA CARMONA[1] and T. KRISTOFFERSEN
Department of Dairy Technology, Ohio Agricultural R& D Center, Columbus, Ohio

PROTEIN DEGRADATION IN CHEDDAR CHEESE SLURRIES

SUMMARY—Preferential degradation of caseins was noted in the ripening of Cheddar curd slurries, with the degradation generally being in the order of para-κ, β-, α_s-caseins. The addition of reduced glutathione to the slurry caused an immediate release of peptides from the protein mass and decreased the initial rate of β-casein degradation. The peptides appeared to be preferentially utilized during the first 4 days of ripening; thereafter rapid degradation of β-casein occurred. In quiescent slurries, α_s-casein degradation exceeded that of β-casein, whereas periodic agitation reversed the degradation rate of the two caseins. The rate and extent of characteristic flavor development appeared to be related directly to β-casein degradation, but not to changes in concentration of para-κ or α_s-caseins.

INTRODUCTION

THE RECENTLY DEVELOPED cheese slurry system of Kristoffersen et al. (1967) provides a means for studying cheese ripening processes in a matter of days rather than in months or years. These workers found that Cheddar curd slurries developed full, characteristic flavor within 5–7 days when stored at 30°C, and that the addition of glutathione to the slurry system resulted in a faster and more uniform flavor development. It was noted that when glutathione was added to the slurry, the rate of protein degradation was increased. The present study was conducted to determine the effect of starter culture, glutathione and the degree of agitation of the slurry during incubation on flavor development, casein hydrolysis and peptide formation.

EXPERIMENTAL

Curd manufacturing

The Cheddar cheese curd was manufactured from pasteurized Grade A quality milk utilizing different starter cultures. The curd was manufactured by standard methods and held without pressing in the salted form overnight at room temperature.

The following cultures were used for curd manufacturing: (a) *Streptococcus cremoris* C_{13}; (b) commercial buttermilk Culture A; and (c) a general purpose cheese Culture B.

Curd slurries

Slurries were prepared from 24-hr old curd essentially by the method of Kristoffersen et al. (1967) by blending 500g of curd with 250 ml of 5.2% sterile NaCl solution. When used, glutathione (100 ppm) was added at the time of slurry preparation. The slurries were stored at 30°C for 7 days, being sampled for flavor and chemical analyses at specified intervals. For analysis, 20g of slurry was removed aseptically and stored at −20°C. Normally the curd was agitated for 2 min on a daily basis.

[1]Present address: Bacteriological Institute, Santiago, Chile.

Acetone preparation of slurry proteins

The changes in the protein fractions of the cheese slurries were determined on acetone-powder samples. The thawed slurry was blended with distilled water (1:2 v/v) and transferred to a Mini-Mill steel cup, containing 20 ml each of 0.05M potassium phosphate butter (pH 7) and 0.1% ethylenediamine tetra-acetic acid, and maintained at 4°C. The Mini-Mill was operated at 22,500 rpm using, in sequence, a rotator setting of 120 for 10 min and 10 for 25 min. The slurry was centrifuged (10,000 rpm for 10 min) and the supernatent filtered (Whatman No. 1 filter paper). The filtrate was combined with 150 ml of acetone and 20g of dissolved dry ice, allowed to stand undisturbed for 15 min and the protein removed by suction filtration (Whatman No. 42 filter paper). The resulting acetone powder was then washed successively with acetone (50 ml) and diethyl ether (50 ml). Traces of acetone and ether were removed by vacuum desiccation over $CaCl_2$. The protein preparation was stored at −20°C in a screw-cap glass bottle, which was placed in a sealed container with $CaCl_2$.

The protein content of the acetone powder was determined by the Folin Ciocalteau phenol method as modified by Lowry et al. (1951).

Polyacrylamide disc gel electrophoresis (PAG)

Essentially, the method developed by Ornstein and Davis (1959) was used to determine changes in the individual caseins during ripening. The gel was formed in a glass tube 5.5 × 50 mm. The gel was prepared in two sections composed of (a) 7.5% polyacrylamide, and (b) 60 μl of a solution containing 0.60–0.70 mg of acetone powder in 100 μl of 0.05M pH 7.0 phosphate buffer containing 0.1% 2-mercapto ethanol and 250 μl of large pore gel (3.5% PAG).

The electrophoretic separation was conducted at 4°C for 45 min using a constant current of 3 milliamps per tube.

After electrophoresis, the gels were placed in 0.1% Amido Black staining solution for 2–24 hr, cleared by immersion in 7% acetic acid for 24 hr, and analyzed with a Photovolt Densicord Recording Densitometer at 525 mu. The area of the peaks was determined by a Gelman planimeter. Standard solutions of pure α_s-casein and β-casein were analyzed in the same manner for comparison.

Gel filtration

For gel filtration, the method of Lindqvist (1962) was utilized. A 1.85 × 25 cm glass column was packed with Sephadex G-25. One ml of a water extract of the cheese slurry was passed through this medium utilizing 0.1N NH_4OH as the eluant. The resulting eluate was analyzed by a UV densitometer at 280 mu.

RESULTS

Sephadex gel filtration

The effect of glutathione and starter cultures on the relative amounts of water soluble proteins and peptides was determined by means of Sephadex G-25 gel filtration immediately after the slurries were prepared.

The results in Figure 1 show that regardless of the starter culture, the GSH-treated slurries contained less high molecular weight soluble protein material and more small molecular weight peptides than the control slurries. The elution patterns of the three control slurries were generally similar irrespective of the starter culture used, whereas in the GSH-treated slurries, the relative concentration of peptide components varied as a function of the starter culture.

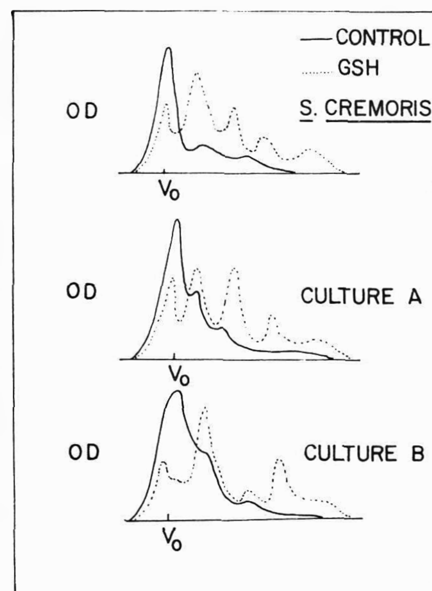

Fig. 1—Culture and GSH effects on Sephadex G-25 elution patterns of freshly made Cheddar curd slurries. (Flow rate was 20 ml/hr; Optical density (OD) determined at 280 mu; V_0 is void volume.)

The differences in the elution patterns of the freshly-made control and GSH-treated slurries suggest that there is an initial dissociation of preformed peptides by the action of GSH on the cheese protein.

Protein changes

Attention was directed next to the changes in cheese proteins during storage of slurries at 30°C as revealed by polyacrylamide electrophoresis, with particular attention to the changes in para-κ, α_s- and β-caseins. Curds made with *S. cremoris* C_{13} and Culture B were utilized for slurry preparation in this phase of study. Water-extractable, acetone-precipitable protein specimens were prepared from the cheese slurries at 0, 2, 4 and 6 days of age. Exploratory trials had revealed that the protein changes which occurred between 0–1 day and between 2–3 days of ripening were insignificant.

The data in Table 1 are averages of three trials. Identical trends were noted in all three trials with variations between trials being less than 1%. The amount of water-extractable, acetone-precipitable protein in the curd slurries generally increased with storage time. The control slurries containing Culture B exhibited higher soluble protein values and slightly greater overall changes than those containing *S. cremoris* C_{13}. However, when GSH was added, the rate and extent of proteolysis was increased for the *S. cremoris* C_{13} slurries in comparison with the control, whereas solubilization of proteins was retarded for Culture B slurries during the early stages of storage.

The water-extractable, acetone-precipitable protein preparations were subjected to PAG electrophoresis. The concentrations of the protein fraction were determined by calculating the percentage of each protein as a function of the total area. The values were corrected for the differences in the concentrations of the total water-soluble, acetone-precipitable material.

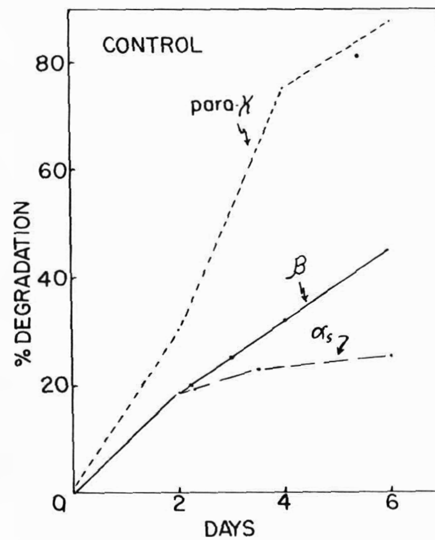

Fig. 2—Percentage degradation of various caseins in control slurry during 6 days of incubation; (starter culture was S. cremoris C_{13}).

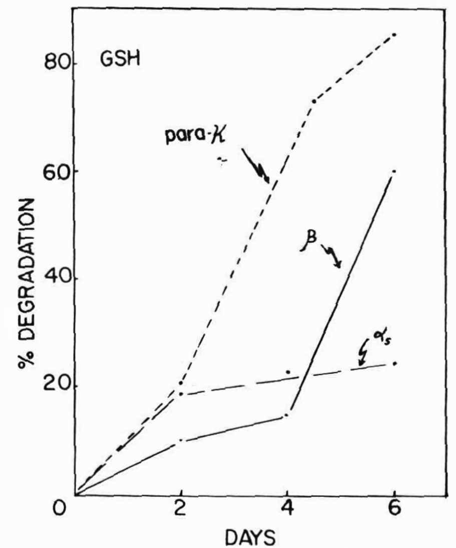

Fig. 3—Percentage degradation of various caseins in GSH-treated slurries during 6 days of incubation; (starter culture was S. cremoris C_{13}).

Four major protein fractions, para-κ, α_s-casein, β-casein, and a proteose fraction were present in all samples (Fig. 2). The decrease in the concentrations of caseins during 6 days of ripening are shown for the control slurry made with *S. cremoris* C_{13}. All three casein fractions were degraded during ripening with from approximately 15%–80% of native protein remaining after 6 days. The degradation of para-κ casein was most extensive and α_s-casein exhibited the least change. The presence of added GSH affected the β-casein fraction only (Fig. 3). Without added GSH β-casein was degraded at a relatively uniform rate during the storage period. In comparison, the addition of GSH resulted in a slower rate of β-casein degradation during the first 4 days of storage which was followed by a rapid and more extensive disappearance of β-casein.

The rate of flavor intensity and defects of the various slurries during ripening are shown in Table 2. Similar results were obtained for slurries made with both starter cultures. Characteristic Cheddar flavor occurred after 2–4 days of storage, with GSH enhancing the rate and extent of characteristic flavor development. After 6 days, the intensity of characteristic flavor in the GSH-treated slurries was about three times that of the controls.

To determine the possible reason for the effect of added GSH on β-casein degradation during ripening of the slurries, a comparison was made of the rate of degradation of the soluble protein fraction which was eluted with the void volume during Sephadex gel filtration of the water-extractable protein and that of β-casein in the acetone powders, with results being presented for *S. cremoris* C_{13} slurries in Figure 4. Similar results were obtained for slurries made with both Culture A and B and *S. cremoris* C_{13}. For the control slurry, the rate of degradation of soluble protein and the degradation of β-casein were similar and relatively low. For the GSH-treated slurry, the rate of soluble protein degradation was very rapid during the first 4 days of ripening, whereas β-casein was not degraded rapidly until after 4 days of storage. The degradation of soluble protein after 4 days was about 85%, and only slight additional degradation oc-

Table 1—Water soluble, acetone precipitable proteins in Cheddar curd slurries with and without added glutathione

Age (days)	Culture	Water soluble protein (g/ml)[a] Control	GSH-treated
0	*S. cremoris* C_{13}	384	422
2	*S. cremoris* C_{13}	434	596
4	*S. cremoris* C_{13}	447	630
6	*S. cremoris* C_{13}	540	714
0	Culture B	520	423
2	Culture B	660	473
4	Culture B	708	825
6	Culture B	720	714

[a]Average of three trials.

Table 2—Flavor characteristics of control and GSH-treated Cheddar curd slurries made with different starter cultures

Ages days	Control CF[a]	Control Defect	GSH-treated CF[a]	GSH-treated Defect
		S. cremoris C_{13}		
0	0	none	0	none
2	0	none	1	none
4	1	none	1	none
6	2	none	5	none
		Culture B		
0	0	none	0	none
2	0	none	0	none
4	0	sl. unclean	1	sl. fermented
6	1	unclean	3	sl. fermented

[a]Characteristic Cheddar flavor intensity: 0 absent; 1–2 very slight; 3–4 slight; 5–6 definite; 7–8 pronounced.

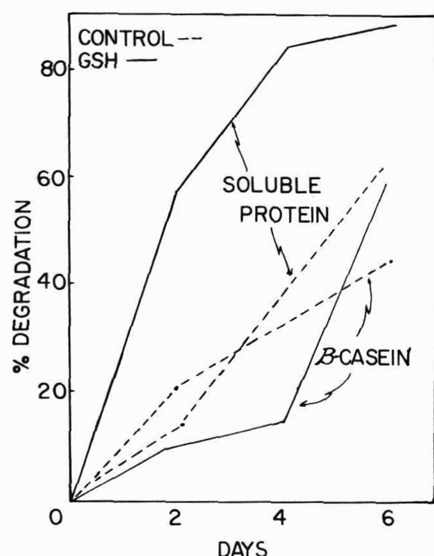

Fig. 4—Comparison of β-casein degradation and Sephadex G-25 separated soluble protein degradation in control and GSH-treated slurries; (starter culture was S. cremoris C_{13}).

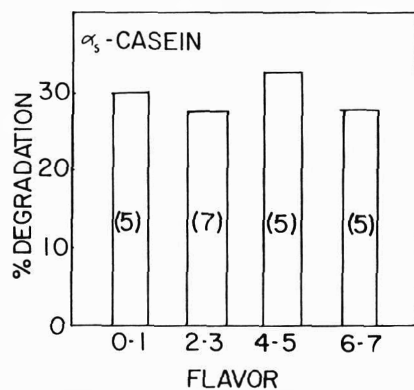

Fig. 5—A comparison of α_s-casein degradation and flavor development in Cheddar cheese slurry systems.

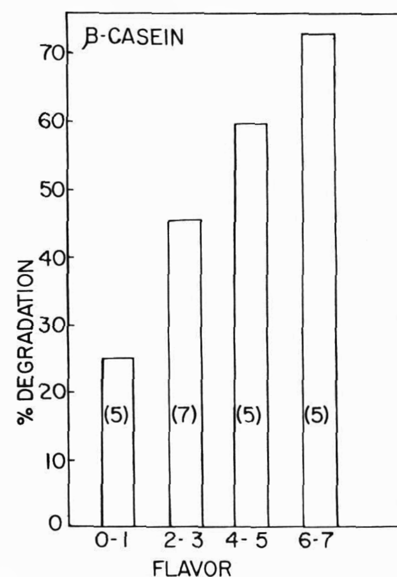

Fig. 6—Comparison of β-casein degradation and flavor development in Cheddar cheese slurries.

curred during the last 2 days of storage. The rapid increase in β-casein degradation after 4 days of storage may be related to the decrease in the concentration of soluble protein. Assuming that the protein materials are utilized by the microorganisms of the slurry system, the results suggest that the materials dissociated from the curd proteins initially by the GSH are utilized more readily by the microorganisms than β-casein, and that the protease attacks β-casein preferentially after the soluble protein fraction has been utilized.

Effect of agitation on protein degradation

Since agitation of the slurry during the ripening process has been shown to affect flavor development (Kristoffersen, 1967), attention was directed to the protein changes in relation to the degree of agitation of the cheese slurries made from curd manufactured with *S. cremoris* C_{13} and Culture B. Protein analyses were made of agitated and quiescent GSH-treated slurries. Table 3 presents the flavor intensities, the concentrations of peptide, α_s-casein and β-casein, and the percent loss in α_s- and β-casein during the ripening process. For both cultures, the flavor intensity of the slurries was improved by daily agitation, with maximum flavor being obtained with agitation for the first 4 days of incubation in the case of *S. cremoris* C_{13} slurry and with agitation for the entire fermentation period in the case of the Culture B slurry. For the *S. cremoris* C_{13} slurry, the concentration of peptide increased slightly with increased agitation, whereas in the other slurry the agitation had no apparent effect on the peptide concentration. In both slurries, agitation changed the relative rate of disappearance of the two caseins. With *S. cremoris* C_{13}, the degradation of α_s-casein decreased and that of β-casein increased with increasing agitation. For the Culture B slurry, increased agitation resulted in an increase in the rate of β-casein degradation, but had only a slight effect on the degradation of α_s-casin. These results suggest that the selective degradation of the two major caseins is controlled by both the culture and the fermentation conditions.

Flavor-casein degradation relationships

In order to more clearly demonstrate the relationship between flavor development and casein degradation in the slurries, the flavor intensities and the percentages of α_s- and β-casein degradation were compared for 22 lots of 4-day and older slurries. Generally no relationship was found between α_s-casein degradation and the development of characteristic flavor (Figure 5), whereas β-casein degradation and characteristic flavor development were directly related (Figure 6).

Table 3—Effect of agitation on the flavor intensity and the protein changes of Cheddar curd slurries with added GSH

Starter culture	Agitation	Age of slurry (days)	CF[a]	μg/750 μg acetone powder[b]			% Loss	
				Peptide	α_s-casein	β-casein	α_s-casein	β-casein
S. cremoris C_{13}	—	0	0	25	187	207	0	0
	none	7	5	103	70	124	51	40
	Daily for 4 days	7	8	109	109	112	21	54
	Daily for 7 days	7	7	140	120	49	13	76
Culture B	—	0	0	30	167	200	0	0
	none	7	3	308	112	170	33	15
	Daily for 4 days	7	4	310	84	161	50	20
	Daily for 7 days	7	7	308	98	35	42	73

[a] See footnote Table 2.
[b] Calculated from polyacrylamide gel electrophoretic patterns of proteins. Average of two trials.

DISCUSSION

KRISTOFFERSEN et al. (1967) have previously reported that one of the effects of added GSH is an increase in the rate of proteolysis in Cheddar curd slurries. The results of the present study suggest that GSH may have more than one role in respect to the degradation of proteins in such slurries.

The first effect of GSH appears to be the disaggregation of protein-bound peptides. Since rennet is known to break κ-casein into several peptide components

which follow the curd mass (Lindqvist and Storgards, 1959), it seems possible that GSH might free these peptides from casein. If this were true, then the ready availability of such peptides might result in a preferential degradation during the slurry ripening and thus account for the more rapid proteolysis which occur in GSH-treated systems in comparison with the untreated system. The polyacrylamide gel patterns of the proteins appear to confirm this, because the para-κ-casein fraction disappeared more rapidly than either the α_s- or the β-casein fractions.

The second role of GSH appears to be associated with the rapid increase in the rate of degradation of β-casein in cheese slurries after 4 days of ripening. The GSH-released peptides appear to stimulate the production of protease which selectively degrades the peptides and soluble protein components. Upon depletion of these materials, the activity of the protease apparently is directed to the β-casein.

Previous investigations, using conventional cheese, have not agreed concerning the relative rate of degradation of caseins during the ripening process. Lindqvist and Storgards (1959) suggested that β-casein is degraded more rapidly than α_s-casein, whereas Melachouris and Tuckey (1966) found no difference in the rate of degradation of the two caseins. Ney et al. (1966) found different results at different temperatures of ripening, with degradation of only α_s-casein at refrigeration temperatures. In the experiments with agitation herein reported, the relative amount of α_s-casein and β-casein degradation varied as a function of both the agitation and the starter culture used. When α_s-casein degradation was greater than β-casein degradation, a relatively low intensity of characteristic flavor developed. Also, it was shown that the extent of β-casein degradation in general was related directly to the intensity of characteristic flavor of aged slurries.

The fact that there appears to be a relationship between flavor formation and β-casein degradation, along with a preference of microbial enzymes for β-casein (Lindqvist, 1962), suggest that the initial degradation products of β-casein (i.e., peptides and amino acids) may be specifically involved in further changes that are related to flavor development.

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S. J. TOMA and S. NAKAI
Department of Food Science, University of British Columbia, Vancouver 8, B.C., Canada

ULTRAVIOLET SPECTROPHOTOMETRIC DETERMINATION OF PROTEIN IN SOME FOOD PRODUCTS

SUMMARY—The spectrophotometric method of estimating the protein content of whole milk at wavelength 280 nm (Nakai and Le, 1970) was modifed for corned beef, flour, bean and egg yolk. A clear solution was obtained by adding 50% sulfuric acid or 2N sodium hydroxide with and without 7–8M urea depending on solubility of the food products. The protein content was calculated from the absorbance at 280, 243 or 215 nm on a spectrophotometer. The correlation coefficient between the absorbance and the protein by Kjeldahl method was 0.99, 0.99, 0.99 and 0.99 with the coefficient of variability of Kjeldahl protein for absorbance of 1.8, 4.1, 3.8 and 4.1% for bean, corned beef, egg yolk and flour, respectively.

INTRODUCTION

ULTRAVIOLET absorption spectrophotometry has been widely used as a simple method for determining protein in biological materials (Webster, 1970). However, the complexity of food products and interferences derived from compounds other than protein in foods present difficulties in the utilization of this technique. Recently the above technique was employed in this laboratory for protein determination in whole milk and an application of this method for other food products was suggested (Nakai and Le, 1970).

In this paper a simple method for determination of protein in flour, bean, rice, egg and meat products is proposed. Urea, 7M, in 50% sulfuric acid was used to dissociate and dissolve all protein, carbohydrate and fat to give a completely clear solution for flour. In the case of rice, bean and egg clear solutions were obtained by adding excess 50% sulfuric acid. Sodium hydroxide, 2N, containing 8M urea was employed for dissolving meat products after preparing a uniform meat suspension with 0.1M citric acid (Ashworth, 1970).

EXPERIMENTAL

Reagents and materials

Reagent grade chemicals were used for preparing the following: 0.1M citric acid, 8M urea in 2N sodium hydroxide, 10% and 50% sulfuric acid (v/v), 7M urea in 50% sulfuric acid.

Spectrophotometer, Unicam SP 800 and Beckman DB were used for measuring absorbances.

Procedures

Flour. To 40 mg of flour passed through a 200-mesh screen was added 0.2 ml of water while mixing, followed by 20 ml of 7M urea in 50% sulfuric acid and mixed again. A 2.5 ml portion was diluted more with 5 ml of 50% sulfuric acid and the absorbance read at 215 nm with a 1-cm cell against a reagent blank.

Bean. To 50 mg of a 200 mesh-passed sample was added 1 ml of 10% sulfuric acid and mixed gently. The mixture was diluted with 33.3 ml of 50% sulfuric acid and mixed for another 5 min. The absorbance was measured at 280 nm.

Egg yolk. A whole egg yolk was diluted with 250 ml of water and mixed thoroughly. A 0.2 ml portion of this solution in a test tube was diluted with 5 ml of 50% sulfuric acid and the absorbance measured at 280 nm within 15 min after dilution.

Corned beef. Mixed meat, 15g, was blended with 250 ml of 0.1M citric acid in a Waring Blendor for 2 to 3 min. To a portion of 0.5g in a test tube was added 9.5 ml of 8M urea in 2N sodium hydroxide, shaken, centrifuged at 5000 rpm for 7 min to separate the fat and the absorbance measured at 243 nm.

RESULTS & DISCUSSION

Dissolution of samples

Solubilization of different food samples were tried until complete dissolution was obtained without adverse color development and with reasonable stability of the absorbance.

For flour 50% sulfuric acid alone was unable to dissolve the sample until 7M urea was added to the sulfuric acid. Increasing the concentration of acid did not improve dissolution and rather yielded a brown color. On the other hand the 50% sulfuric acid alone was able to dissolve and dissociate the bean and egg yolk. Two steps were necessary for dissolving meat products which are the uniform suspension preparation and the dissolution to make clear solutions. Among 0.1M citric acid, 0.2M acetic acid and 0.1N sodium hydroxide, 0.1M citric acid was the best solvent to obtain a uniform suspension of meat. For the dissolution, this suspension was diluted with 8M urea in 2N sodium hydroxide whereas the 50% sulfuric acid caused a continuous increase of the absorbance with time. Lower concentration of urea and sodium hydroxide alone were not effective for dissolving meat suspension. Centrifugation was applied for meat after dissolution to remove the fatty substances which cause turbidity and interfere with absorbance reading. The lipid materials in egg yolk also have some effect on the absorbance. After 15 min following the addition of sulfuric acid to the egg yolk the absorbance started increasing. This is due to turbidity development caused by the lipid materials and the centrifugation was not able to separate these lipids after development of turbidity unless petroleum ether was added. A modification of this method by adding petroleum ether is recommended if a more stable absorbance was required.

Wavelength

The wavelength of 200–220 nm was recommended for protein determination (Wrigley and Webster, 1968) because of high and uniform sensitivity of absorbance for proteins of different origins. And for this reason we tried to use this region for protein measurement; however, the relationship between absorbance and Kjeldahl protein values was not linear due to the shifting of the peak with increasing concentrations of protein except flour. In the case of corned beef there was no peak at the 220 nm region but at the 243 nm.

Comparison with the Kjeldahl method

Samples of various concentrations were prepared for (a) five different commercial brands of corned beef; (b)

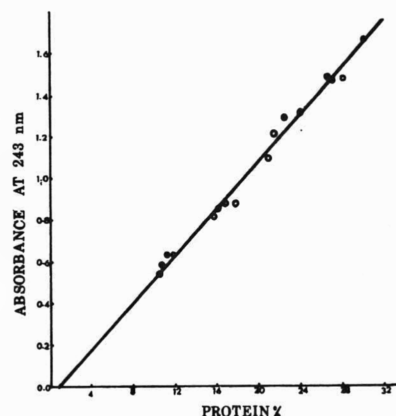

Fig. 1—Comparison with Kjeldahl values for protein in corned beef.

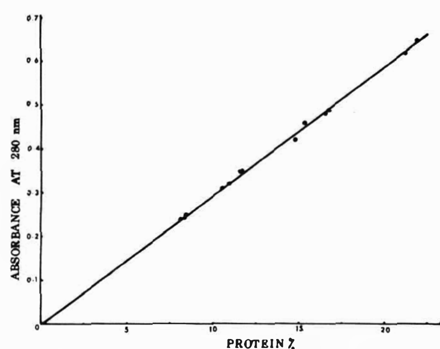

Fig. 2—Comparison with Kjeldahl values for protein in beans.

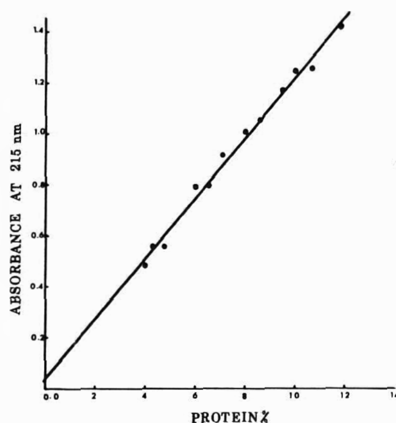

Fig. 3—Comparison with Kjeldahl values for protein in flour.

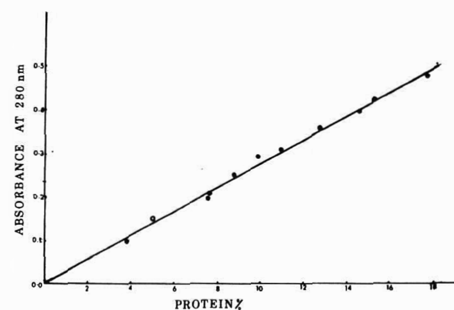

Fig. 4—Comparison with Kjeldahl values for protein in egg yolk.

three different commercial flours; (c) five egg yolks; and (d) five different commercial beans of two varieties: cowpeas and kidney beans. The absorbances by the present method were plotted against the proteins analyzed by the Kjeldahl method for corned beef, bean, flour and egg yolk, Figures 1, 2, 3 and 4. The correlation coefficient between the two methods, the standard error of estimate and the regression equation for each food product are shown in Table 1.

It was possible to apply this method to other foods. Adding the same solvents used for flour and egg yolk to rice and egg white, respectively, resulted in completely clear solutions. The method used for corned beef could be used for fresh beef, lamb and pork when the standard curve for calculation of protein for each meat was drawn separately.

Table 1—The correlation coefficient between the absorbance and Kjeldahl protein, the standard error of estimate and the regression equation for each food product.

Food product	Correlation coefficient	Standard error of estimate	Regression equation[a]
Corned beef	0.99	0.79	y = 17.23x + 1.025
Bean	0.99	0.24	y = 33.78x + 0.065
Flour	0.99	0.30	y = 8.53x − 0.440
Egg yolk	0.99	0.39	y = (738.5/wt(g) of yolk)x − 0.103

[a]y is Kjeldahl protein (N × factor); the factor used for bean, meat and egg yolk was 6.25 and for flour 5.7; x is the absorbance at wavelength described in the text for each sample.

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U. S. ASHWORTH
Food Science Department, Washington State University, Pullman, Wash. 99163

PROTEINS IN MEAT AND EGG PRODUCTS DETERMINED BY DYE BINDING

SUMMARY—Under optimum conditions Acid Orange-12 will bind to the proteins of meat and eggs. The dye-binding capacity is somewhat greater for these products than the previously reported values for wheat and milk products. A standard curve relating free dye concentration to mg of protein in the sample as determined by the Kjeldahl method can be readily prepared for each type of product. The method is rapid and useful for composition control in ground meats, eggs and prepared mixes. Cooking has little effect on the dye-binding capacity but hydrolysis of the proteins to the proteose-peptone stage reduces the dye bound. The coefficient of variation averaged about 3% of the protein content for the samples of meat and eggs tested.

INTRODUCTION

RAPID METHODS for composition control in the food processing industry have become important to assure uniform day-to-day quality in large volume operations. Consumer protection is involved also as more interest in certain nutrients such as protein is stressed. This laboratory was one of the first to apply dye binding to the determination of protein in milk and dairy products (Ashworth et al. 1960; Ashworth, 1966). Modified procedures have now been tested for several other food products including meat and eggs.

Dye-binding methods depend upon the reaction between certain dyes and the intact proteins of foods to form insoluble complexes. These can be removed by filtration and the free dye concentration measured colorimetrically. The dye bound is determined by difference and the dye-binding capacity (DBC) calculated as mg dye bound per mg of protein. Once the DBC is known for the conditions of the test, the protein content in unknown samples can be determined from the dye bound. Previous work has shown, however, that DBC increases with free dye concentration (Ashworth and Chaudry, 1962).

EXPERIMENTAL

CITRIC ACID, 0.1M, was used to emulsify the meat and egg proteins on the acid side of their iso-electric points to facilitate binding of the acid dye. Pure Acid Orange-12 dye was secured from Udy Analyzer Co., Boulder, Colorado (Sherbon 1967). The dye reagent was prepared by dissolving 1.300g of the dye in the buffer described by Sherbon (1967) and diluting to one liter. The absorbancy index of the dye is 59.0 at 0.475 microns.

After considerable preliminary work the following procedure was established as routine.

1. Weigh sample to contain about 4g protein. Measure 0.1M citric acid in 250 ml volumetric flask. Blend the mixture in Waring Blendor for 2-3 min. Assume total weight is 250g + weight of sample.
2. Weigh 4 aliquots of the diluted sample to 0.01g (top loading balance) into 50 ml polycarbonate centrifuge tubes. These samples should be in the range of 2-4g. After recording weight adjust total weight to 5g with water. This can be done volumetrically with a graduated pipet. Assume total volume, after adding 25.0 ml dye reagent, as 30.0 ml. After adding dye reagent, stopper and shake vigorously.
3. Allow 30 min or longer for dye to react with protein binding sites and for aggregation of the complex to occur.
4. If mixture still appears cloudy, centrifuge and filter; otherwise just filter through 11 cm S & S No. 597 paper or its equivalent.
5. Determine free dye concentration from absorbance at 0.475 microns in a special flow-thru cuvette with approximately 0.75 mm light path (Ashworth et al. 1960).
6. Read mg of protein from standard curve relating dye concentration to mg protein as determined by the Kjeldahl method (N × 6.25). Calculate mg of protein per gram of blended sample used. Average all aliquots falling in the middle range of the standard curve (0.3-0.6 mg free dye per ml). See Fig. 1. Calculate average mg protein per g of blended sample.
7. From known dilution of weighed samples with 250 ml of citric acid, calculate protein percentage in original sample.
8. Standard curve must be prepared from meat of approximately the same composition as the unknown.

RESULTS

WHEN THE FINAL volume of dye + sample was held constant, a linear relationship between Kjeldahl protein and free or excess dye concentration could be shown (Fig. 1). The useful range of free dye concentration was 0.3-0.6 mg/ml, where a straight line relation was found with protein content. For routine composition control this standard curve is the most practical. One should emphasize that the amount of reagent dye added must be held constant as well as the final volume.

A commercial sample of sausage containing 12.5% protein by the Kjeldahl method (63% fat) was extracted with acetone to yield a product containing 30.3% protein. Both the original and extracted samples were weighed and blended with citric acid. Aliquots of the blended samples were then prepared in a range allowing a dye:protein (D/P) ratio in the original mixture ranging from 0.6-1.0 mg dye added per mg of protein in the sample. The relation between free dye concentration remaining and the dye-binding capacities is shown in Fig. 2. The curve shows that the proteins of meat products do not react stoiciometrically with the dye. As the D/P ratio increases the dye-binding capacity also increases. Milk proteins have been shown to react in a similar way (Ashworth and Chaudry, 1962). The free dye concentration must be used to find the proper value of the dye-binding capacity before this value can be used to convert dye bound to mg protein in an unknown meat sample. In the D/P ratio range of 0.64-0.92 dye bound could be used as a measure of protein content. The D/P ratio for Amido Black is even more critical (Moss and Kielsmeier, 1967).

Proteins vary in their capacity to react with dyes as brought out in Table 1. The average DBC values were read from curves like the one shown in Fig. 2. Values were taken at 0.4 and 0.6 mg free dye per ml for comparison. The hemoglobin was a commercial powder pre-

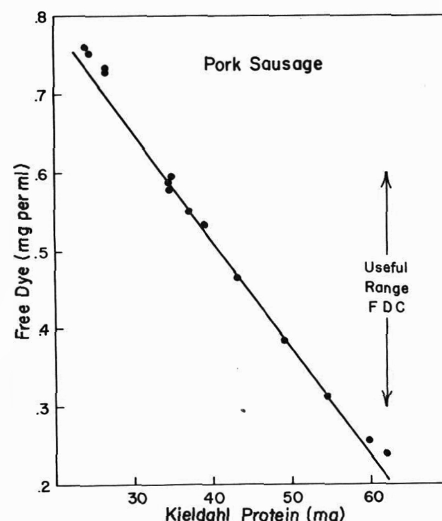

Fig. 1—A standard curve relating free dye concentration to total protein in the sample measured for dye binding. 25.0 × 1.3 mg AO-12 dye added. Final volume, 28 ml.

Table 1—Dye binding capacities of meat and egg products at two levels of free dye concentration

		DBC at free dye concentrations			
		0.4 mg/ml		0.6 mg/ml	
	No. of samples[a]	DBC Mean	SD	DBC Mean	SD
Egg yolk	4	0.44	.003	.48	.006
Egg white	7	0.39	.004	0.41	.009
Whole egg	6	0.41	.010	0.44	.015
Sausage	5	0.42	.013	0.45	.016
Chicken liver	4	0.36	.012	0.39	.0105
Chicken lunch meat	9	0.42	.015	0.45	.017
Chicken meat	6	0.46	.015	0.48	.016
Ground beef	9	0.43	.007	0.44	.017
Frankfurters	4	0.41	.016	0.43	.024
Beef liver	8	0.42	.018	0.44	.022
Gelatin (calfskin)	9	0.31	.021	0.35	.011
Proteose-peptone	3	0.09	.03	.145	.020
Hemiglobin	6	0.56	.019	0.58	.026

[a]Each sample represents a curve prepared as shown in Figure 2 for at least 8 aliquots run on the same blended sample.

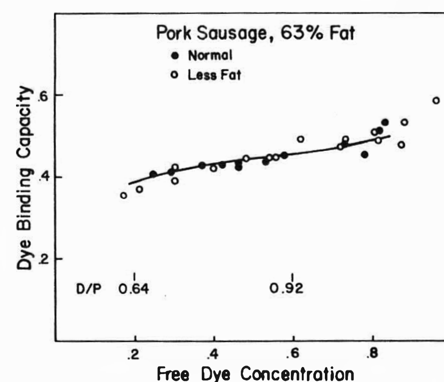

Fig. 2—The relation between dye-binding capacity and free dye concentration expressed as mg/ml. D/P is the ratio of dye to protein before binding had occurred.

pared for an enzyme substrate from bovine blood. The high DBC for this product and its ready availability in a stable form suggests its use as a standard for checking the dye-binding procedure.

Ground beef was heated in an oven allowing the melted fat to drain away from the patties at temperatures up to 160°C for 40 min with no effect on DBC. A loss of about 7% in the DBC was found when the patty was cooked at 250° C for 40 min and had lost 60% of its original weight. These results indicate that normal cooking has very little effect on the DBC

Pork bound the dye to the same extent as beef. Break-down products of meat, such as gelatin and the proteose-peptone fraction, bind less dye than the intact proteins. Previous work (Ashworth 1966) showed that milk fat had no effect on the DBC of milk proteins. Beef and pork fat were without effect on the DBC of meat proteins. The standard deviations shown in Table 1 vary with the product tested. The greatest variation occurs for those proteins which bind less dye as measured by the DBC such as gelatin and the proteose-peptone fraction. When the coefficient of variation was calculated, the meat and egg products showed mean values of 2.7% at a free dye concentration of 0.4 mg/ml and 3.3% at 0.6 mg/ml.

Chicken meat had a higher DBC than pork or beef but there was no significant difference between light and dark meat. Skin and liver proteins bound less dye. Cooked chicken luncheon meat and raw comminuted chicken meat from a mechanical deboner bound less dye than isolated muscle tissue. Egg yolk proteins bound more dye than egg white proteins. Coagulated egg white bound the same amount of dye as the raw white but there was a mechanical problem of dye penetration in the hard cooked white.

The dye-binding procedure is suitable for the determination of protein in mixtures of animal products. For example, when whole eggs and milk were mixed in the proportion commonly used for egg custards the dye-binding capacity was intermediate between that found for whole egg (0.41) and that found for milk proteins (0.34). The egg content of mayonnaise can be estimated from the protein content found from dye-binding data again showing that fats and oils do not interfere. Preliminary data with cooked cereal products containing eggs (noodles) caused turbidity problems after filtration of the dye-protein complex. Prevention of this problem is receiving attention at the present time.

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EDWARD W. TOEPFER and KENNETH MORGAREIDGE[1]
USDA Human Nutrition Research Div., ARS, Beltsville, M. 20705

EFFECT OF MALATHION ON NUTRIENT COMPOSITION OF EGGS AND FLAVOR OF MEAT FROM LAYING HENS

SUMMARY—Malathion in the feed of laying hens did not produce any indications of adverse effects on the flavor of the cooked meat or on the nutrient composition of the egg whites and egg yolks.

INTRODUCTION

FOR THE CONTROL of poultry lice or mites, malathion has been used as 3% in roost paint, a 1% spray of emulsified concentrate or wettable powder for use on the coop or environment, or a 4-5% dust directly on the birds USDA, 1968. Dermal LD_{50} was in excess of 4,444 mg/kg (rats) and orally 1,375 for males and 1,000 for females, indicating a very low toxicity. Malathion fed to hens was slow to accumulate in the tissues according to March et al. 1956. 2-3% was excreted unchanged and 97-98% was excreted as ionic water-soluble metabolites. The study reported here was not intended for toxicological purposes or for tissue accumulation of residues but for the determination of any effects on eating quality of the meat and on nutrient composition of eggs. No similar information had been previously reported on malathion. In order to control dosage levels, malathion was incorporated into the feed at levels determined by preliminary studies.

MATERIALS & METHODS

Management of pullets

A premix was prepared by gradually mixing sufficient technical grade malathion (assay, 96.0%) into the commercial mash to yield a level of 1.0% of the pesticide. This premix was stored in polyethylene-lined fiber drums under refrigeration. The premix was then diluted with fresh mash, as needed, to yield the two test levels; 1:10 and 1:100 for the 1000 and 100 ppm diets, respectively. The feeding dilutions were prepared fresh at intervals of not more than 2 wk. Samples removed from the feed hoppers after 2 wk analyzed 90% or better of the expected malathion content.

Four hundred 21-wk-old single comb white leghorn pullets were distributed into 12 floor pens furnished with 4 in. of fresh chopped corn cob litter over concrete and equipped with self feeders and watering devices. Ample supplies of commercial laying mash were available ad libitum. Lighting, controlled by automatic clocks, was maintained at optimum levels for 14hr/day. The pullets were then culled and randomized among the 12 pens so that average body weight per pen was as uniform as possible, with 30 birds per pen for the test groups and 25 or 26 per pen for the control groups. Test pens 1, 5 and 9 were assigned to 100 ppm level of malathion and pens 3, 7 and 11 to 1000 ppm level. Pens 2, 4, 6, 8, 10 and 12 continued to receive the basal ration.

On the last day of the 2nd, 4th, 6th and 8th wk following initiation of the test feeding, 6 birds per pen from the test groups and 5 per pen from the controls were selected at random and slaughtered at a local poultry dressing establishment. The whole eviscerated carcasses, to which the livers had been returned wrapped in parchment paper, were chilled in slush ice for 4 hr and then individually bagged in heat-shrunk film.

One-half of the carcasses from each treatment were transferred to the Analytical Laboratory where they were stored in deep freezers (−5°) until needed. The remaining birds were sent to the taste panel kitchen. Half of these were frozen for the 6-month storage test and half were prepared for immediate evaluation by the panel.

During the course of the 8-wk feeding period, the entire egg production from the flock was collected. After recording number and weight, the eggs were broken out and the white and yolk separated. Each fraction was then pooled by pen over each 2-wk period in large polyethylene wide-mouth bottles kept in a deep freezer. Subsequently, the egg magmas were thawed briefly, thoroughly mixed, and appropriate aliquots for analyses were frozen in dry ice.

Cooking procedures for taste panel

As described in the preceding section, the plan for flavor evaluation covered four controlled variables, i.e., freshly cooked vs. rewarmed meat from newly dressed birds and freshly cooked vs. rewarmed meat from carcasses which had been stored in a freezer for 6 months. Separate evaluations were also required on birds dressed after each biweekly feeding period and at both levels of malathion feeding. Thus, in all, 32 sittings of the taste panel were employed.

A uniform cooking procedure was adopted in preparing the taste samples for each sitting of the panel. The fresh or thawed carcasses were washed under the tap, patted dry with paper toweling, lightly salted (5g/bird), trussed, and placed on a trivit in a large, open roasting pan. The pans were covered with a double thickness of heavy-gauge aluminum foil molded to the pan rim. A meat thermometer was inserted through the foil into the breast muscle of one bird near the center of the pan. The pans were then placed in an electric oven whose thermostat was set to maintain a temperature of 350°F (calibrated by thermocouple). When the meat thermometer reached a reading of 175°F, the foil cover was removed and the cooking completed to an internal temperature of 185°F.

Immediately on removal from the oven, the two sides of the breast were removed and separated, as were the two thighs. After deboning the thighs, both the white and dark meat (skin removed) from one side of the bird was sliced across the grain with an electric carving knife to yield approximately 1-oz serving pieces. The meat from the other side of the bird was wrapped in new aluminum foil and placed in a refrigerator maintained at 37-43°F.

Taste panel procedure

Sliced meat portions were distributed to small serving dishes in accordance with a pre-selected pattern conforming to the requirements of the triangle test. The order of portions from test and control birds was randomized with respect to the six possible combinations for each judge and presented as the left, center, and right samples, two of which were always identical (i.e., from the same treatment).

The judges were required to taste the samples in the same order (left to right) at each sitting and to mark on a score card that which was judged to be the odd sample. "No difference" scores were not permitted. Since the odd sample might appear in any of the three positions and its position would change from sitting to sitting, the judges were required to guess even if no perceptible difference was detected.

The panel of 18 judges sat twice on each testing day in three groups of six. Each judge was assigned to an enclosed air conditioned cubicle without contact with fellow judges. In the morning session (from 11 a.m. to noon) meat from the controls was compared with that of the low test feeding level; in the afternoon session (3 to 4 p.m.), control meat was compared with that from the high test feeding level. This same pattern was repeated two days later when samples which had been rewarmed after 48 hr in the refrigerator were compared. The meat was reheated for 30 min in an oven at 350°F.

ANALYTICAL PROCEDURES

Proximate composition

The analyses conducted included total solids, fat, protein and ash. Carbohydrate was determined by difference. Total solids were analyzed using the methods of AOAC (1965). Fat, total nitrogen and ash were determined using methods from the same source. Total nitrogen x 6.25 was taken as the protein content.

Fatty acid distribution

Fatty acid distribution in the glycerides and phospholipids were determined using gas

[1]Food and Drug Research Laboratories Inc., Maspeth, N.Y. 11378

chromatography of the respective methylated lipid fractions separated by low temperature fractionation and thin layer chromatography. An approximately 1g sample of egg yolk was extracted with 25 ml of a 1:1 mixture of absolute ethanol and chloroform. The sample was filtered, divided into two portions and each portion was evaporated to dryness under a stream of nitrogen.

The residue from the first portion was taken up in 20 ml of acetone and the resulting solution placed in a freezer. This latter treatment was designed to precipitate the phospholipids. The solution was filtered keeping the entire operation in the cold. The residue on the filter was redissolved in chloroform, taken to dryness, taken up again in acetone, allowed to precipitate in the cold and refiltered. The combined filtrates were taken to dryness and redissolved in 1 ml of acetone. This fraction contained primarily the triglycerides and the cholesterol.

Thin layer chromatography was used to further purify each fraction. Aliquots of the acetone solution from above were streaked on thin layer plates of Silica Gel G (2mm) and developed using propanol:ammonia (3:1) until the solvent front reached half the distance up the plate. The plate was dried and further developed using chloroform:benzene (2:1) until the solvent front reached a point about 1/2 in. from the top of the plate.

A trial plate was run at first to assist in locating the position of the lipid fractions. The trial plate was sprayed with dichlorfluorescein in alcohol, put in a tank filled with iodine vapors, removed, sprayed with sulfuric acid:chromic acid (1:1) and placed in an oven. The spots in the lower portion of the plate were the phospholipids; cholesterol in the center; and near the top, glycerides. Normally there was very little evidence of phospholipids on these plates.

The portion of the actual test plates containing the glycerides was scraped off, dissolved in absolute alcohol, and filtered. A few ml of 60% aqueous KOH was added and the mixture refluxed for 30 min to saponify the fatty acids. The methyl esters of the fatty acids were prepared using AOCS (1964) methods and an aliquot was injected into a gas chromatograph following the procedure in the same manual. The fatty acid distributions of the glycerides were calculated using the peak areas on the resulting chromatograms.

The residue from the filtration of the cold acetone solution above was dissolved in chloroform, taken to dryness and redissolved in 1 ml of chloroform.

Aliquots of this solution were spotted on thin layer plates, subjected to the same development but, this time, the lower protion of the plate was scraped off. The scrapings were extracted as described above, saponifed, derivatized and injected into the gas chromatograph. The fatty acid distributions of the phospholipids were calculated using the peak areas of these chromatgrams.

Cholesterol

The second portion of the original alcohol-chloroform extract of the eggs was used for the cholesterol determination. A weighed portion of this total lipid residue was dissolved and the cholesterol content determined using an Autoanalyzer procedure which is a modification of the method of Sperry and Webb (1950) for total cholesterol. The cholesterol content of the egg yolk was calculated using the total lipid content of the eggs and the cholesterol content found in the total lipids.

Amino acids

The amino acid distribution in both whites and yolks was determined using the procedure of Moore et al. (1958). The results were reported on an N = 16% basis.

Carotene

Carotene was determined using the official methods of AOAC (1965).

RESULTS

Physical observations

Body weights, average weekly feed consumption, egg production, and efficiency of feed utilization for egg production were not affected by dosage level of malathion and there was no evidence of toxicity or adverse effects in the treated birds. At the 1000 ppm level of malathion in the feed, residues were found in raw livers after 8 wk, but only in amounts of 0.11 to 0.14 ppm which are well below the tolerance of 4 ppm (USDA, 1968). Malathion residues in cooked chicken livers were less than 0.1 ppm in control as well as those from hens treated over the entire 8-wk period. This was also true of the cooked chicken fat and raw and cooked chicken muscle, light and dark meat.

Flavor evaluations

Table 1 summarizes that data from the flavor evaluations for high and low levels of the pesticide in the feed for freshly cooked and rewarmed meat. Using a panel of 18 judges, it is obvious that one would expect about 6 correct identifications in each trial if there were actually no difference between samples. On the other hand, Chi-square statistics indicate that if as many as 10 out of 18 judgements are correct, there is a probability of 0.95 that a real difference between samples was detectable. It is obvious that there was no correlation with the length of time during which malathion was fed to the birds. There was a slight, but not significant indication that more correct identifications were achieved when the birds received

Table 1—Summary of total correct identifications by flavor panel out of 18 treated samples

Treatment	Period, weeks 2	4	6	8	Avg
	(Number correct identifications)				
Not stored					
100 ppm					
Fresh	7	8	9	3	6.8
Rewarm	9	3	7	7	6.5
1000 ppm					
Fresh	9	7	7	9	8.0
Rewarm	7	10	5	10	8.0
Stored 6 mo					
100 ppm					
Fresh	7	5	5	9	6.5
Rewarm	5	5	5	6	5.3
1000 ppm					
Fresh	10	5	9	7	7.8
Rewarm	8	4	7	6	6.3
Grand Average					6.9

Table 2—Proximate composition of egg whites and egg yolks

Constituent g/100g	Week	Egg white test group Control	Low level	High level	Egg yolk test group Control	Low level	High level
Total solids	2	10.5	11.2	10.8	53.4	56.2	54.7
	4	13.4	13.8	13.5	52.7	52.4	52.2
	6	12.5	13.6	13.4	53.2	53.5	52.6
	8	11.6	10.8	9.9	54.8	54.8	54.9
Fat	2	0.04	0.03	0.07	33.6	34.4	34.6
	4	0.15	0.14	0.13	33.5	32.8	33.4
	6	0.18	0.19	0.18	34.8	34.4	34.1
	8	0.04	0.04	0.05	34.7	35.8	35.3
Protein (N × 6.25)	2	9.1	9.3	10.6	16.9	16.4	16.4
	4	11.3	11.4	11.4	16.4	16.6	16.6
	6	11.8	11.4	12.6	16.6	16.6	16.4
	8	10.9	9.4	10.0	16.8	16.7	16.6
Ash	2	0.06	0.64	0.66	1.8	1.8	1.6
	4	0.78	0.94	0.91	1.8	1.8	1.7
	6	0.54	0.66	0.66	1.8	1.8	1.6
	8	0.70	0.64	0.74	1.6	1.7	1.7
Carbohydrate (by difference)	2	0.80	1.2	0.0	1.1	3.2	3.1
	4	1.20	1.2	1.1	0.9	1.2	0.4
	6	0.20	1.3	0.0	0.0	0.7	0.5
	8	0.0	0.7	0.0	1.7	0.6	1.3

Table 3—Fatty acid distribution in glycerides of egg yolks

Test group	Fatty acid	Week 2	4	6	8
		(Per cent)			
Control	Myristic	1.2	0.90	0.80	0.60
	Palmitic	26.8	22.2	24.3	23.4
	Palmitoleic	2.3	2.8	4.7	2.6
	Stearic	5.0	4.6	3.0	3.6
	Oleic	53.3	52.2	51.6	53.8
	Linoleic	11.8	14.3	16.5	15.9
Low level	Myristic	0.65	0.83	1.0	0.60
	Palmitic	26.5	20.4	24.5	23.0
	Palmitoleic	2.8	2.8	3.6	2.9
	Stearic	4.4	4.2	3.8	3.2
	Oleic	51.1	54.4	53.3	55.9
	Linoleic	14.7	17.2	13.4	14.5
High level	Myristic	0.36	0.60	0.36	0.40
	Palmitic	25.3	21.2	24.0	24.3
	Palmitoleic	2.7	3.0	3.8	2.8
	Stearic	4.6	4.2	3.7	2.9
	Oleic	52.2	54.3	55.3	56.2
	Linoleic	14.6	16.5	13.6	13.2

Table 4—Fatty acid distribution in phospholipids of egg yolks

Test group	Fatty acid	Week 2	4	6	8
		(Per cent)			
Control	Myristic	0.20	0.3	0.33	0.45
	Palmitic	34.4	37.4	32.6	32.2
	Palmitoleic	—[a]	—	1.6	1.8
	Stearic	19.0	16.6	17.3	18.0
	Oleic	28.8	29.4	32.6	31.2
	Linoleic	15.7	13.9	13.9	14.8
	Arachidonic	2.8	2.4	1.2	2.4
Low level	Myristic	0.16	0.15	0.1	0.1
	Palmitic	35.2	37.2	32.9	31.8
	Palmitoleic	0.6	—	1.8	1.2
	Stearic	20.2	16.7	17.7	18.8
	Oleic	26.0	29.7	31.0	31.6
	Linoleic	14.9	13.2	14.5	14.0
	Arachidonic	3.3	3.0	2.0	2.6
High level	Myristic	0.15	0.25	0.15	0.25
	Palmitic	32.2	37.1	30.2	33.6
	Palmitoleic	1.6	—	1.4	0.93
	Stearic	20.0	16.2	20.2	20.8
	Oleic	31.2	30.6	31.0	29.2
	Linoleic	13.4	13.4	14.8	13.6
	Arachidonic	2.6	2.6	2.2	1.7

[a]—Not detected.

1000 ppm as compared with only 100 ppm. The grand average of the correct scores was 6.90 which supports the null hypothesis, i.e., the feeding of malathion to laying hens had no effect on the flavor of the meat.

Nutrient composition

The proximate compositions of the egg whites and yolks (Table 2) exhibited by variance analysis no significant differences between the control and low and high feeding level groups. In addition, the values compared favorably with published average compositions of eggs. More important is the fact that no significant differences were noted between the control and test groups.

The fatty acid distribution in the glycerides of the yolks (Table 3) remained the same in all groups throughout the entire test. The same was true of the fatty acids in the phospholipids of the yolks (Table 4). Whereas the treatment had no effect on the relative cholesterol level of the yolks, there was a small increase in all groups after the fourth week of feeding (compare Table 5). It was concluded that there was no adverse effect upon the yolk lipids when laying hens are exposed to as much as 1000 ppm of malathion in their diet.

The essential amino acid distribution in the proteins of the egg white and of the egg yolk (Table 6) were unchanged in all groups throughout the entire test. On the basis of no significant change in any of the essential amino acids it must be concluded that there was no effect upon the protein quality of the eggs when malathion comprises up to 1000 ppm of the diet.

The results of the carotene analyses are given in Table 7. Initial analyses of the egg yolks showed no significant amounts of preformed vitamin A present. As a result no further vitamin A analyses were conducted. There were some differences in the carotene values after the 4th wk. These consisted of apparent increases in the carotene level of the eggs from the test groups, particu-

Table 5—Cholesterol content of egg yolks

Week	Test group: Control	Low level	High level
		(Per cent)	
2	1.3	1.3	1.4
4	1.9	1.4	1.1
6	1.5	1.6	1.8
8	1.4	1.6	1.6

Table 6—Amino acid distribution in proteins of egg whites and egg yolks

Amino acids (g/100g protein)	Egg white protein: Control	Malathion Low level	Malathion High level	Egg yolk protein: Control	Malathion Low level	Malathion High level
Aspartic	7.3	7.3	7.8	7.8	8.4	9.5
Threonine	4.1	4.6	4.4	5.0	5.5	5.8
Serine	6.4	7.0	7.0	7.3	7.4	7.6
Glutamic	12.9	13.2	13.4	10.7	11.0	11.4
Proline	3.7	3.4	3.7	4.3	4.6	4.7
Glycine	3.6	3.6	3.7	2.9	2.9	2.9
Alanine	6.8	6.7	6.0	4.9	5.0	5.3
Valine	7.2	7.2	7.4	5.2	5.4	5.1
Cystine	2.9	3.1	3.2	2.5	2.1	2.0
Methionine	4.2	4.2	4.1	2.3	2.3	2.2
Isoleucine	5.8	5.8	5.6	5.1	5.2	5.2
Leucine	8.7	8.8	8.6	8.4	8.8	9.0
Tyrosine	4.3	4.5	4.6	4.4	4.3	4.5
Phenylalanine	6.5	6.6	6.7	4.6	4.5	4.1
Lysine	7.6	7.6	7.7	7.4	7.4	7.7
Histidine	2.5	2.6	2.6	2.5	2.4	2.5
Arginine	6.3	6.3	6.4	7.3	7.3	7.4

Table 7—Carotene content of egg yolks[a]

Week	Test group		
	Control	Low level	High level
		mg/100g	
2	33.8	28.5	30.5
4	34.6	36.9	36.4
6	37.1	46.3	42.5
8	33.7	52.2	42.9

[a]Average of triplicate analyses.

larly the low level (100 ppm) group. In any event, the changes noted do not imply a deterioration of the nutritional value of the eggs from the test groups.

It was concluded that if the level of feed contamination likely to be encountered in normal poultry management, where malathion is used as a dust for parasite control reached 100 ppm, there would be no adverse effect on the eating quality of the meat or the nutrient composition of the eggs. It was further concluded that this lack of adverse effects was also true for a dosage level up to 1000 ppm.

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WILLIAM C. FRANKE and MYRON SOLBERG
Department of Food Science, Rutgers The State University, New Brunswick, N.J. 08903

QUANTITATIVE DETERMINATION OF METMYOGLOBIN AND TOTAL PIGMENT IN AN INTACT MEAT SAMPLE USING REFLECTANCE SPECTROPHOTOMETRY

SUMMARY—A technique for determining the relative quantities of oxymyoglobin, metmyoglobin and total pigment concentration at the surface of an intact meat sample was developed. A Beckman DK-2 spectrophotometer with reflectance attachment was used and spectra were recorded on the R_A scale. The sample port of the spectrophotometer was modified so that a uniform and high intensity light beam measuring 0.5 × 0.6 cm reached the surface being evaluated. A sample holder was constructed so that known proportions of oxygenated and oxidized meat could be exposed to the light beam. A family of curves representing varying known amounts of metmyoglobin and oxymyoglobin were obtained. The height of the peak at 632 nm (ΔR_{A632}) was directly related to the amount of metmyoglobin at the surface of the meat sample. For 100% oxymyoglobin, ΔR_{A632} was at a minimum and equal to R_{A750}. For 100% metmyoglobin, ΔR_{A632} was at a maximum and the height of the response depended upon the amount of total pigment present. A linear relation was obtained when ΔR_{A632} was plotted against percent metmyoglobin or against total pigment determined by the Hornsey (1956) method. The method requires making two readings of the meat samples at a single wave length. One reading of the sample followed by one reading of the same sample after oxidation with $K_3Fe(CN)_6$ provides a quantitative evaluation of the metmyoglobin concentration and the total heme pigment concentration. The accuracy of the method may be improved by making multiple readings.

INTRODUCTION

THE CONSUMER considers the bright red color of oxymyoglobin in fresh meat desirable, while the brown color of metmyoglobin is considered undesirable. The interactions between the environment and the myoglobin–metmyoglobin reaction in post-mortem animal tissue are not completely characterized. An accurate and precise technique for determining the relative quantities of oxymyoglobin, metmyoglobin, and total pigment concentration at the surface of an intact meat sample is required in order to evaluate the influence of the environment upon a meat system.

Mangel (1951), Butler et al. (1953) and Broumand et al. (1958) developed spectrophotometric methods for determining the states of myoglobin contained in extracts of meat surfaces. The extraction process is a source of considerable error in these methods.

Reflectance spectrophotometry eliminates the need for extraction and allows the pigment to be evaluated in its natural environment, the muscle. Qualitative evaluation of metmyoglobin in muscle using reflectance spectrophotometry was reported by Pirko and Ayres (1957) and Naughton et al. (1958). Dean and Ball (1960) developed a quantitative method for measuring meat pigments based upon the absorbancy ratio method of Broumand et al. (1958), but using reflectance measurement of meat surfaces. This technique used K/S ratios (Judd and Wyszecki 1963) to adjust for sample thickness and scatter. Snyder (1965) attempted unsuccessfully to adjust for nonlinear responses of % reflectance measurements by measuring reflectance on the absorbance scale (R_A). Adjustment of all spectra to a value of R_A equal to 1.0 at 525 nm effectively removed scatter due to nonmyoglobin related factors (Snyder 1965). Snyder (1965) noted that as stored beef color changed there were large responses at 632 nm.

Stewart et al. (1965) used reflectance spectrophotometry to determine total pigment and percent metmyoglobin. Total pigment was determined by converting the reflectance response from meat samples at 525 nm to K/S values which were linearly related to total pigment, as determined by the Hornsey method (Hornsey, 1956). Metmyoglobin was determined from the ratio K/S 572 nm/K/S 525 nm. Limiting values for the ratio were established for meat containing 0% and 100% metmyoglobin and a linear relationship was assumed for intermediate values.

Snyder and Armstrong (1967) reported the relative merits of R_A spectra versus K/S ratios when using a model system. They found a linear relationship between the K/S ratios at the isobestic myoglobin–oxymyoglobin wavelengths of 571 nm and 505 nm and the concentration of metmyoglobin and oxymyoglobin in nonfat dry milk using adjustment of all spectra to R_A = 1.0 at 525 nm. This model system tended to confirm the assumption of Stewart et al. (1965) regarding linearity between 0 and 100% metmyoglobin. If the myoglobin–nonfat dry milk model system is truly representative of a meat system, then the percent myoglobin derivative can be determined by using the K/S ratio at 571 nm.

The specific objectives of this study were: to develop an improved method for determining total pigment and the relative amounts of oxymyoglobin and metmyoglobin present at the surface of beef samples by reflectance spectrophotometry and to develop a means for evaluating the precision and accuracy of the method developed through the presentation of meat samples containing known proportions of metmyoglobin and oxymyoglobin to the reflectance spectrophotometer.

MATERIALS & METHODS

TOTAL REFLECTANCE measurements were made using a Beckman DK-2 spectrophotometer with reflectance attachment. The standard reference material was MgO. (Scanning time 2; scale expansion 2×; time constant 0.2; range 0.5–1.5; sensitivity 20.)

The sample ports on the reflectance attachments were modified so that quantitative work could be carried out. The size and position of the light beam were determined by placing a piece of paper over the sample port and tracing the light beam. The size of the light beam was 0.9 cm × 0.7 cm. The intense portion of the light beam was displaced from the center of the sample port. The brightness of the intense beam was not as great toward the edges as it was in the center. In order to get a beam of known size and uniform intensity, a hole (0.6 cm × 0.5 cm) was cut in a piece of black construction paper forming a shield, which was then positioned over a sample port so that the intense part of the light beam passed through it. The sample port was covered with M-mylar polyethylene to prevent meat drippings from entering the integrating sphere. The M-mylar polyethylene film exhibited no measurable absorption of light between 400 nm and 750 nm.

The meat samples were obtained from several sources. USDA Choice top rounds were obtained from a local packing plant while other cuts of beef and pork were purchased from local supermarkets. Samples used to represent 100% metmyoglobin at the surface were painted with 1% $K_3Fe(CN)_6$, covered with saran, and allowed to stand at least 8 hr at 32°F. Samples representing 100% oxymyoglobin were fresh cut and allowed to bloom in air at 32°F for 1–2 hr before mea-

surements were made. All meat samples were cut perpendicular to the fibers.

To determine total pigment concentration, beef and pork sample surfaces were first converted to 100% metmyoglobin. The R_A spectrum of the sample was recorded, after which the total pigment concentration was determined according to the method of Hornsey (1956). During preparation of samples for total pigment analysis (Hornsey, 1956) some extracts, especially pork, were cloudy even after filtration. These extracts were placed in an acetone-dry ice bath for about 2 min and refiltered, thus providing a clear solution.

A sample holder (Fig. 1), which allowed various proportions of oxymyoglobin and metmyoglobin to be exposed to the light beam, was constructed. The sample holder consisted of a piece of thin sheet metal 4 in. × 2 in. with a 2 in. × 1 in. hole cut in the center to allow passage of the light beam. Aluminum guide bars which were slotted to accommodate a razor blade, were mounted on either side of the hole. The sheet metal was fastened to a mechanical microscope stage, which was mounted on the spectrophotometer so that the sheet metal was flush with the sample port. The sample holder was movable vertically.

A razor blade was used to isolate 2-in. cubes of oxymyoglobin and metmyoglobin from one another with a minimum separation distance. The cubes were fastened to the razor blade with a rubber band.

A scale of 0.1 cm divisions was mounted on the microscope stage next to a fixed point corresponding to the bottom of the light beam. When the oxymyoglobin and metmyoglobin samples were placed against the razor blade between the aluminum guide bars, it was possible to expose various proportions of each to the light beam simultaneously. Each division on the scale was equal to 1/6 the area of the light beam.

A spectrum was run with 100% metmyoglobin and 100% oxymyoglobin in the light beam. Intermediate values were obtained by exposing 1/6 (16.7%) metmyoglobin, 5/6 (83.3%) oxymyoglobin; 2/6 (33.3%) metmyoglobin, 4/6 (66.7%) oxymyoglobin; 3/6 (50.0%) metmyoglobin, 3/6 (50.0%) oxymyoglobin; 4/6 (66.7%) metmyoglobin, 2/6 (33.3%) oxymyoglobin; 5/6 (83.3%) metmyoglobin, 1/6 (16.7%) oxymyoglobin to the light beam.

The spectra were obtained by recording reflectance on the absorbance scale (R_A values) without setting the instrument to 0 and 100% transmission. Each scan was started at 750 nm, and each sample was adjusted to the same starting point with the 100% adjusting control of the spectrophotometer.

In order to measure the amount of metmyoglobin present in a sample, ΔR_{A632} was used. ΔR_{A632} is defined as the height of the 632 nm peak measured from the 750 nm starting point to the top of the peak, as illustrated in Figure 3.

In related experiments oxymyoglobin and white fat were used to expose various proportions of oxymyoglobin to the light beam. The same was done with metmyoglobin and fat. Spectra were obtained as previously described.

To test the validity of the linear relationship between the ratio K/S 572/K/S 525 and metmyoglobin concentration in a sample as proposed by Stewart et al. (1965) and Snyder and Armstrong (1967), the spectrophotometer was calibrated to 0 and 100% transmission (range 0–100% T) using magnesium oxide without the shield. The shield was positioned over the light beam of the sample port, the previously described sample holder was put into place, and spectra were obtained using various known proportions of oxymyoglobin and metmyoglobin from eight different samples of a single top round. The instrument range was set at 0.5–1.5 on the absorbance scale. From the data obtained the following were plotted against the proportion of metmyoglobin exposed: R_{A572}, R_{A632}, R_{A572}/R_{A525}, R_{A632}/R_{A525}, K/S 572, K/S 632, K/S 572/K/S 525, K/S 632/K/S 525.

Four different samples from one top round were used to compare the responses from the shielded and unshielded light beam. The instrument was calibrated to 0 and 100% T with magnesium oxide and samples freshly bloomed to give 100% oxymyoglobin were used to obtain shielded and unshielded spectra. The samples were then treated with $K_3Fe(CN)_6$ to obtain intermediate values between 0 and 100% metmyoglobin. Spectra both shielded and unshielded, were obtained at the same intervals as the oxidation progressed to 100% metmyoglobin.

The following values were calculated from the shielded and unshielded spectra and were plotted against % metmyoglobin determined by ΔR_{A632}, R_{A572}, R_{A632}, R_{A572}/R_{A525}, R_{A632}/R_{A525}, K/S 572, K/S 632, K/S 572/K/S 525 and K/S 632/K/S 525.

RESULTS & DISCUSSION

WHEN ΔR_A VALUES for samples of beef and pork, which had been converted to 100% metmyoglobin at the surface, were correlated with total pigment as determined by the Hornsey method (Hornsey, 1956), a linear relationship resulted. The various levels of total pigment concentration were obtained by using pork loin (low level), beef rib eye (intermediate level), and beef round (high level).

Figure 2 shows the regression line and 95% confidence intervals obtained when the reflectance and total pigment data were statistically analyzed. Each point represents a different piece of meat subjected to chemical and spectrophotometric analysis. The regression line had a coefficient of correlation of 0.99. The regression line passes through the origin by definition, since in the absence of

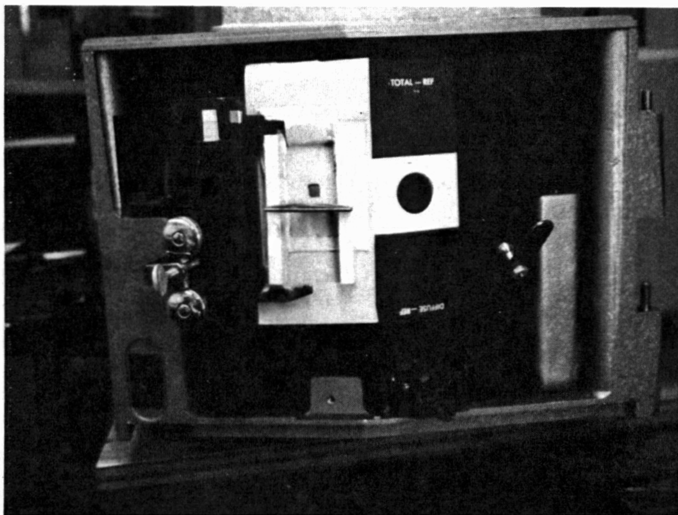

Fig. 1—Modified sample port of spectroreflectometer with sample holder.

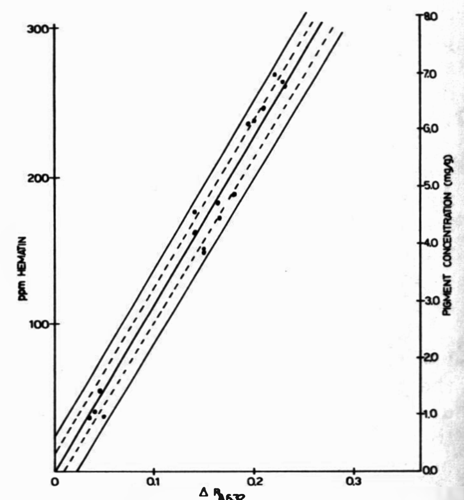

Fig. 2.—Relationship between pigment concentration measured by extraction and by reflectance spectrophotometry plotted as ΔR_{A632}. Equation for the line is mg pigment/g meat = 29.86 (ΔR_{A632}). (—— regression line; —— 95% confidence interval for one reading of each sample; ------ 95% confidence interval for five readings of each sample).

reduced myoglobin ΔR_{A632} measures only pigment in the metmyoglobin form. If no metmyoglobin is present, ΔR_{A632} equals zero and therefore total pigment measured as metmyoglobin must equal zero. The equation for the regression line is y = 1148.35x, where y = ppm of hematin and x = ΔR_{A632}.

In order to predict total pigment concentration from ΔR_A values, a set of 95% confidence intervals for the mean of 1-5 observations were constructed about the regression line. (See Fig. 2). It is evident that as more observations are made, the error decreases.

To obtain pigment concentration in mg pigment/g meat, ppm hematin is multiplied by 0.026. This factor is calculated by dividing the molecular weight of myoglobin ($\approx$17,000) by the molecular weight of hematin (656) $\times 10^3$.

Some of the variance in the correlation of total pigment with ΔR_A values may be attributed to differing amounts of fat and connective tissue at the surface and in the underlying tissue. ΔR_A values evaluate only the surface composition, while total pigment by the Hornsey method combines surface and underlying tissue.

It is important that a sample which is homogeneous be chosen if the pigment concentration of the lean meat is to be determined. The reduced exposure size of the sample port contributes to sample uniformity.

The height of the 632 nm peak is a function of the light energy reaching the sample. Variations in the size of the exposure port, light source age and other factors which affect the amount of total light energy reaching the sample port will alter the response. It is therefore important to calibrate the modified port spectrophotometer and correlate the results with pigment extraction data (Hornsey 1956) to establish a standard curve. Since linearity has been established only one point is needed.

Figure 3 shows the spectra obtained when various proportions of oxidized meat (metmyoglobin) and fat were exposed to the modified light beam of the spectrophotometer. The ΔR_A value at 632 nm increased as the proportion of metmyoglobin exposed to the light beam increased. At 425 nm severe nonlinearity was evident between 0% and 16.7% metmyoglobin. Similar nonlinearities exist at other wavelengths.

Figure 4 represents the spectra obtained by exposing various proportions of oxygenated meat (oxymyoglobin) and fat to the modified light beam. The R_A value at 632 nm remained constant, regardless of the amount of oxymyoglobin exposed to the light beam. Therefore, in a fresh meat system where all of the pigment at the surface is either in the oxygenated or oxidized state, and no reduced myoglobin is present due to microbial deterioration or oxygen restriction, any change in response at 632 nm must be due to the presence of metmyoglobin. Under the conditions of the measuring technique, no other wavelength offered this response.

Figure 5 illustrates a typical family of curves obtained when various proportions of oxygenated and oxidized meat, prepared from a single beef round were exposed to the modified light beam. These curves represent the sum of the previously presented Figures 3 and 4 and further illustrate the absence of true linearity at other wavelengths across the entire visible spectrum.

Figure 6 presents the % ΔR_A values obtained when various proportions of oxygenated and oxidized meat were exposed to the modified light beam. Each point on the curve represents a response obtained from the exposure of the indicated proportion of freshly bloomed and fully oxidized meat taken from the same beef top round. Data from 15 separate experiments are represented. A single point in some cases represents two or more identical results. The data in Figure 6 are from eight different top rounds. Numerous additional data have been collected confirming this slope which is approximately equal to 1.0. It was not possible to obtain duplicate results on a single sample because the oxymyoglobin began to oxidize during exposure to the light beam of the spectrophotometer at room temperature. All data were converted to percentage values (% ΔR_A) so that they could be pooled. This was necessary to nullify the effect of varying total pigment concentration from sample to sample. This was done by subtracting

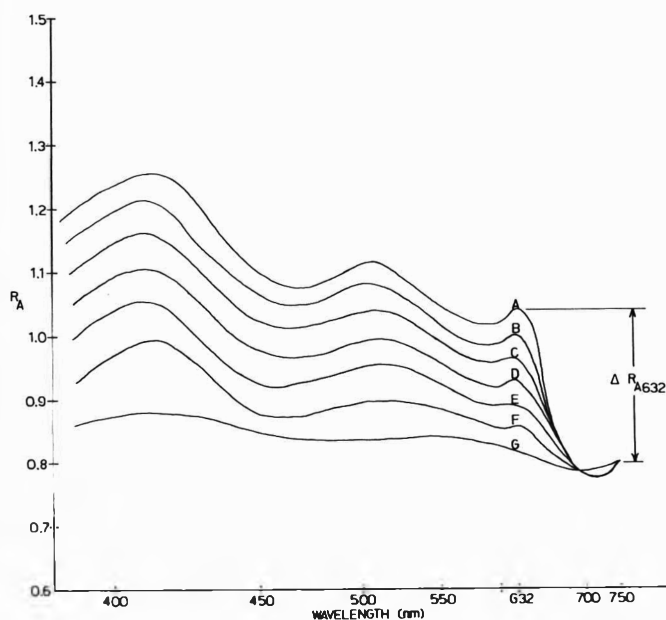

Fig. 3—Spectra obtained when various proportions of oxidized meat (metmyoglobin–Mb⁺) and beef fat were exposed to the spectroreflectometer. (Curve A = 100% Mb⁺; B = 83.3% Mb⁺, 16.7% fat; C = 66.7% Mb⁺, 33.3% fat; D = 50.0% Mb⁺, 50.0% fat; E = 33.3% Mb⁺, 66.7% fat; F = 16.7% Mb⁺, 83.3% fat; G = 100% fat.)

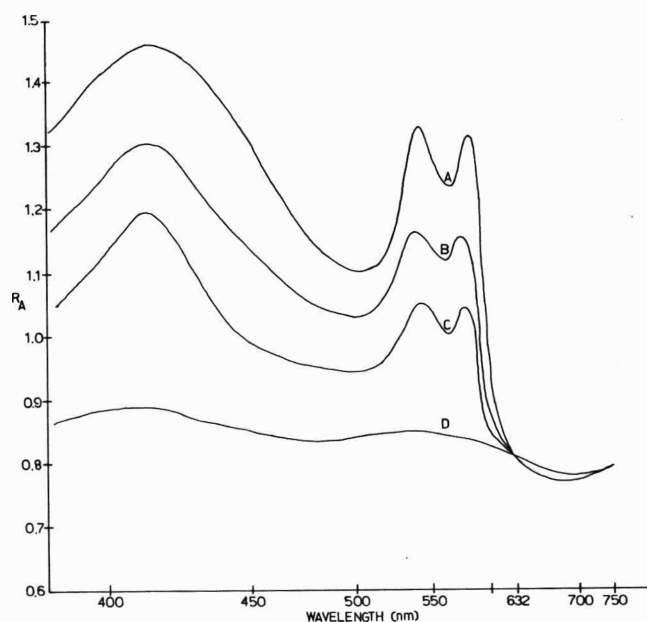

Fig. 4—Spectra obtained when various proportions of oxygenated meat (oxymyoglobin–MbO_2) and beef fat were exposed to the spectroreflectometer. (Curve A = 100% MbO_2; B = 60% MbO_2, 40% fat; C = 30% MbO_2, 70% fat; D = 100% fat.)

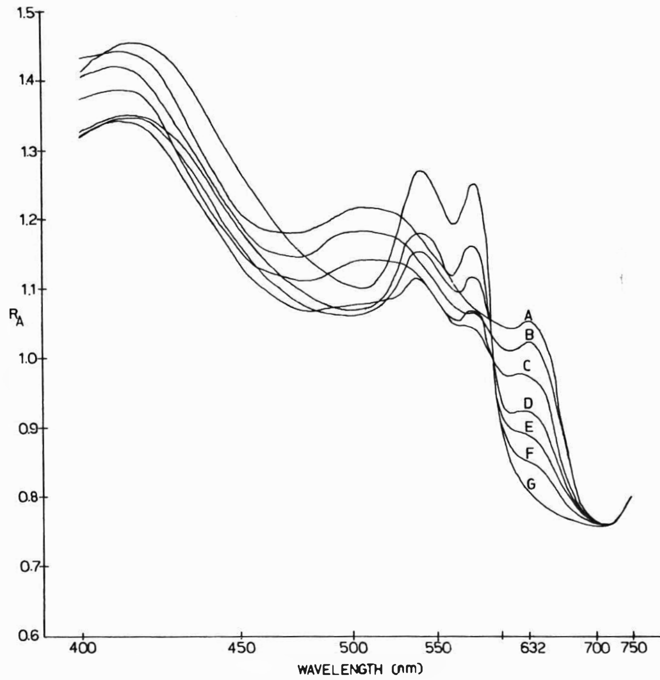

Fig. 5—Spectra obtained when various proportions of oxygenated meat (oxymyoglobin–MbO₂) and oxidized meat (metmyoglobin–Mb⁺) were exposed to the spectroreflectometer. (Curve A = 100% Mb⁺; B = 83.3% Mb⁺, 16.7% MbO₂; C = 66.7% Mb⁺, 33.3% MbO₂; D = 50.0% Mb⁺, 50.0% MbO₂; E = 33.3% Mb⁺, 66.6% MbO₂; F = 16.7% Mb⁺, 83.3% MbO₂; G = 100% MbO₂.)

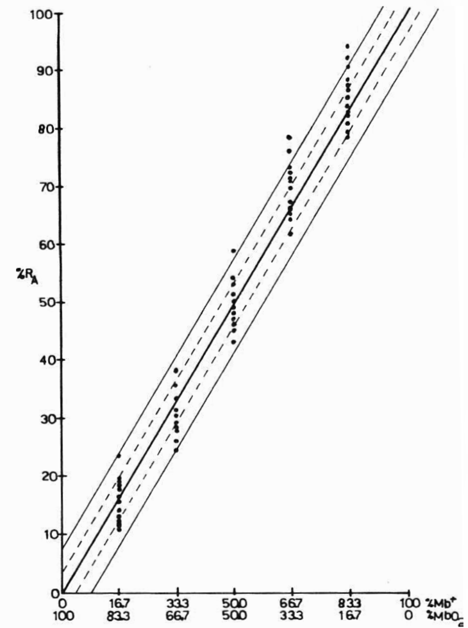

Fig. 6.—Relationship between % ΔR_A values at 632 nm (defined in equation 2) and the percent oxygenated meat (oxymyoglobin—MbO₂) and the percent oxidized meat (metmyoglobin—Mb⁺) presented to the spectroreflectometer (——— regression line; ——— 95% confidence interval for one reading of each sample; ------ 95% confidence interval for five readings of each sample).

the reading from the R_A scale for 100% oxymyoglobin which is equal to the arbitrarily selected R_A value at 750 nm, from all R_A readings to get ΔR_{A632} values and then dividing these values by the ΔR_{A632} value for 100% metmyoglobin.

$$\% \Delta R_A = \frac{R_{A632(Mb^+ + MbO_2)} - R_{A750}}{R_{A632(100\% Mb^+)} - R_{A750}} \times 100 = \frac{\Delta R_{A632(Mb^+ + MbO_2)}}{\Delta R_{A632(Mb^+)}} \times 100$$

The regression line and 95% confidence intervals obtained when the % ΔR_A values were statistically analyzed are presented in Figure 6. The equation for the line is y = 0.9821x where y = % ΔR_{A632} and x = proportion of metmyoglobin exposed to the light beam. Regression analysis showed that the regression line had a coefficient of correlation of 0.99.

In order to predict the percentage of metmyoglobin present at the surface of an intact sample, a set of 95% confidence intervals for the mean of one and five observations were constructed about the regression line. In order to use this figure it is necessary to convert R_A or ΔR_A values to % ΔR_A values as previously described.

The validity of using K/S 572/K/S 525, R_{A572}/R_{A525} or ΔR_{A632} as a measure of the percent oxymyoglobin and metmyoglobin in a meat sample can only be tested by exposing a meat sample containing known proportions of each pigment to the spectroreflectometer. The modified sample port previously described is a necessity in this type of experiment because the light beam is extremely difficult to center and the geometry of a light beam composed of a rectangular intense portion and a circular diffuse portion make it impossible to expose known pigment or sample portions accurately.

Table 1 summarizes the responses obtained when various parameters were plotted against known proportions of oxygenated and oxidized meat exposed to the shielded light beam.

The coefficients of correlation and the qualitative evaluations of linearity show the use of K/S ratios did not improve the results and that ΔR_{A632} deviated least from linearity when the shielded light beam was used.

It appears that the use of ΔR_{A632} system precludes the need for correction due to scatter. The factors involved in the scattering coefficient include the wavelength of the incident light, the particle size of the material being exposed, and the total light reaching the sample.

The ΔR_{A632} system reduces scatter to an apparently insignificant level. The long wavelength (632 nm) with its relatively low energy minimizes scatter. Since the black shield absorbs the lower intensity light, the total light reaching the sample is reduced and the total scattering is also reduced. The particle size is kept relatively constant due to the presentation of all samples with the

Table 1—The degree of linearity of selected spectral parameters obtained from the exposure of known proportions of oxygenated and oxidized meat to a shielded light beam in a Beckman DK-2 spectroreflectometer

Parameter	Degree of linearity	
	Qualitative	Correlation coefficient
K/S 572	poor to fair[a]	-----
R_{A572}	poor to fair	-----
K/S 632	none[b]	-----
R_{A632}	none	-----
K/S 572/K/S 525	very good[c]	0.94
R_{A572}/R_{A525}	very good	0.95
K/S 632/K/S 525	none	-----
R_{A632}/R_{A525}	none	-----
ΔR_{A632}	very good	0.96

[a] A distinct change in slope at the low or high end of the curve.
[b] A reversal of slope direction.
[c] Slight deviations around the line connecting 0 to 100% metmyoglobin.

muscle fibers parallel to the light beam thus equalizing the scatter due to this component. Although the scatter will vary with change in absorbance, the variation is minimal in this system of high coefficient of absorbance (K) and low coefficient of scatter (S).

The meat system is therefore quite different from the low K and high S system using nonfat milk as a base (Snyder 1965). The nonfat milk system would probably make the need for scatter coefficient correction essential.

When freshly oxygenated meat was treated with $K_3Fe(CN)_6$ and spectra with the shielded light beam were recorded at intervals as the oxidation progressed from 0 to 100% metmyoglobin, the results for the parameters listed in Table 1 were similar to those reported for the known pigment proportion studies. The % ΔR_{A632} was used as a measure of the metmyoglobin concentration in these comparisons. The unshielded light beam also produced reasonably linear responses but with slightly increased deviations from the line. K/S 572/K/S 525 data appeared slightly more linear than the R_{A572}/R_{A525} data in this case. This may be the result of the slightly increased scattering coefficient introduced by the increased total light reaching the sample.

The wide range of K/S 572/K/S 525 values reported in the literature for 100% oxymyoglobin and 100% metmyoglobin make it necessary to know both of these values for each piece of meat if an accurate determination of an intermediate metmyoglobin content is to be made. When ΔR_{A632} is used a partially oxidized meat sample can be accurately evaluated for its metmyoglobin content since the only data needed to establish a standard curve for that piece of meat is the 100% metmyoglobin value.

For the proposed method to be valid, it must be assumed that a piece of fresh meat exposed to oxygen will have all of its pigment in the oxymyoglobin form. The dissociation equilibrium constant for oxymyoglobin (2.1×10^{-6}) (Gibson and Roughton, 1955) shows that the equilibrium oxymyoglobin $\rightleftharpoons$ myoglobin + oxygen lies far to the left and therefore it may be assumed that the pigment is either in the oxymyoglobin or metmyoglobin form, as long as no external reducing substances are present. The samples were covered with M-mylar polyethylene during the spectrophotometric analysis. Since the analysis was accomplished almost immediately after the contact between the film and the meat was made, little or no reduced myoglobin should be formed.

The concentration of oxymyoglobin and metmyoglobin may be measured at 632 nm because the ΔR_A value for oxymyoglobin at 632 nm is at a minimum and the ΔR_A value for metmyoglobin is at a maximum.

The method described overcomes some of the difficulties presented in the studies by previous workers (Dean and Ball, 1960; Snyder, 1965; Stewart et al., 1965) as long as the same sample area is presented to the light beam of the spectrophotometer before and after oxidation and that the evaluation takes place prior to the reduction of pigment by microbial action.

CONCLUSIONS

THE METHOD described can be used to determine the relative concentration of metmyoglobin and the total pigment concentration at the surface of meat samples. Only one point (100% metmyoglobin) is needed to establish a standard curve for % metmyoglobin when ΔR_{A632} is used. The results obtained with the model system used are equivalent to those which would be obtained with a sample having metmyoglobin and oxymyoglobin homogeneously dispersed, since the light reflected from the model system was integrated in the integrating sphere of the spectrophotometer.

This method eliminates the need to make readings at two different wavelengths and then to compute and compare the K/S ratios or to adjust all data to a uniform point at 525 nm. In addition, it is not necessary to standardize the instrument before each run, because the values obtained are all relative to 100% metmyoglobin and are not absolute values as they must be when K/S ratios are used. ΔR_{A632} values eliminate the need for K/S ratios which were essential when points intermediate to 0 and 100% metmyoglobin were based upon high scatter coefficient model systems instead of meat.

A linear relationship exists at 632 nm between the ΔR_A values and the concentration of metmyoglobin and oxymyoglobin present at the sample surface. A linear relationship also exists at 632 nm between the total pigment concentration and the ΔR_A value for 100% metmyoglobin.

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E. MAESSO WEDRAL, D. V. VADEHRA and R. C. BAKER
Cornell University, Ithaca, New York 14850

MECHANISM OF BACTERIAL PENETRATION THROUGH THE EGGS OF Gallus gallus. 2. Effect of Penetration and Growth on Permeability of Inner Shell Membrane

SUMMARY—The mechanism of penetration of bacteria into the eggs of domestic fowl (Gallus gallus) was investigated by determining the changes in permeability of the inner shell membranes (i.s.m.) to radioactive amino acids and viable Salmonella typhimurium LT2 cells after bacteria had penetrated the i.s.m. It was found that penetration and growth of bacteria in eggs did not cause any significant changes in the permeability of the i.s.m. for up to 8 days of incubation after exposure to spoilage. Bacteria, however, were found to penetrate the exterior structures within 36 hr after exposure. These results indicate that the actual penetration of bacteria through the i.s.m. does not permanently alter its permeability.

INTRODUCTION

THE MECHANISM of penetration of bacteria into the eggs of domestic fowl *(Gallus gallus)* has been of interest to food scientists, poultry biologists and microbiologists for several years. Numerous research workers (Elliott, 1954; Kraft et al., 1958; Lorenz et al., 1952) have demonstrated that the egg shell membranes are more important barriers to bacterial infection of eggs than the shell. The inner shell membrane (i.s.m.) in particular has been shown to offer the greatest resistance to bacteria (Garibaldi and Stokes, 1958; Lifshitz et al., 1964; Trussel and Florian, 1957). It has been suggested by several workers (Brown et al., 1965; Lifshitz et al., 1964; Lorenz et al., 1952) that bacterial penetration into the egg is mediated by a digestive enzymatic process. Part 1 of this study investigated the effect of enzymes on the permeability of the inner shell membranes (Wedral et al., 1970). It was observed that most of the enzymes did not produce any change in the permeability of the membranes as determined by radioactive lysine and penetration of *Salmonella typhimurium.* The present study was undertaken to determine if bacterial penetration and eventual spoilage of eggs actually causes a permanent change in the i.s.m. in regard to its permeability or ability to act as a barrier to bacteria. The permeability changes in the i.s.m. as affected by bacterial infection and spoilage were investigated by studying diffusion of uniformly labelled C^{14} lysine and penetration of viable *Salmonella typhimurium* LT2 using model systems.

MATERIALS & METHODS

Eggs

2-day old eggs from a single strain of White Leghorns from the Cornell University poultry farm were used. The eggs were washed in a commercial immersion type washer with a sanitizer detergent for 5 min and rinsed with water at 40°C to remove any excess detergent.

Test organisms

Salmonella typhimurium LT2 and *Pseudomonas aeruginosa* were used as the test organisms for penetration and spoilage studies. The *P. aeruginosa* culture was isolated in this laboratory from spoiled eggs, and its identity was confirmed using cultural and biochemical characteristics including growth at 42°C. The organisms were grown in nutrient broth (5g peptone, 3g beef extract/1 water) for 24 hr. *S. typhimurium* was grown at 37°C and *P. aeruginosa* at 32°C. After 24 hr of incubation, the postulations were approximately 2–5 × 10^7/ml.

Exposure of eggs to bacteria

The broth culture was diluted 1:100 with sterile distilled water (15°C) and the washed eggs at approximately 40°C were dipped in contaminated water for 5 min. The eggs were removed and placed on sterile flats to dry. The control eggs were dipped similarly in sterile distilled water. The artificially contaminated eggs and control eggs were incubated at room temperature (25°C) for a max of 15 days.

Microbial examination

The penetrability of *S. typhimurium* and *P. aeruginosa* into the interior of the egg was determined by aseptically breaking the eggs, 1, 2, 3, 6, 12, 18, 24 and 36 hr after exposure to infection according to the procedure of Williams et al. (1968). The egg contents were transferred into sterile nutrient broth and incubated at 37°C for 24 hr. The broth was then tested for *Pseudomonas* and *Salmonella. Pseudomonas* was tested by its ability to produce characteristic pigments (Breed et al., 1957). Viable cell counts were determined using nutrient agar. The plates were incubated at 32°C for 48 hr. *Salmonella* was tested and enumerated by AOAC (1967) procedures.

Isolation of inner shell membrane

In order to study the changes in permeability of the inner shell membrane following infection and incubation, model systems were used. The inner shell membrane from the infected eggs was very carefully removed by a technique similar to that reported by Lifshitz et al. (1964). The isolated membranes were tested for breaks and handled very carefully to avoid any stress on the structure.

Determination of permeability of the i.s.m. to radioactive amino acids

The inner shell membranes from artificially infected eggs after 2, 4, 6, 8, 10 and 15 days of incubation at 25°C were removed and tested for the rate of permeability of radioactive amino acids. The experimental apparatus used for permeability studies is shown in Figure 1. The socket portion of the apparatus contained 72 ml of phosphate buffer pH 7.0 (40 μg KH_2PO_4/ml) and uniformly labelled C^{14} lysine (4 μc/l). To prevent bacterial growth the buffer also contained 330 mg/l of each of the following: penicillin G. streptomycin sulfate, chlorotetracycline and aureomycin. (Preliminary experimentation showed that these antibiotics did not affect i.s.m. permeability). The opening of the ball portion

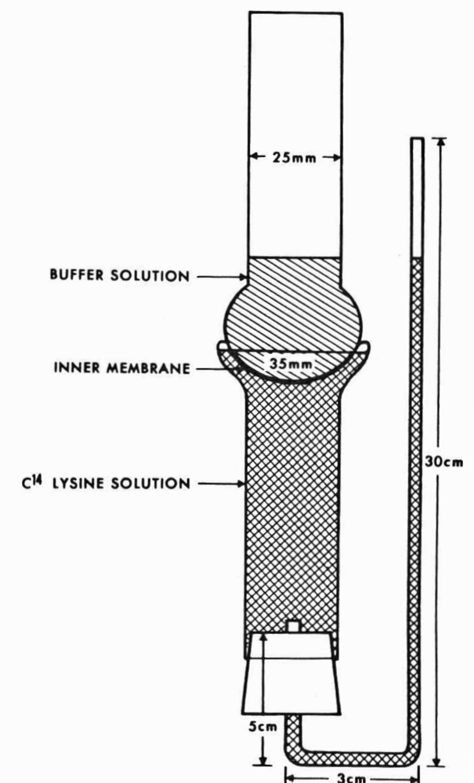

Fig. 1—Experimental apparatus for the determination of permeability of the inner shell membrane.

of the joint was covered with the test membranes and these were placed on the socket making sure that no air bubbles or breaks in the membranes occurred. The joints were clamped in place with a #35 spring clamp. 10 ml of a sterile solution of 40 μg/ml KH_2PO_4 at pH 7.0, the diluent, was placed above the membrane. Sampling was accomplished by drawing 0.5 ml of the solution from the top of the i.s.m. at 100 min intervals up to a max of 700 min. The samples were placed in bottles containing 10 ml of Bray's Scintillation Fluid and counted in a Nuclear Chicago well counter model 6804 (D55 probe with lead shield) connected to a Nuclear Chicago 161 A Binary Scaler. This was compared with a 0.5 ml portion of the original uniformly labelled C^{14} lysine solution. Corrections were also made for normal environmental radiation. After sampling, 0.5 ml of buffer was used to replace the withdrawn sample. The count was adjusted for radiation loss due to the constant dilution of radioactivity.

At the end of each permeability study, plate counts were made of the solutions above and below the membrane. If contamination was found, results were disregarded.

The permeability studies were also done using uniformly labelled C^{14} glycine and C^{14} aspartic acid. The results of these experiments were similar to those observed with C^{14} lysine.

Determination of penetrability by bacteria

An apparatus similar to that in Figure 1 was used. Sterilized pyrex ball and socket joints (12 mm I.D. and 28 mm O.D.) were used. Self annealing thin rubber stoppers were placed in the bottom of the socket portion and then the socket was filled with sterile 0.9% NaCl. The i.s.m. isolated from eggs which were artificially infected and incubated for 0, 4, 8 and 15 days at 25°C were placed over the ball portion and the joints were then clamped into place with #28 clamps. 10 ml of a suspension of *S. typhimurium* LT2 (2×10^7/ml) in 0.9% NaCl was then placed above the i.s.m. The solution did not contain any antibiotics. The system was closed by covering with parafilm. The penetration of test organisms was tested by drawing 0.2 ml samples aseptically using a sterile needle and syringe at 0, 3, 6, 9, 12, 18, 24 and 36 hr. The same quantity of sample was replaced by sterile 0.9% NaCl. The 0.2 ml sample was placed into 10 ml of nutrient broth and incubated 24 hr at 37°C. The nutrient broth samples were tested for *Salmonella* by the standard AOAC methods (1967).

Statistical analysis

The data on the permeability of amino acids and bacteria through the i.s.m. was analyzed at each sampling period and compared to the controls. The analysis of variance procedure of Steel and Torrie (1962) was used to statistically analyze the data and the significance of differences between treatments and controls were tested.

RESULTS & DISCUSSION

THE RATE of penetration of *S. typhimurium* and *P. aeruginosa* into the egg contents was studied and the results are summarized in Table 1. It was observed that the penetration patterns for the two organisms were very similar. At 36 hr after exposure to infection, almost 100% eggs showed the presence of viable penetrating bacteria. The organisms could be detected as early as 2 hr after exposure to infection indicating that penetration of the organism into the egg is a fairly rapid process. However, the eventual growth and spoilage take a longer period of incubation. These results are in agreement with previous work done on hatching eggs by Williams et al. (1968).

Table 1—Rate of penetration of Salmonella typhimurium and Pseudomonas aeruginosa into egg interior after exposure to bacterial challenge[a]

Time elapsed after exposure (hr)	Test organism: *S. typhimurium*	*P. aeruginosa*
	(Number positive out of 48)	
0	0	0
1	0	1
2	3	4
3	9	11
6	19	19
12	22	22
18	25	29
24	39	42
36	47	48

[a]Bacterial challenge was 3×10^5/ml.

Results of studies on the effect of penetration and eventual spoilage on the permeability of the inner shell membrane are shown in Figure 2 and Table 2. Data in Figure 2 were obtained when the spoilage organism was *S. typhimurium;* data in Table 2 are representative of *P. aeruginosa.* It was observed that incubation of eggs up to 8 days after infection did not produce any significant change in the permeability of C^{14} lysine through isolated i.s.m. as compared to the controls. The number of organisms in the albumen after 8 days of incubation at 25°C was approximately 3×10^5/ml; however, longer periods of incubation showed a significant change in the permeability of i.s.m. and the permeability data after 15 days of incubation was statistically significant at the 0.01 probability level. The number of bacteria had increased to 5×10^6/ml at 10 days and 8×10^8/ml at 15 days. The organisms were retested (AOAC, 1967) and found to be *Salmonella* positive. These results indicate that penetration of bacteria (Table 1) through the egg exteriors does not cause any significant changes in the permeability of the inner shell membrane (Fig. 2) as the penetration is almost complete by 36 hr and permeability changes do not take place until 10 days of incubation. However, the increase in permeability at 10 and 15 days of incubation may be due to growth and metabolism of bacteria on the inner shell membrane. It has been shown by Stokes and Osborne (1956) and Lifshitz et al., (1965) that the bacteria can utilize the shell membrane material for growth. The data shown in Figure 2 and Table 2 were obtained with C^{14} lysine; however,

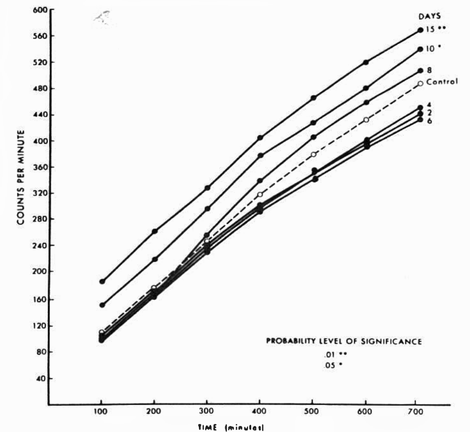

Fig. 2—Changes in the permeability of the inner shell membrane from eggs following bacterial exposure and incubation at 25°C.

Table 2—Rate of penetration of C^{14} lysine through the isolated inner shell membrane (i.s.m.) from eggs exposed to Pseudomonas aeruginosa and incubated at 25°C for a max of 15 days

Test organisms	Incubation time post infection (days)	100	200	300	400	500	600	700
		Sampling time (min) (counts per plate)						
P. aeruginosa	2	128	179	225	290	352	396	444
	4	134	182	230	300	355	400	450
	6	139	181	235	288	342	390	438
	8	130	185	240	335	399	461	503
	10*	175	218	300	370	425	477	532
	15**	190	260	330	400	465	520	566
None[a]		150	188	245	318	381	437	492

[a]Average control counts for all incubation time periods.
*$P < 0.05$.
**$P < 0.01$.

Table 3—Effect of length of incubation after exposure to infection on the penetration of Salmonella through the isolated inner shell membrane in a model system

Test organism used for infection of eggs	Incubation time post infection (days)	Salmonella penetration through i.s.m. isolated from exposed and control eggs Sampling time (hr)							
		0	3	6	9	12	18	24	36
		% positive for *Salmonella*[a]							
Salmonella typhimurium	0	0.00	0.00	6.25	25.00	43.75	75.00	100.00	100.00
	4	0.00	6.25	6.25	18.75	50.00	62.50	100.00	100.00
	8	0.00	25.00	37.50	56.25	75.00	100.00	100.00	100.00
	15	0.00	50.00	68.75	81.25	100.00	100.00	100.00	100.00
Pseudomonas aeruginosa	0	0.00	0.00	6.25	18.75	43.75	62.50	100.00	100.00
	4	0.00	0.00	6.25	18.75	50.00	62.50	93.75	100.00
	8	0.00	25.00	37.50	56.25	75.00	100.00	100.00	100.00
	15	0.00	43.75	56.25	81.25	100.00	100.00	100.00	100.00
None (control)	0	0.00	0.00	31.25	43.75	50.00	75.00	100.00	100.00
	4	0.00	0.00	31.25	43.75	62.50	75.00	93.75	100.00
	8	0.00	0.00	37.50	50.00	75.00	81.25	93.75	100.00
	15	0.00	25.00	43.75	56.25	75.00	87.50	100.00	100.00

[a]16 i.s.m. were used for each treatment tested; bacterial challenge (2×10^7/ml).

C^{14} glycine and C^{14} aspartic acid were also used to confirm that the penetration patterns were not unique for C^{14} lysine. Results in these cases were very similar.

The penetration studies were extended to include bacteria which represent a more natural system and the results are shown in Table 3. The inner shell membranes from eggs after 0, 4, 8 and 15 days of infection were removed and used to study the penetration of *S. typhimurium*. The infected membranes did not facilitate the penetration of *S. typhimurium* for up to 8 days after inoculation; however, when the membranes were removed from eggs which were inoculated and incubated for 8 to 15 days, it appeared that *S. typhimurium* could penetrate these membranes more rapidly than control membranes or membranes from eggs infected and incubated for 8 days. These data were statistically significant at the 0.05 probability level. This would strengthen the hypothesis that penetration of bacteria through the membranes (which occurs within 36 hr) does not enhance the permeability of i.s.m.; however, their growth and metabolism does bring about changes in the membranes such that the permeability is increased.

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W. A. MOATS, ROGER DABBAH and V. M. EDWARDS
USDA, Market Quality Research Division, ARS, Beltsville, Md. 20705

INTERPRETATION OF NONLOGARITHMIC SURVIVOR CURVES OF HEATED BACTERIA

SUMMARY—*Complex survivor curves of heated bacteria are interpreted to be composites of several convex survivor curves that represent populations of different heat resistances in a single culture of bacteria. The variation in heat resistance appears to be physiological rather than genetic since subcultures of heat-resistant cells were no more heat resistant than the parent culture. Composite curves can appear to be nearly exponential. Results support a multiple-site hypothesis of thermal death. Tailing of survivor curves, with small numbers of cells surviving extended heating, was frequently noted when curves were carried through 6–9 log cycles. Such tailing might be of practical importance because it would predict that small numbers of cells might survive much longer heating than would be predicted from D-values calculated from curves carried through 4–5 log cycles.*

INTRODUCTION

IT IS WELL documented that a plot of log survivors vs. time for bacteria heated at constant temperature may give a straight line (logarithmic death rate) or various types of concave or convex curves (Hansen and Riemann, 1963; Schmidt, 1957; Vas and Proszt, 1957). Various explanations have been advanced for the shape of experimental survivor curves. The logarithmic death rates have been attributed to inactivation of a single molecule or gene per cell (Charm, 1958; Chick, 1930; Rahn, 1929; Rahn, 1945; Stumbo, 1965) in a homogeneous population. Clark (1933) objected strongly to the monomolecular theory stating that "its application leads to conclusions that are so absurd that they are difficult to discuss." Chick (1930), Rahn (1929), Charm (1958), and others arguing in support of the monomolecular theory, showed that an extremely skew distribution of heat resistance in the population must be assumed to explain exponential survivor curves solely on the basis of differences in heat resistance in the population. They further argue that a skew distribution is contrary to what is known in other instances of biological variation, Chick (1930), however explains deviations from exponential curves by assuming variations in heat resistance. Rahn (1929) explained curves with an initial lag in death rate by assuming that more than one molecule per cell was inactivated in these cases but assumed a homogeneous population. Withell (1942) argued that observed survivor curves could be explained by assuming that heat resistance of the bacterial population followed a log normal distribution, a distribution known to occur in biological systems. Vas and Proszt (1957) have calculated several hypothetical curves on the assumption of log normal distributions with various mean heat resistances and standard deviations. Their curves resemble some experimental survivor curves. Jordan and Jacobs (1944) were the first to point out that a strictly log normal distribution cannot result in an exponential decline in viable cells. Stumbo (1965) attributes deviations from exponential death rates to clumping, either before heating in the case of lags in death rate, or flocculation during heating which would cause apparent death rates to fall more rapidly initially than would be expected from the exponential curve.

The interpretation of survivor curves is of considerable importance from both a theoretical and a practical standpoint. Theories of thermal death of bacteria must be compatible with all valid experimental observations. At the same time, one must be prepared to deal with all types of experimental survival of bacteria in practical sterilization problems.

In the present paper, we will consider the interpretation of some typical examples of more complex nonexponential survivor curves selected from a large number of experimental curves which we have prepared in our laboratory.

EXPERIMENTAL

Bacterial cultures

The following bacterial cultures were used: *Salmonella anatum* (ATCC 9270); *Salmonella sentfenberg* (775W); *Streptococcus faecalis* (from Dr. A. J. Mercuri); and *Escherichia coli* (ATCC 26).

Preparation of culture

All cultures were carried on Trypticase Soy Agar (TSA) slants, then passed once in Trypticase Soy Broth (TSB) before being inoculated in TSB for the indicated length of time at 35°C. After incubation, they were centrifuged, washed twice with 0.1M phosphate buffer, (pH 7.0) and resuspended in the heating medium used.

Heating procedure

For experiments 1-4 (Figures 1-4), heating was done in sealed 1- or 5-ml sterile ampules completely immersed in a thermostated (±0.01°) hot water bath. Heating time was determined with a Thermistor thermometer (Yellow Springs Instrument Co.) with a thermocouple sealed in an identical ampule immersed in the water bath. Heating time was recorded from the time the contents of the ampule reached 5° below the holding temperature which gave a reasonable correction for come-up and cooling time. Ampules were removed at intervals and immediately cooled in an ice water bath.

For experiment 5 (Fig. 5), 300 ml of heating medium were placed in a sterile 3-necked round bottom flask with a magnetic stirrer and thermometer. The flask was completely immersed except for the necks in the thermostated heating bath and held with stirring until the temperatures in the flask and heating bath were the same. For the experiment, 5 ml of a concentrated suspension of the washed bacteria were injected into the 300 ml of heating medium. Samples were removed aseptically with a pipette at intervals and cooled immediately in a sterile capped test tube in an ice bath. Experiment 6 (Fig. 6) was carried out in an identical manner and successive 5 ml portions of a washed suspension of *E. coli* were added an hour apart.

Temperatures from 55°C–62.5°C were used as indicated.

Enumeration of survivors

The cooled ampules were opened and appropriate dilutions in 0.1% peptone water were pour plated in triplicate on the indicated agar. Plates were read after incubation for 48 hr at 35°C. MacConkey agar (BBL) and/or Trypticase Soy Agar (BBL) were used for the *Salmonella* tested; TSA (BBL) and/or M-Enterococcus Agar (Difco) were used for *S. faecalis;* and TSA for *E. coli.* Results are reported as the average of the three plates per dilution.

RESULTS & DISCUSSION

FIGURE 1 shows survivor curves at 55° C of *Salmonella anatum* grown in trypticase soy broth at 35°C for 18, 24, and 36 hr and heated in the growth medium. Both the shape of the survivor curves and the overall heat resistance change with age of the culture. This effect of age of the culture was first observed by Reichenbach (1911) (data redrawn by Rahn, 1930) who found that the shape of survivor curves changed from concave at 5 hr to logarithmic or convex at 18–24 hr with *"Bact. paratyphosum."* The 18 hr culture gives a convex curve with the beginning of a tail. Moats (1971) has shown that convex curves, ignoring the tail, can be explained on the assumption that the bacterial population is homogeneous with regard to heat resistance and

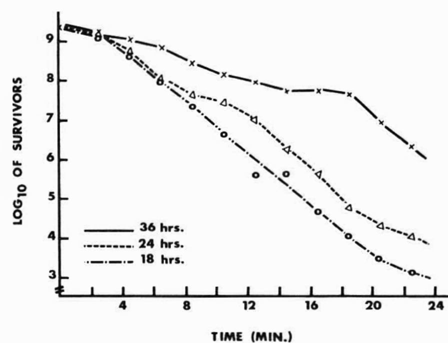

Fig. 1—Survivor curves of Salmonella anatum illustrating variation of heat resistance with the age of the culture. Cultures grown at 35°C for 18, 24, and 36 hr in trypticase soy broth (TSB), heated at 55°C directly in 1-ml sealed ampules, and plated on trypticase soy agar for counting after heating.

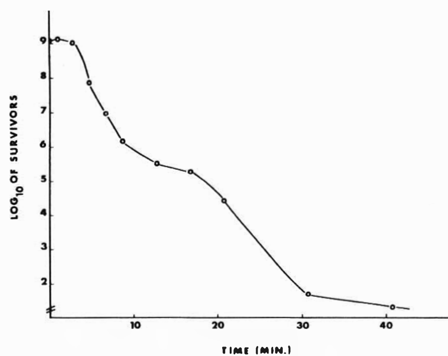

Fig. 2—A complex survivor curve representing three distinct populations of cells of differing heat resistance. Streptococcus faecalis grown 24 hr at 35°C in trypticase soy broth (TSB), washed, heated at 60°C in 0.1N pH 7.0 phosphate buffer in sealed 1-ml ampules, and plated on trypticase soy agar (TSA).

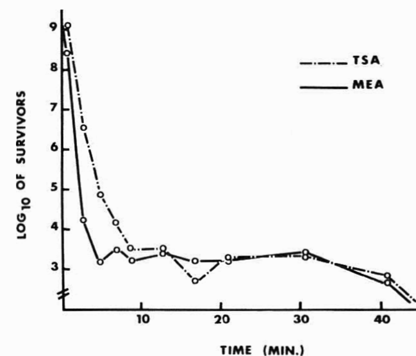

Fig. 3—Tailing of a survivor curve, Streptococcus faecalis, grown 24 hr at 35°C in trypticase soy broth, washed and heated at 62.5°C in 0.1N pH 7.0 phosphate buffer in sealed 1-ml ampules. Aliquots from each ampule were plated on M-Enterococcus Agar (MEA) and trypticase soy agar (TSA). Differences in counts were found between the two agars for the heat-sensitive portion of the population but not in the heat-resistant tail.

that death occurs from inactivation of some fraction of multiple critical sites, a model first proposed by Rahn (1929). The 24 hr curve is similar but has a hump in the middle. This curve can be interpreted as a complex or two convex survivor curves representing two populations differing slightly in heat resistance with a third population represented in the beginning of the tail. The 36-hr culture is more heat resistant and again shows a hump in the middle. All of these curves could be interpreted as being logarithmic by crediting any deviation from linearity to experimental error as is often done.

Figure 2 shows results with *Streptococcus faecalis* heated at 60°C in neutral phosphate buffer. This curve shows clearly the complex of two convex survivor curves with a heat resistant tail and could not be reasonably interpreted as logarithmic.

Figure 3 shows another set of results with a culture of *S. faecalis* heated at 62.5°C in which a small (1 in 10^6) very heat-resistant population was present. Two plating media were used: one selective (M-Enterococcus Agar) and one nonselective (TSA). Differences were noted between the two agars in recovery of the heat-sensitive populations but not in recovery of the heat-resistant cells. No survivors were detected on either type agar plate after 60 min heating, but, when the heated bacteria were inoculated in trypticase soy broth and incubated for 24 and 48 hr at 35°C, "recovered" bacteria were detected even after 80 min at 62.5°C. A more extreme sample of tailing is shown in Figure 4 with *Escherichia coli.* The bulk of the population was killed in less than 4 min. A few survivors were unaffected by heating even after 80 min at 62.5°C.

Another example of drastic tailing of a survivor curve of *Salmonella senftenberg* 775W heated at 60°C is shown in Figure 5. Differences of recovery between a selective agar (MacConkey's) and a nonselective agar (TSA) were conspicuous.

Changes in overall heat resistance of a culture, depending on the age, indicate that differences in heat resistance of bacterial cells in a homogeneous culture are determined physiologically. The shapes of the curves also change with the age of the culture, suggesting that the relative proportion of cells of different heat resistances also changes. Our experimental data in Figure 5 show a smooth concave curve over most of the range indicating some sort of regular distribution (i.e., normal, log normal, etc.) of heat resistance in that part of the population. However, as previously discussed, the curves in Figures 1 and 2 suggest the presence of two or three discrete populations of different heat resistances, rather than a continuum of different heat resistances, with no indication of any regular pattern of distribution. The abrupt breaks in curves in Figures 3 and 4 also show the occurrence of discrete very heat resistant populations which cannot be explained by assuming any orderly distribution of heat resistance. Bacteria in the stationary phase some-

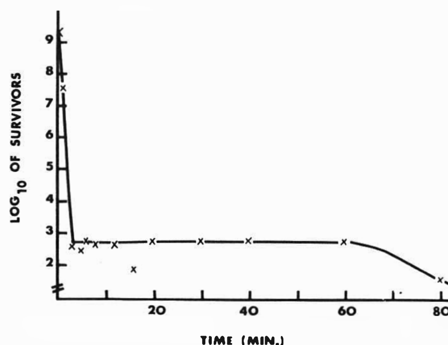

Fig. 4—An extreme sample of tailing. Escherichia coli grown 24 hr at 35°C in trypticase soy broth, heated at 62.5°C in 0.1N pH 7.0 phosphate buffer in 1-ml sealed ampules, and plated on trypticase soy agar (TSA). After an initial reduction of count of 6 log cycles in 2.7 min, no further kill occurred until after 60 min heating and survivors were still found after 80 min when the experiment was terminated.

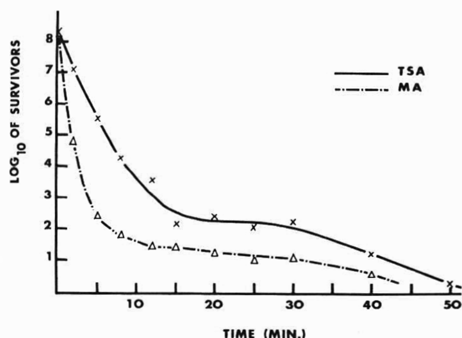

Fig. 5—Differences in survival of Salmonella senftenberg after heating at 60°C using a selective and nonselective medium. The curves both show significant tailing. Culture grown 24 hr at 35°C. A concentrated suspension of washed cells was injected into preheated 0.1N pH 7.0 phosphate buffer. Aliquots were plated on trypticase soy agar (TSA) and MacConkey's agar (MA) to enumerate survivors.

times give simple convex survivor curves and these have been interpreted as indicating a population homogeneous with regard to heat resistance (Moats, 1971). On the other hand, curves calculated by Vas and Proszt (1957) for various log normal distributions resemble some experimental survivor curves encountered. Thus, experimental evidence indicates that the heat resistance of a genetically homogeneous bacterial population may follow a regular distribution, an irregular distribution or may be homogeneous, depending on physiological factors which are not readily controlled.

Stumbo (1965) has explained deviations from the "logarithmic law" solely on the basis of clumping. Convex curves with an initial lag in death rate are explained on the assumption that the population is clumped at the start of heating. Clumps of two or more cells would produce a colony as long as one cell in the clump is viable. Riemann (Hansen and Riemann, 1963) found that clumping was the factor primarily responsible for the initial flat portion of a curve of *Streptococcus faecium* heated in milk since it was almost competely eliminated by treatment of the suspension in a Waring Blendor prior to heating. Stumbo (1965) also suggests that concave curves (a rapid initial death rate which falls off with time) result from flocculation of bacteria during heating. According to this hypothesis, the rapid initial reduction in counts results from bacteria aggregating into clumps rather than from actual death of the bacteria. To explain observed curves on this basis, formation of clumps would have to be rapid and the clumps would have to be sufficiently stable that they would not be broken up during normal dilution procedures for counting. We have observed microscopically the behavior of *E. coli* heated in neutral phosphate buffer (said by Stumbo to flavor flocculation) and observed little evidence of clumping. Further, such slight flocculation as occurred was completely dispersed by normal dilution techniques. Such slight flocculation might cause erratic counts at low levels of survival, however, and this was observed. Moats (1961), in a study of direct microscopic counting procedures, found no decrease in clump counts after heating several types of bacteria in milk while a decrease would be expected if significant flocculation occurred. There is, therefore, no evidence that flocculation during heating affects the shape of survivor curves and variation in heat resistance remains the only acceptable explanation of concave survivor curves.

Alderton et al. (1964) reported experiments which purported to show adaptation of spores to heat in response to heat treatment. However, they used *Bacillus megaterium* spores sensitized to heat by removal of calcium ions. Since incubation of the spores at lower temperatures in the presence of calcium ions also restored heat resistance, the increase in heat resistance noted during heating would appear to result from uptake of calcium by the spores and cannot be considered a true heat-induced adaptation. The question of whether bacteria can acquire increased heat resistance as an adaptive response to heat therefore remains open. While such an effect cannot be completely ruled out, there is no evidence to support this hypothesis.

The occurrence of very heat resistant tails in survivor curves has been occasionally reported by other workers. Jordan et al., (1947) found that a small fraction of a population of *E. coli* heated in nutrient medium at 49°C and 53°C survived "indefinitely." Farmiloe et al., (1954) observed that the decimal reduction time of spores of *Bacillus subtilus* heated in pH 6.5 phosphate buffer at 100°C increased from 1 min initially to about 1 hr at the end of the experiment. Vas and Proszt (1957) observed very heat resistant tails in populations of *Bacillus cereus* spores. Migaki and McCulloch (1949) found small abnormally resistant populations in several types of bacteria treated with surface active agents. In our experiments and in those of Vas and Proszt (1957) and Migaki and McCulloch (1949), subcultures of organisms surviving in the resistant tails were no more resistant than the original populations, indicating that the resistant populations are not resistant mutants or, if they are, the mutations are very unstable. Since we heated bacteria in sealed ampules completely immersed in a water bath, it is difficult to see how any could have escaped the full effects of the heat. It is possible that high concentrations of vegetative bacteria or spores may somehow have a protective effect.

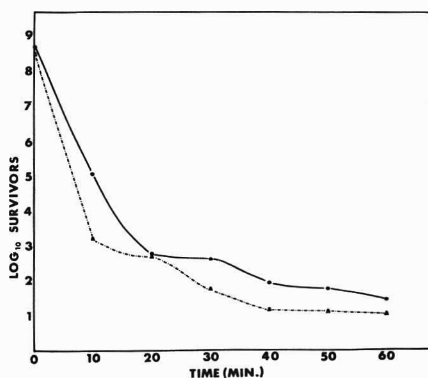

Fig. 6—Effect of heat-killed bacteria on survival of a fresh suspension of bacteria. Successive portions of a washed suspension of E. coli were added to preheated trypticase soy broth held at 60°C with stirring. Solid line, first addition; dashed line, second addition 60 min after the first.

An experiment was conducted to determine if bacteria killed by heat protected the few survivors. A washed suspension of *E. coli* was divided into two portions. One portion was injected into preheated TSB at 60°C and the survivors determined at intervals. After heating for an hour, the second portion of the suspension was added and survival again determined. The results (Fig. 6) show that the dead bacteria in the heating menstruum have no protective effect and in fact slightly increase the death rate of the fresh suspension. Therefore, tailing of survivor curves cannot be explained on the basis of a protective effect of the heat-killed cells.

The abnormally resistant cells occur at a level of 1 in 10^6–10^8 which is the same as found by the other investigators who observed extreme tailing. Vas and Proszt (1957) point out that the beginnings of flat heat resistant tails have been reported by many investigators, but they have failed to continue their experiments long enough to determine how resistant the few survivors were. Furthermore, investigators starting with low initial populations or carrying survivor curves through only 4 or 5 log cycles, as is frequently done, would completely miss such small heat resistant populations. The limited evidence available suggests that the occurrence of small numbers of very heat resistant cells may be a normal characteristic of bacterial populations. Such resistant cells could be of great importance in situations where total destruction of a bacterial population is desired. They would be of less importance where a reduction in bacterial load by a factor of 10^6 is satisfactory.

The idea that thermal death of bacteria is exponential has remained attractive to bacteriologists and remains the basis of thermal process calculations used for food processing (Ball and Olson, 1955; Schmidt, 1957; Stumbo, 1965).

The theoretical basis for assuming that thermal death of bacteria is logarithmic is based on two assumptions: (1) Populations of single strains of bacteria are homogeneous with regard to heat resistance; and (2) Thermal death of bacteria is unimolecular, that is to say that death occurs from inactivation of a single molecule or critical site per cell.

Experimental evidence indicates that assumption (1) is seldom true and that assumption (2) is incompatible with evidence of sublethal injury (Ball and Olson, 1955; Moats, 1971). Therefore, there is no theoretical basis for the common practice of assuming that survivor curves are logarithmic and crediting any deviation from linearity to experi-

mental error. In fact, published curves which are interpreted as logarithmic are seldom very precisely so and sometimes deviate considerably from linearity when experimental points are connected. Experimental curves which are nearly logarithmic probably result from a fortuitous mixture of cells of different heat resistances.

Figure 5 shows a clear example of the differences in number of survivors observed when a heated culture is plated on two different media. Differences in plate counts of several log cycles are observed. If death occurred from inactivation of a single-site per cell, the cells should be either dead or alive and the counts on the two agars should be the same. If death occurs from inactivation of some fraction of multiple critical sites, difference in counts on two agars can readily be explained on the assumption that the cells can survive and repair greater injury (i.e., more critical sites inactivated) on one medium than the other.

If thermal death of bacteria is not truly logarithmic, decimal reduction times (D-values) are meaningless (Vas and Proszt, 1957). Although authors such as Rahn (1945) and Schmidt (1957) have argued that the assumption that thermal death is logarithmic is convenient in comparing survival at different temperatures or survival of different bacteria under the same conditions, it is evident that the same end could be accomplished by the use of thermal death point measurements or F-values, that is to say, direct measurement of times required for a given probability of kill at a given temperature. This would give valid comparisons without introducing unwarranted assumptions as to the logarithmicity of the death rate. Ott et al. (1961) have pointed out the advantages of such an approach and found F-values were more reproducible than D-values. At the same time, allowance should be made for variability in heat resistance of the same culture manipulated in the same manner (Olson et al., 1952) and the possibility of resuscitation of heat shocked bacteria (Dabbah et al., 1969). It is evident from the examples shown in Figures 3, 4, and 5 that extrapolation of D-values, determined from survivor curves carried through 4 or 5 log cycles, to higher probabilities of kill could be seriously misleading.

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C. P. THOMAS, P. S. DIMICK and J. H. MacNEILL
The Pennsylvania State University, Div. of Food Science & Industry, University Park, Pa. 16802.

POULTRY PRODUCT QUALITY. 4. Levels of Carbonyl Compounds in Fresh, Uncooked Chicken and Turkey Skin

SUMMARY—The complex nature of poultry flavor prevents any single, complete characterization of its source. The contribution of the sulfur compounds to the flavor of the meat has received attention and recently the carbonyl-related off-flavor development in further processed products has become a problem. In order to determine the carbonyl compounds available in raw poultry skin, the hexane soluble extract of ground skin was reacted with DNPhydrazine. The derivatives formed were fractionated by column and thin layer chromatography and quantified spectrophotometrically. Male and female chickens and turkeys were raised on standard diets to selected ages to study the influence of age, sex and group of birds on the level and composition of carbonyl compounds. The particular carbonyls investigated were the total carbonyls, the total monocarbonyl fraction and the specific aliphatic monocarbonyl classes. Data showed higher levels of total carbonyl and total monocarbonyl compounds in male turkeys than in females until both were quite mature. Within the chicken samples, there was no appreciable difference due to either sex or age. The only methyl ketone found in any of the samples was acetone. The male turkeys had an increase in acetone concentration from 20 wk to a max at 24 wk, the same time at which lipid composition was the lowest. During the same time the female turkey maintained a relatively constant methyl ketone level. At 30 wk both sexes exhibited the lower concentration of acetone. Chicken skin data consistently displayed low acetone concentrations similar to that of the female turkeys.

INTRODUCTION

THE FLAVOR of poultry has been one of many flavors studied. General causes of flavor development of precursors of flavor in poultry have been studied (Dawson, 1968; Pippen, 1967; Minor et al. 1965; Mecchi et al. 1964; Lineweaver and Pippen, 1961; Ballance, 1961; Pippen and Nonaka, 1960; Pippen et al. 1954; Klose et al. 1952; Bouthilet, 1951; Crocker, 1948; Asmundson et al. 1938) but not resolved. The sources of poultry flavor were recently reviewed by Thomas et al. (1971).

Our particular interest was in the off-flavor development in further processed products using poultry skin. Chicken skin has been used satisfactorily in further processed products in forming emulsions and providing a chicken flavor (Baker et al. 1968; Baker and Darfler; 1967). Attempts have been made to utilize turkey skin in various further processed products, since the skin represents 5% or more of the weight of the bird (MacNeil and Buss, 1968). Unfortunately the off-flavors which develop have made such products difficult.

The aliphatic monocarbonyl compounds, namely methyl ketones, alkanals, 2-enals and 2,4-dienals, are known to influence the ultimate flavor of heated, stored dairy products (Kinsella et al. 1967). Previous work in our laboratory involved the aliphatic monocarbonyls in off-flavor development in cooked, stored turkey skin (Dimick and MacNeil, 1970). After cooking, the oil was pressed from the skin and the two fractions analyzed separately. The oil was expected to develop high levels of carbonyls, but actually was more stable than the residue. The saturated aldehydes were not evident in these samples but both 2-enals and 2,4-dienals as well as the methyl ketones were definitely present and could be measured. The methyl ketone fraction was further separated by thin layer chromatography and found to contain ketones of odd carbon chain length from 3 through 13. Carbonyl analyses of stored samples showed striking increases in the quantities of unsaturated aldehydes in the residue fraction. Concurrent organoleptic analyses revealed the development of objectionable flavors as these chemical changes took place in the skin residue of 29–31 wk old turkeys. Whether the monocarbonyls, namely the methyl ketones, were present in the raw skin or were formed only during the cooking process was unknown from this first study. The cooked chicken oil and residue had lower concentrations of carbonyls and did not develop the potent off-flavors. Carbonyl differences in the raw skin of turkeys and chickens might account for differences in off-flavor development between the groups of birds. Age and sex were additional factors whose influence as related to carbonyls had not been investigated but could be important.

This study was undertaken to determine the concentration of carbonyls in raw skin of chickens *(Gallus domesticus)* and turkeys *(Meleagris gallopavo)* which might account for differences in off-flavor development between the groups of birds.

Specifically the purposes of this study were (a) to determine the concentration of carbonyl compounds, including total carbonyls, total monocarbonyls and specific aliphatic monocarbonyl classes present in the fresh raw skin of chickens and turkeys; and (b) to determine the influence of age and sex on the concentration of these carbonyl fractions. The results should help determine the reason for the difference in off-flavor development in products using the skin of the two groups of birds. This should contribute to the development of either new processing methods or birds with skin characteristics which will remain acceptable during processing and subsequent storage.

The carbonyl compounds were analyzed as their 2, 4-dinitrophenyl (DNP) hydrazone derivatives and quantified spectrophotometrically. Individual classes of aliphatic monocarbonyls were separated by thin layer chromatography.

EXPERIMENTAL

Selection and preparation of the birds

Chickens and turkeys were hatched and raised in the poultry facilities of The Pennsylvania State University. The chickens were an Indian River broiler cross and the turkeys were a large white strain, developed and maintained by the Poultry Science Department. The turkey ration consisted of a standard commercial starter, grower, finisher program (Thomas, 1970). The broiler ration consisted of corn (54%), wheat (5%), oats (5%), alfalfa meal (2.5%), soybean meal (17.5%), fish meal (5.0%), meat scrap (5.0%), dried whey (2.5%), yeast (2.5%), limestone (0.5%), mineral supplement mix (0.28%) and vitamin mix (0.21%) with antioxidant added. Birds were raised to three ages, selected to represent those younger than usual market age, typical market age, and an age beyond that at which birds are usually slaughtered for market. Specifically these ages were 8, 10, 12 wk for chickens and 20, 24, and 30 wk for turkeys. On each designated day, 10 chickens and 5 turkeys of each sex were selected from flocks of approximately 200 broilers and from two pens each containing 30 turkeys of both sexes. All birds were visibly healthy at the time of selection.

The birds were slaughtered by severing the jugular vein, bled, scalded in 85°C water, machine picked, pinfeathers removed and singed. The main body skin was immediately removed, leaving the skin on the legs and wings of each bird. The body skin was air-blast frozen for 10 min at −35°C for easier handling. Time elapsed between slaughter and freezing was approximately 45 min for chickens and 30 min for turkeys. The skin was cut

into small pieces and ground twice in a Hobart meat grinder equipped with a 1/8-in plate. A representative sample of the ground skin was removed for analysis which was begun within 30 min after grinding.

Extraction of lipids for Carbonyl analysis

A 50g portion of the ground skin was homogenized in pure hexane for hexane-extractable lipid determination. Hexane, rendered carbonyl free according to Schwartz and Parks (1961) was used for extraction of the carbonyls. The tissue was homogenized in a Waring Blendor in 150 ml pure hexane for 15 min at a rheostat setting of 40. The extract was decanted and filtered through Whatman No. 1 filter paper. The blender jar was rinsed with 100 ml pure hexane and the homogenate was blended an additional 5 min. The entire mixture was then added to the filtration system. When most of the filtrate had passed through the filter, a final 50 ml pure hexane was rinsed through the residue in the filter. The filtrate was collected for reaction with DNPhydrazine.

Efficiency of hexane extraction procedure

Another portion of fresh, raw, ground skin was homogenized exactly as described above after which the filtrate was dried under vacuum and weighed to determine the quantity of hexane-extractable lipid present. A third 50g portion of ground skin was homogenized in chloroform-methanol (2:1, v/v) for total lipid extraction, using the method of Folch et al. (1957). The total lipid phase was dried under vacuum and weighed to determine total lipid content. All extractions described above were carried out in triplicate for age, sex, and group of birds.

Analysis of carbonyls as their DNPhydrazones

The triplicate samples used for carbonyl analyses were treated according to the procedure used by Dimick and MacNeil (1970) for cooked stored poultry skin samples, a modification of the methods described by Schwartz et al. (1962). Each sample was transferred quantitatively onto a reaction column (2.8 mm I.D. x 21.2 mm) composed of analytical Celite (Fisher Scientific Co.) impregnated with DHPhydrazine, phosphoric acid and water (Schwartz and Parks, 1961). The carbonyls present were converted to their respective DNPhydrazones for qualitative identification and quantification by spectrophotometric procedures. The eluate was collected and the column rinsed with 200 ml pure hexane to insure the complete removal of all the carbonyls and lipid. The eluate was brought to known volume in a volumetric flask and quantified using a Beckman DB-G spectrophotometer. The DNPhydrazone concentration was determined by reading the absorbance of the solution compared to pure hexane at 350 mμ and converting to μmol using A_m = 22,500 (Jones et al. 1956). These data were referred to as total carbonyl concentration and expressed as μmol per 10g hexane-extractable lipid.

Removal of the lipid from DNPhydrazones

Lipid material was removed from the DNPhydrazones by passing the eluate from the reaction column through a magnesia column (14 mm I.D. × 370 mm) containing 20g Celite 545-SeaSorb 43 (1:1, w/w) (Dimick and Walker, 1968). The Celite had been activated at 150°C for 24 hr. Both the Celite 545 and the Sea Sorb 43 were obtained from Fisher Scientific Co. The lipid was removed by percolating with pure hexane after which the absorbed carbonyl DNPhydrazones were eluted with 140 ml chloroform-nitromethane (3:1, v/v). The eluate was evaporated on a steam bath to approximately 10 ml and stored until further analysis.

DNPhydrazone class separation

The monocarbonyl derivatives were fractionated into their respective classes on a 10g Celite 545-SeaSorb 43 (1:1, w/w) column (14 mm I.D. × 370 mm) by means of selective eluting solvents in 100 ml quantities. The monocarbonyl fraction was investigated more thoroughly because of the flavor potential of these compounds. When the monocarbonyl fraction, dissolved in pure hexane, had percolated into the column, elution was begun with chloroform-pure hexane (20:80, v/v). The eluents which followed contained increasing proportions of chloroform: 40:60, 60:40, 80:20 and finally 150 ml chloroform. By the time the last solvent had percolated through the column, no visible carbonyl derivative bands remained or could be eluted with methanol. Eluate was collected using an LKB automatic recording fraction collector (LKB-Produkte, Stockholm, Sweden).

The contents of the collector tubes exhibiting distinct color and/or recording absorption on the spectral chart were checked spectrophotometrically to determine the class of monocarbonyls present. The absorbance maxima are given in Table 1. Tubes with the same absorbance maximum were grouped and the contents pooled, taken to dryness on a steam bath, brought to known volume in volumetric flasks and quantified in chloroform.

Identification by thin layer chromatography

The methyl ketone class was further fractionated using thin layer chromatography on Kieselguhr G (E. Merck Ag, Distributed by Brinkmann Instruments, Inc., Great Neck, N.Y.) plates developed in methylcyclohexane (Badings and Wassing, 1963). The 8 × 8 in glass plates were spread with a slurry of 8g Carbowax 400 (Applied Science Laboratories, Inc., State College, Pa., 15g Kieselguhr G and 30 ml distilled water, to 0.25 mm thickness. Plates were air dried for 2 hr, heated for 30 min at 100°C and stored in a desiccator over calcium sulfate. Plates were activated for 20 min at 100°C immediately before use. The methyl ketone fraction, dissolved in hexane, was spotted on the plates and developed in approximately 1 hr. Known 2, 4-DNPhydrazones of methyl ketones of chain lengths from C_3 to C_{15} and samples of the cooked turkey skin methyl ketone fraction were run on the same plates as standards.

Table 1—Spectrophotometric constants for DNPhydrazone derivatives in selected carbonyl fractions found in poultry skin

Carbonyl fraction	Absorptivity[a]	Wavelength, mμ max adsorption
Total carbonyl	22,500	350[b]
Total monocarbonyl	22,500	365[c]
Methyl ketone	22,500	365[c]
2-enals	27,500	373[c]
2,4-dienals	37,500	390[c]

[a] A_m values from Jones, et al. 1956.
[b] In hexane.
[c] In chloroform.

Statistical analyses

Data were analyzed by three factor analysis of variance to determine the interaction of the sex, age and group on carbonyl content of the skin. The effect of individual factors and interactions among factors were determined.

Correlation coefficients between the three carbonyl values were calculated to determine whether changes in concentration were correlated.

RESULTS & DISCUSSION

Lipid analysis

Lipid content of the skin for all ages, sexes and groups of birds is shown in Table 2. The point of particular interest was the decreased quantity of lipid in the turkeys at 24 wk. At 20 wk, for example, male turkey skin contained about 35.8% hexane-extractable lipid compared to 55.6% in the females. At 24 wk the hexane-extractable lipid content had declined to 22% and 44% for males and females respectively. 6 wk later the hexane-extractable lipid content of both had increased beyond the 20 wk level to

Table 2—Quantities[a] of lipid extracted by two processes from 50g poultry skin

	Total Lipid	Hexane-extractable Lipid
	g/50g skin	
Chicken		
Male		
8 wk	18.2	16.2
10 wk	19.2	12.9
12 wk	22.4	16.0
Female		
8 wk	21.8	15.0
10 wk	24.6	16.6
12 wk	23.2	19.4
Turkey		
Male		
20 wk	19.8	17.4
24 wk	13.4	11.0
30 wk	37.9	21.7
Female		
20 wk	28.0	27.8
24 wk	26.8	22.0
30 wk	36.7	29.0

[a] Mean of triplicate samples which were within one standard deviation.

43.4% for males and 58% for female turkeys. This pattern was observed in the Folch extraction of total lipids as well as in the hexane extraction. The diet of the birds beyond 8 wk contained a minimum of 3.5% fat. This lipid level is designed commercially to cause poultry to develop a pleasing fat layer under the skin, commonly known as finish. The total lipid data indicate that the male turkeys were not continuously laying down fat but instead actually had a decreased quantity of lipid under the skin at 24 wk, a typical market age. Although the decrease was minimal in the total lipids of female turkeys, again there was a noticeable decline in the hexane-extractable lipid. The lipid composition requires more extensive study, but the decrease in both total lipids and hexane-extractable lipids demonstrated in this study is unique.

Male turkeys at 24 wk had a desirable appearance and finish. The question arises as to the reason for the disparity in apparent and real lipid content. Perhaps this physical characteristic at 24 wk is due to factors other than lipid content. The skin of turkeys frozen slowly appears thin and transparent, revealing an undesirable, bluish muscle tissue below (MacNeil, 1970, Private communication). The skin of fast frozen birds, in contrast, develops an opaque characteristic due to rapid freezing of intercellular water which alters the index of refraction. It is not impossible to suspect that a physical condition at 24 wk causes the less-fat bird to appear well-finished. This area needs more investigation.

Total carbonyl and monocarbonyl concentration in chickens and turkeys

Total carbonyls and monocarbonyls from chicken skin were quantified as their DNPhydrazone derivatives. Mean values are shown in Table 3 as μmol/10g hexane-extractable lipid from chicken. Standard deviations were computed for all data. Each of the triplicate values was within one standard deviation of the mean for the data.

Decreasing total carbonyl concentration with increasing age was apparent for both sexes except in the 8-wk old male birds whose total carbonyl content of 46.5 μmol/10g lipid was less than the 49.2 μmol in 10-wk old males. By 12 wk, however, both had continued their decline, reaching about 36 μmol/10g lipid.

The monocarbonyl data exhibited a uniform decline in concentration with age. As with total carbonyl compounds, the female chicken had a higher concentration of monocarbonyl compounds at both 8 and 10 wk but at the age of 12 wk the skin of the male birds contained 3.6 μmol/10g lipid compared to 2.9 μmol in the skin of female birds.

The difference between the total carbonyl content and the monocarbonyl concentrations represents the ketoglyceride fraction and the dicarbonyl compounds as well as perhaps other currently unstudied compounds. The role of the dicarbonyl fraction in flavor has received little attention because of the difficulty in eluting and quantifying them. Thin layer chromatographic systems were developed by Cobb (1964) for separation of DNPosazones of dicarbonyls, but Ronkainen (1967) reported interference from the DNPhydrazine when he attempted to isolate DNPhydrazones of dicarbonyls. The dicarbonyls could be active in Strecker degradtion but this mechanism has not been shown in poultry (Pippen, 1967). Attempts were made to characterize the ketoglyceride fraction. Although no specific components could be separated, the presence of two types of ketoglycerides was definitely indicated and has not previously been noted.

The concentrations of DNPhydrazone derivatives of turkey total carbonyl and monocarbonyl compounds are shown in Table 3. Distinct sex differences were observed in both fractions. Both male and female turkeys showed increasing concentrations of total carbonyls from 20–24 wk of age but males increased from 263 μmol/10g lipid to nearly 400 μmol contrasted with increases from 30–50 μmol for females. The female turkeys showed a continued increase in total carbonyl content to 69 μmol/10g lipid. The males conversely decreased to a total carbonyl concentration of 50 μmol/10g lipid by 30 wk.

The male turkey monocarbonyl concentration followed the same pattern, rising from 112 μmol at 20 wk to 312 μmol by 24 wk and then dropping to 5.4 μmol/10g lipid at 30 wk of age. The female turkeys also increased in monocarbonyl concentration from 2.6–11.6 μmol/10g lipid at 20 and 24 wk respectively. At 30 wk, however, the monocarbonyl concentration decreased to 3.2 μmol/10g lipid rather than increasing as had the total carbonyl concentration.

As with the chicken skin, the carbonyl compounds present in turkey skin may contribute to both desirable and undesirable flavor characteristics. Dimick and MacNeil (1970) found levels of total carbonyl and monocarbonyl compounds in cooked chicken skin residue to be at least three times as great as the levels shown in Table 3 for raw skin. Cooking caused some increase in total carbonyl concentration but much more markedly increased the monocarbonyl levels in skin residue to a level at which the flavor of the residue was rated unacceptable by a trained taste panel (Dimick and MacNeil, 1970; MacNeil and Dimick, 1970). The high levels of monocarbonyls in the 20- and 24-wk old male turkeys may result in a higher flavor potential as compared to the female turkey and chickens of both sexes.

Separation of monocarbonyl classes

When the monocarbonyls were fractionated, only the methyl ketone class appeared in the samples of raw skin, regardless of age, sex or group of bird. Only one band was apparent on the magnesia column and only one absorbance maximum was recorded by the LKB fraction collector. Repeated determinations on the spectrophotometer revealed no fractions with absorbance maxima other than at 365 mμ in chloroform, indicating that methyl ketones were predominantly present. No alkanals were detected, as had been the case with cooked, stored skin (Dimick and MacNeil, 1970) but also absent were the 2-enals and 2, 4-dienal classes.

The methyl ketone derivatives were then spotted on Kieselguhr thin layer plates with standard derivatives of known carbon chain lengths from C_3–C_{15} and derivatives of the methyl ketone fraction of cooked poultry skin samples,

Table 3—Concentration[a] of total carbonyl and total monocarbonyl derivatives present in male and female poultry skin at three ages

Sex	Chickens			Turkeys		
	Age in wk					
	8	10	12	20	24	30
	μmol/10g hexane-extractable lipid					
Total carbonyl						
Male	46.5	49.2	38.8	263.4	397.5	50.0
Female	65.3	55.2	33.1	30.4	48.6	69.3
Total monocarbonyl						
Male	9.9	4.9	3.6	111.9	312.2	5.4
Female	10.3	5.9	2.9	2.6	11.6	3.2

[a]Mean of triplicate samples which were within one standard deviation.

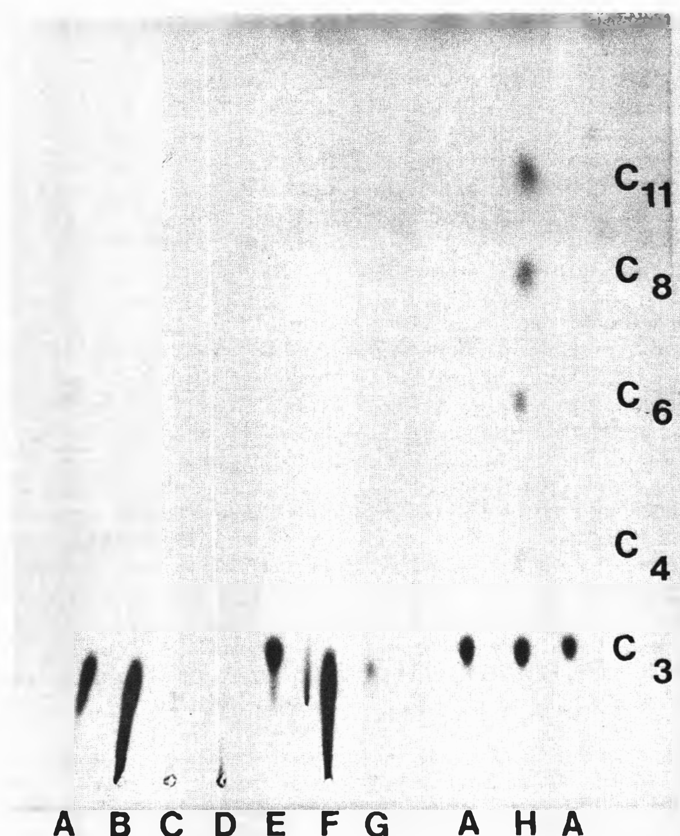

Fig. 1—Thin layer chromatographic separation of the DNPhydrazone methyl ketones from fresh chicken and turkey skin. A = acetone; B = chicken skin (8 wk); C = chicken skin (10 wk); D = chicken skin (12 wk), E = turkey skin (20 wk); F = turkey skin (24 wk); G = turkey skin (30 wk); H = mixture of known DNPhydrazones (C_3 through C_{11}).

Table 4—Concentration[a] of methyl ketone[b] derivatives in male and female poultry skin at three ages

Group	Males	Females
	μmol/10g hexane-extractable lipid	
Chickens		
Age (wk)		
8	2.8	1.2
10	1.1	0.9
12	0.6	1.1
Turkeys		
Age (wk)		
20	110.1	2.0
24	182.2	4.3
30	2.9	2.7

[a]Mean of triplicate samples which were within one standard deviation.
[b]Acetone.

which were known to contain odd numbered chain lengths from C_3-C_{13}. Even when concentrated spots of unknown derivatives were applied, the only methyl ketone present after development of the chromatogram was acetone (Fig. 1).

To confirm that the only methyl ketone derivative present was the DNPhydrazone of acetone, melting points were determined on recrystallized samples of the methyl ketone fraction, according to the method of Shriner et al. (1956). The melting point of 126°C confirmed the exclusive presence of acetone in the methyl ketone fraction.

Acetone contamination in the solvents could cause high levels of acetone to appear erroneously. To eliminate this possibility, both benzene and hexane were rendered carbonyl-free, as outlined by Schwartz and Parks (1961). Also, samples of the solvents were monitored along with the poultry skin samples and no important contamination was shown. Total carbonyl values for the solvents were within a range of 2-5 μmol/400 ml solvent when percolated through the reaction column and only about 0.1-0.2 for monocarbonyl contamination.

Between groups, had the various methyl ketones appeared in the monocarbonyl fraction or had classes besides methyl ketone been present, the interrelationships among these components would have been of particular concern. However, since the monocarbonyl fraction was found to be composed only of the methyl ketone acetone for both groups of birds, the influence of age and sex on the concentration of the carbonyl fractions became the primary emphasis of this study.

The differences in the methyl ketone concentrations for chickens of both sexes and all three ages were minor and in the range of 0.6-1.2 μmol/10g lipid, except for the youngest males. These data are shown in Table 4. The 8-wk old male chickens had a methyl ketone concentration of 2.8 μmol/10g lipid which was more than two standard deviations greater than the mean of the other data. This high level of methyl ketone could indicate that these birds were undergoing some physiological development or change which caused a greater concentration of acetone than was typical of female chickens or more mature males.

Age produced significant variations in the methyl ketone concentration in turkey skin. An increased concentration of acetone from 20-24 wk was seen in both sexes. The quantity of acetone doubled in the females and increased by 70% in the males; however, at 24 wk the concentration in the males was 182.2 μmol/10g lipid compared to 4.3 μmol in the same amount of female tissue. By 30 wk of age acetone in the skin of both males and females had dropped to similar low levels. These were 2.9 μmol/10g lipid for males and 2.7 μmol for female turkeys.

The sex differences were particularly striking in the turkeys. The level of acetone in the female turkeys was not high at any age although there was an increase in acetone content at 24 wk. In male turkeys the acetone level was significantly higher than that of the females at both 20 and 24 wk. Only by 30 wk were the levels similar regardless of sex.

Analysis of variance for total carbonyls, monocarbonyls, and methyl ketones indicated that each variable (age, sex, and group, i.e. chicken or turkey) was significant at the 1% level as was the interaction among the factors.

Relationship between flavor and age

More complete data are needed to determine the specific sources of poultry flavor. This study has shown that definite differences exist between the carbonyl levels of chicken and turkey skin. Age is a factor which influences the concentration of carbonyl levels. The high concentration of all carbonyl classes in 24-wk old male turkey skin probably contributes to the off-flavor development when this skin is used in further processed products. The decrease in total

carbonyl and total monocarbonyl content at 30 wk may indicate a physiological maturation which eliminates the characteristic high carbonyl concentrations. While carbonyl compounds are a part of off-flavor development, their entire contribution to flavor may not be detrimental. However, if the processor were to use turkey skin, the selection of a more mature bird or the use of the skin of the females only would appear advisable for these products. On the other hand, if more were known about the cause of these high levels of carbonyls, birds could be bred, fed and/or slaughtered so that the off-flavors were minimized or possibly never developed.

In some foods the development of flavor during aging is important for a high quality product. Fruits and vegetables usually develop their best flavor when they have been allowed to ripen before harvesting. Particularly interesting is the contrast between the flavor of veal and that of mature beef in which age definitely influences the flavor. Perhaps the desirable old-fashioned flavor reportedly missing from today's birds could be parallel. Since this study was conducted, broilers are being slaughtered earlier to provide the size pieces desired by the ready-to-eat and heat-and-eat chicken establishments. If interpolations were to be made, the 6-7 wk old bird now common for this use may yield a flavor response quite different than birds slaughtered at later ages.

At the moment only speculation on the source of the acetone is possible. Radioactive tracers may be useful in elucidating the sources of the carbonyl compounds or their synthetic pathways. Presently it can be said that acetone is available in fresh skin of both chickens and turkeys. Sex and age are influential in regulating the specific level but particularly high concentrations are available in skin of maturing male turkeys. Once the causes of this carbonyl development are known, the interaction of these compounds with other flavor sources will bear consideration.

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S. J. BISHOV, A. S. HENICK, J. W. GIFFEE, I. T. NII, P. A. PRELL and M. WOLF
Food Lab, U.S. Army Natick Laboratories, Natick, Mass.

QUALITY AND STABILITY OF SOME FREEZE-DRIED FOODS IN "ZERO" OXYGEN HEADSPACE

SUMMARY—Excellent retention of fresh flavor quality in a series of freeze-dried foods of plant and animal origin was achieved in "zero" oxygen headspace, using an atmosphere of 5% hydrogen in nitrogen with a palladium catalyst. Freeze-dried, precooked carrots, sweet potatoes, green beans, peas, spinach, white potatoes, peaches, apricots, chicken, pork, beef, beef stew, chicken stew and shrimp were investigated for oxygen uptake, flavor quality and some for consumer acceptability. The rate of oxygen uptake during storage was greater in the animal products than in the plant products studied. For oxygen-sensitive products such as carrots and sweet potatoes, loss of quality was observed in packs with headspace containing as little as 0.5% oxygen within 1 month at 100°F reflected in the lower scores given by a technological panel. A flavor profile panel reported that all the foods packed with 5% hydrogen in nitrogen with palladium catalyst had aroma and flavor amplitudes after storage at 100°F for 6 months comparable to the original products. Randomly selected untrained consumer test panels preferred the foods packed in "zero" oxygen to those in 2% oxygen after brief storage at 100°F. This preference persisted throughout the test year.

INTRODUCTION

DETERIORATION of initial quality in freeze-dried foods has long been attributed to reactions with headspace oxygen. Harper and Tappel (1957) found improved stability in nitrogen packs. The relationship between headspace oxygen, residual moisture, time and temperature of storage and deterioration was described by Thompson et al. (1962). That oxygen was most critical in initiating deterioration was reported by Roth et al. (1965) and by Bishov and Henick (1962). Even items which absorb oxygen very slowly, such as freeze-dried stews, become unacceptable in time if enough oxygen is available (Tuomy et al., 1968).

Oxygen sensitive dehydrated food items have been packaged with "inert gas" headspace, usually achieved by nitrogen flushing or by vacuum closing. Residual oxygen not greater than 2% is a customary standard and has been adopted in military specifications (US Army Natick Lab., 1967). The present study was designed to answer the question, what improvement in quality could be achieved in freeze-dried oxygen-sensitive foods if they were processed and stored in the total absence of oxygen? The recently developed method of gas displacement described by Hu and Breyer (1970) can achieve residual oxygen of the order of 0.1%. More effective still is the technique of removing traces of oxygen by reaction with hydrogen on a palladium catalyst described by Abbot et al. (1961) and employed by Tamsma et al. (1960) to increase the shelf life of whole milk. In the headspace of a package filled with 5% hydrogen in nitrogen (a nonflammable, nonexplosive mixture), residual oxygen quickly falls below 0.001%. This technique, which achieves "zero" oxygen, was applied to freeze-dried carrots and cooked beef with great success (A. F. Mabrouck, D. F. McMorran and J. K. Jarboe, Personal Communication). It was used in this study to compare quality retention during storage with that in 2% oxygen packs.

EXPERIMENTAL

Materials

Plant products. Included carrots, spinach, sweet potatoes, green peas, green beans, white potatoes, apricots and peaches; peeled and diced (if suitable); blanched (except fruits) in water at 212°F for 5 min, blast frozen at −30°F and freeze-dried in glass to a final moisture content of ca. 1.5%.

Animal products. Included pork loin, beef (longisimus dorsi), chicken dark meat, chicken white meat and shrimp; cooked, diced (except shrimp); blast frozen at −30°F and freeze-dried in glass to a final moisture content ca. 2.0%.

Gases

Gases were analytical grade nitrogen; oxygen, 2% v/v in nitrogen; and hydrogen, 5% v/v in nitrogen. Catalyst was palladium, powdered metal, 0.5% w/w on kaolin as pellets.

Equipment

Used a laboratory freeze-drier with stainless steel condenser, cooled with solid CO_2 in alcohol and manifold holding six 3L, r.b. flasks. Drying proceeded at 2×10^{-2} Torr. Glove box, 90 × 65 × 70 cm, using a balloon to assist in expelling the atmosphere, was operated at a positive pressure of 0.3 Torr with the selected gas atmosphere. See flow diagram, Figure 1.

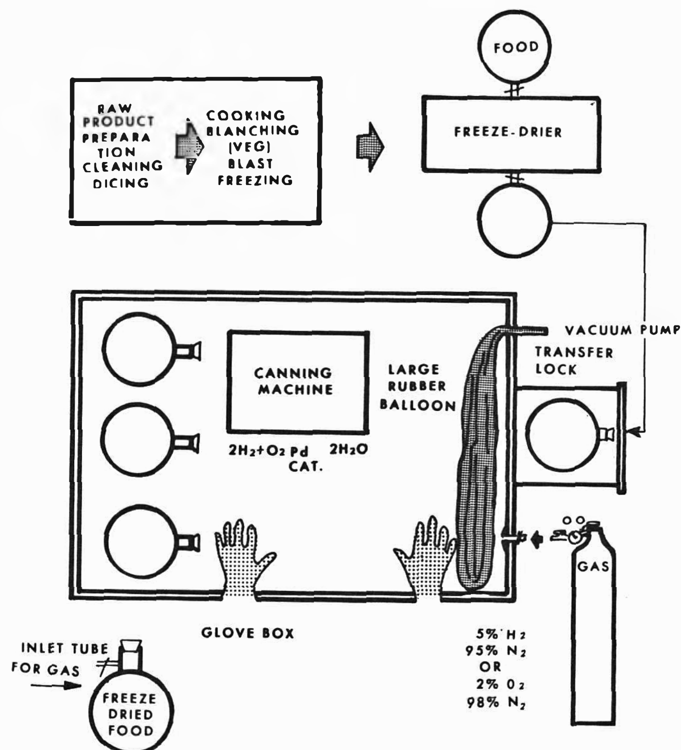

Fig. 1—Flow diagram for "zero O_2" pack process of freeze-dried foods.

Packaging

Vacuum in each freeze-drier flask was broken individually with nitrogen gas at a slightly positive pressure. The flasks were stoppered tightly and introduced through the air lock into the glove box, which had an atmosphere of 5% hydrogen in nitrogen. The contents of all flasks were blended together and then filled into 300 × 304 cans. Palladium catalyst, 250 mg (3-4 pellets), wrapped in a tissue, was added to 1/2 of the cans, which were then covered and sealed with a portable machine already in the glove box. Glove box atmosphere was then displaced and replaced by 2% oxygen in nitrogen. The remaining cans were covered and sealed. After removal from the glove box both ends of all cans were dipped in molten paraffin wax, a treatment found necessary to prevent gas leakage through the double seam seal. All cans were stored at 100°F.

Headspace gas analysis

Residual oxygen was determined by the gas chromatographic method of Bishov and Henick (1966). Sampling was achieved with a can-lid piercing needle on a gas tight syringe; the sampling area on the can lid was first covered with a self-adhesive tape of closed-cell polyurethane foam.

Panel testing

Three different panels were used to evaluate stored samples:

(1) A flavor profile panel established according to Sjostrom and Caul (1963) consisting of 6 judges who reported overall aroma and flavor amplitudes and defined specific flavor notes together with their intensities and the order perceived. The scale used by this panel was 0 - 3 by half-steps.

(2) A technological panel of 12 selected and trained judges who reported normative judgments on color, flavor, and odor on a 9-point scale (Peryam and Shapiro, 1955).

(3) Consumer panels of 30 judges randomly selected for each session who reported their preference on a 9-point scale (Pilgrim and Peryam, 1958). All food items were freshly rehydrated and served warm.

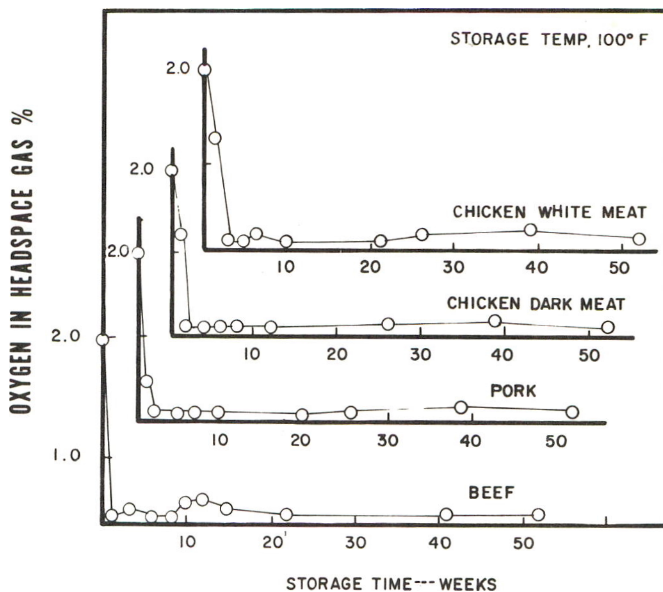

Fig. 2—Oxygen uptake of some freeze-dried meat items.

RESULTS & DISCUSSION

Oxygen uptake

Most rapid uptake of oxygen was observed in the meat items, decreasing from beef to chicken white meat (Fig. 2). Most of the available oxygen was consumed in from 1-4 wk. The vegetable items took up oxygen chiefly as a function of pigment content (Fig. 3). Those with a high carotene content (sweet potatoes, spinach and carrots) underwent a fairly rapid uptake during the first 15-40 wk, and had consumed all available oxygen at the end of 1 year. Lesser pigmented vegetables with a lower lipid content (green beans and potatoes) showed a slow, steady uptake, which may have been complete at the end of 1 year. The two fruit items, peaches and apricots (Fig. 4) displayed a very slow uptake, using only 30% to 50% of available oxygen during the first year. It will be seen later that flavor changes were quite well correlated with rate and amount of oxygen consumed.

Flavor profile panel

This panel evaluated 13 freeze-dried foods for aroma and flavor. They reported both pleasant and unpleasant notes. Initially, pleasant notes were dominant, but during storage in the oxygen containing packages, unpleasant notes developed. Amplitude, which may be correlated with overall quality, is a useful indicator of this change. Figure 5 shows flavor amplitude of these foods after 1 year at 100°F. In all cases, the items stored in "zero" oxygen retained more of the desirable notes than those packed under 2% oxygen. The extent of difference between the two amplitudes was generally related to oxygen uptake.

The panel also recorded specific flavor notes. Table 1, in which the foods are arranged in decreasing order of stability, summarizes the first appearance of a significant unpleasant note in the 2% oxygen packs, and the overall description at the end of 1 year (6 months for green beans and peaches, 8 months for pork) in the "zero" oxygen packs. In all cases, the "zero" oxygen items were fresh-like, whereas those in 2% oxygen had undergone significant deterioration in from 1 week for the carrots to 8 months for the white potatoes.

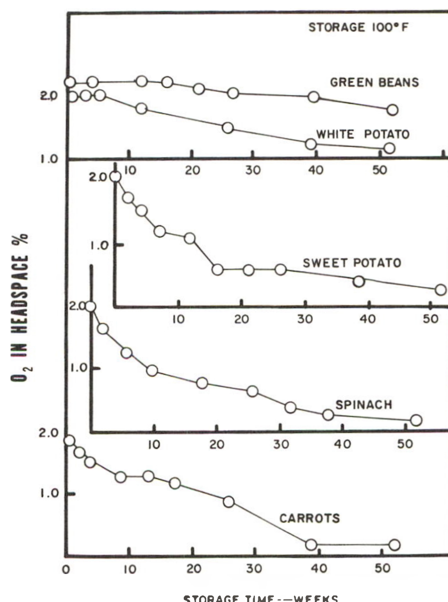

Fig. 3—Oxygen uptake of some freeze-dried vegetables.

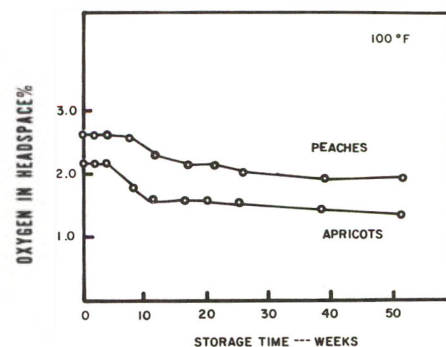

Fig. 4—Oxygen uptake of freeze-dried peaches and apricots.

Table 1—Influence of oxygen on flavor of freeze-dried foods stored at 100°F; flavor profile panel evaluation

Food	First detection of flavor changes (mo) 2.0%	"Zero"	Flavor description at time of first detectable change 2.0%	"Zero"
White potato dice	8	12	sl. oxidized	fresh, toasted
Green peas	6	12	sl. oxidized	fresh green pea
Green beans	5	12	oxidized	pleasant, jasmine
Spinach	3/4	12	stale washed out	fresh tasting
Sw. potato dice	1/2	12	violet ionone	pleasant sw. potato
Carrot dice	1/2	12	oxidized ionone	fresh flavor odor
Peaches, sliced	1	6	haylike washed out	peachy, good
Apricot slices	3	12	haylike washed out	apricoty, good
Chicken white meat (cubes)	5	12	washed out tasteless but not rancid	fresh chicken taste
Chicken dark meat (cubes)	2	12	rancid stale	fresh chicken taste
Pork (cubes)	2	8	stale	fresh, good flavor
Beef (cubes)	1	6	rancid washed out	good beef taste

Technological panel

Freeze-dried carrots were packed under four initial levels of headspace oxygen: (1) "zero" oxygen—hydrogen and catalyst; (2) 0.5% oxygen—vacuum closing; (3) 1% oxygen—triple nitrogen flush; and (4) 2.2% oxygen—mixed gases ($O_2 + N_2$).

Storage was at 100°F and lasted for 1 year. Summarized results of this study are shown in Figure 6. The general increase in attribute scores at the end of 12 months (as compared to those at 6 months) can be explained only as a probably unconcious change in standard on the part of the panel. The data were subjected to analyses of variance, and results for each quality attribute will be considered separately.

Color. No significant changes occurred during 12 months' storage in the carrot samples in "zero" and 0.5% oxygen. Under 1% oxygen, fading was observed at 2 months, and at 3 months rated significantly lower than both "zero" and 0.5% oxygen. In the sample packed at 2% oxygen, fading and bleaching was observed at 2 months and rated consistently lower than all other samples throughout the study.

Odor. Storage for 12 months at 100°F had no significant effect on the odor of samples packed at "zero" and 0.5% oxygen. Although no significant differences were measurable between these two samples, some panelists noted a moderate level of oxidized carotene odor (β-ionone) in the 0.5% pack after 9 and 12 months. Samples packed at 1% oxygen became signficantly poorer than those at "zero" oxygen after 2 months and remained so throughout the study. Under 2% oxygen headspace, significant changes had occurred within 2 wk of storage, at which time the odor score was below fair (4.7). Comments of oxidized carotene odor were also made. After 2 months of storage there was no significant difference between the 1% and 2% oxygen packs, both of which were rated below fair.

Flavor. No significant changes in flavor occurred during 12 months' storage in the samples packed under "zero" and 0.5% oxygen. Differences from the "zero" oxygen pack were significant after 2 months under 1% and after 2 wk under 2% oxygen. These differences persisted throughout the study. The relationship between flavor score and initial oxygen content of the headspace is seen in Figure 7.

Consumer panel

Randomly selected consumer panels rated freeze-dried carrots packed under "zero", 1% and 2% oxygen, stored at 100°F. Results are shown in Figure 8. Evidently, even the very brief holding

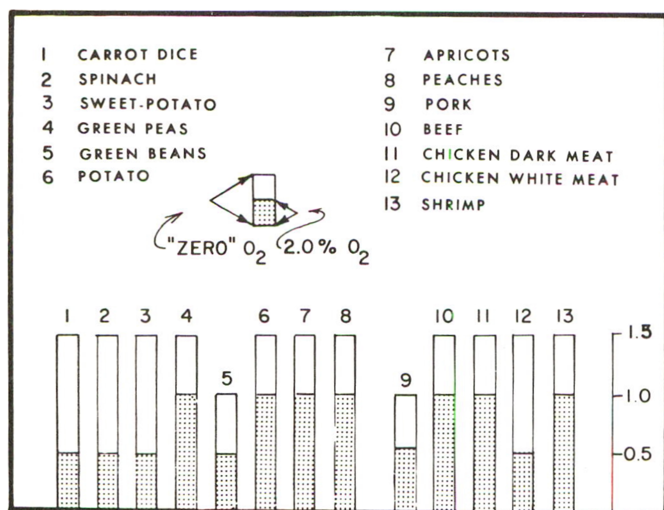

Fig. 5—Profile panel flavor amplitudes of freeze-dried foods in "zero" O_2 and 2.0% O_2 for 1 year at 100°F.

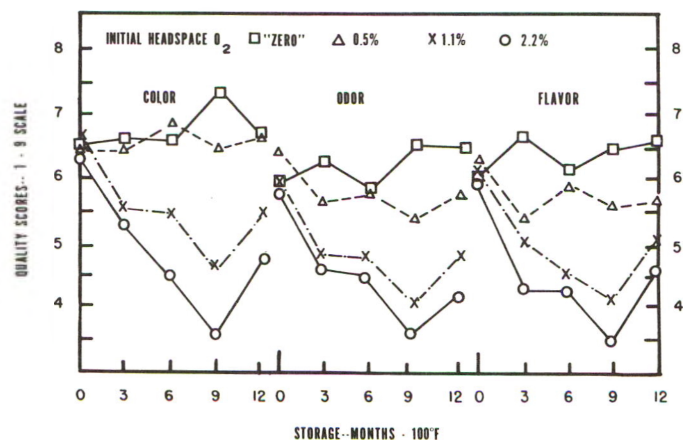

Fig. 6—Effect of oxygen on storage quality of freeze-dried carrots; a technological panel evaluation.

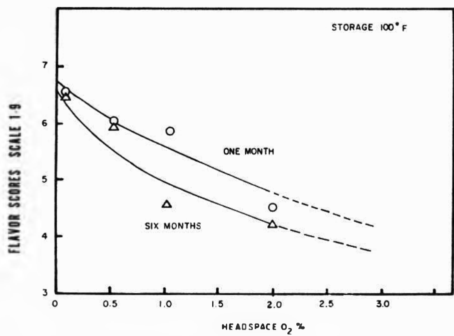

Fig. 7—Relation of flavor scores and initial oxygen levels of freeze-dried carrots in storage at 100°F.

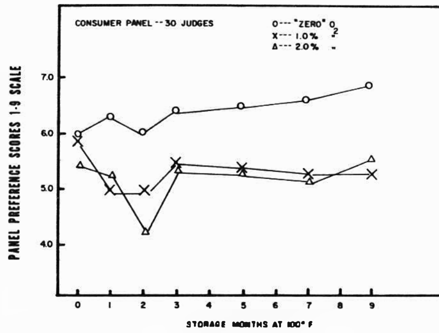

Fig. 8—Consumer panel preference scores of freeze-dried carrots.

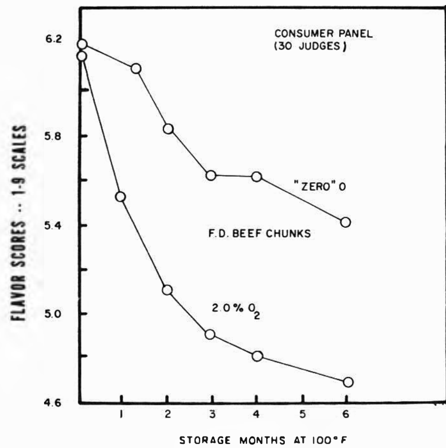

Fig. 9—Consumer panel preference scores of freeze-dried beef.

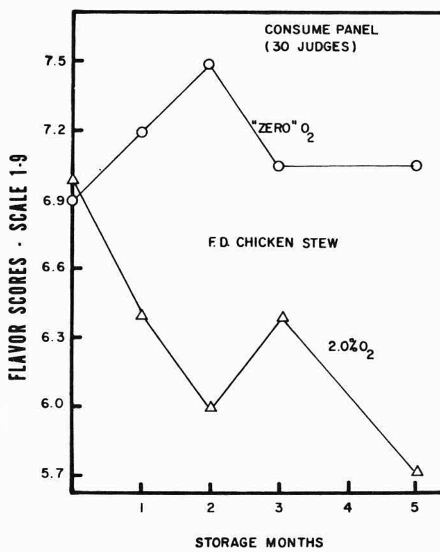

Fig. 10—Consumer panel preference scores of freeze-dried chicken stew.

time, ca. 5 days, before the test caused a significant decline in the scores of the carrots packed under 1% and 2% oxygen. These lower scores persisted throughout the storage period.

Another oxygen sensitive item evaluated was the freeze-dried beef chunks. The "zero" oxygen pack of this product was preferred to beef chunks packed in 2% oxygen at each monthly withdrawal period, although all ratings of flavor declined over 6 months (Fig. 9).

Chicken stew, a composite freeze-dried item containing carrots, was also rated by the consumer panel. The panel showed a distinct preference for chicken stew under "zero" oxygen as compared to 2% (Fig. 10), which was retained throughout the storage period at 100°F. Analyses of variance of mean score values of the chicken stews evaluated by the consumer panel indicated a significant preference for the "zero" oxygen pack.

CONCLUSIONS

IN THESE studies the value of very low oxygen in preserving the fresh odor and flavor quality of freeze-dried food has been confirmed. Every food item studied benefited by "zero" oxygen packing; those known to be susceptible to oxidation retaining acceptable fresh-like flavor long after those in other packs had failed.

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RESEARCH NOTES

M. R. SEVENANTS[1] and W. G. JENNINGS
Dept. of Food Science and Technology, University of California, Davis, Calif. 95616

A Research Note
OCCURRENCE OF 6-PENTYL-α-PYRONE IN PEACH ESSENCE

IN A PREVIOUS study, Sevenants and Jennings (1966) isolated and identified a number of components in a steam distillate of the Red Globe variety of freestone peaches. One compound exhibited an infrared spectrum characteristic of an α-pyrone (e.g. split C = O absorptions at 1740 and 1725 cm^{-1}, pronounced C = C stretching bands at 1635 and 1553 cm^{-1}; Nakanishi, 1962). Beyond establishing that its ultraviolet absorption was quite broad (250–340 nm) and relatively nondescriptive, and that hydrogenation produced an aliphatic carboxyllic acid as the only product, the authors were unable to further characterize this compound.

Recently Nobuhara (1969) synthesized a series of unsaturated lactones. The infrared spectrum of his 6-pentyl-α-pyrone appeared to match precisely that of our unknown pyrone. A sample of the authentic compound was chromatographed under the same conditions, and exhibited Kovats Indices of 1430 on OV101, and 2124 on Carbowax 20M columns, values which agree with those for the unknown compound. The infrared spectrum of the pyrone, isolated from the gas chromatograph, was the same as that of the compound from peach. Accordingly, we are confident that the unknown pyrone isolated from peach is 6-pentyl-α-pyrone. Nobuhara (1969) reported that the substance exhibited a "butter or butter cake" aroma; several of our co-workers, familiar with aroma evaluation, have described the aroma as "coconut-like," or "lactonic."

[1]Present address: Procter and Gamble, Winton Hill Technical Center, 6071 Center Hill Ave., Cincinnati, Ohio 45224.

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W. MALEWSKI and P. MARKAKIS
Department of Food Science, Michigan State University, East Lansing, Mich. 48823

A Research Note
ASCORBIC ACID CONTENT OF THE DEVELOPING TOMATO FRUIT

INTRODUCTION

PREVIOUS REPORTS on the relationship between stage of maturity and the ascorbic acid content of tomatoes are conflicting. Genetic, environmental and methodology factors may be responsible for the discrepancies. Clow and Marlatt (1930) and Jones and Nelson (1930) found by bioassay that the ascorbic acid content of tomatoes increased as the fruit matured. Maclinn et al. (1944) concluded that the stage of maturity had no effect on the ascorbic acid concentration. Hamner et al. (1945) reported a small but continuing increase in ascorbic acid content through the overmature stage, while Lo Coco (1945) found a rather large (88%) increase up to the red-mature stage with a subsequent decrease as the fruit overmatured. Fryer et al. (1954) detected a definite rise in ascorbic acid content as the fruit developed from mature-green to mature-red, the increase (30%) being twice as large in the field-grown as in the greenhouse-grown tomatoes.

The objective of the present study was twofold: to reexplore the relationship between ascorbic acid content and maturity by defining the latter as time from anthesis and not merely on the basis of color, and to compare under similar growth conditions several current cultivars regarding this relationship. With the introduction of new varieties it is generally necessary to reevaluate the nutritional qualities of food commodities.

METHODS & MATERIALS

SEEDS of four tomato cultivars, Fireball, New Yorker, (supplied by Harris Seed Co.) and VF-13L and VF-145B (supplied by Ferry-Morse Seed Co.), were sown in flats. On June 2, 1970, 2-in seedlings were transplated on the Michigan State University Horticultural Farm. Approximately 50 plants were grown for each cultivar. Anthesis was at its maximum on July 15 for Fireball and New Yorker and July 31 for the VF-13L and VF-145B. Blossoms which appeared before or after those dates were removed. Late blossoms were left on the vines but the fruits developed from them were easily distinguished and were not included in the study.

The first sampling of fruits began 2 wk after anthesis. Subsequently, samples were obtained weekly up to 9 wk after anthesis. At least one fruit was picked from at least ten plants at random for each cultivar, at each sampling date. The fruits were weighed, their average color assessed visually and one radial section corresponding to 1/8 or 1/4 of the fruit, depending on size, was removed. The sections were placed in a Waring Blendor, covered with metaphosphoric acid-acetic acid stabilizing mixture and blended for 3 min at high speed. Nitrogen gas was introduced to the head space of the blender jar during blending. The slurry was filtered and three aliquots of filtrate, 5 or 10 ml each, were titrated according to the AOAC (1965) method.

RESULTS & DISCUSSION

RESULTS of the ascorbic acid determination along with the data on color and average fruit weight are shown in Table 1. A definite trend is discernible in the biosynthesis of ascorbic acid in the developing tomato fruit. In all four cultivars the ascorbic acid content decreased slightly after the second week from anthesis, reached a maximum at the fifth or sixth week and again decreased thereafter. All cultivars tested exhibited the highest ascorbic acid concentration at the stage just before full red color development, or full maturation on the vine. From the previous reports on the relationship between ascorbic acid content and maturity the results of Lo Coco (1945) are in closest agreement with the present findings. New Yorker is an early cultivar while VF-13L and VF-145B are late under Michigan growth conditions. VF-13L also appears to be a little less rich in ascorbic acid than the other three cultivars.

Table 1—Ascorbic acid content (mg/100g fruit), color, and average fruit weight (g) of four tomato cultivars harvested at various periods from anthesis

Tomato cultivar		Weeks from anthesis							
		2	3	4	5	6	7	8	9
New Yorker	Asc. A.	10.7	7.6	10.9	20.7	14.6	10.1	—	—
	Color[a]	gr	gr	gr-yel	yel-red	red	red-om	—	—
	Avg wt.	33.4	57.2	102.5	145.7	159.9	167.6	—	—
Fireball	Asc. A.	13.6	7.9	11.0	17.9	22.6	15.2	13.6	—
	Color[a]	gr	gr	gr	gr-red	yel-red	red	red-om	—
	Avg wt.	37.3	88.5	108.1	152.6	169.0	170.0	165.4	—
VF-13L	Asc. A.	14.7	10.5	11.3	14.3	17.1	15.6	12.8	12.2
	Color[a]	gr	gr	gr	gr-yel	yel-red	red	red-om	red-om
	Avg wt.	17.2	32.2	51.0	57.6	70.4	80.2	84.6	83.7
VF-145B	Asc. A.	10.1	7.1	9.7	19.1	20.5	15.2	13.6	12.1
	Color[a]	gr	gr	gr	gr-yel	yel-red	red	red-om	red-om
	Avg wt.	19.9	37.8	72.9	86.4	105.3	115.3	112.6	121.7

[a]Color: gr = green; yel = yellow; om = overmature.

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W. Y. COBB
North Carolina Department of Agriculture, Raleigh, N.C. 27611
and H. E. SWAISGOOD
Department of Food Science, North Carolina State University, Raleigh, N.C. 27607

A Research Note
ROASTED PEANUT FLAVOR AND ITS RELATION TO GROWTH ENVIRONMENT

INTRODUCTION

RECENTLY, it has been shown that the unique flavor and aroma of roasted peanuts is due primarily to the interaction of reducing sugars and amino acids at high temperatures (Mason and Waller, 1964; Newell et al., 1967). During the 1968 peanut-growing season we were presented with samples of the same selection which had been grown in different soil types under varying moisture conditions. Peanut samples from two of the locations were found to differ widely in flavor upon roasting as judged by an expert flavor panel. The following is a presentation of amino acid and sugar composition of the samples, the correlation with roasted flavor quality and the growth environment.

EXPERIMENTAL

SAMPLES were grown under accepted cultural conditions on selected test locations in Virginia and North Carolina. Maturity at harvest was estimated by inspection of the pericarp interior and oil color (Emery and Gupton, 1968; Holley and Young, 1963). Following curing, only medium and extra-large size grades (Woodroof, 1966) were used for flavor panel and chemical evaluation. The numerical flavor scores were generated from results of the flavor panel at Corn Products Company, Alameda, Calif., by their "CLER" technique. In this evaluation higher scores (maximum 100) indicate better overall roasted flavor. A flavor score of 40 is considered as the lower borderline for use in peanut butter.

Sugars and free amino acids were extracted and purified in a manner similar to that described by Newell et al. (1967). Amino acids were assayed with a Beckman Model 116 Amino Acid Analyzer using the standard 4-hr hydrolysate method (Beckman Instruction Manual). UR-30 (acidics and neutrals) and PA-35 (basics) resins were employed. Silylethers of sugars were analyzed by gas-liquid chromatography using 5% SE-52 as stationary phase.

RESULTS & DISCUSSION

TABLE 1 lists agronomic and flavor score data of samples from experimental selection 15714, a cultivar of Virginia-type peanuts. The samples from the Chowan, N.C. location, grown on a fine, well-drained sandy soil were judged to possess considerably better roasted flavor than the samples from the Nansemond (Va.) location. In contrast, samples from the latter site, a fine sandy loam of heavier composition than that at Chowan, gave higher projected yields of peanuts. The rainfall at Chowan in 1968 was extremely low, showing a negative deviation of over 12 in. from the 36-yr mean during the months of July through September. Only a portion of normal peg formation occurred, but those pegs which were set were placed in the ground within a short time; thus, the resultant peanuts matured at approximately the same time. Harvest Date I (9/20) was judged to be moderately mature, while the second date (10/4) was satisfactory and possibly overmature, as the projected yield and flavor score dropped. The Nansemond location experienced adequate rainfall during July, thus allowing pegs to be set over several weeks. Subsequent rainfall was very low. These peanuts were thus not uniform in maturing, and were consequently judged as immature at the first harvest date

Table 1—Agronomic data and flavor evaluation of experimental selection 15714 (1968 season)

Sample	Harvest date	Yield (lb/acre)	Maturity[a]	CLER score	Quality comments	Soil type	1968 Rainfall (in.)	Deviation from 36-yr mean (in.)
Chowan (N.C.)								
1st Harvest (CI)	9/20	2423	Moderate	74	General flavor quality good, nut texture crisp and good; satisfactory for product use	Norfolk fine sand, deep Phase, well-drained, dries out fairly rapidly	July 2.95	−3.89
							Aug. 0.90	−5.38
2nd Harvest (CII)	10/4	2268	Satisfactory	66	Very little off-flavor; "low peanut flavor;" borderline "good peanut flavor"		Sept. 1.69	−3.06
Nansemond (Va.)								
1st Harvest (NI)	9/28	3769	Immature	—[b]	"Low peanut flavor;" borderline "low level off-flavor"	Woodstown fine sandy loam, fairly well-drained, heavier than Chowan, better water-holding capacity	July 7.77	+1.62
2nd Harvest (NII)	10/11	3763	Satisfactory	42	Generally "low peanut flavor;" borderline "low level off-flavor;" not recommended for product use		Aug. 1.69	−4.48
							Sept. 2.45	−2.01

[a]Based on optical density of cold-pressed oil at 450 nm and color of interior surface of pericarp.
[b]Flavor evaluation discontinued due to exceptionally poor quality.

Table 2— Concentrations of sugars[a] appearing in extracts from experimental selection 15714 (1968 season)

Sample	Fructose	Glucose	Inositol	Sucrose
CI	0.68	0.28	6.75	70.3
CII	2.88	0.33	0.49	137.0
NI	1.70	0.94	0.87	48.7
NII	7.49	6.87	1.54	174.0

[a] μm/g fat-free meal.

Table 3—Amino acid composition[a] of samples from experimental selection 15714 (1968 season)

Compound	CI	CII	NI	NII
Lysine	0.88	0.64	0.92	Tr
Histidine	2.00	0.57	1.40	Tr
Arginine	4.31	2.70	1.16	0.58
Tryptophan	1.32	4.43	0.74	0.35
Aspartic acid	4.95	3.09	3.16	0.86
Threonine, Serine, Glutamine, Asparagine	4.77	2.80	3.21	1.60
Glutamic acid	9.97	6.12	5.04	1.99
Proline	2.39	3.80	0.73	Tr
Glycine	1.91	0.72	0.80	0.31
Alanine	3.15	1.36	1.14	0.75
Half Cystine	Tr	Tr	Tr	—
Valine	2.27	0.65	Tr	Tr
Methionine	Tr	Tr	Tr	Tr
Isoleucine	0.62	Tr	0.07	Tr
Leucine	1.40	Tr	Tr	Tr
Tyrosine	0.54	Tr	Tr	Tr
Phenylalanine	2.46	0.88	0.81	Tr

[a] μm/g fat-free meal.

(9/28). At the second date the samples were characterized as "satisfactorily" mature. No flavor score was given the peanuts from the first harvest date due to their exceptionally poor roasted flavor quality; samples from the second harvest date were given a CLER score of only 42.

Sugar concentrations are listed in Table 2. As expected, sucrose was the major saccharide. Fructose and glucose concentrations did not exceed 7.5 μm/g meal. Inositol ranged from 0.5–6.75 μm/g meal. NI, the sample of poorest roasted quality had a sucrose content of less than 50 μ/g; CI, the sample of highest quality exhibited slightly over 70 μm/g meal. Concentrations of sucrose increased between harvest dates, with NII containing slightly less than 175 μm/g. meal. The overall levels of sugars were considerably lower than found by Mason et al. (1969) and Holley and Hammonds (1968). Sucrose hydrolysis has been noted to furnish the reducing sugar components for roasted flavor development (Mason et al., 1969). By virtue of the low flavor: high sucrose content of NII, the flavor potential in these samples must rest primarily elsewhere.

Higher concentrations of certain amino acids have been correlated with "typical" roasted flavor (Newell et al., 1967). These included aspartic acid, asparagine, glutamine, glutamic acid, phenylalanine and histidine. It was not possible to resolve asparagine and glutamine from serine and threonine using the UR-30 resin and the 4-hr hydrolysate technique. An attempt at such by quantitation of aspartic and glutamic acids before and after hydrolysis of purified extracts was not successful, possibly due to presence of peptide material. The four acids are thus expressed as a sum total. Sample CI had a generally higher concentration of all amino acids, but particularly of histidine, aspartic acid, glutamic acid and phenylalanine. The higher concentrations of glutamic acid in CI and CII are of particular interest in that this compound is the most heavily-destroyed amino acid during roasting (Mason et al., 1969). The concentration of phenylalanine, the precursor of the very significant "bouquet" compound phenylacetaldehyde, is particularly high in CI. Mason et al. (1969) noted at least two peptides which were hydrolyzed during roasting and most likely served as amino acid pools for roasted flavor development reactions. The level of these peptides present was positively correlated with the level of phenylalanine. While peptides were not studied herein, the higher concentrations of phenylalanine in samples CI and CII would suggest the more abundant presence of these flavor-precursor peptides. The effect of soil type and moisture is difficult to predict in a study of this brevity. Such would result from extensively-planned plot studies. It should be mentioned, however, that yields in 1969 were again significantly higher at Nansemond (NI-3937 lb/acre, NII-4463, CI-3148, CII-2622). The flavor scores for these samples were NI-59, NII-59; CI-68, CII-71. Rainfall at both locations was somewhat above the total for the 36-yr mean. Thus, the effect of location appears in evidence.

These data are presented to emphasize that the food chemist and plant breeder may, in working together under optimum growing conditions, select a high-yielding, high-flavor potential variety. In the end, however, environmental conditions such as weather and soil type at the planting site may be the primary determinants of year-to-year quality, rather than genetics.

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M. S. BAXTER and A. WALTON
Chemistry Department, University of Glasgow, Glasgow W.2., Scotland, U.K.

A Research Note
CARBON-14 CONCENTRATIONS IN RECENT WINES AND SPIRITS

INTRODUCTION

THIS NOTE represents the results of carbon-14 analyses of wines and spirits and describes a possible application of these data. The naturally-occurring radioactive isotope carbon-14 (radiocarbon) enters all living plant and animal life through photosynthetic activity and the food cycle. The hypothesis was therefore entertained that the concentration of the isotope in wines and spirits should be closely related to the mean atmospheric level during the growth period of the original plant, i.e. grape, barley. The assumptions required by this hypothesis are (1) that plant growth involves rapid uptake of primarily atmospheric carbon and (2) that manufacturing processes do not introduce any significant source of noncontemporaneous carbon. The validity of these assumptions was tested through radiocarbon analyses of post-1950 Scottish malt whiskies and various vintage wines of accurately known age and origin. Close cooperation with manufacturers ensured that samples were derived from single year's plant crops and that only authentic specimens were analyzed.

MATERIALS & METHODS

SINCE 1950 atmospheric carbon-14 levels in the northern hemisphere have been directly measured and shown to follow the irregular profile of the solid line plot in Figure 1 (Broecker and Walton) 1959; Broecker and Olson, 1960; Nydal 1963, 1968). The variability of post-1955 atmospheric carbon-14 levels is due to sporadic release of large quantities, about 10^{29} atoms, of artificial carbon-14 from nuclear weapon tests. In the figure, carbon-14 activities are expressed as the percentage deviation from the natural level which is therefore represented as zero on this scale. Atmospheric concentrations shown for each year are averaged over the plant "growth" period (spring to summer) and the corresponding "winter" period with brackets indicating the overall activity range during the growth season. Since with few exceptions, the excess carbon-14 concentration in the atmosphere was different for every year, analyses of wines and spirits permitted a reliable assessment of these materials as short-term indicators of atmospheric levels of the isotope.

The "boxes" shown in the plot represent the radiocarbon levels in the nuclear era samples such that their ordinates represent the standard deviations associated with the measurements. The radiocarbon analytical techniques based on methods originally outlined by Libby (1955) and Arnold (1954), are described in full elsewhere (Baxter 1969; Baxter et al., 1969). About 40 ml of each sample was distilled to 10 ml of alcohol/water mix to remove coloring matter prior to standard conversion to methane and gas proportional counting for at least 20,000 counts in a 0.5 liter detector. A number of data points derived from analyses by L'Orange and Zimen (1968) are included in the figure. Excellent agreement is observed between atmospheric and sample carbon-14 levels. Indeed for several preliminary whiskey samples, analyses were completed without prior knowledge of the sample ages (Walton et al., 1967). By fitting each sample carbon-14

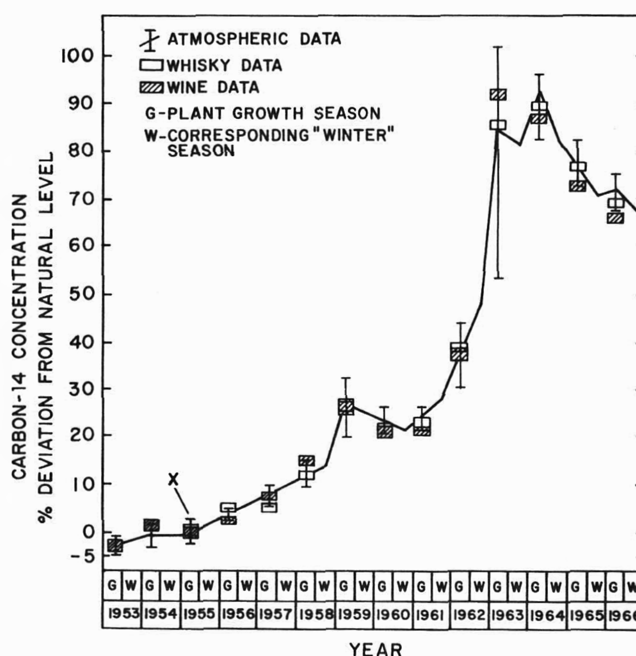

Fig. 1—Correlation between carbon-14 concentrations in atmospheric samples and those in whiskies and wines.

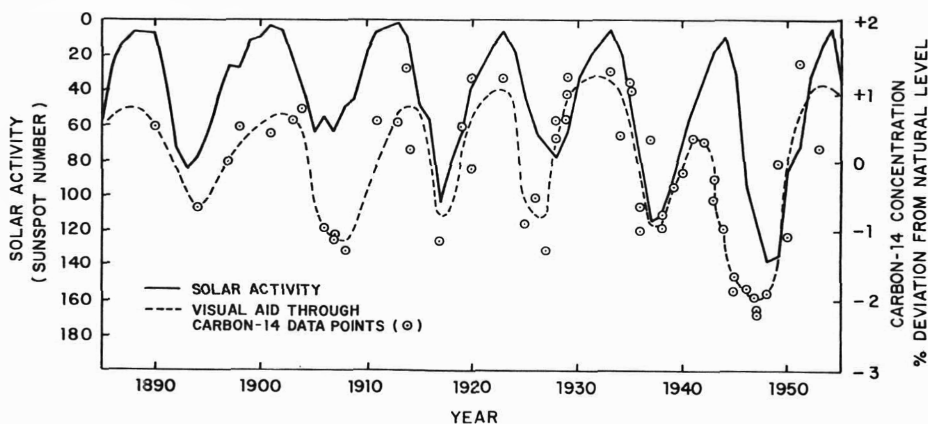

Fig. 2—Natural atmospheric carbon-14 concentrations (1890-1950).

activity onto the atmospheric plot to attain best agreement a probable year of barley growth was allocated. In all cases the carbon-14 age agreed with the true age. The experimental data therefore confirm that malt whiskies and vintage wines are accurate measures of atmospheric carbon-14 levels and that samples of unknown age from the nuclear era may be dated using this correlation. For samples of certain consecutive growth years, however, e.g. 1956 and 1957, a unique age assignment is difficult as atmospheric carbon-14 levels did not change significantly during these years. One sample of interest (sample X in Fig. 1) was a blend of predominantly three individual whiskies: some 1939, some 1951 but mainly 1955. That this sample was attributed a 1955 growth year implies that the method is sufficiently sensitive to detect the major component of blended spirits of certain compositions.

The correlation between atmospheric and alcohol carbon-14 activities permitted the use of these sample types to measure atmospheric carbon-14 levels during the unstudied period 1890-1950. By analyses of a large number of French and Portuguese wines and Scottish whiskies and correction of carbon-14 activities for radioactive decay and contamination by industrial fossil fuel dilution, the natural carbon-14 profile for this period was obtained (Fig. 2). A highly significant fluctuation of atmospheric carbon-14 concentrations was observed in direct anticorrelation with solar activity. Thus analyses of wines and spirits of accurately known age have enabled detection of an effect which has proved to be of importance both to a geophysical/meteorological understanding of the dynamic carbon cycle and also to the radiocarbon dating method used in archaeology. The details of these implications are discussed elsewhere (Baxter 1969).

CONCLUSIONS

IN CONCLUSION one markedly anomalous result should be mentioned: A supposedly 1918 wine sample had a carbon-14 content greater than 90% above the known 1918 atmospheric level and typical of 1963 activities. Since sample concentration to this degree was not feasible it seems likely that the discrepancy was the result of mistaken identity of the sample. This example further illustrates the value of carbon-14 analysis as a dating technique and underlines its application to the detection of illicit blenders and manufacturers.

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S. M. TANDON and K. G. GOLLAKOTA
Department of Microbiology, U.P. Agricultural University, Pantnagar, India

A Research Note
CHEMICALLY DEFINED LIQUID MEDIUM FOR THE GROWTH AND SPORULATION OF *Bacillus stearothermophilus* 1518 ROUGH VARIANT

INTRODUCTION

SO FAR, only complex liquid and solid media have been reported for sporulation of *Bacillus stearothermophilus* (Long and Williams, 1960; Downey, 1962; Kim and Naylor, 1966; Yao and Walker, 1967; Thompson and Thames, 1967; Hill and Fields, 1967). Therefore, an attempt was made to develop a chemically defined liquid medium which could support germination, vegetative growth and sporulation of *B. stearothermophilus* 1518 rough variant.

MATERIALS & METHODS

Organism

B. stearothermophilus 1518 rough variant was supplied by the USDA, Washington, D.C. and maintained on nutrient agar slants.

Medium

The composition of the medium (V-medium) is as follows: 0.1g glucose, 0.0105g L-arginine HCl, 0.0045g L-histidine, 0.0192g L-leucine, 0.014g DL-isoleucine, 0.006g L-methionine, 0.0144g L-valine, 0.238g L-glutamic acid monosodium salt, 0.292g L-lysine HCl, 0.00005g $FeSO_4 \cdot 7H_2O$, 0.0005g $CuSO_4 \cdot 5H_2O$, 0.005g $MnSO \cdot H_2O$, 0.0005g $ZnSO \cdot 7H_2O$, 0.02g $MgSO_4 \cdot 7H_2O$, 0.2g $(NH_4)_2SO_4$, 0.008g $CaCl_2 \cdot 2H_2O$, 0.05g K_2HPO_4, 15 μg thiamine HCl, 150 μg nicotinic acid, and 1 μg biotin in 100 ml of distilled water (final concentration).

Stock solutions of sodium glutamate, glucose, phosphate, $CaCl_2$, amino acids and vitamins were sterilized separately at 15 lb pressure for 15 min and stored aseptically at low temperature. The stock mineral solution was diluted 1:100 with distilled water, after making allowances for the subsequent addition of ingredients, and autoclaved. To this sterile mineral mixture, 1 ml of each of the stock nutrient solutions was added to give 100 ml of the medium. The volume of the medium in the flask was kept at 10% of the total volume of the flask. The final pH of the medium was 6.75 without adjustment.

Cultivation of the organism

The active culture technique of Collier (1957) and Halvorson (1957) was used with suitable modifications. To 9 ml of medium was added 1 ml of spore suspension (10^6/ml) heat shocked at 110°C for 10 min. This starter culture was incubated at 60°C ± 1° in a reciprocating water-bath shaker with 155 strokes per min. After good growth, as evidenced by a significant lowering of the pH, a 10% transfer was made into fresh medium. Routinely 3 such transfers were made into fresh medium (Master culture). The first transfer was made after 8 hr and subsequent transfers after every 2 hr incubation. The time of the last transfer was taken as zero hr.

At periodic intervals the following determinations were made on aliquots of the master culture: optical density (Spectronic-20-440 mμ); pH—Metrohm AG (Swiss) pH meter; and extent of sporulation by examining samples either stained by aqueous crystal violet or by using Wirtz's technique as modified by Bartholomew and Mittwer, (1950).

At the end of the experiment, the following determinations were also made on the master culture. Total viable cells/ml (TVC-spores and vegetative cells) were made by plating appropriate dilutions in nutrient agar. Octyl stable cells/ml (OSC-total spores) were obtained by treating a 1:100 dilution of culture with octyl alcohol (0.05 ml/10 ml) for 15 min with vigorous shaking at 5 min interval, then plating appropriate dilutions on nutrient agar. Heat stable cells/ml (HSC-heat stable spores only) were obtained by heating the appropriate dilution sample at 110°C for 10 min prior to plating. The plates were incubated at 60°C for 24-36 hr and the colonies counted.

RESULTS & DISCUSSION

IN THE MEDIUM consisting of minerals of modified G-medium (Gollakota and Halvorson, 1960), arginine, histidine, leucine, isoleucine, methionine, valine, biotin, nicotinic acid and thiamine, growth was good but sporulation was poor (10-20%) (O'Brien and Campbell, 1957). Addition of glutamate increased the sporulation to about 80-90%. However, active culture could not be built up in either of the above media. They supported rapid germination and good vegetative growth of the starter culture, but growth was slowed down when transfers were made into fresh media. This suggested a requirement of some substance for growth. When autoclaved spore extract was added to the above media, active cultures could be obtained in the normal way. Lysine was found to be a satisfactory substitute for the autoclaved spore extract.

Although 55°C was reported as optimum for the growth of this organism, in our experience when the organism was grown at 55°C, the timings for the first and subsequent transfers were 10 and 3 hr respectively. Also sporulation took more than 30 hr for completion. In an effort to reduce the time needed for completion of sporulation, higher temperatures of incubation were tried.

Table 1—Growth and sporulation of B. stearothermophilus 1518 in V-medium

Age of culture in hr	pH	O.D.	Slide studies
0.00	6.85	0.16	Vegetative cells in chains of two cells.
1.00	6.55	0.42	—
1.30	6.35	0.52	—
2.00	5.5	0.80	—
2.15	5.2	0.95	—
2.30	5.1	0.95	—
3.00	5.2	1.092	—
3.15	5.35	1.05	Vegetative cells in chains of 2-4 cells.
6.00	7.1	1.15	Vegetative cells in chains of 2 cells and bulging starts at each end.
7.00	7.40	1.28	Clear endospores start appearing.
8.00	7.9	1.5	—
20.00	8.3	2.0	60-70% endospores. 30-40% free spores.
24.00	8.4	2.0	90-95% free spores.
Plate counts after 24 hr		TVC/ml	1.24×10^8
		OSC/ml	1.29×10^8
		HSC/ml	1.28×10^8

Incubation at 60°C considerably speeded up growth and sporulation without any adverse effect on maximum growth. Hence the organism was routinely incubated at 60°C.

The growth characteristics of the master culture are given in Table 1. The pH steadily fell reaching a minimum about 2-1/2 hr; it then started to rise, reaching neutrality by the 6th hr. The pH after 24 hr of incubation was 8.4.

The O.D. rose steadily till 2-1/2 hr. After remaining nearly stationary for 1 hr, the O.D. started to rise sharply again and almost doubled itself by the end of the experiment.

Granulation and bulging of one end of the cells could be seen after the 6th hr of incubation and endospores started to appear by the 7th hr. Sporulation was completed by the 24th hr.

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A RECOMMENDATION BY THE
IFT Committee on Sensory Evaluation

AUTHOR GUIDELINES for IFT Research Papers Reporting SENSORY EVALUATION DATA

☐ THESE GUIDELINES have been prepared for use by persons writing research articles which include sensory evaluation data. While they may be helpful to authors preparing papers concerned with new metbods (sensory or statistical), it is recognized that these authors may want to report their work in greater detail than suggested by these guidelines.

The value of these guidelines is fourfold: (1) They will make preparation by the author easier in that he would have a checklist as a guide; (2) they will facilitate the review of a technical manuscript prior to its acceptance for publication; (3) the likelihood of changes by the author would be lessened; and (4) they could lead to consideration of the checklist factors in the planning of the experiment.

The *IFT Style Guide for Research Papers*[a] provides instructions for the preparation of research papers submitted for publication. In line with the Style Guide specifications, "Sensory Evaluation" procedures should be the "Experimental" section of the paper.

The IFT Style Guide specifies that the author should "provide sufficient detail of the method and equipment to allow the work to be repeated." Methods for which there are adequate references need not be described in detail in the paper, but the proper references should be cited.

It is recommended that the following guidelines be used as a check on content—rather than as a specific format.

[a] Food Technology 21: 483–486; revised 1970. Copies of Revised Style Guide available from: Director of Publications, Institute of Food Technologists, Suite 2120, 221 N. LaSalle St., Chicago, IL 60601 USA.
[b] 1964. Sensory testing guide for panel evaluation of foods and beverages. IFT Committee on Sensory Evaluation. Food Technology 18(8): 25–43.

—GUIDELINES—

Checklist for Content

METHOD USED FOR SENSORY EVALUATION[b]

1—Single sample
2—Paired comparison
3—Duo-trio
4—Triangle
5—Rank order
6—Scalar difference from control
7—Scalar scoring
8—Scalar hedonic
9—Descriptive analysis
10—Sensitivity
11—Other (specify)

PANEL

1—Type
  (a) Source (in house, recruited from outside organization)
  (b) Consumer
  (c) Experienced (degree and type)
2—Selection criteria (physiological acuity, product usage, etc.)
3—Composition (age, sex)
4—Changes in panel members (if any)

MATERIAL EVALUATED

1—Preparation
  (a) Boiling, baking, broiling, opening fresh container, etc.
  (b) Carrier (if used)
2—Presentation
  (a) Coding
  (b) Serving order (randomized/nonrandomized)
  (c) Sample size
  (d) Temperature and method of control
  (e) Sample container and utensils used
  (f) Time of day
  (g) Special instructions to panelists
  (h) Special conditions (time interval, mouth rinsing, use of crackers or other materials, samples swallowed or expectorated, etc.)

STATISTICAL DESIGN

1—Type of experiment (Randomized block, Latin square, Factorial, Confounded, Split plot, Incomplete block, or other)
2—Replications

ENVIRONMENTAL CONDITIONS

(Booth, store, home, bus, isolated, presence or absence of distractions, temperature, lighting control, special conditions)

DATA ANALYSIS

1—State how reported data were derived from actual responses (assignment of numerical scores to rating scale points, proportion of choice in paired comparison, average ranks, etc.)
2—Identify type of analysis used and degree to which underlying assumptions are met

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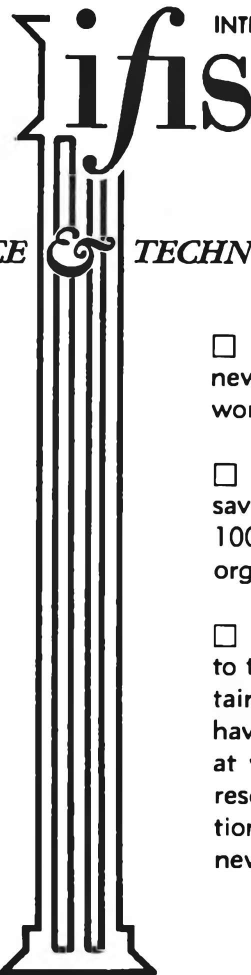

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