3 March-April 1973
Volume 38 : Number 3
Coden: JFDSAZ 38(3) 369-552 (1973)

JOURNAL of FOOD SCIENCE

BASIC SCIENCE

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IFT *A PUBLICATION OF THE INSTITUTE OF FOOD TECHNOLOGISTS*

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APPLIED SCIENCE and ENGINEERING

RESEARCH NOTES

March-April 1973
Volume 38 : Number 3

JOURNAL of FOOD SCIENCE

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 JOURNAL OF FOOD SCIENCE (formerly *Food Research*) is published 7 times a year (bimonthly except during January and February) by Institute of Food Technologists, Suite 2120, 221 N. LaSalle Street, Chicago, Illinois 60601 USA. Printed in USA. Second class postage paid at Chicago, Ill. and at additional mailing offices.

ABSTRACTS:
IN THIS ISSUE

A STATISTICAL APPROACH TO THE SUBJECTIVE AND OBJECTIVE MEASUREMENTS OF ODORS INDUCED BY γ-IRRADIATION OF BEEF FAT. N. KOSARIC, T.B. DUONG & W. Y. SVRCEK. *J. Food Sci.* **38**, 369–373 (1973)–Beef fat, extracted from commercial beef, was subjected to γ-irradiation and the intensity of the odor induced by irradiation was studied. A statistical approach was developed to evaluate the organoleptic data obtained by taste panel evaluation and to correlate this data with the results obtained by chromatographic analysis of the irradiated samples. For this purpose, a step-wise multiple regression analysis was developed and this technique was analyzed for validity and applicability.

γ-IRRADIATION OF BEEF FAT. EFFECTS ON ODOR INTENSITY AND RANCIDITY. N. KOSARIC, T.B. DUONG & W.Y. SVRCEK. *J. Food Sci.* **38**, 374–376 (1973)–Odor intensity and rancidity of irradiated beef fat were studied at different experimental conditions. Temperature was found to be an important factor in reducing the odor intensity and in increasing the stability of fat. There exists a correlation between the odor intensity and the peroxide value of fat if antioxidants are not incorporated. The addition of antioxidants protects the fat from induced odor and the rancidity developed during and after irradiation.

AROMA OF CANNED BEEF: GAS CHROMATOGRAPHIC AND MASS SPECTROMETRIC ANALYSIS OF THE VOLATILES. T. PERSSON & E. von SYDOW. *J. Food Sci.* **38**, 377–385 (1973)–Volatile compounds in the headspace gas of canned minced beef products were determined using both a sampling technique and an open tubular column gas chromatographic technique, making it possible to analyze large volumes (up to 500 ml), and identified on a combined gas chromatograph-mass spectrometer, 95 compounds were identified. Among these 21 were sulfur compounds, 12 aldehydes, 16 ketones and 11 furans. No new compounds were formed or eliminated when fat and/or carbohydrate were added or when the heating time was varied, but there were large quantitative differences. On increasing heating time, concentrations of sulfur compounds and some aldehydes increased markedly, particularly hydrogen sulfide, methyl mercaptan, ethyl mercaptan, 2-methyl propanal, 2-methyl butanal and 3-methyl butanal. These compounds were present in large amounts ranging from 100–6000 times the corresponding odor threshold values. In general, concentration in the headspace gas decreased when fat was included in the formulation. Concentration for some of the compounds increased when carbohydrate was added but the influence was less pronounced than when fat was used.

AROMA OF CANNED BEEF: SENSORY PROPERTIES. T. PERSSON, E. von SYDOW & C. ÅKESSON. *J. Food Sci.* **38**, 386–392 (1973)–Triangle tests, preference tests and assessment of the intensity fo 28 odor qualities were performed by trained panelists on canned beef differing in composition and heating times. A specially designed statistical procedure showed that most of the odor qualities could be measured by uni-dimensional scales. Introducing a standard reference sample improved the panel's discrimination and reproducibility. Increasing heating time resulted in beef with increasing intensities of heat-related terms, such as "retort flavor, canned beef off-flavor" and "burnt, smoky," while odor qualities which decreased included "like blood, raw meat." The addition of fat decreased odors associated with heat treatment and increased "sickly," "musty, mouldy," and "oily, fatty" odors. Addition of starch caused reductions in intensities of most odors. Correlation coefficients between the 28 odor qualities are given for all samples.

ISOLATION AND IDENTIFICATION OF VOLATILE FLAVOR COMPOUNDS IN BOILED BEEF. C. HIRAI, K.O. HERZ, J. POKORNY & S.S. CHANG. *J. Food Sci.* **38**, 393–397 (1973) Volatile flavor compounds were isolated from boiled beef by flash evaporation and vaporization from a continuous thin heated film under vacuum. The beef used was the semitendinous muscles from steers of the same feeding lot, with known history. The isolated volatile flavor compounds were separated into broad fractions by gas chromatography. Two of these broad fractions had an aroma reminiscent of that of boiled beef. The broad fractions were further separated by repeated gas chromatography into pure components with the use of different stationary phases. The pure gas chromatographic fractions were then identified by infrared and mass spectrometry. The identification was finally confirmed with the use of authentic compounds. A total of 54 compounds (seven hydrocarbons, 10 alcohols, one ester, one ether, one lactone, nine aldehydes, five ketones, three acids, four sulfides, seven aromatic compounds and six heterocyclic compounds) was identified. Many of the compounds identified have not been previously reported as components of boiled beef flavor.

THE INHIBITION OF WARMED-OVER FLAVOR IN COOKED MEATS. K. SATO, G.R. HEGARTY & H.K. HERRING. *J. Food Sci.* **38**, 398–403 (1973) Retorted beef, pork and turkey meats do not develop warmed-over flavor (WOF). An active substance(s) is produced during heat treatment which has inhibitory or antioxidative properties. The inhibitor is not developed in meat which has been extracted with water prior to retorting, nor is the inhibitor present in retorted beef which has been water extracted. It is suggested that a nonenzymatic browning-type reaction occurs during retorting of meat which results in formation of reducing-type substances which act as inhibitors of WOF.

INFLUENCE OF pH AND FIBER CONTRACTION STATE UPON FACTORS AFFECTING THE TENDERNESS OF BOVINE MUSCLE. P.E. BOUTON, F.D. CARROLL, P.V. HARRIS & W.R. SHORTHOSE. *J. Food Sci.* **38**, 404–407 (1973) Cold shortened and stretched bovine muscles, with ultimate pH values ranging from 5.4–7.0, have been obtained by using controlled pre-slaughter injections of adrenaline. Mechanical measurements have been used to show that connective tissue strength, as well as shear force, was increased as fiber contraction increased. The effect of contraction on tenderness was reduced as pH and water-holding capacity increased. It has also been demonstrated that muscles containing predominantly long sarcomeres have better moisture retention properties and contract significantly more with cooking than other samples containing mainly shorter sarcomeres.

FATTY ACID COMPOSITION OF BOVINE INTRAMUSCULAR AND SUBCUTANEOUS FAT AS RELATED TO BREED AND SEX. A.T. GILLIS, N.A.M. ESKIN and R.L. CLIPLEF. *J. Food Sci.* **38**, 408–411 (1973) 36 animals representing six crossbreeds and two sexes were used in this study. The breeds represented the F_1 generation produced by crossing Limousin and Simmental bulls with Hereford, Shorthorn and Aberdeen Angus cows. Intramuscular fat from the longissimus and biceps femoris muscles as well as a portion of the corresponding subcutaneous fat were extracted by the chloroform-methanol method of Bligh and Dyer. Methyl esters of the total intramuscular and subcutaneous fat were separated by gas liquid chromatography. Bulls had significantly ($P < 0.05$) more C14:0 and C18:2 and significantly ($P < 0.05$) less C18:1 than steers in both subcutaneous and intramuscular lipids. A further significant ($P < 0.05$) sex effect was evident for C16:0 in the subcutaneous lipid and

C16:1 in the intramuscular lipid. There were no other significant sex differences among the six major fatty acids. A significant ($P < 0.05$) breed difference was observed for C14:0, C16:1 and C18:0 in both the intramuscular and subcutaneous lipids. A further significant ($P < 0.05$) breed effect for C16:0 was found only in the subcutaneous lipid. Significant ($P < 0.05$) sex × location interactions were observed for C16:0, C18:0 and C18:2 together with a crossbreed × sex × location interaction for C14:0 in the intramuscular lipid. In the subcutaneous lipid significant ($P < 0.05$) sex × location and crossbreed × sex interactions were observed for C16:0 together with crossbreed × sex and crossbreed × location interactions for C16:1.

RADIANT ENERGY-INDUCED CHANGES IN BOVINE MUSCLE PIGMENT. C.S. SETSER, D.L. HARRISON, D.H. KROPF & A.D. DAYTON. *J. Food Sci.* 38, 412–417 (1973)–Loss of red color (MbO_2) in bovine semitendinosus muscle during 3-hr exposure to 254, 405 and 577 nm of radiant energy in 100, 20 and 0% oxygen was followed by spectral reflectance. In 100% oxygen, differences in metmyoglobin between control (20% O_2, dark) and exposed samples were not significant. In 20 and 0% oxygen, differences between control and exposed samples generally were significant at 474, 525, 571, 600, 630, 650 and 685 nm; visual color scores indicated those differences were not of practical importance. Radiant energy per se at 254, 405 or 577 nm did not cause major discoloration of muscle.

EFFECT OF LIGHT, pH AND BUFFER STRENGTH ON THE AUTOXIDATION OF PORCINE, OVINE AND BOVINE MYOGLOBINS AT FREEZING TEMPERATURES. N.Y. ZACHARIAH & L.D. SATTERLEE. *J. Food Sci.* 38, 418–420 (1973)–The stability of the oxymyoglobins isolated from bovine, ovine and porcine muscle was studied with respect to freezing temperature, light, pH and buffer strength. At freezing temperatures, both buffer strength and short wavelength fluorescent light affect the autoxidation of all three oxymyoglobins. Storage temperature also affects the oxymyoglobin stability, with temperatures of −11 to −12°C having the greatest effect. The information described in this study indicates that the preservation of red color in beef, lamb and pork can be enhanced by storage in the absence of fluorescent light and at temperatures below −11 to −12°C.

EFFECT OF POSTMORTEM MUSCLE CHANGES ON POULTRY MEAT LOAF PROPERTIES. F.B. WARDLAW, L.H. McCASKILL & J.C. ACTON. *J. Food Sci.* 38, 421–423 (1973)–Pectoralis muscle tissue excised from 8-wk and 10-wk old broilers showed a significant ($P < 0.05$) decrease in muscle pH and myosin extractability during 0–24 hr postmortem aging. Extractable actomyosin and muscle water-holding capacity significantly increased while sarcoplasmic and nonprotein fractions remained constant during this same aging period. Loaves prepared with the meat at each postmortem period showed no change in cooking loss or binding strength during the 24 hr of aging. Loaves from muscle tissue frozen after 24 hr aging had significantly less cooking loss and a higher binding strength in comparison to loaves at prerigor intervals (0–6 hr). Shear values of loaf cubes were significantly ($P < 0.05$) lower for loaves of postrigor tissue and those prepared with muscle tissue of 8-wk old broilers.

EFFECT OF BINDING TO MUSCLE PARTICULATE FRACTIONS ON GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE ACTIVITY. S.M. DAGHIR & H.O. HULTIN. *J. Food Sci.* 38, 424–425 (1973)–Under certain conditions, glyceraldehyde-3-phosphate dehydrogenase of chicken breast muscle binds to the particulate fraction of the homogenized tissue. When binding occurs, the kinetic properties of the enzyme can be markedly changed. With the experimental conditions used in this study, the maximal velocity of glyceraldehyde-3-phosphate dehydrogenase (5 μM at pH 6.5) decreased from 29 nmoles of NAD^+ reduced per sec for the soluble enzyme to 7 nmoles per sec for the bound. The K_M (glyceraldehyde-3-phosphate) decreased from 0.2 mM for the soluble to 0.07 mM for the bound enzyme. Thus, the bound enzyme retains a greater proportion of its activity at low substrate concentrations than does soluble glyceraldehyde-3-phosphate dehyrogenase. Substrate inhibition of glyceraldehyde-3-phoshate dehydrogenase, which is marked for the soluble enzyme, is eliminated by binding of the enzyme to the subcellular particulate fraction. The changes in kinetic properties observed could have important implications in studies utilizing homogenized muscle tissue.

CONTROL OF Staphylococcus aureus IN SAUSAGE BY STARTER CULTURES AND CHEMICAL ACIDULATION. C. DALY, M. LA CHANCE, W.E. SANDINE & P.R. ELLIKER. *J. Food Sci.* 38, 426–430 (1973)–Three commercially available starter cultures were examined for their ability to control *S. aureus* during sausage fermentation at 21°C, 30°C and 37°C. The three starters contained *Pediococcus cerevisiae* or *Lactobacillus plantarum* and a mixture of the two. These cultures inhibited 30,000–190,000 initial *S. aureus*/g greater than 99% after 50 hr of incubation at 37°C; inhibition of 30,000/g was more than 99% and 97% at 30°C and 21°C, respectively. Chemical acidulation (glucono-delta-lactone plus citric acid) yielded good initial control of *S. aureus* but extensive growth of the pathogen occurred by 50 hr. A combination of starter cultures and chemical acidulation gave approximately 99.99% inhibition of *S. aureus*.

BIOCHEMICAL AND MICROBIAL STUDIES ON SHRIMP: VOLATILE NITROGEN AND AMINO NITROGEN ANALYSIS. B.F. COBB III, I. ALANIZ & C.A. THOMPSON JR. *J. Food Sci.* 38, 431–436 (1973)–Analysis of trimethylamine (TMN), dimethylamine (DMN) and total volatile nitrogen (TVN) by the Conway microdiffusion procedure was investigated. Interference of NH_3 and DMN in TMN analysis was reduced by the use of a saturated Na_3PO_4 · KOH solution as releasing agent rather than K_2CO_3 which is usually employed. The following analytical procedures were developed: (1) a procedure for simultaneous analysis of DMN and TMN; (2) a method for obtaining TVN values reflective of NH_3 + TMN content of shrimp; (3) a rapid method of estimating TVN in shrimp extracts; and (4) a rapid and simple colorimetric test for amino nitrogen (AA-N). TVN/AA-N ratios appeared to be more reflective of shrimp quality than TVN alone.

SENSITIVITY OF Vibrio parahaemolyticus TO COLD IN OYSTERS, FISH FILLETS AND CRABMEAT. H.C. JOHNSON & J. LISTON. *J. Food Sci.* 38, 437–441 (1973)–The halophilic food poisoning organism *Vibrio parahaemolyticus* dies off rapidly in laboratory media when held at refrigerator temperatures or frozen. Preliminary studies have indicated that the organism may survive better in food stuffs. Strains of *V. parahaemolyticus* and the related organism *V. alginolyticus* were inoculated into shucked oysters, picked crabmeat and fish fillets which were packaged in Polymylar pouches. Depurated shellstock oysters were also contaminated directly by exposing them to seawater containing *V. parahaemolyticus*. Oysters were then shucked aseptically and packaged in Polymylar pouches. Oysters were stored at 11°, 8°, 5°, 1°, −15° and −30°C, and fish fillets and crabmeat at 1°, −15° and −30°C. Samples of all lots were removed from storage at intervals and surviving vibrios enumerated, using selective and nonselective media. Counts decreased during storage, but viable organisms could be detected up to 40 days at 1°C and 130 days (the end of the testing period) at −15° and −30°C in oysters. In fish fillets, viable organisms were detectable after approximately 60 days at −15° and −30°C and after 15 days at 1°C. In crabmeat, viable organisms could be recovered after approximately 30 days at 1°, −15° and −30°C. *V. parahaemolyticus* can thus be expected to survive in contaminated seafoods during normal conditions of commercial handling.

VITAMIN E CONTENT OF SELECTED BABY FOODS. K.C. DAVIS. *J. Food Sci.* 38, 442–446 (1973) Strained baby foods were assayed by Florisil column chromatography and paper chromatography or by thin-layer chromatography on silica gel to differentiate the forms of tocopherol present. The spots were eluted and measured colorimetrically using $FeCl_3$ and 2,2'-bipyridine. Fruits and desserts contained 0.17–1.33 mg alpha-tocopherol/100g. Spinach contained 1.55 mg alpha-tocopherol/100g. Other vegetables contained from 0.12–0.84 mg/100g. In fruits and vegetables the ratio of vitamin E to fat met or exceeded the National Re-

ABSTRACTS:
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search Council recommendation. Meats, high-meat dinners, and egg yolks had from 0.13–0.73 mg/100g. The mixed dinners and breakfasts contained from 0.12–0.36 mg/100g. The meat products, mixed dinners and breakfasts had borderline ratios of vitamin E to fat. These values compared favorably with reported vitamin E values for similar raw, frozen, or canned products. Spinach, squash, sweet potatoes, apricots and peaches are very good sources of vitamin E in terms of both a large quantity of vitamin E and a high vitamin E to fat ratio.

FLUOROMETRIC DETERMINATION OF VITAMIN A IN FOODS. J.W. ERDMAN JR., S-H.F. HOU & P.A. LACHANCE. *J. Food Sci* 38, 447–449 (1973)–The official AOAC method for vitamin A analysis is the colorimetric Carr-Price reaction. This method is considered by many investigators to be insensitive and nonspecific because a number of non-vitamin A compounds interfere with the colorimetric procedure. This paper describes a modification of the fluorometric determination of vitamin A in human blood and liver (J.N. Thompson et al., Biochem. Med. 5:67, 1971) for use in food analysis. Food samples are ground, saponified, homogenized and extracted with hexane for direct fluorometric measurement. The method is accurate, sensitive and specific for vitamin A when the appropriate correction formula for phytofluene is utilized. Vitamin A has been satisfactorily assayed in several types of foods, including: cereals, margarines, instant breakfast powders and fortified entrees.

SOME MINOR VOLATILE COMPONENTS FROM THERMALLY DEGRADED THIAMINE. B.K. DWIVEDI, R.G. ARNOLD & L.M. LIBBEY. *J. Food Sci.* 38, 450–452 (1973)–Thiamine solutions adjusted to pH 5.0, 6.0, 7.0 and 8.4 with 1N NaOH were autoclaved in sealed bottles for 1 hr at 121°C. Heated thiamine solutions were extracted with ethyl ether. Ether extracts were subjected to gas chromatography-rapid scan mass spectrometry. On the basis of gas chromatographic and mass spectral data, 2-methyl thiophene, 4,5-dihydro-2-methyl thiophene and 4-methyl-5-(β-hydroxyethyl) thiazole, which had previously been reported in thiamine degradation studies, were identified. Additionally, 2-methyl sulfide-5-methyl furan, 4,5-dimethyl thiazole, 2-methyl-3-oxy-tetrahydrothiophene, and 2-acetyl tetrahydrothiophene were tentatively identified. These compounds had not been reported previously.

SOLANUM ALKALOIDS: BIOSYNTHESIS AND INHIBITION BY CHEMICALS. S.J. JADHAV, D.K. SALUNKHE, R.E. WYSE & R.R. DALVI. *J. Food Sci.* 38, 453–455 (1973)–Incorporation of radioactive carbon from β-hydroxy-β-methylglutaric acid (HMG)-3-^{14}C, L-leucine-U-^{14}C, L-alanine-U-^{14}C and D-glucose-U-^{14}C into the glycosidic steroidal alkaloids, solanine and chaconine of potato sprouts, was 9, 15, 24 and 20 times less than that of mevalonic acid (MVA)-2-^{14}C, respectively. The efficiency ratio revealed that HMG was incorporated via acetate or acetoacetate. The distribution of radioactivity originated from D-glucose-U-^{14}C was nearly 9 times higher in the glycoside moiety than that in the aglycone part of the glycoalkaloids. Apparently, Alar® (succinic acid, 2,2-dimethylhydrazide), Ethrel® (2-chloroethylphosphonic acid) and Telone® (1,3-dichloropropene and related chlorinated hydrocarbons) significantly reduced the rate of incorporation of HMG into the alkaloids.

PHYSICAL, RHEOLOGICAL AND CHEMICAL PROPERTIES OF BANANAS DURING RIPENING. R.J. CHARLES & M.A. TUNG. *J. Food Sci.* 38, 456–459 (1973)–During ripening at 16 ± 1°C and 25 ± 1°C bananas were compared with regard to peel color, pulp-to-peel ratio, tissue strength, softening, sugars and starch content. At the higher temperature ripe fruit failed to develop a full yellow color. Pulp tissue became increasingly soft throughout ripening at 25°C, while at 16°C softening increased during initial ripening then remained relatively constant. Total and reducing sugars tended to be lower whereas starch content was generally higher in fruit ripened at 16°C. Good correlations among rheological and chemical properties were obtained at both ripening temperatures. The effects of ripening temperatures on the quality of bananas are discussed.

EFFECT OF METAL IONS ON THE COLOR OF STRAWBERRY PUREE. R.E. WROLSTAD & J.A. ERLANDSON. *J. Food Sci.* 38, 460–463 (1973)–Anthocyanin concentration and Hunter color difference meter (CDM) values were measured for strawberry puree containing tin and aluminum salts at 50°C. Red color was stabilized by aluminum, stannous and stannic ions; however, anthocyanin stability was not improved. Strawberry puree containing stannous ions remained purplish-red after treatment with bisulfite whereas the control was readily bleached. Strawberry juice treated with stannous chloride formed a red precipitate; the supernatant had an anthocyanin concentration of the same order as the control juice. The results suggest that color stability of stannous-treated puree is due to interaction of metal ions with constituents other than anthocyanins. The phenomenon may be similar to that occurring in canned pears where leucoanthocyanin oxidation intermediates form insoluble colored complexes with tin.

ION EXCHANGE PURIFIED ANTHOCYANIN PIGMENTS AS A COLORANT FOR CRANBERRY JUICE COCKTAIL. C.D. CHIRIBOGA & F.J. FRANCIS. *J. Food Sci.* 38, 464–467 (1973) Anthocyanin and flavonol pigments recovered from cranberry press cake were used to color samples of pale cranberry juice cocktail. Color stability was not significantly changed by the addition of pigment. The taste of added pigment at low levels was difficult to detect and the taste threshold level was found to be 29 mg of crude pigment per 100 ml cocktail. These pigments appeared to be suitable for coloring cranberry juice cocktail as well as other food products where a red color is desired.

CONTINUOUS DIFFUSION OF CHLOROGENIC ACID FROM SUNFLOWER KERNELS. F.W. SOSULSKI, M.A. SABIR & S.E. FLEMING. *J. Food Sci.* 38, 468–470 (1973)–Continuous diffusion of chlorogenic acid from sunflower kernels was more rapid than consecutive batch extractions with 0.001N HCl. However, over 4 hr of continuous extraction at 80°C was required to produce a light-colored meal when adjusted to pH 10. Diffusion rates were not greatly enhanced by increasing the solvent-to-kernel ratio but the diffusion time was reduced to one-half by pearling the kernels. A split kernel technique for testa removal is recommended for rapid diffusion under high or low temperature conditions. Losses of oil from diffusion extraction and testa removal were negligible but the yields of meal were reduced from 45 to 30g/100g of kernels. However, this product contained 70% protein and would qualify as a protein concentrate.

METHIONINE SUPPLEMENTATION OF SOY MILK TO CORRECT CYSTINE LOSS RESULTING FROM AN ALKALINE SOAKING PROCEDURE. A.F. BADENHOP & L.R. HACKLER. *J. Food Sci.* 38, 471–473 (1973) A study was conducted to evaluate the use of methionine supplementation for improving the nutritive value of alkaline-extracted soy milk. Samples of soy milk of four different pH's were prepared by soaking soybeans in water and in three conc of NaOH; half of each sample was heat-processed for 10 min at 121°C. Amino acid analy-

ses showed cystine was the only amino acid which decreased with pH in both the unprocessed and processed samples (15 and 25%, respectively, at pH 8.97) while tryptophan decreased 14% at pH 8.97 in only the heat-processed soy milk. Animal feeding studies using the soy milk as the protein source indicated supplementation with 0.35% L-methionine improved nutritive value in both the heat-processed and nonprocessed samples. The PER of nonprocessed samples was improved ≈ 50% at each pH, and in the heat-processed samples 28, 32, 27 and 92%, respectively, at pH 6.50, 7.21, 7.81 and 8.97. In no case was methionine supplementation alone found as effective as a combination of heat processing and methionine supplementation for improving the nutritive value of soy milk.

GROWTH AND ENTEROTOXIN PRODUCTION BY VARIOUS STRAINS OF Staphylococcus aureus IN SELECTED FOODS. D.L. SCHEUSNER & L.G. HARMON. *J. Food Sci.* **38**, 474–476 (1973)–Various strains of *Staphylococcus aureus* were inoculated into commercial foods and incubated at 21°C for 48–119 hr. Foods in which measurable enterotoxin was produced during incubation for 68–119 hr had an initial pH of 5.51–6.60 and a terminal *S. aureus* population ranging from 1.7×10^7 to 5.7×10^8. The initial total count of these same foods varied from < 10 to 3.0×10^2/g and the terminal total count varied from 7.9×10^7 to 4.3×10^9. During incubation periods extending to 48–71 hr, inoculated foods with an initial pH below 5.0 showed a decrease in staphylococcal population, and the total population, including inoculum, either decreased on remained stable. *S. aureus* strains produced enterotoxins A, B, C and D in vanilla pudding with an initial pH of 6.37 over a temperature range of 19–45°C.

EFFECT OF CARBOXYMETHYLCELLULOSE ON THE PROTEOLYSIS OF ALPHA$_S$-CASEIN BY IMMOBILIZED PEPSIN. M. VALARIS & W.J. HARPER. *J. Food Sci.* **38**, 477–480 (1973)–The effect of the presence of carboxymethylcellulose (CMC) on the activity of pepsin covalently attached on alkylamino-glass derivatives was investigated. CMC above 0.01% concentration was found to inhibit the action of pepsin on α_S-casein, the inhibition being independent of substrate concentration. Further experimentation with C^{14}-carboxy-labeled CMC showed that the inhibitor binds directly on the enzyme and not on the porous glass beads. The CMC binding capacity of immobilized pepsin was found to be unaffected by the presence of the substrate α_S-casein. Treatment of this column with hydrochloric acid resulted in some dissociation of the enzyme-inhibitor (EI) complex but did not restore any of the proteolytic activity. Subsequent washing of the acid-treated immobilized pepsin with pH 5.0 acetate buffer had no effect on either the EI complex or the enzymatic activity. The activity of the bound enzyme, however, was almost doubled after treatment with 1M NaCl although no further release of inhibitor from the column was detected.

EFFECT OF CARBOXYMETHYLCELLULOSE ON THE PROTEOLYSIS OF ALPHA$_S$-CASEIN BY IMMOBILIZED TRYPSIN. M. VALARIS & W.J. HARPER. *J. Food Sci.* **38**, 481–483 (1973)–The effect of the presence of carboxymethylcullulose (CMC) on the activity of trypsin covalently attached on arylamine glass derivative was investigated. CMC, above concentrations of 0.02%, was found to inhibit the action of trypsin on intact α_S-casein, the inhibitory effect decreasing with increasing substrate concentration. However, CMC did not inhibit tryptic proteolysis of α_S-casein partially hydrolyzed by pepsin. Experiments with C^{14}-carboxy-labeled CMC solution recycled through a column packed with immobilized trypsin derivative showed that CMC binds directly to the enzyme in the absence of substrate protein. The presence of 0.28% α_S-casein prevented the enzyme-inhibitor interaction. It was concluded that the inhibition of trypsin by CMC is of the competitive type. The results of this investigation suggest that CMC would have no effect on the tryptic digestion of casein, and that any nutritional significance of CMC would relate only to its effect on pepsin.

SOME EFFECTS OF BETA AMYLOLYTIC DEGRADATION OF PASTES OF WAXY MAIZE STARCH. L. DAHLE, V. BRUSCO & G. HARGUS. *J. Food Sci.* **38**, 484–485 (1973)–A shortening of the unbranched chains of amylopectin by beta amylase action is accompanied by a loss of cohesive and extensible properties, measurable on an Instron Instrument. Measurement of maltose permits calculation of average chain length shortening. This chain length shortening can be related to the diminishing absorbance of the amylopectin-iodine complex which occurs as beta amylase action proceeds. Decreasing viscosity of amylopectin in neutral and alkaline solutions accompanies beta amylase action.

FLUORESCENT PRODUCTS IN A GLUCOSE-GLYCINE BROWNING REACTION. H.R. ADHIKARI & A.L. TAPPEL. *J. Food Sci.* **38**, 486–488 (1973)–Fluorescent products from a glucose-glycine reaction system that was heated at 100°C for various time intervals were analyzed. The molar ratio of glucose to glycine was 2:1. Fluorescence spectra of the products and effects of pH and metal chelation on the fluorescence indicated that the fluorescence could be largely due to a Schiff-base in conjugation with an electron donating group. Gel chromatographic analysis of the fluorescent products showed that they were mainly polymers. The presence of similar fluorescent products in brown foods that undergo carbonyl-amine reactions during processing was observed. The application of fluorescence measurements to monitor browning is discussed.

RHEOLOGICAL PROPERTIES OF SYRUPS CONTAINING GUMS. J.L. COLLINS & B. DINCER. *J. Food Sci.* **38**, 489–492 (1973)–Three syrups of 50% solids were treated with CMC and xanthan gums. Apparent viscosity was measured at 26.7–71.1°C and at four rates of shear. The gums thickened all syrups; however, corn syrup become thicker than sucrose syrup and the sucrose-corn syrup blend with increasing gum level. Syrups with xanthan were more resistant to heat-thinning than syrups with CMC. Xanthan-containing syrups became pseudoplastic while CMC-containing syrups tended to remain Newtonian.

VITAMIN RETENTION IN BEAN PRODUCTS: COOKED, CANNED AND INSTANT BEAN POWDERS. C.F. MILLER, D.G. GUADAGNI & S. KON. *J. Food Sci.* **38**, 493–495 (1973)–Thiamine, pyridoxine, niacin and folacin were determined in raw, canned and drum-dried pinto beans. Significantly larger amounts of thiamine were retained in the drum-dried powder than in the canned beans. The retention of pyridoxine, niacin and folacin varied from 79–81% of the raw bean values. The canned bean retention values for these vitamins varied from 52–92% depending on treatment before canning and retort time at 250°F. Corrected protein efficiency ratios for drum-dried powders were 0.91–0.92, while those for canned beans varied from 0.84–1.06 depending on treatment before canning. From a nutritional standpoint, drum-dried pinto bean powder is equal or superior to canned beans.

SPLIT PEELED BEANS: PREPARATION AND SOME PROPERTIES. S. KON, A.H. BROWN, J.G. OHANNESON & A.N. BOOTH. *J. Food Sci.* **38**, 496–498 (1973)–Peeled beans were produced by the use of an abrasive debranning machine. Whole peeled beans required about half the cooking time of unpeeled beans when neither was presoaked and 64–74% of unpeeled beans when both were presoaked over night. Protein efficiency ratio (PER) and other nutritional characteristics were unchanged by peeling.

BLOATER FORMATION IN BRINED CUCUMBERS FERMENTED BY Lactobacillus plantarum. H.P. FLEMING, R.L. THOMPSON, J.L. ETCHELLS, R.E. KELLING & T.A. BELL. *J. Food Sci.* **38**, 499–503 (1973)–Fermentation of brined cucumbers by *Lactobacillus plantarum* resulted in bloated cucumbers when most of the CO_2 produced was retained in the brine. Bloater damage began within 2 days after inoculation and increased progressively over a 2 wk period. The damage was more severe in larger cucumbers, although the maximal concentrations of CO_2 that accumulated in the brines were the same for large and small sizes, 80 mg/100 ml brine. Fermentations at 32°C resulted in severe bloater damage, but damage was less at 27°C and only slight at 21°C. A rise in the brine level was noted during the bloating stage and was attributed to gaseous expansion inside the cucumbers. The rise in brine level, termed "expansion volume," was used as an indication of bloater development during the entire fermentation. Relationships between the expansion volume and concentration of dissolved CO_2 in the brine were studied. Removal of CO_2 from the brine by purging with nitrogen during fermentation reduced or prevented bloater formation. Piercing the fruit or removal of the skin prior to brining also prevented bloater development.

ABSTRACTS:
IN THIS ISSUE

CARBON DIOXIDE PRODUCTION IN THE FERMENTATION OF BRINED CUCUMBERS. H.P. FLEMING, R.L. THOMPSON, J.L. ETCHELLS, R.E. KELLING & T.A. BELL. *J. Food Sci.* **38,** 504–506 (1973)–Dissolved CO_2 in the brine of fermenting cucumbers originated from the cucumbers and from bacterial activity in the brine. CO_2 diffused from the cucumbers during the first two days after brining. A further increase then occurred due to growth of the natural microflora. When cucumbers were brined to equilibrate at 7% NaCl, about 40 mg CO_2/100 ml brine diffused from the cucumbers. Upon subsequent microbial activity, a maximal concentration in the brine of 82 mg CO_2/100 ml was reached. Higher concentrations of CO_2 accumulated when cucumbers were brined at lower NaCl concentrations. In pure culture fermentations with *Lactobacillus plantarum*, a total of 175 mg CO_2/100g of cucumbers was produced. About one-half of this amount was produced by *L. plantarum* and the remainder came from the cucumbers. Of the lactic acid bacteria tested, two strains of *L. plantarum* produced 35 and 46 mg CO_2 and *Pediococcus cerevisiae* produced 75 mg CO_2 per gram of sugar fermented in pure culture fermentations.

FIELD TESTS OF SALT RECOVERY SYSTEM FOR SPENT PICKLE BRINE. E.L. DURKEE, E. LOWE, K.A. BAKER & J.W. BURGESS. *J. Food Sci.* **38,** 507–511 (1973)–A salt recovery system first used to reclaim sodium chloride from spent olive brine was successfully applied to "strong" pickle brine. The process involves the use of a submerged combustion crystallizer to produce a slurry containing approximately 60% salt and incineration of the slurry to remove organic contaminants. The experimental data indicate a portion of the potassium in the fresh cucumber is leached out into the storage brine to raise the potassium level in the recycled salt. Repeated recycling should in time increase the amount of potassium in the reclaimed salt until the potassium level in the spent brine reaches equilibrium with the potassium in the fresh cucumber. The spent pickle brine foamed when heated by submerged combustion, but foaming was controlled by the use of a defoamer. Nevertheless, the evaporative capacity of the crystallizer was derated by about 20% because of the foaming properties of the spent brine. Vapor exhaust from the crystallizer was contaminated with brine droplets and organic volatiles. A 4-stage system was used to clean up the exhaust but results were not as good as had been expected. The vapor-gas stream discharged into the atmosphere contained an excessive amount of salt carryover.

EFFECTS OF DIFFERENT SUBMERGENCE TIMES IN HOT CALCIUM CHLORIDE ON PEELING EFFICIENCY OF TOMATOES. T.S. STEPHENS, G. SALDANA & H.E. BROWN. *J. Food Sci.* **38,** 512–515 (1973)–Tomato cultivars were submerged in boiling $CaCl_2$ for 20, 25, 30, 35 and 40 sec to determine an ideal submergence time for maximum percentage peel removal with minimum calcium absorption. The percentage of tomatoes that ruptured and peeled increased as the submergence time in the hot $CaCl_2$ increased. The fruit of one cultivar that ruptured and peeled at one submergence time interval was not the same for the other cultivars. The peeling percentage was not uniform from one submergence time to the next. The amount of calcium taken up by the tomatoes could not be controlled by regulating the length of time the tomatoes were submerged in the peeling solution.

AQUEOUS PROCESSING OF FRESH COCONUTS FOR RECOVERY OF OIL AND COCONUT SKIM MILK. R. HAGENMAIER, C.M. CATER & K.F. MATTIL. *J. Food Sci.* **38,** 516–518 (1973)–Fresh, husked coconuts were shelled, the meats ground with coconut water and the mixture pressed to separate the solids (residue) from the liquid emulsion. The liquid emulsion was centrifuged to give three phases: a precipitate (insoluble protein), an aqueous phase and a creamy emulsion. The creamy emulsion was diluted with oil and agitated to produce coconut oil and more aqueous phase. The combined aqueous phases (coconut skim milk) were dried to give a product that contains 25% crude protein, 5.1% fat, 45% soluble carbohydrates, 9% ash and 0.0% crude fiber. The cost of production of dry skim milk fraction is estimated to be $0.16/lb.

DEMINERALIZATION OF UNTREATED COTTAGE CHEESE WHEY BY ELECTRODIALYSIS. S.V. D'SOUZA, D.B. LUND & C.H. AMUNDSON. *J. Food Sci.* **38,** 519–523 (1973)–An Ionics electrodialyzer unit of 18 cell pairs with external staging was used to study the demineralization of cottage cheese whey. Experiments with 1–4% NaCl solutions indicated that flow rate and temperature in the range studied had little effect on the extent and rate of demineralization. During demineralization of cottage cheese whey, it was observed that the pH of the whey changed continuously. The extent and rate of demineralization for cottage cheese whey was pH dependent and exhibited a maximum rate at pH 4.65. Above pH 4.65 there appeared to be a precipitation with a resulting fouling of the anion membranes. Minimum water transfer from the diluate into the concentrate was observed at pH 4.65.

DIFFERENCE TASTE THRESHOLDS FOR SODIUM CHLORIDE AMONG YOUNG ADULTS: AN INTERLABORATORY STUDY. B. JOHANSSON, B. DRAKE, R.M. PANGBORN, N. BARYLKO-PIKIELNA & E. KÖSTER. *J. Food Sci.* **38,** 524–527 (1973) Difference thresholds expressed as jnd values or Weber ratios for the taste of sodium chloride at two standard concentrations were determined at four laboratories in four different countries using a method of constant stimuli. The total jnd values (Weber ratios) for the four laboratories were: 0.021% (0.070), 0.022% (0.073), 0.026% (0.087) and 0.031% (0.103) at the standard concentration of 0.30% NaCl; and 0.049% (0.062), 0.042% (0.053), 0.058% (0.073) and 0.097% (0.121) at the standard concentration of 0.80% NaCl. At both standard concentrations, the jnd values were in more or less close agreement for three of the four laboratories, whereas the values for the fourth laboratory were significantly higher. The jnd values for females were generally slightly lower than for males. Difficulties encountered in interlaboratory comparisons are briefly discussed.

FLAVOR THRESHOLDS FOR FATTY ACIDS IN BUFFERED SOLUTIONS. R.E. BALDWIN, M.R. CLONINGER & R.C. LINDSAY. *J. Food Sci.* **38,** 528–530 (1973) Paired comparisons revealed that increased concentrations of butanoic, hexanoic, octanoic and decanoic acids were required for flavor recognition as pH of the citrate-phosphate buffer carrier changed from 3.2 to 4.5 and to 6.0. Therefore, the contribution of a given level of these fatty acids to flavor of foods may be expected to increase as pH of the product decreases. Thresholds for ethanoic acid were not established because the amount exceeded the buffer capacity of the system. Since aroma and flavor thresholds were more similar than taste and flavor thresholds for butanoic and decanoic acids, aroma apparently contributed more than taste to the flavor of these acids.

PRESERVATION OF CHANNEL CATFISH WITH SOME SELECTED CHEMICALS. L.R. BEUCHAT, E.K. HEATON & T.S. BOGGESS JR. *J. Food Sci.* **38,** 531–535 (1973) Dressed channel catfish were treated with dips containing various concentrations of pentasodium diethylenetriaminepentaacetic acid (Na_5DTPA), 2,4,4'-trichloro-2'-hydroxydiphenyl ether (THDE) and methyl 2-chloroacetoacetic acid (MCA) individually or in combination in an attempt to increase storage life. Organoleptic evaluations, surface microbial counts, shear press firmness measurements, pH and moisture determinations were performed after 0,

3, 6, 8, 10, 13, 16 and 22 days storage at 0°C. Treatments containing Na_5 DTPA and MCA were shown to preserve the organoleptic qualities of catfish over the controls while THDE did not. Microbial populations, however, were suppressed by treatments containing Na_5 DTPA and THDE but not by MCA. There were no statistically significant differences in texture, pH and moisture as a result of chemical treatments. All agents employed show potential for extending the shelflife of channel catfish.

MUSCLE QUALITY, COOKING METHOD AND AGING VS. PALATABILITY OF PORK LOIN CHOPS. M.E. BENNETT, V.D. BRAMBLETT, E.D. ABERLE & R.B. HARRINGTON. *J. Food Sci.* **38**, 536–538 (1973)–10 pork loins each of pale, soft, exudative (PSE), normal and dark, firm, dry (DFD) musculature were cut into two sections and the sections aged for either 48 or 144 hr postmortem. Chops were either oven broiled or deep fat fried. When oven broiled, PSE chops cooked most slowly, had the greatest cooking loss, were most tender and least juicy. DFD chops gave opposite results. When fried, PSE chops cooked most quickly and were least juicy. Oven broiling required longer cooking times; losses for the two methods were similar. Oven broiling produced significantly more tender chops than frying. Muscle quality differences were more apparent in oven-broiled chops. Palatability did not vary between loin sections nor was it affected by aging treatment.

QUALITY CHANGES IN PRE-RIGOR BEEF MUSCLE AT −3°C. J.R. BEHNKE, O. FENNEMA & R.G. CASSENS. *J. Food Sci.* **38**, 539–541 (1973) Quality attributes (percent drip loss, tenderness and percent shortening) were determined for sternomandibularis (neck) muscles which had been: freshly excised from cutter-grade cows, frozen pre-rigor, held at −3°C for various times, stored in crushed dry ice, thawed and cooked. Drip losses increased rapidly as shortening exceeded a critical value of about 35%. A plot of toughness versus percent shortening was approximately bell shaped, with maximum toughness occurring at 43% shortening. Samples that were frozen immediately following slaughter, then stored at −3°C for 24 hr, did not differ significantly in tenderness from samples that were chilled for 24 hr at 4–7°C prior to freezing.

STORAGE BEHAVIOR OF ARTIFICIALLY WAXED GREEN SNAP BEANS. R.P. SINGH, F.H. BUELOW & D.B. LUND. *J. Food Sci.* **38**, 542–543 (1973) Rapidly respiring vegetables such as snap beans have a very short storage life. Wax emulsions provide a thin coating on the vegetable thus checking the respiration process. A pilot test was conducted to measure the effect of two emulsions, namely an emulsified vegetable oil (Sta-Fresh 800) and an anionic polyethylene emulsion (Sta-Fresh 715) on the CO_2 permeability of waxed filter paper. Sta-Fresh 800 significantly reduced CO_2 permeability. Samples of green snap beans were waxed with these two emulsions and stored at 4.4 and 7°C. Significant reduction in respiration was observed for the beans waxed with Sta-Fresh 800 and stored at 4.4°C. The waxed beans were generally in better condition than the unwaxed. This suggests that certain waxy coatings applied to vegetables could extend storage life and maintain product quality.

EFFECTS OF HEAT PROCESSING ON THE RETENTION OF VITAMIN B_6 IN LIMA BEANS. C.A. RAAB, B.S. LUH & B.S. SCHWEIGERT. *J. Food Sci.* **38**, 544–545 (1973) The vitamin B_6 content of dry large lima beans (*Phaseolus lunatus*) was determined microbiologically with *Saccharomyces carlsbergensis* before and after heat processing. Two varieties of beans, one of which was grown in three California localities, were analyzed. Chromatography of the bean samples indicated no changes in the relative proportions of the three vitamin B_6 components as a result of heat processing. The vitamin loss of 15–25%, which occurred during blanching, was comparable for both steam and water blanching. Following blanching, the vitamin B_6 was stable to three heat treatments in the range used commercially.

COMPOUNDS CONTRIBUTING TO HEAT-INDUCED BITTER OFF-FLAVOR IN AVOCADO. G. BEN-ET, A. DOLEV & D. TATARSKY. *J. Food Sci.* **38**, 546–547 (1973)–Components contributing to the heat-induced bitter off-flavor in Ettinger avocado have been isolated. The lipids were extracted from the bitter fruit with absolute ethanol, and nonbitter triglycerides were precipitated by storage at −28°C and removed by centrifugation. The ethanol-soluble bitter mixture was then fractionated by column chromatography with silica gel and fluorosil. Among the compounds isolated, 1-acetoxy-2,4-dihydroxy-n-heptadeca-16-en (1) and 1,2,4-trihydroxy-n-heptadeca-16-en (2) were identified as mildly bitter by a trained taste panel. The avocado off-flavor is assumed to be caused by a synergistic effect involving several compounds, including (1) and (2).

ALPHA-AMYLASE IN SWEET POTATOES. A Comparison Between the Amyloclastic and Chromogenic Starch Methods of Analysis. W.M. WALTER JR. & A.E. PURCELL. *J. Food Sci.* **38**, 548–549 (1973)–A newly developed chromogenic starch method for assay of α-amylase activity has been adapted to measure the enzyme levels in several varieties of sweet potatoes. The procedure involves incubating sweet potato juice (or dilutions) for 15 min with a 2% suspension of a blue chromogenic starch substrate, Amylopectin Azure. The blue color released is related to the α-amylase activity of the sample. Enzyme activities obtained by the chromogenic starch method correlate well with those measured by the standard AOAC amyloclastic procedure. The newly developed method is precise, relatively simple and sensitive, representing a significant improvement over amyloclastic procedures. This new procedure could be of use in processing plants where enzyme conversion is necessary to prepare good quality precooked dehydrated sweet potato flakes.

ASCORBIC ACID CONTENT OF ARTIFICIALLY RIPENED TOMATOES. C.E. PANTOS & P. MARKAKIS. *J. Food Sci.* **38**, 550 (1973)–Tomatoes of two cultivars were either harvested mature-green and ripened at 55°, 60°, 65° and 70°F or left to ripen on the vine. Artificially ripened tomatoes contained one-fourth to one-thrid less ascorbic acid than when ripened on the vine.

THE USE OF SQUID IN MEAT EMULSIONS. R.L. SAFFLE. *J. Food Sci.* **38**, 551–552 (1973)–A study was undertaken to determine the potential of squid as an emulsifying agent and the effect of varying amounts on the characteristics of various fish sausage formulations. Results show there is almost twice as much (69.8%) salt-soluble protein available to act as an emulsifier for squid as compared to (38.2%) cow meat. Binding efficiency of squid is 25.66 as compared to 13.98 for cow meat, indicating squid should have high fat emulsifying properties in actual meat emulsions; however, textural properties of all formulations were quite poor.

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A STATISTICAL APPROACH TO THE SUBJECTIVE AND OBJECTIVE MEASUREMENTS OF ODORS INDUCED BY γ-IRRADIATION OF BEEF FAT

INTRODUCTION

SENSORY TESTS are essential procedures used in the food industry to establish the quality of a product. These tests are very subjective and time consuming, hence, statistical interpretations of these organoleptic data would be very useful. In fact, for routine use as a quality control, a high correlation between analytical data and sensory evaluation is necessary.

The objective of this paper is to present a methodology for the analysis of the organoleptic evaluations of food and the means for correlating these results with the analytical data. The selected model for study was beef fat, which produces off-odors during γ-irradiation.

It has been shown by Merritt (1966) that volatile components originated from lipids are responsible for the off-odor and off-flavor of irradiated meat which is a great obstacle in the use of this method of food preservation. The mechanisms of lipid damage (Merritt, 1966; Katri et al., 1966; Champagne, 1968; Morre, 1969) were proposed and comprised a rupturing of fatty acids to yield smaller volatile molecules. It was expected that the changes in odor intensity of irradiated beef fat can be detected by a fatty acid analysis.

MATERIALS & METHODS

Preparation of irradiated beef fat

Beef fat taken from three different animals (specimens) was individually extracted with chloroform-methanol (2:1). The solvent was evaporated to dryness under vacuum and nitrogen. Each specimen (labelled 'j') was divided into eight samples (labelled 'i') which were irradiated at 0.1, 0.5, 1, 3, 6, 10 and 20 megarads. The irradiation was performed in evacuated sealed ampules using γ-cell 220 at the Cancer Research Laboratory, The University of Western Ontario. A temperature of 30°C was maintained during irradiation. The control samples were also kept at the same temperature. In order to minimize any post-irridation changes, the samples were stored at −10°C prior to further analysis.

Organoleptic tests

These tests were performed using a 19-member panel which established the scores and the threshold values for the irradiated samples. Points on the score test were assigned to samples from 1–8 according to the odor intensity ranging from nondetectable to extremely strong. For the threshold test, the irradiated fat was mixed in various concentrations (at 10% increments) with odorless mineral oil. This set of increments would limit the mis-specification error to 5% compared to the true threshold concentration.

To find an acceptable concentration with statistical significance the null hypothesis test was used. The null hypothesis (H_0) states that the results were due to chance when the probability (p) to choose the correct level is 50%. If the probability is greater than 50%, the result (H_1) was considered to be correctly chosen. A normal distribution was assumed. Data were analyzed at 99% confidence.

Preselection of methyl ester peak ratios

The odor index, which is the reverse of the acceptable threshold concentration of the sample, was correlated with peak ratios of fatty acid methyl esters. The fatty acids present in the control and irradiated samples were trans-methylated (Carroll et al., 1968). The resulting methyl esters were analyzed by GLC. Standard methyl ester mixtures (NIH and beef fat standards) were used to standardize the instrument. The quantity of individual components in the chromatography was calculated using the method described by Carroll (1961).

The peak ratios were preselected using the six non-irradiated specimens as references. All possible peak ratios in a sample were formed and those ratios which did not vary (± 20%) within the six control specimens were selected as the peak ratios to be calculated after irradiation. This approach was adopted as a reasonable means of minimizing the variations in peak ratios not due to irradiation.

Regression analysis

The accepted peak ratios were then correlated with the odor intensity profile developed from threshold results as outlined previously. The form of the multiple regression equation used is as follows:

$$y_t = b_{1t}\, x_{1t} + b_2 x_{2t} + \ldots + b_n\, x_{nt} + u_t$$

$$y_t = \sum_{i=1}^{n} b_i\, x_{it} + u_t \qquad (1)$$

where y_t = dependent variable of observation t (e.g., odor);
x_{it} = independent variables (e.g., methyl ester peak ratios);
b_i = regression coefficient to be estimated;
n = number of independent variables;
t = observation; and
u_t = the deviation of the regression model from the observed y_t.

In matrix form, the equation (1) becomes

$$\begin{bmatrix} y_1 \\ \cdot \\ \cdot \\ \cdot \\ y_T \end{bmatrix} = \begin{bmatrix} x_{11}\ x_{21} & \cdot\ \cdot & x_{n1} \\ \cdot & & \\ \cdot & & \\ \cdot & & \\ x_{1T}\ x_{2T} & & x_{nT} \end{bmatrix} \begin{bmatrix} b_1 \\ \cdot \\ \cdot \\ b_n \end{bmatrix} + \begin{bmatrix} u_1 \\ \cdot \\ \cdot \\ \cdot \\ u_T \end{bmatrix}$$

and in vector notation, it reduces to

$$Y = X \cdot B + U \tag{2}$$

To solve equation (2), the ordinary least square (OLS) procedure appears to be the simplest and is most commonly used.

The basic assumptions of OLS are:

(1) the expected value of the error term U is equal to zero; and
(2) the variances of the error terms are constant and equal from one observation to the other. The observations are randomly independent.

Mathematically stated, this implies equations (3) and (4).

$$E(U) = 0 \tag{3}$$

$$COV(U) = \sigma^2 I = \begin{bmatrix} \sigma^2 & 0 & \cdot & \cdot & 0 \\ 0 & \sigma^2 & \cdot & \cdot & 0 \\ \cdot & \cdot & & & \cdot \\ \cdot & \cdot & & & \cdot \\ 0 & 0 & & & \sigma^2 \end{bmatrix} \tag{4}$$

where σ^2 = the variance of each observation; and
I = the identity matrix.

The least square estimate of the coefficient vector B is given by Equation (5)

$$\hat{B} = (X'X)^{-1} X'Y \tag{5}$$

where $\hat{B}$ = the calculated value of B; and
X' = the transpose of matrix X.

It can be shown that $\hat{B}$ in equation (5) is the unbiased estimator of B as in equation (6)

$$E(\hat{B}) = B \tag{6}$$

The covariance matrix is accordingly given as follows:

$$COV(\hat{B}) = \sigma^2 (X'X)^{-1} \tag{7}$$

With the specifications of U as in equations (3) and (4), Gauss Markov theorem (Golberger, 1966; Wonnacott and Wonnacott, 1970; Johnston, 1972) proves that the least square estimators of B in equation (5) have the least variance.

Stepwise regression

Stepwise regression is considered as multiple regression in that each variable is added one at a time. Because of this procedure, the method tends to discriminate against the last regressors and may lead to a biased estimation of the regression coefficient (Wonnacott and Wonnacott, 1970). The computer program that was used for stepwise regression analysis in this work was written by Thornber (1966) and modified by the computing center of The University of Western Ontario (1967). An important property of this technique incorporated into the program is based on the following:

(1) A variable may be indicated to be significant in any early stage and enters the correlation equation; and
(2) After several other variables are added to the regression equation, the initial variable may become insignificant. This insignificant variable will then be removed from the regression before the program enters another variable.

For that reason, only significant variables are included in the final equation.

Limitation of the OLS

In the above model, if assumptions shown as equations (3) and (4) are violated, the OLS procedure breaks down at three points (Durbin and Watson, 1950):

(1) The estimate of the regression coefficients, though unbiased, need not be the most efficient estimator (i.e., need not have the least variance).
(2) The usual formula for the variance of the estimation, equation (7), is no longer applicable.
(3) The t and F Distribution, used for making the confidence test lose their validity.

Depending on various situations, different violations of the assumptions in equation (4) may arise:

(a) The most general case can be shown in matrix form as follows:

$$COV(U) = \begin{bmatrix} \sigma_1^2 & a & \cdot & \cdot & c \\ a & \sigma_2^2 & & & \cdot \\ \cdot & \cdot & & & \cdot \\ \cdot & \cdot & & & \cdot \\ c & \cdot & \cdot & \cdot & \sigma_T^2 \end{bmatrix} \tag{8}$$

If Ω is known, the Gauss Markov theorem proves that the general square (GLS) can be used to give unbiased and most efficient estimators in equation (9).

$$\hat{B} = (X' \Omega^{-1} X)^{-1} X' \Omega^{-1} Y \tag{9}$$

However, Ω is usually not known and we have to specify the model as in the following cases (b and c).

(b) The variance of each observation is equal and constant but any two observations are not independent:

$$COV(U) = \begin{bmatrix} \sigma^2 & a & \cdot & \cdot & c \\ a & \sigma^2 & & & \cdot \\ \cdot & \cdot & & & \cdot \\ \cdot & \cdot & & & \cdot \\ c & \cdot & \cdot & \cdot & \sigma^2 \end{bmatrix} \tag{10}$$

This situation may arise if the organoleptic intensity of one sample is high; the evaluation of the next sample may be affected by the remaining effect of the previous one. This is referred to as a serial correlation. In its simplest form, the error of each observation follows the first order Markov scheme which is given in equation (11).

$$u_t = \sigma u_{t-1} + v_t \tag{11}$$

where σ is constant and the distribution of v_t's is assumed as follows:

$$E(V) = 0 \tag{12}$$

$$COV(V) = \sigma^2 I \tag{13}$$

It can be shown that the covariance matrix of U in equation (10) becomes

$$COV(U) = \sigma^2 \begin{bmatrix} 1 & \rho & \cdot & \cdot & \rho^{T-1} \\ \rho & 1 & \cdot & \cdot & \cdot \\ \cdot & \cdot & & & \cdot \\ \cdot & \cdot & & & \cdot \\ \rho^{T-1} & \cdot & \cdot & \cdot & 1 \end{bmatrix} \tag{14}$$

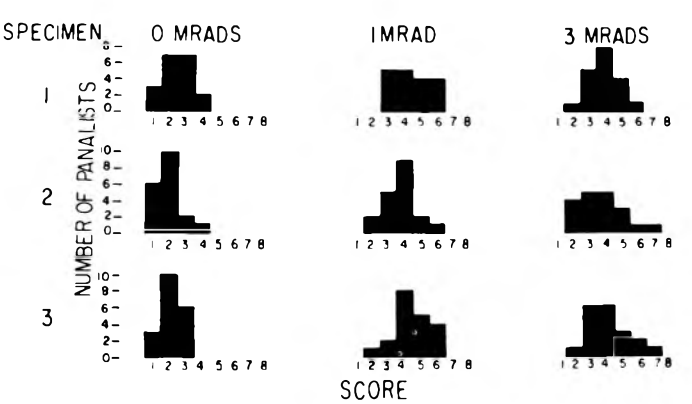

Fig. 1—Taste panel scores for irradiated samples.

Table 1–Summary of organoleptic results

Dose, megarads	Acceptable threshold level (percent)	Organoleptic odor index	Panel score
0		1.0	2
0.1	19.32	5.2	3
0.5	10.38	9.6	3
1	5.26	19.0	4
3	3.06	32.7	4
6	2.50	40.0	5
10	1.47	68.0	5
20	0.8	125.0	6

Table 3–Significant test of the adding variable at each step

Step	degrees of freedom left	R^2	t	t_c at 95% confidence
1	6	0.86095		
2	5	0.98359	6.1129	2.015
3	4	0.99600	3.5228	2.132
4	3	0.99989	10.4185	2.353
5	4	0.99989	0[a]	
6	3	0.99990	0.5477	2.353

[a]One variable was sorted out from the regression equation at this step.

When all variables are transformed to a new set of variables as follows

$$y_t^* = y_t - \rho y_{t-1} \tag{15}$$

$$x_t^* = x_t - \rho x_{t-1} \tag{16}$$

OLS can be applied to this modified set of variables:

$$y_t^* = \sum_{i=1}^{n} b_i x_{it}^* + v_t \tag{17}$$

The problem is how to estimate ρ. Practical procedures recommended for this kind of situation are

(1) Apply OLS to equation (2) to get unbiased estimation of b_i's (by equation 5)

(2) Calculate $\hat{u}_t = y_t - \Sigma b_i x_{it}$

(3) Apply OLS to $u_t = \rho \hat{u}_{t-1} + v_t$ to get ρ

(4) Transform variables to y_t^* and x_t^* according to equations (15) and (16)

(5) Apply OLS to equation (17) to get $\hat{b}_i$

Repeat the above procedure from step (2) to step (5) until the estimation of coefficient b_i's converge.

This approach is fine as long as the assumption in equation (11) holds. The question is how can one know that the data are serially correlated. The solution to this problem was given by Durbin and Watson (1951). That is, by constructing the statistic d as given in Equation (18).

$$d = \frac{\sum_{t=2}^{T} (\hat{u}_t - \hat{u}_{t-1})^2}{\sum_{t=1}^{T} \hat{u}_t^2} \tag{18}$$

The lower value d_L and the upper value d_u were tabulated for testing the two hypotheses:

H_0 : the errors of different observations are independent

H_1 : the errors are positively correlated ($\rho > 0$)

If $d > d_u$: accept H_0

If $d < d_L$: reject H_0

(The alternative hypothesis H_1 for $\rho < 0$ can also be tested by using $d^* = 4 - d$).

If $d_L < d < d_u$, the test is indeterminate. Theil and Nagar (1961) adjusted beta distribution to the distribution of Durbin and Watson's statistic d and presented a value for Q. Therefore, the test of the above two hypotheses is as follows:

If $d > Q$: accept H_0

If $d < Q$: reject H_0

The values of Q are tabulated for 0.01 and 0.05 level of significance.

In summary, the suggested procedures for obtaining the unbiased and efficient estimations of the b_i's are:

(1) Apply OLS to equation (2);

(2) Calculate $\hat{u}_t = y_t - \Sigma \hat{b}_i x_{it}$;

(3) Construct the Durbin Waston statistic d;

(4) Test the hypothesis of the independence of the error term;

(5) If the test favors the hypothesis that the error terms are independent, OLS in step (1) can be used. If the test indicates the disturbance terms are serially correlated, ρ needs to be estimated as previously described.

(c) The observations are independent but the variance of each observation is different from one to the other. In matrix form, it states that

$$\text{COV}(U = \begin{bmatrix} \sigma_1^2 & 0 & . & . & 0 \\ 0 & \sigma_2^2 & . & . & . \\ . & . & & & \\ . & . & & & \\ 0 & . & . & . & \sigma_T^2 \end{bmatrix} \tag{19}$$

This situation is called the problem of heteroscedasticity. The "weighted" least square can be used in this case to get the unbiased and efficient estimation of b_i's. That is, all variables are "weighted" by the standard deviation before OLS can be used. Detailed discussion of this part is referred to Johnston (1972).

Table 2–Accepted peak ratios used in the regression analysis

Accepted peak ratios	Peak used as numerator	Peak used as denominator
x_1	1	10
x_2	5	4
x_3	8	13
x_4	9	6
x_5	9	8
x_6	9	10
x_7	9	11
x_8	9	12
x_9	10	5
x_{10}	10	4
x_{11}	10	8
x_{12}	11	7
x_{13}	11	9
x_{14}	11	12
x_{15}	12	5
x_{16}	12	7
x_{17}	13	9

If the sensory tests are organized among different panels, the regression coefficients can be treated as a variable which is the characteristic of each panel. In this way the discrepancies between different groups can be adjusted. For discussion of the appropriate approach to this situation, the reader is referred to the original papers (Rubin, 1950; Hildreth and Houck, 1968; Theil, 1971).

RESULTS & DISCUSSIONS

THE SCORES given by the taste panel were presented in a histogram on which frequency or the number of people who gave a certain score to the sample was plotted against the score. Results of the score test were the modes or the scores which occurred with greatest frequencies. These values were judged from several specimens at the same dose of irradiation. Some typical results are shown in Figure 1.

The acceptable threshold level, odor indexes and panel scores of samples irradiated at different doses are shown in Table 1. According to this table, the acceptable threshold level is inversely proportional to the dose and consequently the odor intensity increases with the applied dose. The odor indexes, having more ordinal, cardinal and physical significance than the panel score, were used for interpretation. The ordinal significance stems from the fact that the odor indexes correspond to the odor intensity of irradiated beef fat. This characteristic is essentially the same as the organoleptic score or the indexes developed thus far by other workers (the indexes derived from discriminant analysis by Powers, 1968; Powers et al., 1968; Mulutinovic et al., 1970; and Young et al., 1970). The physical and cardinal significance arise from the threshold odor approach with the assumption that the odor intensity of the mixtures of highly nonvolatile mineral oil and irradiated fat depended on the concentration of the fat. Therefore, the stronger the odor intensity of the sample, the more dilution is necessary.

Using the panel score only, one could conclude that the sample irradiated at 6 megarads gave a stronger odor than those irradiated at 1 and 3 megarads. This information does not tell us anything about the rate of increase of odor intensity relative to the increase of irradiation dose. Moreover, when the variance of the test is high, the same score is usually given to the samples which are only slightly different. For example, samples irradiated at 0.1 and 0.5 megarads could not be distinguished from the result of panel scores as performed in the course of this work. In fact, the panel scores represent the "band" of odor intensity whose range can only be detected from experiment as shown in Figure 2. When these organoleptic evaluations were compared with our odor indexes, some interesting features were observed. First, the range of the descriptive words "litttle odor" was less than that for "moderate" and in turn less than that for "lightly strong." This showed that the variance of the panel evaluation was getting larger at higher odor intensity. This observation is intuitively perceptible and is usually referred to as the inertness of the sensory senses. Secondly, Figure 2 also reveals that there exists a certain intensity where there is an overlap in descriptive terms, which makes the evaluation more difficult.

Figure 3 shows spectra of 3 and 6 megarad irradiated beef fat samples. Some differences in the releative peak ratios can be observed; peak 8 and 13, 4 and 10, 5 and 10.

The preselection of variables resulted in 17 peak ratios as shown in Table 2. These were used in the step-wise multiple regression analysis and were regressed against the odor indexes developed in this work. Since there is no reason for the odor intensity to be only a linear function of peak ratios, input data (x's) were converted to general exponential form (x^n) before they were used as regressors. The selected values of n were ½, 1, 2 and 3. This procedure gave all-together 68 regressors and one regressand (odor index) for the analysis.

The computer program was "left free" to choose the most sensitive variables for a regression equation of the odor profile. At each step, the regression equation was printed out with the square of multiple correlation coefficient (R^2) and the Durbin-Watson statistic so that the improvement of the model caused by the entering variable could be followed.

To test the significance of the last regressor the following equation (20) (Wonnacott and Wonnacott, 1970) was used:

$$t^* = \sqrt{\frac{R_n^2 - R_{n-1}^2}{1 - R_n^2}\,(T-1-n)} \qquad (20)$$

(*t-distribution with T-1-n degrees of freedom)

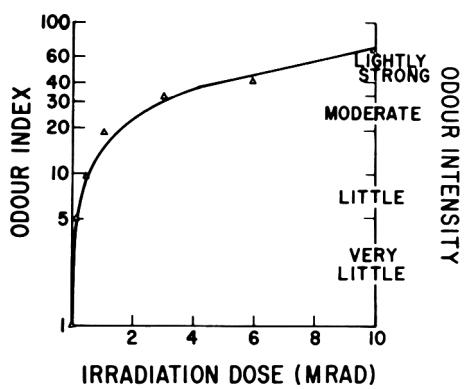

Fig. 2—Odor index of irradiated fat in vacuum at 30° C.

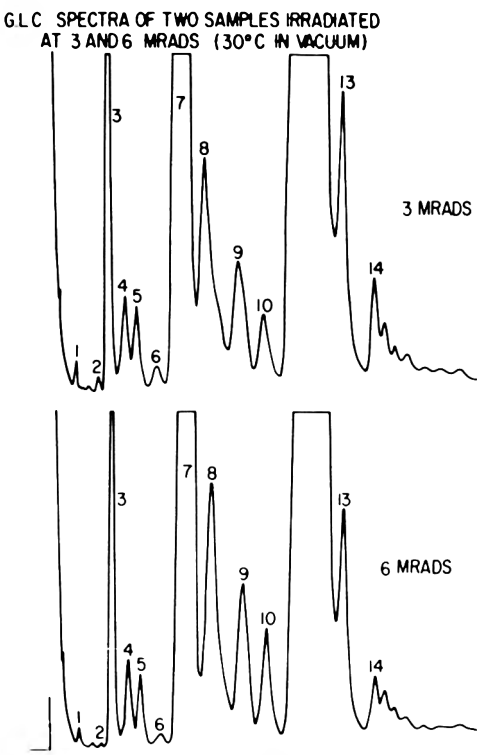

Fig. 3—GLC spectra of two samples irradiated at 3 and 6 megarads (30° C in vacuum).

R^2 at each step and the t-value calculated from equation (21) are tabulated in Table 3.

As the t_c at 90% confidence gives a value greater than the t-value at step 6, the equation in step 5 was accepted for further testing of the validity of the OLS procedure. The Durbin-Watson test for the equation given by step t was d = 1.88131. The statistic d as well as the Theil and Nagar statistic Q are not tabulated for a small number of observations ($T < 15$). However, the result can be confirmed from the following derivation.

If the errors are serially correlated, the serial correlation is given by a first order Markov scheme (equation (11)).

It was shown from OLS that,

$$\hat{\rho} = \frac{\sum \hat{u}_t \hat{u}_{t-1}}{\sum \hat{u}_{t-1}^2} \qquad (21)$$

With the assumption that u_1^2 and u_T^2 are approximately the same, it can be shown that

$$\hat{\rho} = 1 - \tfrac{1}{2} d \qquad (22)$$

In our case, $\hat{\rho} = 1 - \frac{1}{2}(1.88131) = 0.05935$ which shows that the error terms are, for all practical purposes, assumed to be independent. Thus, the predicted odor intensity y is given as follows:

$$y = 237.3 - 42.3 (X_3)^3 - 10.62 (X_9)^2 + 0.0237 (X_{10})^3 \qquad (23)$$

The predicted odor intensity given by equation (23) has been rechecked for samples irradiated at 1, 3 and 6 megarads. In those cases, the difference between predicted values and indexes derived from experimental threshold results were less than 30%.

The regression analysis discussed above has been widely recognized and used in data analysis for predicting or explaining the contribution of certain factors to a variable. In fact, it has been used by Bednarczyk and Kramer (1971) to preselect the important components for the development of synthetic ginger flavor and flavor-fortified ginger oil.

In applying this technique, we should realize that the regression analysis is only a mathematical tool, therefore it is subjected to all restrictions within its framework. A good understanding of the technique and a solid knowledge of the problem are absolutely required.

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γ-IRRADIATION OF BEEF FAT. EFFECTS ON ODOR INTENSITY AND RANCIDITY

INTRODUCTION

IT IS WELL KNOWN that fat, when subjected to γ-irradiation will produce distinctive off-odors (Merritt, 1966; Champagne and Nawar, 1969; Dubravcic and Nawar, 1969; and others). The odor intensity and the rancidity of fat vary according to conditions during and after irradiation. The odor intensity and the rancidity of beef fat at different conditions of irradiation and storage have been studied.

The objective of this work is to study the effect of various conditions on odor intensity and rancidity of irradiated beef fat. The variables under study were temperature, medium of irradiation and antioxidants. The destruction of antioxidants during and after irradiation was also studied.

MATERIALS & METHODS

BEEF FAT was extracted according to the procedure mentioned previously (Kosaric et al., 1973). The odor index of each sample was determined from the experimental threshold level as outlined in the above publication.

To study the effect of temperature on odor intensity of irradiated beef fat, four temperatures were tested: 30 ± 2°C, 0 ± 1°C, −78 ± 4°C and −196 ± 4°C. Before irradiation, the extracted fat samples were maintained at the selected temperature for about 5 min. The selected temperatures were maintained during the whole course of irradiation.

Rancidity of fat was measured by peroxide number according to Jacobs (1958). The peroxide number of each sample was expressed as milliequivalent per kilogram of fat. Since the peroxide number at the start of rancidity depends on the iodine value of fat, normalized peroxide number is used throughout this work. Peroxide number of nonirradiated fat at the beginning of the experiment was used as the base and the peroxide values were expressed relative to that number.

Effects of three antioxidants propyl gallate (PG), α-tocophenol (TC) and butylated hydroxyanisole (BHA) at concentrations of 0.01% and 0.05%–added to the fat sample before and after irradiation were investigated.

Antioxidants present in the samples were determined by the ferric chloride-dipyridyl method (Welcher, 1963) which was applicable to all antioxidants under study.

For the study of post-irradiation effects, the samples were irradiated at three different doses (1, 3 and 6 megarads) in air and vacuum at two temperatures (0°C and 30°C). For further study, the samples were kept in air and vacuum at room temperature and −10°C.

RESULTS & DISCUSSION

AS SHOWN in Figure 1, the rate of odor index decrease was much greater for temperatures above 0°C than for the lower temperatures (0 to −196°C). The reflection seems to appear at the freezing point. These results are in agreement with empirical observations reviewed by Harlan and Kauffman (1967).

The relationship between odor indexes and the normalized peroxide number of the irradiated beef fat is shown on Figure 2. A good correlation between odor indexes and the peroxide number exists. The correlation is presented by the least square line:

$$Y = 27.0719 + 26.6049\ X$$

where Y = the odor index and X = the corresponding normalized peroxide number.

This characteristic was not observed when antioxidant was added to the fat before or after irradiation. This observation will be discussed later in this paper.

Figure 3 shows the comparison between the effects of temperatures (0 and 30°C) and medium (air and vacuum) during irradiation on the peroxide number of fat. At the same temperature, the irradia-

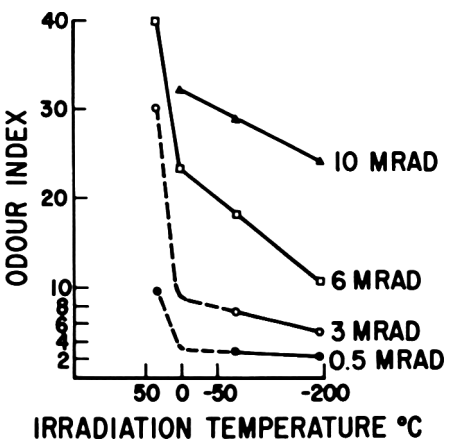

Fig. 1–Effect of irradiation temperature on odor intensity.

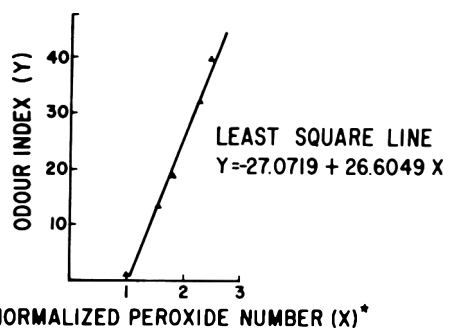

Fig. 2–Correlation of odor index and normalized peroxide number of irradiated fat (without antioxidant).

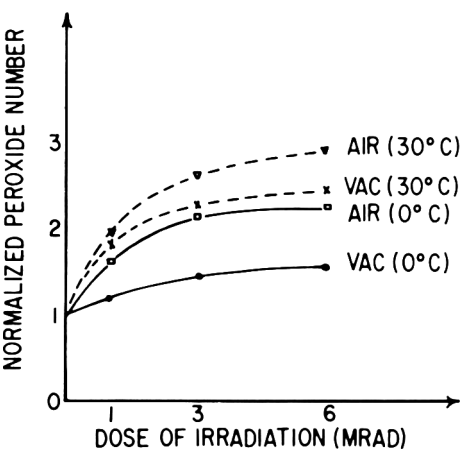

Fig. 3–Effects of temperature and medium on the peroxide numbers of fat (without antioxidant), measured at 0 day after irradiation. ($P.O._t \sim 1.5\ P.O._m$; $O.I._t \sim 2.0\ O.I._m$, based on nonsynergistic effects. $P.O._t$ = the change of peroxide number due to temperature; $P.O._m$ = the change of peroxide number due to medium; $O.I._t$ = change of odor index due temperature; and $O.I._m$ = the change of odor index due to medium.)

Table 1—Odor intensity of peroxide number due to medium of irradiation (at 30°)[a]

Dose of irradiation	Air	Vacuum	% Decrease	Average
	Odor Intensity			
1 Mrad	24.9	19.	23.7	
3 Mrad	42.4	32.5	23.4	22%
6 Mrad	49.7	39.9	19.8	
	Normalized Peroxide Number			
1 Mrad	1.953	1.824	6.6	
3 Mrad	2.612	2.258	13.6	12%
6 Mrad	2.887	2.440	15.5	

[a]Data at 0 day after irradiation

Table 2—Odor intensity and peroxide number due to medium of irradiation (at 0°C)[a]

Dose of irradiation	Air	Vacuum	% Decrease	Average
	Odor Intensity			
1 Mrad	16.4	5.1	68.9	
3 Mrad	29.5	11.0	62.7	63%
6 Mrad	32.3	13.6	57.9	
	Normalized Peroxide Number			
1 Mrad	1.635	1.21	26.0	
3 Mrad	2.128	1.43	32.8	30%
6 Mrad	2.233	1.55	30.6	

[a]Data at 0 day after irradiation

tion in vacuum reduces the peroxide value of the sample and the odor intensity. This result is expected because the peroxide formation and the rancid odor intensity are proportional to the level of oxygen present. One distinctive result observed from Figure 3 is that the irradiation in air at 0°C yields a lower peroxide value than in vacuum at 30°C. This shows that the effect of temperature (from 30°C to 0°C) in reduction of peroxide number and the odor intensity is greater than the effect of the change from air to vacuum. The result is quantitatively demonstrated in Tables 1 and 2. At 30°C, the reduction of peroxide number due to the change from air to vacuum is 12% and that of odor intensity is 22%. At 0°C the corresponding reduction of peroxide number and odor intensity is 30% and 63%, respectively. If the effects of the temperature and the medium during irradiation are independent, the decrease of the temperature from 30°C to 0°C will be responsible for the reduction of 18% of peroxide number and of 41% of odor intensity. The relative efficiency in terms of peroxide number is 1.5 and in terms of odor intensity is around 2. This relative effect between temperature (a decrease from 30°C to 0°C) and the medium (a change from air to vacuum) also holds for the post-irradiated fat.

Figure 4 shows the effect of irradiation on the rancidity of fat and hence the stability of fat. The effect of irradiation as shown in Figure 4 results in a higher peroxide number at zero day. During storage, the increase of peroxide number by autoxidation becomes greater for the nonirradiated sample. Comparing to fat irradiated and stored in air at 30°C, the transition occurs at 1 and 2 days for samples irradiated at 1 and 6 megarads, respectively. This first transition point occurred at a shorter period of time when comparing to 1 and 6 megarad irradiated samples at 0°C. For samples stored at 30°C in air, as shown in Figure 4, there is another transition point at about 30 days. At this time, the peroxide number of the irradiated samples exceeds that of the nonirradiated sample. The existence of the two transition points was also observed for samples irradiated at two different doses: 1 and 6 megarad irradiated samples in air at 30°C; 1 and 6 megarad irradiated samples in air at 0°C. In any event, the odor intensity at the second transition point is unacceptable for all samples at 0 and 30°C.

The effect of the medium and temperature on the rancidity of irradiated fat is shown in Figure 5 for the 1 megarad samples. As expected, the sample irradiated and stored in vacuum showed a lower peroxide value than the one in air. However, about 30 days after irradiation, the peroxide value of the sample in vacuum exceeded that of the sample in air. We cannot find an explanation for this phenomenon although it was suggested by Chipault and Mizuno (1966a) that this might be due to higher susceptibility to oxidation of the samples that were previously irradiated and kept under vacuum. The oxidation apparently arises when the sample is opened for analysis and exposed to air.

Effects of antioxidants added to fat before and after irradiation are shown in Figures 5, 6 and 7. As can be seen from Figure 5, the fat samples conditioned with antioxidants resisted autoxidation up to 42 days after irradiation. The addition of antioxidants also reduces the odor intensity of irradiated fat (Fig. 6). Comparing the activities of different antioxidants, the overall efficiency in terms of blocking the oxidation of irradiated fat and reducing the odor intensity is greatest with propyl gallate, following BHA and then tocopherol. This results from the apparently more efficient antioxidation effect of propyl gallate (Fig. 5 and 6) and its greater resistance during irradiation (Fig. 7).

The advantages of adding antioxidants before rather than after irradiation were observed. The antioxidants do not only

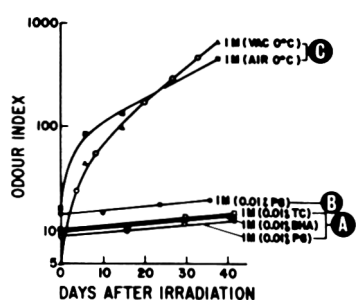

Fig. 4—Rancidity changes in post-irradiated sample (as a function of irradiation dose).

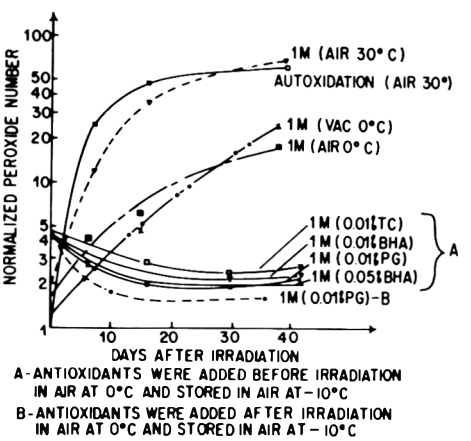

Fig. 5—Rancidity changes in post-irradiated samples (as a function of temperature, medium and antioxidant).

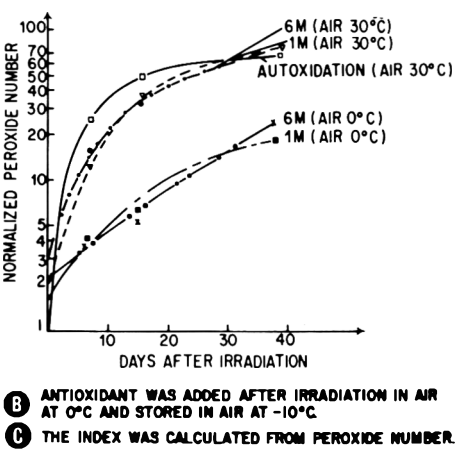

Fig. 6—Odor index changes in post-irradiated samples (as a function of medium and antioxidants).

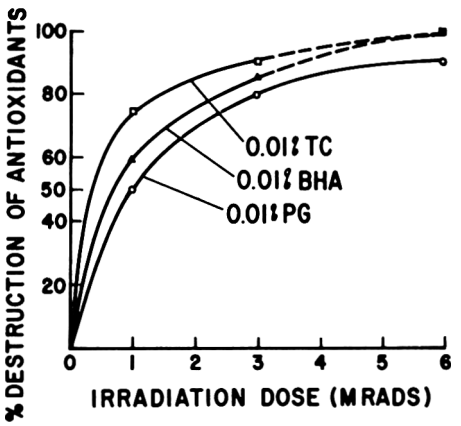

Fig. 7–Destruction of antioxidants during irradiation (irradiation in air at 0° C).

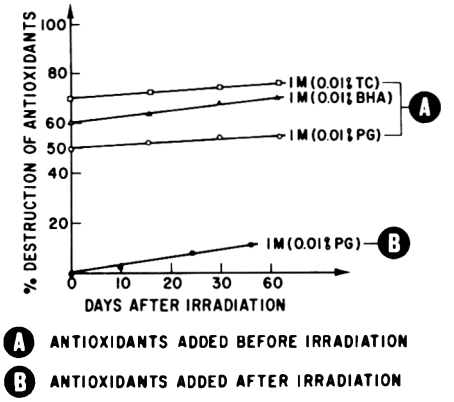

Fig. 8–Destruction of antioxidants after irradiation (irradiation at 0° C and stored at −10° C in air).

protect the samples from irradiation damage but also reduce the odor intensity of irradiated fat. For a 1 megarad irradiation in air at 0°C and stored at −10°C, the addition of antioxidants before irradiation made the odor intensity of the irradiated samples still acceptable 42 days after irradiation. The addition of antioxidants at the same level after irradiation resulted in the samples being unacceptable after 36 days.

Figure 7 shows that the 6 megarad irradiation dose in air at 0°C will completely destroy α-tocophenol, BHA and almost all of propyl gallate. The rate of destruction of antioxidants in post-irradiation is less when the same level of antioxidants was added before irradiation than after irradiation (Fig. 8). This may be due to the higher level of antioxidants present in the sample.

At the level of antioxidants studied in this work, the rate of increase of odor intensity (Fig. 6) was the same for antioxidants added before or after irradiation. This shows that the efficiency of an antioxidant is proportional to its concentration to a certain level at which it attains a maximum effectiveness. Beyond that, there is no improvement in its useful effect.

Comparing Figures 6 and 8, the rate of destruction of antioxidants in post-irradiated fat seems to have some correlation with the rate of increase of odor intensity. After the addition of antioxidants (either before or after irradiation) as shown in Figures 5 and 8, the peroxide number and the odor intensity could no longer be correlated. Antioxidants could only slow down the rate of increase of odor intensity whereas they were able to bring down the level of peroxide number for a certain period of time before the total peroxide value can again increase. This action of antioxidants upon the peroxide number was also observed by other workers (Chipault and Mizuno, 1966) and the mechanism is not clearly understood. The peroxide group, formed during oxidation, may be destroyed by the antioxidant at a higher rate than the autoxidation. This will result in a lowering of peroxide number for a certain period of time after irradiation. When the antioxidant level becomes lower due to its utilization, the rate of formation of peroxide will increase above the rate of the dissociation of peroxide group which will lead to an increase in total peroxide number.

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Ms received 7/20/72; revised 10/24/72; accepted 10/25/72.

Research supported by the National Research Council of Canada.

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AROMA OF CANNED BEEF: GAS CHROMATOGRAPHIC AND MASS SPECTROMETRIC ANALYSIS OF THE VOLATILES

INTRODUCTION

CANNING OF MEAT products affords excellent opportunities for widespread distribution and extended shelflife of such products. However, conventional sterilization processes often cause undesirable changes in such quality factors as flavor, thus limiting use of a particular preservation technique. The resulting flavor is different not only from that of the raw product but also from the flavor of the ready-to-eat product when cooked from the raw material. This is often referred to as "retort flavor" or in this case to "canned beef off-flavor." The reason for the appearance of this off-flavor is that the heat treatment necessary for sterilization is more severe than in normal cooking procedures and that the product is hermetically enclosed in the can during heating, causing the volatiles formed to remain in the product. It is well known that aseptic packing of foods sterilized in an open system, such as the Flash-18 system, often renders products with less pronounced off-flavor.

The literature on the flavor of meats was recently reviewed by Herz and Chang (1970) and Solms (1968). It is obvious from their accounts that emphasis so far has been on the chemical aspects of meat flavor rather than the sensory properties. The more specialized problem of canned meat off-flavor was first discussed by Luh et al. (1964) in relation to high temperature-short time (HTST) sterlization. They found that the content of H_2S in the product was three times higher in the conventional process than in the HTST one. The sensory properties of the latter were judged as more "raw" than in the other which had the typical flavor of canned meat. Brennan and Bernhard (1964) analyzed the headspace constituents of canned beef heated at 122°C for 90 min. They concluded that propyl mercaptan and butyl mercaptan might be responsible for the typical off-flavor as they were not present in cooked beef. This was not justified sensorically, however.

Canned minced pork was investigated chemically and sensorically in two interesting papers by Przeździecka and Żótowska (1967) and Żótowska (1967). They processed at temperatures between 95–121°C and for times varying between 0–30 min at product center. Hydrogen sulfide and methyl mercaptan were determined and found to increase with increasing heat treatment, temperature being a more important factor than time. They found a significant, negative correlation between odor and quality scores on one side and the concentrations of H_2S and methyl mercaptan on the other.

Methyl mercaptan was found to have a stronger influence on the sensory scores than hydrogen sulfide. The changes in free amino acids were also determined. Among others methionine and cysteine were found to decrease when increasing heating. As these are the main precursors for methyl mercaptan and hydrogen sulfide, respectively, the breakdown of these amino acids can be related to the formation of the undesirable canned meat off-flavor. This was confirmed sensorically in other experiments where methionine and cysteine were added to the formulations.

Ziemba and Mälkki (1971) determined the content of hydrogen sulfide, methyl mercaptan and dimethyl disulfide in canned beef specimens obtained by sterilizing at 121°C for times corresponding to F_C values from 5.2–67.3. The compounds were found to increase with increasing processing time, the major changes taking place during the first period of heating. Hydrogen sulfide was dominating and found to go through a maximum, which was explained by a condensation reaction involving other volatiles. The specimens were also analyzed sensorically, using an olfactometric technique, in which various fractions of the volatiles were adsorbed in different chemical reagents. Thus they were able to distinguish between the following odors: "meaty," "ammonia," "hydrogen sulfide" and two or more "off-odors." They indicate that the concentration of H_2S has a significant negative correlation to the acceptability score.

Bryant (1970) analyzed the headspace gas of a heated meat sample using a sulfur specific detector. Seven compounds were detected, one of which was hydrogen sulfide. Golovnya et al. (1972) describe some preliminary work with meat aroma. They precipitated the sulfur compounds with heavy metal salts. After separation the volatiles were regenerated. Using retention data only 21 sulfur-containing compounds were suggested to be present. The paper also includes a critical evaluation of the analytical procedure involving precipitation of sulfur compounds with heavy metal salts.

Of importance for beef aroma is of course the contribution of volatiles, not only from the protein fractions but also from other fractions such as: lipids, carbohydrates and volatiles, formed as a result of the interaction among the various components. Thus Watanabe and Sato (1971) analyzed beef fat heated at 145°C for 10 min. They identified 70 compounds and also evaluated the sensory properties of eluated fractions from the gas chromatograph.

Through these investigations more is now known about the chemical and sensory background on the problem of canned meat off-flavor. However, from a technological and a consumer oriented point of view the problem cannot be regarded as solved. The off-flavor has not yet been defined well enough sensorically

Table 1 – Investigated samples of canned beef

Heating time (min at 121°C)	F_c value[a]	B[b]	BF[c]	BC[d]	BFC[e]
15	0.4	+			+
30	10.5	+	+	+	+
45	24.2	+			+
60	38.6	+			+
75	51.9	+			+

[a]The samples containing fat had about 15% lower values.
[b]B = 79.3% beef + 20% H_2O + 0.7% NaCl.
[c]BF = 66.3% beef + 13% fat + 20% H_2O + 0.7% NaCl.
[d]BC = 74.3% beef + 5% carbohydrate + 20% H_2O + 0.7% NaCl.
[e]BFC = 61.3% beef + 13% fat + 5% carbohydrate + 20% H_2O + 0.7% NaCl.

which implies that a chemical explanation to the phenomenon cannot be given. This means that so far technological solutions to the problem cannot be suggested based on rational conclusions from the chemical and sensory data.

It was felt that a more detailed sensory approach was necessary in order to describe in sufficient detail the sensory changes taking place during heat sterilization of beef. An odor quality assessment technique first described by Harper et al. (1968a, b) and later used by von Sydow et al. (1970) was therefore applied to this problem simultaneously with similar investigations on unconventional proteins and berries, such as black currants (von Sydow and Karlsson, 1971).

On the chemical side the methodological improvements involve usage of large volume headspace technique combined with open tubular column technique (von Sydow et al., 1970). A sulfur specific flame photometric detector was also applied. An attempt was made to quantify the chemical changes occurring by use of calibration curves and mixing the known amounts of various compounds into the specimens.

With these new prerequisites a number of psychophysical and mathematical models were tested for their ability to correlate quantitative, instrumental and sensory data in a meaningful way (von Sydow, 1971).

The parameters of a technological nature in this investigation were: formulation, sterilization time—temperature, heating in closed or open cans and storage of canned product. In this first part of the investigation qualitative and quantitative chemical data will be presented for four different formulations processed for different heating times. The formulations were representative for products based on minced beef and of a typical European composition with regard to other basic ingredients. Thus pork fat represented a typical lipid and starch a typical carbohydrate.

EXPERIMENTAL

Materials

For all experiments involving quantitative determinations one homogenous lot of beef raw material was used. Top side round steaks (Biceps femoris and Vastrus labiralis) from 2 yr old bulls were trimmed to a fat content of 2.5–3%, minced, divided and mixed with the ingredients (Table 1) at a local slaughterhouse. The material was stored at −90°C in flat, stainless steel boxes until used. The fat used was one homogenous batch of minced pork back fat. The carbohydrate used was commercial potatoflour (80% starch and 20% H_2O, from Swedish Starch Producers Association). The salt was commercial table salt (from K.N.Z. Henzelo, Holland). The water used was distilled and filtered through activated charcoal. The formulation BFC (in Table 1), although a model, represents a typical European meat product based on

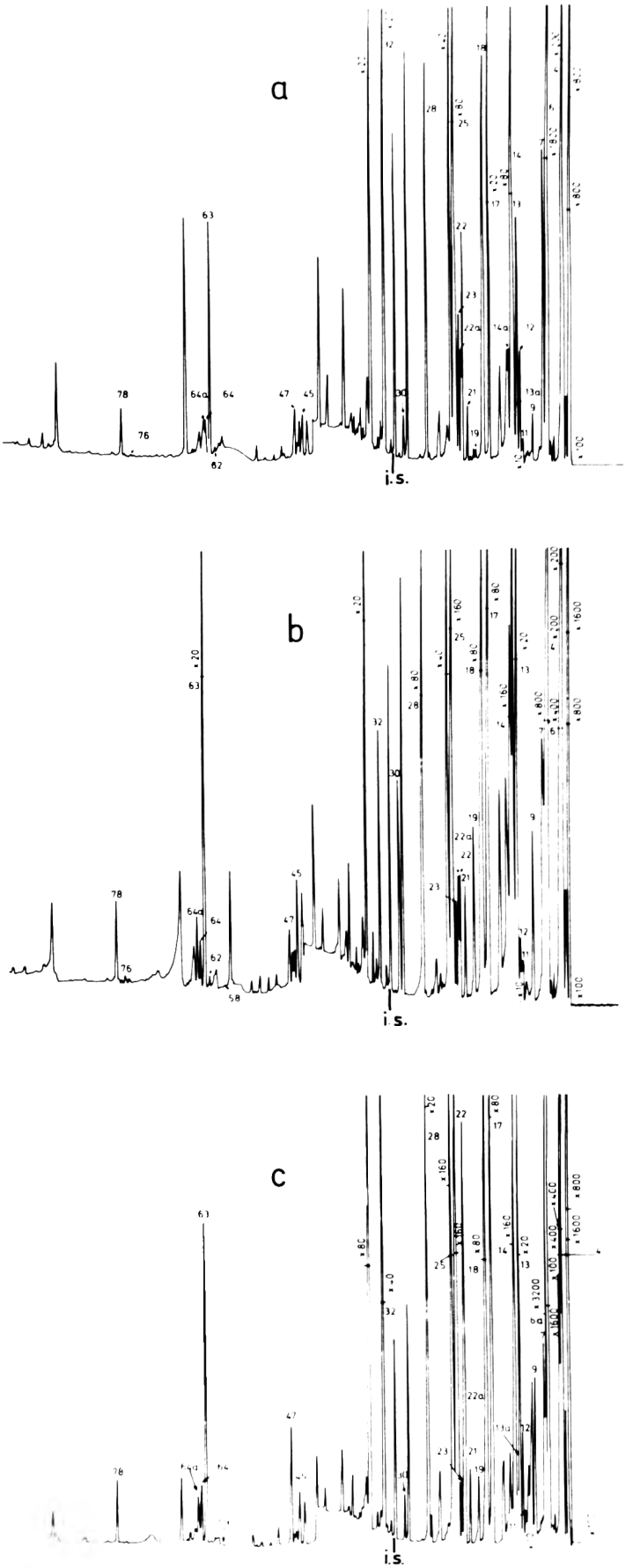

Fig. 1—Gas chromatograms (FID) of 500 ml headspace samples of (a) canned beef 121°C, 15 min; (b) canned beef 121°C, 75 min; and (c) canned beef + fat + carbohydrate 121°C, 75 min.

minced beef. The cans used for experiments involving quantitative measurements were deep drawn from electrolytical tinplate (1.00/0.50 lb/bb) size 73 × 28 mm holding 95g of material.

Processing

The processing was done in a retort assembly designed for thermal death time (TDT) investigations (National Canners Association, 1968). The pressure and temperature of the vapor were controlled by a Honeywell Pneumatic controller (type 152 Brown Elektronik). This system gives a very high degree of control during the heating and cooling period and also a homogenous temperature in the retorts. The processing variables are given in Table 1. The temperature in the retorts and in the can centers were measured with thermocouples manufactured by Ellab Instruments, Copenhagen, Denmark. The tip of the thermocouple was sheathed by a 50 mm long stainless steel needle, 1.0 mm thick (Type TCK 8). In order to insert the needle into the can through the can wall, a packing gland manufactured by the same company (Type TCG 24) was used. The temperature was registered on a Honeywell Temperature Recorder (type Elektronik 15–695–1658). The thermocouples were calibrated by comparing them with accurate (± 0.15°C) thermometers. To measure the F_c values a Telemetric Sterimeter (Telemetric Instruments, Sundbyberg, Sweden), which automatically integrates the time-temperature curve, was used. The accuracy after integration is claimed to be + 3%. The processed cans were stored at −90°C until analyzed.

Concentrates

For mass spectrometric identification and sensory evaluation of the chromatographic eluate (see below) it was necessary to concentrate the components in the headspace gas. A meat slurry was prepared by mixing 1.5 kg meat sample and 1.5 liter deodorized H_2O. The meat sample was obtained from a sufficient number of cans (see Materials) containing one of the formulations described in Table 1. Volatiles from the meat slurry were concentrated by low temperature distillation according to Forss et al. (1967) at 7 mm Hg and 20°C after initial degassing with the meat slurry stirred at 0°C. A mechanical stirrer was used. The distillation column temperature was 6°C and the volatiles were condensed in a cold trap cooled by liquid nitrogen. Several batches were distilled until about 1 liter concentrate was obtained for the mass spectrometric analysis, and about 0.5 liter, for the sensory evaluation of the eluates. These concentrates were redistilled giving in both cases about 100 ml of distillate. This technique is suitable for qualitative determinations rather than for quantitative ones.

Headspace sampling techniques

The sampling technique described by von Sydow et al. (1970) was used with the following modifications: the contents of one can, 95g, were homogenized for 5 min at 0°C with 95 ml deodorized water in a 750 ml flask with stainless steel lid, which also was used as the headspace sampling flask.

In this way transfer of the material from a homogenizer flask to a headspace flask was avoided, resulting in better reproducibility. The flask was rotated for 45 min in an inclined position in a water bath held at 25 ± 0.1°C, to obtain equilibrium. A known amount of 3-hex-

Table 2–Compounds identified by mass spectrometry and their absolute concentrations in the headspace gas of canned beef (121°C, 30 min; F_c value = 10.5) and qualitative and quantitative odor data of gas chromatographic eluates (SF96 column, FID)

Peak No.	Compound	Abs conc (ppb, v/v)	Odor of eluates	
			Quality	Intensity
4b	Methanol	trace		
8a	Ethanol	trace		
25a	1-Butanol	trace		
36	1-Pentanol	1.0		
47a	1-Hexanol	0.2		
26c	1-Penten-3-ol	0.1		
64	1-Octen-3-ol	0.5	Mushroom, sickly, sweet	3.5
4	Ethanal	850	Pungent	5
12	n-Butanal	2.8	Burnt, green, nasty-smelling	2.5
22	n-Pentanal	3.4	Burnt, green	3
32	n-Hexanal	7.0	Green, sickly, pungent	3
47	n-Heptanal	0.9	Green, burnt, sickly	3.5
78	n-Nonanal	0.8	Green, mouldy	2
9	2-Methyl propanal	32	Green, pungent, sweet	2.5
18	2-Methyl butanal	37	Burnt, sickly	4
17	3-Methyl butanal	28	Burnt, green, sickly	4
59	Benzaldehyde	0.5	Sweet, metallic	2
6	2-Propanone	230		
13	2-Butanone	14	Sickly	1
21	2-Pentanone	1.7	Burnt, green	1.5
31	2-Hexanone	0.2		
45	2-Heptanone	1.0	Green, pungent, sickly	2.5
62	2-Octanone	0.3	Green, mushroom	2
76	2-Nonanone	0.2	Green	1.5
83	2-Decanone	trace		
27a	4-Methyl-2-pentanone	0.2	Cooked cabbage	2
22a	3-Pentanone	2.2	Burnt, sickly	2
61	3-Octanone	trace		
55	An unsaturated methyl ketone	trace		
54	An unsaturated methyl ketone (MW = 122)	trace		
13a	2,3-Butandione	1.4	Butter, sickly	3.5
23	2,3-Pentandione	2.4	Butter, sickly	3
6a	Furan	750	Sickly, nasty-smelling	2
14	2-Methyl furan	61	Sickly	1
31a	2-Propyl furan*	trace		
46b	2-Butyl furan	trace		
63	2-Pentyl furan	4.7	Green, pungent, sweet	3
77	2-Hexyl furan	0.2		
15a	3-Methyl furan	0.2		
26	2,5-Dimethyl furan	25		
35	2-Methyl-5-ethyl furan	22		
75a	A furyl ketone (MW = 152)	0.1		
46a	A furyl derivate (MW = 124)	trace		
48	A furyl derivate (MW = 122)	trace		
	Hydrogen sulfide	See Table 4		
4a	Methyl mercaptan	See Table 4	Sickly, sulfurous, cooked cabbage	5
7	Dimethyl sulfide	See Table 4	Sulfurous, sickly	5
11	Ethylene sulfide	See Table 4	Sickly, cooked cabbage, pungent	2.5
14b	Propylene sulfide	See Table 4		
	Carbonyl sulfide	See Table 4		
	Carbon disulfide	See Table 4		
28	Dimethyl disulfide	See Table 4	Sulfurous, sickly, cooked cabbage	5
58	Dimethyl trisulfide	See Table 4	Sulfurous, burnt, cooked cabbage	2.5

Table 2—Continued

Peak No.	Compound	Abs conc (ppb, v/v)	Odor of eluates	
			Quality	Intensity
19	Thiophene	See Table 4	Sickly, pungent	2.5
30	2-Methyl thiophene	See Table 4	Green, sweet	1
41a	2-Methyl thiophene*	See Table 4		
64a	A terpene (MW = 136)	0.4	Fruity, flowery, green, sweet	3
6a	Pentane	160		
14a	Hexane	29		
26	Heptane	15		
35a	Octane	7.5		
69	3-Nonyne	trace		
18a	Benzene	2.0		
29	Toluene	5.9		
41	m-Xylene	0.5		
42	p-Xylene o-Xylene	2.1		
53	n-Propyl benzene	0.2		
58b	l,ethyl,2 methyl benzene	0.1		
54	1,ethyl,3 methyl benzene	0.1		
55	1,ethyl,4 methyl benzene	trace		
58a	1,3,5-Trimethyl benzene	trace		
67	1,2,3-Trimethyl benzene	0.2		
63a	1,2,4-Trimethyl benzene	trace		
66	2-Methylpropyl benzene	0.1		
75a	1-Methyl-2-n-propyl benzene	0.2		
75	1-Methyl-3-n-propyl benzene	0.2		
84	An alkyl benzene (MW = 134)	0.1		

* Tentatively identified

Table 3—Compounds identified on the UCON column, FID, but not on the SF96 column in the headspace gas of canned beef (121°C, 30 min; F_0 value = 10.5)

Benzyl alcohol	Methyl ethyl sulfide
An alcohol	Bismethyl thiomethane*
	3-Methyl thiophene
Methyl acetate	2-Pentyl thiophene
Ethyl acetate	2,5-Dimethyl thiophene*
Ethyl butyrate	2,3-Dimethyl thiophene*
Propanal	2-Formyl thiophene*
n-Decanal	3,5-Dimethyl-1,2,4-trithiolane (cis- and trans-isomers)
6-Methyl-5-hepten-2-one	
4-Nonanone	Four unknown sulfur compounds
2-Methyl-3-oxalanone	Styrene
2-Methyl-propenyl-furan*	Indene
2-Hexenyl furan	
Two unknown furan derivates	

* Tentatively identified

anone was added and used as internal standard (denoted i.s. in Fig. 1). A 500 ml sample of headspace gas was conveyed to the cold trap, which contained 70 mesh beads in the lower U-shaped part. For analysis of the distillation (25 ml samples) a 200 ml flask and a 150 ml headspace sample were used.

When analyzing the sulfur compounds with a Melpar Flame Photometric Detector a sampling system entirely made from glass and Teflon was used. After homogenization, the specimen was transferred to a 750 ml headspace flask with a glass lid. The volatiles were transferred to the trap as described. All connections in the valve oven were made of Teflon tubing and the eight-port switching valve was substituted with two four-port Teflon Kel-F valves (Hamilton No. 2). One of the stainless steel cold traps was replaced by a glass trap, having otherwise the same configuration as the original one. This cold trap was used in the same way as described by von Sydow et al. (1970) and was used for analyzing sulfur compounds. The sample size was 400 ml and the valve oven was held at 60°C. The other cold trap was replaced by a 5 ml sample loop, made of Teflon tubing (2.2 mm OD × 1.2 mm ID). This was held at room temperature and was used for analyzing hydrogen sulfide, methyl mercaptan and dimethyl sulfide which were present in such large amounts that the responses exceeded the working range of the FPD, when the 400 ml samples were analyzed.

Gas chromatography.

The equipment consisted of a Perkin-Elmer model 900 with flame ionization detector and, when analyzing the sulfur compounds, with a Melpar sulfur specific Flame Photometric Detector (see below). The pre-column concentration equipment was connected to the chromatograph. When using the flame ionization detector the headspace gas was analyzed on two open tubular columns, a 0.76 mm ID × 170m stainless steel tube coated with SF96/Igepal CO 880 (95/5) and a 0.76 mm ID × 170m stainless steel tube coated with UCON50HB2000. The temperature was programmed 20–140°C and 40–140°C, respectively, at 2°C/min after an initial isothermal period of 5 min. The helium carrier gas flow rate was 12 ml/min.

For the absolute quantitative determinations of the components in the headspace gas the FID response factors were determined for the various compounds by experiment and from literature data (Dietz, 1971; Kaiser, 1962). The peaks were considered to have the same width (Fig. 1). Therefore, the peak heights were used as a measure of the peak area. Using the absolute concentration of 2-methyl thiophene, determined in the headspace gas according to the method described below, and the response factors, the absolute concentrations of the different compounds in the headspace gas were determined by measuring the peak heights.

The absolute concentrations of the volatile compounds in the canned beef were determined by adding known amounts of the pure compounds to the meat slurry. All determinations were made in duplicate. The concentration was chosen somewhat lower than the one in the canned beef, the purpose being to keep a constant distribution coefficient during the determinations. The concentrations were calculated following simple distribution deductions. The peak heights were determined as above.

When analyzing the sulfur compounds, the sulfur-specific Melpar Flame Photometric Detector (FPD) was used in conjunction with a Perkin-Elmer 900 gas chromatograph, to which the glass-Teflon sampling system (described above) was connected. The column used was a 3.7 mm ID × 6.3m glass column packed with Chromosorb G AW DMCS 80/100 mesh coated with 4% Igepal CA 630. The column end and the detector were connected with a Teflon tube (0.8 mm ID × 1.6 mm OD). For the 5 ml and for the 400 ml sample the temperature was programmed 20–160°C at 10° and 4°C/min, respectively, after initial isothermal periods of 15 and 10 min, respectively. The helium carrier gas

Table 4–Sulfur-containing compounds and their absolute concentrations in the headspace gas of canned beef[a]

Peak No.	Peak No. in Table 2	Compound	Abs conc (ppb)
S2		Hydrogen sulfide	1100
S4		Methyl mercaptan	1800
S5		Ethyl mercaptan	190
S6	7	Dimethyl sulfide	600
S7		Methyl ethyl sulfide	6.4
S8	11	Ethylene sulfide	35
S11	28	Dimethyl disulfide	2.9
S10	19	Thiophene	3.7
S12	30	2-Methyl thiophene	3.7
S13		3,5-Dimethyl-1,2,4-trithiolane	3.0

[a](121°C, 30 min; F_c value = 10.5; Packed glass column with Igepal CA 630; S-specific Melpar Flame Photometric Detector

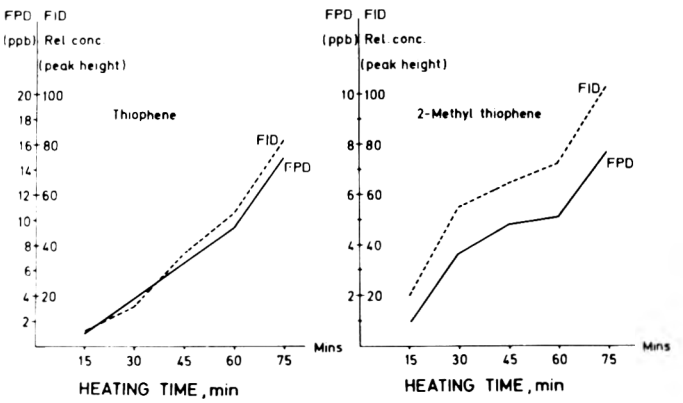

Fig. 2–Response data for two detectors as a function of the heating time for (a) thiophene; (b) 2-methyl thiophene in canned beef heated at 121°C. FID response measured as relative concentration (peak height) and S-FPD response is given as absolute concentration.

flow was 45 ml/min. To calibrate the FPD with various sulfur compounds the procedure with permeation tubes calibrated gravimetrically was used (O'Keefe and Ortman, 1966; Scarangelli et al., 1970; Stevens et al., 1971).

Mass spectrometry.

The samples were analyzed in a combined gas chromatograph–mass spectrometer, Perkin-Elmer 990 (FID)–LKB 9000 with parallel detection in the gas chromatograph and the mass spectrometer. The headspace pre-column equipment described above was connected to the gas chromatograph. The gas chromatographic separation was made on the SF96 open tubular column and on the UCON open tubular column (see above). Mass spectra were recorded at 70 eV. The separator temperature was 200°C and the ion source temperature 270°C. The compounds in the concentrate were identified by comparing with our own reference spectra or spectra given in the literature.

Sensory evaluation of eluate.

The odor properties of the components in a low temperature distillate of canned beef were assessed by sniffing the gas chromatographic eluate from the SF96 column. The gas stream was split approximately 1:10, the smaller part going to the flame ionization detector. The larger part was fed to the human detector through a 0.76 mm ID tube heated to approximately 100°C. The end of the tubing was provided with a Teflon sheild for the protection of the nose. A tape recorder was used for recording the verbal odor assessments and the corresponding retention times, as read from a chronometer in front of the judge. Odor assessments were made at the maximum of each discernible chromatographic peak or shoulder, and besides that, every 15 sec. The judges, experienced in sensory aroma analysis, assessed both odor quality and intensity, the latter by use of a scale from 0 (no odor) to 5 (very strong odor). The maximum continuous sniffing period allowed for each judge was 20 min in order to avoid fatigue. For this reason four judges alternated in each chromatographic run. The sensory analysis of the distillate was repeated three times. The judges sniffed different portions in each run. An odor quality was considered adequate if it was used in two of the three runs and the mean intensity value for this quality was calculated.

RESULTS

Chemical data

The samples investigated are described in Table 1. The qualitative analysis was done using the samples with an F_c value of 10.5. To obtain sufficient amounts for the mass spectrometric identification the headspace of a low temperature distillate of volatiles was investigated using two different columns. The compounds identified are presented in Table 2 and 3. Besides, ethyl mercaptan was identified using retention data from two columns and also by adding the pure compound to the headspace condensate. The only possible alternative using the Igepal column is carbon disulfide which however has a completely different retention time on a polyphenylether column (Stevens et al., 1971) used to check the presence of ethyl mercaptan. To verify the mass spectrometric identification of the compounds, retention data were checked for the sulfur compounds (Table 4) and for most of the nonsulfur compounds. Peak No. S9 in Figure 3 has not been identified but retention studies indicate that it is allyl mercaptan.

Quantitative data for sulfur compounds in the headspace gas of samples of canned beef with an F_c value of 10.5 was determined by using a sulfur specific flame photometric detector. For calibration, a procedure with permeation devices calibrated gravimetrically was used. The data are presented in Table 4. To determine the corresponding data for the nonsulfur compounds, gas chromatographic response factors were used, and by the known concentration of 2-methyl thiophene in the headspace gas and the peak areas (peak heights), the absolute concentrations were determined, see Table 2. The relevance of using 2-methyl thiophene or thiophene for determining absolute concentrations is demonstrated in Figure 2. For all samples investigated, the absolute concentrations in the headspace gas were determined for 32 compounds. These were chosen according to their possible sensory importance as determined by sniffing the chromatographic eluate (see below) and from literature data for odor thresholds. The results are given in Table 5.

The total absolute concentrations of 21 of the volatiles in the canned beef, determined by adding known amounts of the pure compounds, are found in Table 6. Figures 1 and 3 show gas chromatograms for three samples using flame ionization detector and sulfur-specific flame photometric detector, respectively.

Sensory data

The chromatographic eluate of a low

Table 5—Absolute concentrations ppb, v/v of 32 volatile compounds in the headspace gas of specimens of canned beef heated at different times and of specimen of different formulations[a]

Peak No.	Compound	Absolute concentrations (ppb, v/v)												Odor thresholds ppb(v/v)	References
		B_{15}	B_{30}	B_{45}	B_{60}	B_{75}	BF_{30}	BC_{30}	BFC_{15}	BFC_{30}	BFC_{45}	BFC_{60}	BFC_{75}		
64	1-Octen-3-ol	0.5	0.5	0.6	0.5	0.6	1.2	0.8	0.7	0.9	1.2	0.6	0.9		
4	Ethanal	660	850	890	1100	1100	1200	750	750	960	1200	850	730	210	Leonardos et al. (1969)
12	Butanal	3.4	2.8	2.2	2.1	1.5	4.7	3.5	5.1	6.0	5.6	4.2	3.8		
22	Pentanal	5.4	3.4	3.7	3.8	3.4	12	5.9	18	18	14	1.0	13		
32	Hexanal	13	7.0	6.1	6.4	4.1	24	8.8	36	27	22	14	21		
47	Heptanal	0.8	0.9	0.9	0.8	0.8	2.2	1.3	1.4	2.1	2.6	1.5	2.2		
78	Nonanal	0.6	0.8	0.6	0.6	0.9	1.7	1.1	0.7	1.1	1.1	0.6	0.9		
9	2-Methyl propanal	17	32	47	59	61	38	43	15	30	53	53	63		
18	2-Methyl butanal	11	37	43	64	76	34	35	7.3	21	40	47	66		
17	3-Methyl butanal	14	28	56	78	79	51	46	13	37	66	79	97		
59	Benzaldehyde	0.3	0.5	0.1	0.5	0.3	0.5	0.5	0.5	0.5	0.6	0.1	0.3	9960	Pliška and Leisenauer (1961)
13	2-Butanone	9.2	14	19	24	29	13	19	9.6	13	17	20	24	10000	Leonardos et al. (1969)
21	2-Pentanone	1.2	1.7	1.9	2.5	2.9	1.3	1.4	0.9	1.1	1.4	1.5	1.8		
45	2-Heptanone	0.6	1.0	1.4	1.5	1.6	0.8	0.8	0.3	0.5	0.7	0.6	0.9	217	Weurman (1963)
62	2-Octanone	0.2	0.3	0.4	0.5	0.5	0.1	0.3	0.1	0.2	0.2	0.1	0.2		
76	2-Nonanone	0.1	0.2	0.3	0.4	0.4	0.3	0.3	0.1	0.3	0.3	0.1	0.1		
22a	3-Pentanone	2.7	2.2	2.3	2.8	3.2	3.0	2.6	3.4	3.3	3.3	2.8	3.4		
13a	2,3-Butandione	1.1	1.4	1.6	2.2	2.2	1.3	1.1	1.6	1.8	2.2	2.4	1.6	0.82	Appel (1969)
23	2,3-Pentandione	3.6	2.4	2.9	3.2	2.7	1.9	3.0	1.9	2.0	2.0	1.4	1.6		
6a	Furan	930	750	760	960	870	3700	670	2800	3900	3700	980	1400		
14	2-Methyl furan	9.2	61	82	150	130	100	89	76	110	120	110	140		
63	2-Pentyl furan	2.6	4.7	8.7	7.6	8.1	3.0	5.0	0.7	1.6	3.0	2.2	3.8		
S2	Hydrogen sulfide	870	1100	1300	2000	2900	1200	1100	640	1100	1300	1900	2700	0.47	Leonardos et al. (1969)
S4	Methyl mercaptan	1100	1800	2400	3300	4500	1800	2100	470	1700	2800	3500	4400	2.1	Leonardos et al. (1969)
S5	Ethyl mercaptan	170	190	200	230	280	170	290	110	180	170	170	190	1.0	Leonardos et al. (1969)
S6	Dimethyl sulfide	570	600	610	670	700	240	610	230	360	400	450	420	1.0	Leonardos et al. (1969)
S7	Methyl ethyl sulfide	4.4	6.4	6.5	6.9	8.3	5.5	8.6	2.9	5.3	6.2	6.7	6.9		
S8	Ethylene sulfide	35	51	48	43	45	86	51	22	56	67	59	64		
11	Dimethyl disulfide	1.3	2.9	3.7	4.2	4.3	1.4	3.0	0.7	1.3	2.4	2.7	2.7	7.6	Wilby (1969)
S10	Thiophene	1.0	3.7	6.6	9.4	15	2.3	3.7	0.7	2.1	3.9	5.5	6.7		
S12	2-Methyl thiophene	1.0	3.7	4.8	5.1	7.7	1.0	3.9	0.3	1.4	2.2	2.4	2.1		
S13	3,5-Dimethyl-1,2,4-trithiolane	2.2	3.0	4.7	5.3	5.4	3.2	6.1	0.8	2.5	3.5	4.4	5.0		

[a]Heating temperature: 121°C; Heating time in min given as subscript to capital letter, B = beef; F = fat; C = carbohydrate; (cf. Table 1).

Table 6–Absolute concentrations of 21 volatile compounds in canned beef heated at 121°C for 60 min; can size 73 × 52 mm; F_C value = 8

Peak No.	Compound	Abs conc ppb (w/w)	Odor threshold ppb (w/w)	Odor threshold Reference
26c	1-Penten-3-ol	49		
12	Butanal	14	9.0[a]	Guadagni et al. (1963)
32	Hexanal	55	4.5[a]	Guadagni et al. (1963)
47	Heptanal	25	3.0[a]	Guadagni et al. (1963)
9	2-Methyl propanal	140	0.9[a]	Guadagni et al. (1963)
17	3-Methyl butanal	130	0.15[a]	Guadagni et al. (1963)
59	Benzaldehyde	29		
6	2-Propanone	650	500000[a]	Weurman (1963)
13	2-Butanone	110	50000[a]	Weurman (1963)
21	2-Pentanone	13		
31	2-Hexanone	1		
45	2-Heptanone	15		
62	2-Octanone	14		
76	2-Nonanone	10		
23	2,3-Pentandione	85		
6a	Furan	81		
14	2-Methyl furan	15	27000[b]	Evans et al. (1971)
7	Dimethyl sulfide	150	0.3[a]	Guadagni et al. (1963)
11	Ethylene sulfide	2		
19	Thiophene	6		
30	2-Methyl thiophene	2		

[a] Odor threshold determined in H_2O
[b] Odor threshold determined in cottonseed oil

temperature distillate of canned beef was evaluated by human subjects assessing odor quality and intensity. The data obtained for the various peaks is given in Table 2.

DISCUSSION

AT THIS STAGE of the investigation the results of the sensory evaluation of the eluate are only used to focus attention on the important fractions of the volatile complex to be investigated instrumentally in more detail.

A headspace technique has been used as it was assumed that the problem with off-flavor in canned beef is due to volatile compounds rather than to nonvolatiles. Using this technique acids and nonvolatiles cannot be analyzed. 95 compounds have been identified, and among these were 21 sulfur compounds, 12 aldehydes, 16 ketones, 8 alcohols and 11 furans. Some of the compounds have not previously been reported in beef aroma, e.g., ethylene sulfide, propylene sulfide, 2-methyl furan and some thiophene derivates. However, some of them have been reported in chicken aroma, e.g., 2-methyl furan and 2-methyl thiophene (Nonaka et al., 1967). Of these new compounds ethylene sulfide and perhaps some of the furans may be of importance for the aroma.

Recently Liebich et al. (1972) identified 2-methyl butanal, which also has been found in our investigations and is assumed to be one of the more important compounds. 3,5-dimethyl-1,2,4-trithiolane, which has been reported to be of importance for meat aroma (Chang et al., 1969), has also been identified here. The concentration found was very low, 0.84–5.00 ppb (v/v) and probably this compound does not to any great extent contribute to the aroma.

Other previously reported compounds such as 5-thiomethyl furfural, thiophencarboxy-2-aldehyde, 2,4,5-trimethyl-oxazoline, 4-hydroxy-5-methyl-2-dihydrofuran-3-one, 4-hydroxy-2,5-dimethyl-furan-3-one (Herz and Chang, 1970), pyrazines (Watanabe and Sato, 1971) and 2-acetyl-2-thiazoline (Tonsbeek et al., 1971) could not be found, perhaps due to the sampling technique used in this work. Acids could not be found for the same reason. Ammonia and amines, which have been found in the meat aroma (Herz and Chang, 1970), were not analyzed. The unsaturated aldehydes reported by Herz and Chang (1970) and Watanabe and Sato (1971) have not been detected. No lactones have been identified although some of them have been found by the same authors. Applying the sampling technique used in this work, it was possible, however, to detect in model experiments γ-decalactone and more volatile lactones at odor threshold concentration. Based on retention data, Brennan and Bernhard (1969) suggested the presence of propyl mercaptan and butyl mercaptan in the headspace gas of canned beef broth, but none of these compounds have been identified in our samples. Brinkman et al. (1972) have recently reported the presence of 1-methylthio-ethanethiol in the headspace of simmering beef broth. The compound has not been found in our investigation but a similar compound has been identified tentatively, namely bismethyl thio-methane which was present in trace amounts (see Table 3). The two compounds have the same molecular weight but their mass spectra differ in important respects.

No new compounds were formed or disappeared when fat and/or carbohydrate were added or when the heating time was varied, but there were large quantitative differences (see Table 5). When the heating time was increased the concentrations of the sulfur compounds increase drastically, particularly hydrogen sulfide and methyl mercaptan. The way these two components change with heating time does not correspond entirely to the results obtained by Ziemba and Mälkki (1971). This discrepancy may very well be due to factors discussed by Golovnya et al. (1972). Other compounds which increase strongly with increased heating are 2-methyl propanal, 2-methyl butanal and 3-methyl butanal. Compounds which decrease with increasing heating time are butanal, pentanal and hexanal.

There are great differences in the concentrations of many compounds when comparing the different formulations (see Table 5). In general, the concentration in the headspace gas decreased when fat was included in the formulation. This is probably due to the fact that fat dissolves the compounds thus lowering the vapor pressure. Examples of such compounds are dimethyl sulfide, methyl ethyl sulfide, dimethyl disulfide, ethyl mercaptan, thiophene, 2-methyl thiophene, 3,5-dimethyl-1,2,4-trithiolane, 2-methyl butanal and 2-pentyl furan. Straight chain aldehydes, furan and 2-methyl furan had a higher concentration in the samples with added fat, probably due to the fact that fat is a precursor for these compounds. When carbohydrate was included in the formulation most components were unaffected. However, the concentrations of some compounds increased when adding carbohydrate, e.g., 2-methyl furan, ethyl mercaptan, methyl ethyl sulfide, 3,5-dimethyl-1,2,4-trithiolane and some of the aldehydes.

Odor threshold data, available in the literature, has been included in Tables 5 and 6. By comparing these values with the actual concentrations of the corresponding compound in the various samples it is possible to predict something about the sensory importance of the compound for the particular sample. Of course, it must be considered that the threshold data has been obtained in

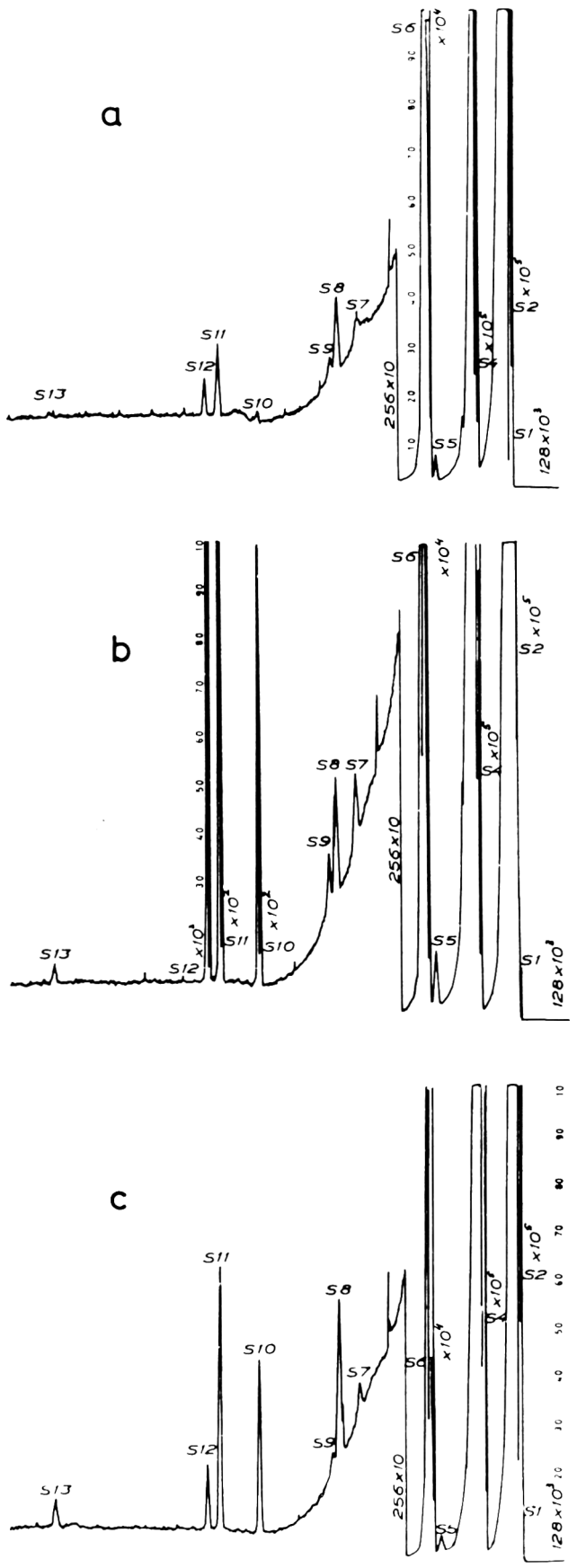

Fig. 3—Gas chromatograms (S-specific FPD) of 400 ml headspace samples of (a) canned beef 121°C, 15 min; (b) canned beef 121°C, 75 min; and (c) canned beef + fat + carbohydrate 121°C, 75 min.

model systems and that in such samples like food items, interactions occur among the various odor components. However, in cases where the concentration is several hundred or thousand times higher than the corresponding odor threshold value, the conclusion that this particular compound is important for the total odor of the sample can be safely drawn.

From Table 5 it can be seen that hydrogen sulfide is present in concentrations which are up to 6000 times higher than the threshold value, methyl mercaptan up to 2200 times higher, dimethyl sulfide up to 700 times higher and ethyl mercaptan up to 300 times higher. Of the remaining sulfur compounds ethylene sulfide and possibly methyl ethyl sulfide and 3,5-dimethyl-1,2,4-trithiolane may contribute to the aroma. Of the nonsulfur compounds the aldehydes form the most important group and among them especially 2-methyl propanal and 3-methyl butanal (see Table 6) which are present in concentrations highly exceeding the threshold values (about 150 and 1000 times higher, respectively). The odor threshold value of 2-methyl butanal is not known but it is assumed that this compound also contributes to the flavor. Of the remaining aldehydes ethanal, butanal, pentanal, hexanal and heptanal may also contribute but not nonanal and benzaldehyde. Except for 2,3-butandione and 2,3-pentandione none of the ketones seems to contribute to the aroma. The concentration of 2-methyl furan is much lower than the threshold value, however, it is possible that furan and 2-pentyl furan can have influence on the aroma. 1-octen-3-ol is probably without importance.

The correlation of these instrumental data with sensory data will be the subject of a later paper.

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Ms received 7/3/72; revised 10/5/72; accepted 10/6/72.

Grants from the Swedish Board for Technical Development are gratefully acknowledged. Advice on, and for the used packaging material has been kindly supplied by Mr. G. Stark, AB Plåtmanufaktur, Malmö, Sweden. Thanks are due to Dr. J. Andersson for helpful discussions, and to Miss R. M. Jansson and Mrs. A. Moore for skillful, technical assistance.

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AROMA OF CANNED BEEF: SENSORY PROPERTIES

INTRODUCTION

THE CHEMICAL composition of the headspace gas of different samples of canned beef was determined and described by Persson and von Sydow (1973). Thus both processing parameters and formulation of product were varied. The present part deals with the sensory properties of the aroma of the same samples analyzed by different techniques.

Luh et al. (1964) compared HTST-sterilized beef with conventionally sterilized beef and found the flavor of the first one more "raw" than the latter. Przeździecka and Żółtowska (1967) investigated canned meat samples which were processed at different time-temperature relations. The sensory properties evaluated were the following: odor quality, palatability and overall quality. They found that within the limits of the investigation, change of temperature had a more pronounced effect on the sensory properties than a change of processing time. Ziemba and Mälkki (1971) analyzed canned beef heated at 121°C for various time periods. The headspace gas was fractionated using different absorbents prior to sensory evaluation. They were thus able to distinguish between the following odors: "Meaty," "ammonia," "hydrogen sulfide" and some more "off-odors." Odor acceptability was also judged; e.g., "off-odor" increased with increasing F_C-values.

In order to relate the problem of off-odor development in canned meat to the processing and formulation variables in a more obvious way, it was felt that a more detailed sensory analysis of the odor was necessary. An odor quality assessment technique originally suggested by Harper et al. (1968a, b) and used by us in other aroma investigations (von Sydow et al., 1970; von Sydow and Karlsson, 1971) was applied. (In the following, the expressions "odor quality" and "odor note" will be used as equivalent concepts.) A somewhat similar descriptive technique has been used on soysause by Tanaka and Saito (1969).

The 36 different odor qualities were also investigated by a special statistical procedure in order to test the metrical assumption that the intensities of these qualities could be represented by a unidimensional, 10-point scale. This is a prerequisite for correlating these qualities with instrumental data (to be published later) in a meaningful way. A correlation matrix for the estimated intensities of all odor qualities was also computed.

EXPERIMENTAL

Materials and processing

Four different formulations have been used, namely minced beef from top side round steaks (Biceps femoris and Vastrus labiralis) with:

(B) 20% H_2O + 0.7% salt

(BF) 13% minced pork back fat + 20% H_2O + 0.7% salt

(BC) 5% potato-flour + 20% H_2O + 0.7% salt

(BFC) 13% minced pork back fat + 5% potato-flour + 20% H_2O + 0.7% salt.

The material was packed into deep drawn electrolytical tinplate cans (size 73 × 28 mm), holding 95g of material. The cans were processed in a retort at 121°C for 15, 30, 45, 60 and 75 min, respectively. For further details, see Persson and von Sydow (1973).

Sensory methodology

Triangle tests. Before the odor quality investigation of the samples in the main experiment was started, triangle tests were performed to establish if there were any significant sensory differences between the samples. The investigated combinations are given in Table 1. One combination was assessed per session and colored light was used to conceal the differences in appearance between samples, which were served in cognac snifters, covered by a watch glass.

Table 1—Significance levels for difference from triangle tests with two formulations heated at four different times at 121°C and three formulations heated at 121°C for 30 min (F_C value = 8)

Formulation[a]	$B_{30\ min}$	$B_{45\ min}$	$B_{75\ min}$
$B_{15\ min}$	0.001	0.01	0.05
$B_{30\ min}$		n.s.	0.05
$B_{45\ min}$			(0.20)

	$BFC_{30\ min}$	$BFC_{45\ min}$	$BFC_{75\ min}$
$BFC_{15\ min}$	0.001	0.001	0.001
$BFC_{30\ min}$		n.s.	0.05
$BFC_{45\ min}$			0.05

	BF	BC
B	0.01	0.001

[a]B = beef; F = fat; C = carbohydrate.

About 15 persons, of whom only few were members of the odor quality panel, participated. Two sessions were used for each comparison. Significance levels for difference were calculated.

Odor quality assessment: Training of the panel; choice of odor qualities, intensity scale and panel members. The panel was selected from a group of 18 persons trained in sensory evaluation. The panel started by assessing two samples of canned beef, one with severe and one with mild heat treatment, using the general odor quality list designed by Harper et al. (1968a, b). Finely divided samples in 25g portions were presented at room temperature in 200 ml cognac snifters covered with a watch glass. The judges were asked to indicate which notes they considered applicable in describing the odor. They were also asked to add those notes they found necessary when describing the total aroma. At the same sessions a similar experiment with black currants was performed. After 10 sessions a frequency study of the terms used was performed, and a preliminary list of 36 notes for canned beef was obtained (Table 2). Some qualities from the general list like: "fruity," "woody," "vanilla-like," "floral," "like petrol, solvents" and "minty" were eliminated, and some were added, e.g., "retort flavor, canned beef off-flavor," "cooked cabbage" and "nasty-smelling." For each odor quality in this list an intensity scale from 0 to 9 was introduced. The scale was defined so that 9 represented the strongest intensity one could expect to meet in normal, everyday life, and 0 was absence of that particular odor quality. The panel members were asked to evaluate the same two samples of canned beef using the intensity scale. This was done for another 10 sessions. For all odor qualities means and ranges were computed for every judge and for the whole panel. Comparisons showed different use of odor quality notes and intensities. The odor quality list and the intensity scale for each quality were discussed with each judge to investigate whether the individual members used the intensity scale in the prescribed manner and if they used the same word to denote a special odor note.

During the discussions, examples of different odor qualities were demonstrated. After another training period of 10 sessions, using the same samples as before, the panel consistently showed a higher degree of consensus, the standard deviations had decreased and significant differences for several odor qualitites were obtained between the samples.

To investigate the metrical structure of the subjective estimates of the different odor qualities and to find out whether the judges could use the odor notes and the scale in a consistent way, a statistical analysis was performed which is described under "Test of metrical structure" (see below). On the basis of this analysis a defi-

nite list of 28 terms was obtained (see Table 3).

When gathering the individual subjects into an optimal panel, there are many criteria for selection. When using the estimation procedure applied, one obvious, fundamental requirement is that the subjects use an intensity ratio scale. By applying the test of metrical structure described below it is possible to exclude those panel members whose scaling behavior is inconsistent. Another reasonable criterion is that the panel members should be able to discriminate adequately. This can be determined by applying, e.g., individual t-tests for difference. These criteria may not be sufficient, however, for obtaining an optimal panel. One should always consider the possibility of existing sub-groups within the panel, each sub-group having a particular response structure with respect to e.g., mean values. In order to test this possibility, a regression analysis between sensory data and chemical data was carried out for the whole group of subjects.

Applying a stepwise procedure it was possible to find the individuals who showed the largest deviations from the regression line. Those particular subjects were excluded. The procedure was repeated until no significant, individual deviations could be found, indicating that the remaining group could be considered as homogeneous. The optimal panel thus obtained, consisting of six persons, was used in the main experiment which included the samples described in "Materials and processing."

Odor quality assessment: Main sessions. In one sub-experiment samples of canned beef heated at 121°C for 30 min were assessed both "by nose" and "by mouth." When assessing "by mouth" the samples were presented in 25g portions in small, flat stainless steel boxes. In both cases the same odor quality list was used.

With the purpose to improve the panel members' ability to discriminate, a reference sample was introduced: canned beef (B) heated at 121°C for 30 min. Every panel member evaluated the reference five times. A personal "profile" was calculated for the reference sample for each judge. To investigate the effect of introducing the reference, two samples [canned beef (B) and canned beef + fat + carbohydrate (BFC)] were assessed with and without the reference. At all subsequent sessions the reference was used and presented at the same time as the sample to be estimated. Each judge was given his personal reference intensities on the odor

Table 2—Results from metrical analysis of odor quality estimation of canned beef[a] Numbers refer to relative average differences (in percent) between metrical and nonmetrical representation

Odor quality	10-point scale – Individuals A[b]	B[b]	F[b]	I[b]	J[b]	G	L	Group values for panel members
Odor strength	+	+	+	+	+	+	3–5	+
Aromatic	0	2–3	0	25–30	0	0	15–20	17–19
Meaty (cooked)	+	+	+	+	+	0	8–10	+
Sickly	2–3	+	+	+	+	10–13	10–25	+
Musty, mouldy	+	0	2–3	+	+	16–20	3–5	1–2
Sharp, pungent	+	2–3	+	1–3	+	11–15	7–10	1–2
Light	0	0	0	0	0	0	4–5	0
Cool, cooling	0	0	0	0	0	0	0	0
Warm	+	+	+	10–12	0	3–5	17–20	4–5
Metallic	7–9	30–35	15–18	17–20	1–3	40-50	35–45	18–20
Cooked cabbage	3–4	1–2	+	1–3	1–2	0	6–10	2–3
Putrid, decayed	0	0	0	0	0	50	0	0
Soapy	0	0	0	0	0	0	30–40	0
Garlic, onion	+	0	+	1–2	+	0	12–15	+
Animal, goat	+	2–3	+	+	2–4	35	0	1–2
Dung-like	0	0	0	2–3	0	0	0	2–3
Nasty-smelling	+	+	+	+	+	+	7–10	+
Fragrant	+	0	2–3	1–2	+	0	15–20	1–2
Earthy	0	0	+	12–15	10–13	25–30	17–20	10–11
Sweaty	11–13	0	2–5	3–4	0	0	10–15	7–8
Burnt, smoky	+	+	+	+	+	13–15	7–10	+
Hay, dried herbs	0	0	0	0	0	0	0	0
Sour, acid, etc.	+	+	2–4	+	+	10–15	2–5	+
Like blood, raw meat	4–5	+	25–30	11–13	15–18	35–40	0	20–23
Dry, powdery	0	0	0	0	0	0	35–40	0
As ammonia	0	0	0	0	0	0	0	0
Oily, fatty	0	6–9	+	12–15	2–4	0	20–25	6–9
Sweet	+	0	+	0	+	0	15–20	+
"Retort flavor, canned beef off-flavor"	+	+	+	+	+	3–5	+	+
Fishy	0	0	0	0	0	0	0	0
Spicy	0	+	0	0	0	15–20	0	+
Rancid	0	0	0	0	0	0	33–38	0
Sulfurous	+	+	+	1–3	+	12–15	3–5	+
Green, cut grass etc.	0	0	0	0	0	15–35	0	0
Heavy	+	+	+	+	+	+	26–30	+
Cooked vegetables	2–3	7–8	2–5	+	+	0	15–20	2–4

[a](+) indicates no statistically significant errors could be found; (0) means the quality in question was considered as absent or irrelevant by the subjects.
[b]Denotes subjects included in the panel

Table 3–The correlation matrix for the preference value and 28 odor qualities (12 samples)

	Preference value	Odor strength	Aromatic	Meaty, (cooked)	Sickly	Musty, mouldy	Sharp, pungent	Light	Heavy	Cool
Preference value	1.000	−0.057	0.443	0.531	−0.643	−0.677	−0.107	0.002	−0.271	−0.375
Odor strength		1.000	0.487	0.744	−0.130	−0.316	0.893	−0.477	−0.327	−0.001
Aromatic			1.000	0.687	−0.756	−0.546	0.316	−0.038	−0.638	0.108
Meaty (cooked)				1.000	−0.589	−0.733	0.734	−0.274	−0.627	−0.244
Sickly					1.000	0.689	−0.065	0.020	0.681	0.069
Musty, mouldy						1.000	−0.342	−0.025	0.527	0.104
Sharp, pungent							1.000	−0.447	−0.366	−0.187
Light								1.000	−0.299	0.221
Heavy									1.000	0.124
Cool										1.000

	Warm	Metallic	Cooked cabbage	Garlic, onion	Animal, goat	Dung-like	Nasty smelling	Fragrant	Earthy	Sweaty
Preference value	0.002	0.369	0.099	−0.253	−0.561	−0.544	0.699	0.558	0.256	−0.579
Odor strength	0.320	0.365	0.768	0.758	0.534	0.069	0.106	0.362	0.561	0.021
Aromatic	0.016	0.533	0.167	0.044	−0.076	0.196	−0.679	0.741	0.501	−0.422
Meaty (cooked)	0.390	0.633	0.645	0.476	0.110	−0.106	−0.410	0.605	0.700	−0.159
Sickly	−0.010	−0.641	−0.032	0.181	0.486	−0.080	0.931	−0.815	−0.386	0.527
Musty, mouldy	−0.489	−0.419	−0.273	−0.033	0.453	0.160	0.579	−0.607	−0.465	0.287
Sharp, pungent	0.641	0.502	0.781	0.903	0.576	0.061	0.208	0.180	0.574	0.281
Light	−0.057	−0.283	−0.626	−0.653	−0.367	0.097	−0.117	−0.289	−0.084	0.111
Heavy	−0.244	−0.516	−0.052	−0.055	0.106	−0.165	0.583	−0.525	−0.430	0.217
Cool	−0.266	−0.380	−0.082	−0.313	−0.190	0.677	−0.051	0.100	−0.090	0.195
Warm	1.000	0.378	0.454	0.581	0.091	−0.073	0.173	−0.018	0.424	0.402
Metallic		1.000	0.385	0.431	−0.005	−0.101	−0.417	0.411	0.444	−0.133
Cooked cabbage			1.000	0.798	0.342	−0.135	0.124	0.331	0.599	0.155
Garlic, onion				1.000	0.691	−0.060	0.433	0.002	0.387	0.318
Animal, goat					1.000	0.213	0.642	−0.390	0.234	0.496
Dung-like						1.000	−0.077	0.030	0.140	0.471
Nasty smelling							1.000	−0.809	−0.234	0.586
Fragrant								1.000	0.357	−0.644
Earthy									1.000	0.226
Sweaty										1.000

	Burnt, smoky	Sour, acid, etc.	Like blood, raw meat	Oily, fatty	Cooked vegetables	Sweet	Retort flavor	Rancid	Sulfurous
Preference value	−0.102	−0.022	0.118	−0.452	0.032	−0.407	0.040	−0.618	0.015
Odor strength	0.805	−0.037	−0.644	−0.527	0.763	−0.492	0.842	−0.198	0.779
Aromatic	0.270	−0.171	−0.040	−0.868	0.358	−0.538	0.297	−0.527	0.357
Meaty (cooked)	0.731	−0.303	−0.598	−0.786	0.768	−0.816	0.779	−0.480	0.776
Sickly	−0.162	0.507	−0.023	0.880	−0.311	0.617	−0.139	0.701	−0.309
Musty, mouldy	−0.383	0.230	0.439	0.620	−0.573	0.806	−0.507	0.515	−0.444
Sharp, pungent	0.962	−0.167	−0.869	−0.435	0.882	−0.625	0.956	−0.191	0.921
Light	−0.356	−0.150	0.249	0.107	−0.378	−0.059	−0.354	0.489	−0.352
Heavy	−0.516	0.642	0.325	0.807	−0.488	0.733	−0.458	0.193	−0.575
Cool	−0.232	0.038	0.088	0.082	−0.005	0.134	−0.309	0.358	−0.143
Warm	0.653	−0.016	−0.798	−0.125	0.631	−0.676	0.686	−0.202	0.628
Metallic	0.537	−0.472	−0.313	−0.746	0.537	−0.651	0.474	−0.748	0.633
Cooked cabbage	0.699	0.096	−0.677	−0.299	0.692	−0.491	0.710	−0.324	0.740
Garlic, onion	0.834	0.030	−0.742	−0.169	0.696	−0.336	0.815	−0.204	0.771
Animal, goat	0.493	0.101	−0.341	0.125	0.219	0.133	0.423	0.306	0.359
Dung-like	0.112	−0.351	−0.103	−0.108	0.172	−0.030	−0.105	0.317	0.163
Nasty smelling	0.113	0.392	−0.233	0.718	−0.073	0.435	0.133	0.571	−0.053
Fragrant	0.199	−0.159	−0.051	−0.782	0.377	0.493	0.226	−0.656	0.341
Earthy	0.559	−0.076	−0.451	−0.568	0.459	0.751	0.531	−0.309	0.627
Sweaty	0.289	−0.088	−0.467	0.416	0.183	0.054	0.140	0.528	0.244
Burnt, smoky	1.000	−0.386	−0.902	−0.490	0.915	−0.702	0.952	−0.157	0.972
Sour, acid, etc.		1.000	0.268	0.495	−0.431	0.423	−0.201	0.024	−0.456
Like blood, raw meat			1.000	0.216	−0.900	0.668	−0.884	−0.010	−0.868
Oily, fatty				1.000	−0.544	0.731	−0.463	0.620	0.602
Cooked vegetables					1.000	−0.742	0.886	−0.254	0.935
Sweet						1.000	−0.698	0.419	−0.784
Retort flavor							1.000	−0.214	0.896
Rancid								1.000	−0.264
Sulfurous									1.000

quality list. Two different samples and two references were presented in pairs at each session. On the average, 12 judges took part in each session. The samples were presented in a randomized order within each subexperiment. Every sample was assessed four times. The panel members were not familiar with the nature of processing and formulation parameters of the samples. The intensity values were marked by the judges on a sheet for optical reading on an IBM 1232 Optical Mark Page Reader, which transforms the data into punched cards for computer analysis.

Odor quality assessment: Statistical treatment of panel data. A computer program was constructed which calculates mean and standard deviation and performs t-test between all possible pairs of samples. The analyses were carried out for all preselected panel combinations. Mean and standard deviation values for the panel can be directly obtained on punched cards for further investigations, e.g., regression analysis together with chemical data. The correlation matrix for all the odor qualities and the preference value (see below) were calculated by help of a computer program from Biomedical Computer Programs (1968).

Preference test. The samples in the main experiment were also assessed for preference of odor by the odor quality panel, using a 10-point scale, where 0 = extremely poor and 9 = extremely good. A reference sample, the same as in the odor quality investigations, was used. It was assigned the value 6 for all panel memebers. The reference together with four or five samples were presented at every session in a randomized order. Every sample was evaluated twice.

Test of metrical structure of the intensity estimation data

Åkesson (1972a, b) has presented a statistical procedure for testing the possibility of constructing sensory scales of intensity from numerical estimation data, taking into consideration different types of possible disturbance factors like stimulus interaction (assimilation-dissimilation), sequential dependencies, errors due to tendencies to use only a limited number of the available scale categories, etc.

The general structure of the statistical model used in the metrical analysis of the numerical judgment data is given by the following:

$$q_{ij} = \mu_{ij} + F(q_{ij}; p) + \epsilon\ ; \qquad \epsilon \sim N(0, \sigma_{ij}) \qquad (1)$$

where q_{ij} denotes the reported numerical estimate of the perceived ratio between a reference sample (S_i) and a comparison sample (S_j) with respect to some quality under consideration. μ_{ij} denotes the mean value of the q_{ij}-data; ϵ is a normally distributed random variable having zero mean and a variance, σ_{ij}, depending on the different sample combinations. The ϵ-term in the model is introduced to account for random errors in the judgement data. F denotes a function on q_{ij}, and p is the parameter vector of F. This function is introduced in order to handle different types of systematic errors of the numerical estimates. One important type of systematic error is generated by the phenomena of assimilation and dissimilation, which can be described as tendencies to underestimate small differences in intensity (assimilation) and tendencies to overestimate large differences (dissimilation).

A necessary condition for constructing a ratio scale of sensory intensity from the q_{ij}-data, as defined by the above model, is that the following parametric reduction is possible,

$$[\mu_{ij} + F(q_{ij};p)] \rightarrow [\mu_i/\mu_j + F(q_{ij};p)] \qquad (2)$$

If $F(q_{ij};p) = 0$ for some choice of p, the reduction can be carried out further as,

$$[\mu_{ij} + F(q_{ij};p)] \rightarrow [\mu_i/\mu_j + F(q_{ij};p)] \rightarrow (\mu_i/\mu_j) \qquad (3)$$

With n different samples there are $n(n-1)/2$ μ_{ij}-parameters to describe the q_{ij}-data, but only n μ_i-parameters. A number of (asymptotically) equivalent test statistics can be formulated for the parametric reductions above. If (2) or (3) can be demonstrated to be valid, the numerical values of the μ_i-parameters constitute the sensory scale values of the derived ratio scale. If the metrical reductions induce errors (bias) on the q_{ij}-data, the magnitude of these errors can be expressed in terms of μ_i-values (scale values).

Analogously, the assumption that the q_{ij}-data represent perceived intervals of intensity rather than ratios can be tested. Applying this approach, the metrical structure of the judgments for the 36 different odor qualities was investigated. The judgments for each individual subject were analyzed separately in order to select those subjects who gave the most consistent estimates. Also, the analysis was performed for pooled judgments.

The 10-point scale used was compared with an enforced 6-point scale.

The material investigated included the following six samples: two formulations (B; BFC) heated at 121°C for 15, 45 and 75 min, respec-

Table 4—Mean panel intensities and standard deviations (s) for 22 odor qualities of canned beef of two formulations, evaluated with and without reference (heat treated beef) (eight judges × four replicates)

	Without reference					With reference				
	B[a]		Significant level for	BFC[a]		B[a]		Significant level for	BFC[a]	
Odor qualities	Intensity	s	difference	Intensity	s	Intensity	s	difference	Intensity	s
Odor strength	6.89	0.17	0.05	6.20	0.45	6.21	0.35		6.22	0.59
Aromatic	2.51	0.41		1.95	0.73	2.15	0.55	0.10	1.53	0.39
Meaty (cooked)	6.20	0.38		5.80	0.50	5.33	0.82	0.10	4.48	0.57
Sickly	2.34	0.27		2.85	0.56	2.75	0.77	0.05	3.98	0.82
Musty, mouldy	1.69	0.43		2.02	0.14	2.28	0.84		2.69	0.32
Sharp, pungent	3.33	0.41	0.05	2.62	0.32	3.10	0.52	0.05	2.52	0.19
Heavy	2.42	0.29		2.33	0.11	2.35	0.55		2.58	0.40
Warm	1.89	0.26		1.62	0.33	1.40	0.18		1.35	0.44
Metallic	1.22	0.50		1.09	0.42	1.00	0.32		0.92	0.30
Cooked cabbage	3.05	0.29		3.09	0.41	2.78	0.22	0.01	1.80	0.48
Garlic, onion	1.42	0.13		1.51	0.13	0.80	0.26		0.65	0.01
Animal, goat	1.85	0.29	0.10	1.56	0.08	1.85	0.24		1.88	0.33
Nasty-smelling	2.06	0.57		2.15	0.38	2.80	0.55	0.10	3.29	0.16
Fragrant	1.87	0.40	0.05	1.22	0.25	1.38	0.44	0.10	0.85	0.27
Sweaty	1.42	0.26		1.02	0.34	1.20	0.23		0.80	0.50
Burnt, smoky	3.60	0.46	0.01	2.54	0.12	3.58	0.68	0.001	1.88	0.19
Sour, acid, etc.	1.36	0.24		1.73	0.23	1.60	0.57		1.70	0.42
Like blood, raw meat	1.04	0.25	0.10	1.47	0.48	1.30	0.57	0.10	2.05	0.53
Oily, fatty	1.24	0.20	0.10	1.91	0.66	1.08	0.47	0.10	1.83	0.58
Sweet	0.49	0.17		0.62	0.35	0.58	0.17	0.10	0.85	0.27
"Retort flavor, canned beef off-flavor"	5.40	0.31	0.01	4.18	0.32	4.22	0.40	0.01	2.85	0.47
Sulfurous	3.80	0.37	0.05	3.07	0.36	2.77	0.39	0.05	2.10	0.38

[a]B = beef; F = fat; C = carbohydrate

tively. All possible pairs of samples were estimated using one sample in each pair as a reference. Two replicates of judgments were obtained for each subject. The necessary computations for the metrical analysis were carried out on an IBM 360/65 computer (Åkesson, 1972b).

RESULTS & DISCUSSION

THE RESULTS from the triangle tests are given in Table 1. This panel showed significant differences for all investigated combinations except for the samples heated at 30 and 45 min, respectively. This shows that the samples can be considered as perceptually different, which is a necessary condition for using the odor quality assessment technique in a meaningful way.

The outcome of the metrical test is given in Table 2. The nonzero numbers in the table are measures of the average, systematic error obtained when trying to represent the numerical judgments by a uni-dimensional ratio scale. The calculated errors can thus be defined as relative errors between nonmetrical and metrical representations and be expressed in terms of percent values. E.g., the interval 2–3 in Table 2 indicates that the metrical representation induces an error of 2-3% compared with the best nonmetrical representation. Due to the limited number of observations, only approximate values of the average relative errors can be calculated. The results suggest that there are substantial differences between subjects with regard to their ability in giving intensity judgments in a consistent way. Thus, subjects A, B, F, I and J are in general superior to subjects G and L.

From Table 2 it also follows that the different odor qualities can be grouped into:

(a) Qualities like: "meaty," "warm," "retort flavor" for which the given estimates are consistent with the applied metrical model; these properties can be regarded as quantifiable and measurable by uni-dimensional scaling techniques;

(b) Qualities like "metallic," "like blood, raw meat," "earthy," which cannot generally be expressed by the ratio model with any high degree of accuracy, indicating that these properties might be more complex and not possible to represent by a uni-dimensional intensity scale;

(c) Qualities like "cool," "as ammonia," "soapy," which were considered as irrelevant.

In conclusion, the result from the metrical investigation clearly shows that it is possible to represent most of the odor

Table 5—Mean panel intensities and standard deviations (s) for 15 odor qualities of beef heated for 30 min at 121°C, evaluated "by nose" and "by mouth" (six judges X four replicates)

Odor qualities	"By nose" Intensity	s	"By mouth" Intensity	s
Odor strength	6.67	0.28	5.19	0.30
Meaty (cooked)	6.08	0.60	5.72	0.21
Sickly	2.11	0.35	1.47	0.28
Sharp, pungent	4.17	0.27	2.00	0.27
Heavy	2.69	0.32	1.69	0.22
Metallic	1.11	0.23	1.22	0.28
Cooked cabbage	3.06	0.16	2.85	0.16
Garlic, onion	1.11	0.12	1.54	0.12
Animal, goat	1.91	0.37	0.97	0.19
Nasty-smelling	2.87	0.15	2.06	0.50
Burnt, smoky	4.65	0.17	2.47	0.24
Sour, acid, etc.	1.80	0.22	1.91	0.32
Like blood, raw meat	0.72	0.27	1.57	0.26
"Retort flavor, canned beef off-flavor"	4.89	0.12	3.85	0.36
Sulfurous	3.06	0.22	2.19	0.08

Table 6—Mean panel intensities and standard deviations (s) for preference value and 15 odor qualities for four different formulations heated at 121°C for 30 min (six judges × four replicates)

Odor quality	BF Intensity	s	Sign. level for dif	B Intensity	s	Sign. level for dif	BC Intensity	s	Sign. level for dif	BFC Intensity	s	Sign. level for dif	BF Intensity	s
Preference value	4.5	1.5	0.01	5.9	0.7	0.02	4.6	1.6	(0.20)	3.7	1.2	(0.20)	4.5	1.5
Odor strength	5.5	0.6		5.5	0.3	0.01	4.3	0.2	0.10	5.0	0.6		5.5	0.6
Meaty (cooked)	4.6	0.4	0.05	5.2	0.2	0.01	4.1	0.5	(0.20)	3.7	0.2	0.01	4.6	0.4
Sickly	3.5	0.2	0.01	2.7	0.3		2.6	0.5	0.05	3.8	0.7		3.5	0.2
Sharp, pungent	2.3	0.6		2.7	0.3	0.01	1.9	0.1		2.2	0.6		2.3	0.6
Musty, mouldy	3.1	0.2	0.01	2.3	0.3	0.05	2.7	0.2		2.8	0.6		3.1	0.2
Cooked cabbage	2.9	0.3		2.7	0.1	0.02	2.3	0.3	0.10	2.7	0.3		2.9	0.3
Garlic, onion	1.5	0.3		1.5	0.2		1.4	0.2		1.5	0.2		1.5	0.3
Animal, goat	1.9	0.5		1.7	0.1		1.5	0.5		1.7	0.2		1.9	0.5
Nasty-smelling	2.9	0.5		2.6	0.2		2.4	0.6	0.05	3.4	0.5		2.9	0.5
Fragrant	1.9	0.2		1.9	0.4		1.7	0.2		1.7	0.5		1.9	0.2
Burnt, smoky	2.1	0.6		2.3	0.2		2.1	0.5		1.9	0.6		2.1	0.6
Oily, fatty	2.3	0.7	0.05	1.3	0.3	0.01	1.9	0.2	0.01	3.5	0.1	0.10	2.3	0.7
Cooked vegetables	2.4	0.3		2.5	0.2		2.5	0.1		2.4	0.3		2.4	0.3
"Retort flavor canned beef off-flavor"	3.0	0.4	(0.20)	3.4	0.4	0.10	2.9	0.4		3.1	0.3		3.0	0.4
Sulfurous	2.6	0.4		2.7	0.2		2.8	0.3	0.10	2.3	0.2		2.6	0.4

qualities by uni-dimensional intensity scales, which is a positive evidence for the validity of the numerical judgment procedure used. For most purposes a 10% systematic average error may be accepted. It must be pointed out, however, that the measurability and the meaning of a specific odor quality may vary for different types of materials.

An attempt was made to improve the degree of consistency by interpreting the response data as interval estimates of intensity rather than ratio estimates. However, the "interval interpretation" proved to be inferior, i.e., the magnitudes of the average relative errors increased when imposing an interval structure on the response data. Also, an attempt was made to obtain some improvement by partitioning the judgments into a 6-point scale, which did not yield any significant reduction of errors, however. It was, therefore, concluded that the 10-point scale should be preferred because it permits a higher degree of discrimination between the samples.

When introducing the reference, the number of significant differences increased from 7 to 13 (Table 4), demonstrating a conspicuous improvement in the panel's ability to discriminate. Also, when performing experiments for a long period of time, one might expect that by introducing explicit reference samples, the potential time-dependent changes ("drifts") in the intensity values can be reduced. This is because memory effects can thereby be eliminated. To reduce possible anchoring effects, the reference (canned beef heated at 121°C for 30 min) was chosen so that it should not fall in any extreme point but within the range of the comparison samples.

When comparing the odor quality intensities assessed "by nose" and "by mouth" (see Table 5), it can be seen that the two sets of data are highly correlated ($r = 0.89$). The intensity values obtained "by mouth" are somewhat lower than those obtained "by nose," except for a few qualities, e.g., "metallic," "sour, acid etc." and "like blood, raw meat." The fact that the "by nose" and "by mouth" intensities are closely related indicates that it is possible to evaluate the samples in this investigation "by nose" only to get an adequate picture of the flavor changes occurring in canning.

In Table 6 the various formulations are compared. When fat is added, the intensity for several odor qualities increased, e.g., "sickly," "musty, mouldy," "animal, goat," "nasty-smelling" and "oily, fatty," while e.g., the intensity for "meaty (cooked)," "burnt, smoky" and "retort flavor, canned beef off-flavor" decreased. The preference value decreased markedly. These changes may be referred to the fact that fat may have two different functions. It may act as a solvent for aroma compounds and it may be a precursor for volatiles. Considering the chemical data (Persson and von Sydow, 1973), the first function seems to be the dominating one.

When carbohydrate (starch) was added, the preference value and "odor strength" and the intensity for some of the odor qualities, e.g., "meaty (cooked)," "sharp, pungent" and "retort flavor, canned beef off-flavor" decreased, while "musty, mouldy" and "oily, fatty" increased.

When both fat and carbohydrate were included in the formulation, the lowest preference value was obtained. If the formulations BC-BFC-BF (see Table 6) are compared, one can see that fat has a more powerful influence on the aroma than carbohydrate in the formulation BFC.

Figure 1 shows how the preference value and 10 of the odor qualities varied with the heating time for samples of canned beef (B). "Odor strength," "retort flavor, canned beef off-flavor," "sulfurous," "burnt, smoky," "sharp, pungent" and "cooked cabbage" increased. "Meaty (cooked)" increased only between 15 and 30 min and were then constant, which shows that the full meat aroma had not been developed at the shorter time, but well at the longer. "Sickly" and "musty, mouldy" showed a minimum, probably depending on the fact that the full meat aroma had not been developed after 15 min and that these intensities increase with increasing heat treatment. For the same reason the preference value showed a maximum at 30 min heat treatment. Analogous results were obtained with the most composite samples (BFC).

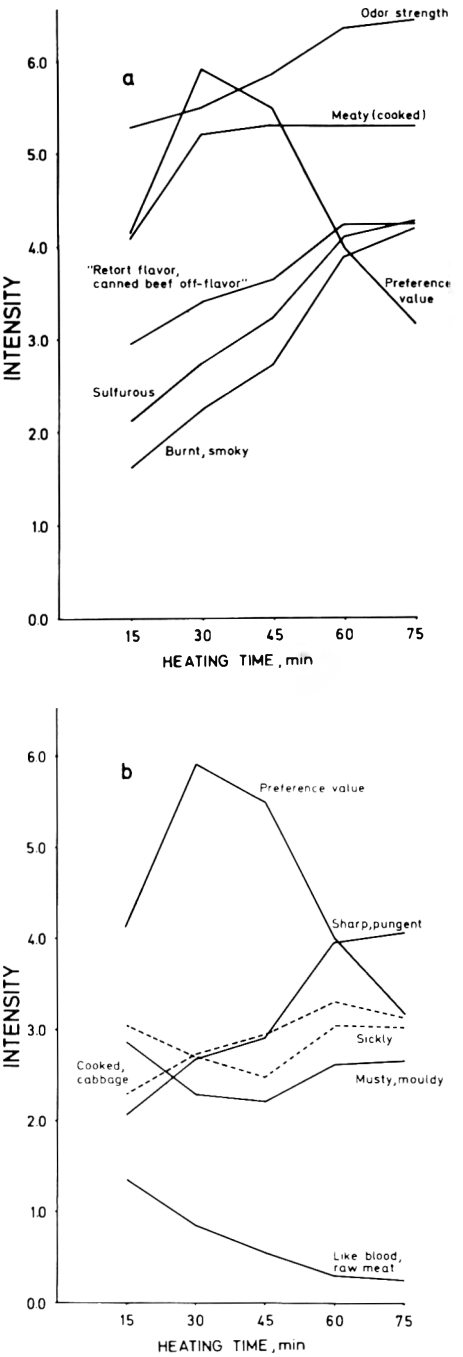

Fig. 1–Mean panel intensities for some odor qualities, and preference value in canned beef, as a function of heating time at 121°C. (a) Preference value; odor strength, meaty (cooked); "retort flavor, canned beef off-flavor;" burnt, smoky; sulfurous; (b) Preference value; like blood, raw meat; sickly; musty, mouldy; cooked cabbage; sharp, pungent.

In the correlation matrix for the preference value and the intensities of the odor qualities (Table 3) it can be seen that the preference value has a positive correlation with "meaty (cooked)" and "fragrant," and a negative correlation with "sickly," "musty, mouldy," "animal, goat," and "nasty-smelling." Thus, the odor qualities which, on the first hand, influence the preference value ought to be found among these.

The matrix may be used also to cut down the number of odor qualities necessary to describe the total aroma, using only those which are independent from each other, i.e., showing low correlation. Among the odor qualities there are a smaller number that are highly correlated but most of them are more or less independent, implying that only a smaller number of terms can be excluded.

The presence of some high inter-correlations in the matrix suggests that, by applying some multidimensional analysis procedure it might be possible to demonstrate that some odor qualities are expressible as combinations of more simple ones. For example, "retort flavor, canned beef off-flavor" might be composed of odor qualities like "sharp, pungent," "burnt, smoky," "cooked vegetables" and "sulfurous."

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Ms received 10/1/72; revised 11/21/72; accepted 11/23/72.

Grants from the Swedish Board for Technical Development are gratefully acknowledged. We thank Mr. B. Vidhög for help with the computer programs; Dr. J. Andersson and Mrs. B. Johansson for helpful discussions; and Miss A. C. Ljungqvist for skillful technical assistance.

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ISOLATION AND IDENTIFICATION OF VOLATILE FLAVOR COMPOUNDS IN BOILED BEEF

INTRODUCTION

LITERATURE on the chemical structure of the volatile compounds responsible for the flavor of boiled beef was practically nonexistent prior to 1960. Many attempts have since been made to reveal the chemical composition of this flavor (Bender, 1961; Bender and Ballance, 1961; Hornstein and Crowe, 1960; Kramlich and Pearson, 1960; Sanderson et al., 1966; Self et al., 1963; Yueh and Strong, 1960). A number of carbonyl compounds, mercaptans, alcohols, sulfides, carboxylic acids, ammonia, hydrocarbons, esters and aromatic compounds have been identified. However, none of these common compounds identified is of particular importance to the genuine flavor of boiled beef. The literature has recently been reviewed by Herz and Chang (1970).

Recently, a few novel cyclic compounds containing nitrogen, sulfur or oxygen have been reported as components of boiled beef flavor. Tonsbeck et al. (1968) identified 4-hydroxy-5-methyl-3(2H)-furanone and 4-hydroxy-2,5-dimethyl-3(2H)-furanone. Chang et al. (1968) reported the identification of 2,4,5-trimethyl-3-oxazoline and 3,5-dimethyl-1,2,4-trithiolane. More recently, Brinkman et al. (1972) isolated and identified 1-methylthioethanethiol and 2,4,6-trimethyl-perhydro-1,3,5-dithiazine from the headspace gas of simmering beef broth. Unfortunately, the compounds identified so far represent neither a complete spectrum of volatile flavor compounds in boiled beef, nor the unique compound or compounds with the characteristic boiled beef flavor. The present paper reports an attempt to systematically characterize the volatile flavor compounds in a sample of boiled beef, using only the semitendinous muscle.

EXPERIMENTAL

Material used

Semitendinous muscles of 22-month-old prime and choice Hereford steers from the same feeding lot and of known history were used for this experiment. The 86 pieces of the semitendinous muscle from 43 steers were aged for 6–7 days at 35°F. They were then freed of external fat and nonsemitendinous meat by knife. Each lean muscle after removal from the carcass was at once wrapped in aluminum foil and placed in the direct stream of cold air from the fan inside a blast freezer box. The muscles were allowed to freeze solid over a period of not less than 75 hr.

As soon as the semitendinous muscles were frozen completely, they were cut into ½ in. cubes by a clean steamed band saw. 7 lb of the composite cubes were defrosted overnight in a refrigerator and dropped into 7 liters of boiling water inside a 12-liter resin reaction flask. The cooking was continued for 1 hr from the time the thermometer indicated that the vapor temperature in the flask was again 100°C. The contents in the resin reaction flask were then allowed to cool. A total of 56.5 lb of beef cubes was thus cooked.

The exit of the resin reaction flask was connected in series to two traps, one cooled with dry ice and the other cooled with liquid nitrogen to collect the volatile compounds evaporated with the water during the boiling process. These volatile compounds were analyzed first by gas chromatography and then combined with those obtained by flash vaporization for further fractionation and characterization.

Isolation of volatile flavor compounds

The cooked beef cubes, at refrigeration temperature, were passed through a Hobart meat grinder. The ground beef was then made into a fine water slurry with the beef broth by using a Waring blender. The volatile flavor compounds were then isolated from the slurry with the use of the apparatus reported by Herz and Chang

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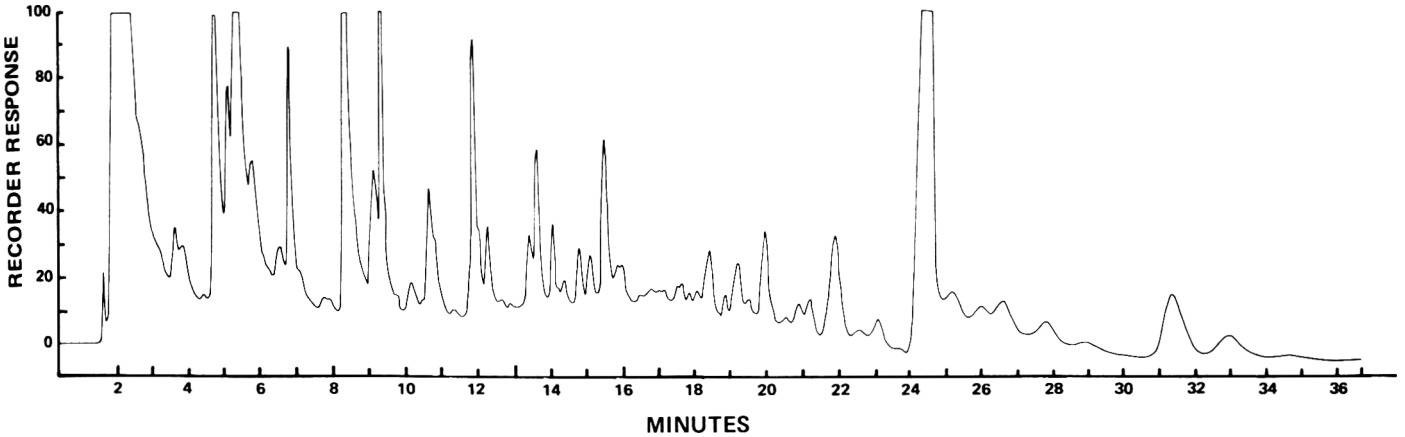

Fig. 1—Gas chromatogram of the nonacidic volatile compounds isolated from boiled beef.

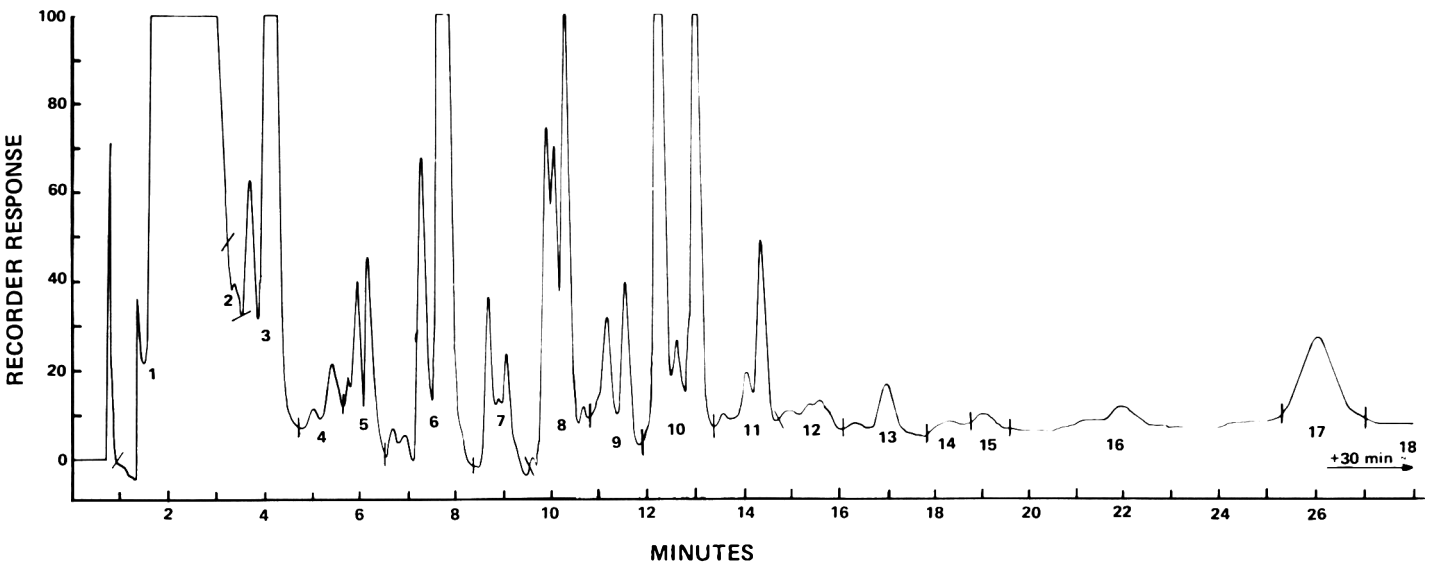

Fig. 2—Gas chromatogram of the nonacidic volatiles of boiled beef used to collect the broad fractions.

(1966), utilizing the principle of flash vaporization followed by evaporation from a continuous heated thin film under vacuum. The slurry, maintained at 70–75°C in the reservoir flask, was pumped continuously into the vaporizer under a vacuum of 0.03 mm Hg at a rate of 70–80 ml/min. The wall of the vaporizer was heated to 105–110°C by circulating heated glycerine in the outer jacket. Water and volatile flavor compounds were flash evaporated from the slurry as it was pumped from atmospheric pressure into a vacuum. Vaporization continued as the residue of the slurry flowed down the vaporizer as a film. The residue collected at the end of the two vaporizers was heated again to 70–75°C for 1 hr, to further generate the desired boiled beef flavor. The recooked slurry was passed through the vaporizer once again at a rate of 35–40 ml/min.

The volatiles were collected in eight traps connected in series: the first five cooled by dry ice and the last three by liquid nitrogen. The condensate collected in these traps was combined, saturated with sodium chloride and extracted with ethyl ether. The ethyl ether extract was concentrated to approximately 400 ml with a 10-plate Oldershaw column. It was then separated into acidic and nonacidic compounds by extracting with 10% aqueous sodium carbonate solution. The ethyl ether solution of the nonacidic volatiles was finally concentrated to a volume of 4 ml with the use of a 6-plate Oldershaw column. The acidic compounds in the ethyl ether solution were converted to their methyl esters with the use of diazomethane (Schlenk and Gellerman, 1960) and then similarly concentrated.

Gas chromatography

The nonacidic volatiles were gas chromatographed with a Varian Aerograph 1520, fitted with a hydrogen flame ionization detector. A 20 ft × 1/8 in. stainless steel column, packed with 15% Carbowax 20M on 60/70 mesh Anakrom ABS, was temperature programmed from 65 to 215°C at a rate of 4°C/min for the first 2 min, then 8°C/min for another 2 min, and 12°C/min thereafter. The helium flow rate was 35 ml/min.

In order to collect pure gas chromatographic fractions for identification, the nonacidic volatiles were separated into 18 broad fractions with the use of a 10 ft × 3/8 in. aluminum column, packed with 15% methyl silicone SE-30 on 70/80 mesh Anakrom ABS, in a Varian Aerograph A-90-P. The temperature was programmed from 50 to 210°C, with a helium flow rate of 120 ml/min. The gas chromatography was repeated until the sample was exhausted. Each broad fraction was accumulatively collected in one cold trap, according to the method of Deck et al. (1965). The 18 broad fractions thus collected were each chromatographed for a second time, using a 6 ft × 1/4 in. aluminum column, packed with 15% Carbowax 20M on 70/80 mesh Anakrom ABS, in a Varian Aerograph 202. The temperature of the column was programmed from 50 to 200°C at a rate of 6°C/min, with a helium flow rate of 60–100 ml/min. Each of the sub-fractions was again collected and gas chromatographed for the third time, either with a Methyl Silicone SE-30 or Carbowax 20M column, to yield pure gas chromatographic fractions which were suitable for identification.

The acidic volatiles in the ethyl ether solution were similarly separated into nine broad fractions, with a 10 ft × 3/8 in. column packed with methyl silicone SE-30 on 70/80 mesh Anakrom ABS. The broad fractions were collected and rechromatographed in a manner similar to the nonacidic volatiles.

Identification of the gas chromatographic fractions

The pure gas chromatographic fractions were identified by the combination of infrared and mass spectrometry, according to the procedure reported previously (Kawada et al., 1966; Smouse and Chang, 1967).

RESULTS & DISCUSSION

THE NONACIDIC volatile compounds isolated from boiled beef were, undoubtedly, a complex mixture as shown by

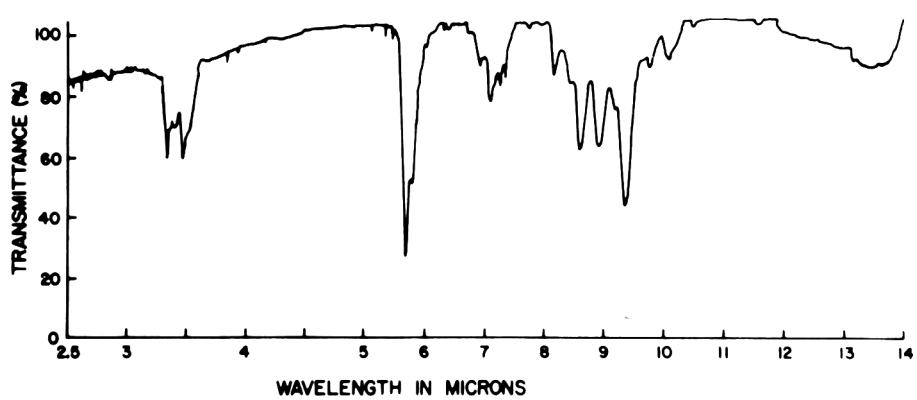

Fig. 3—Infrared spectrum of the fraction identified as 2-methyltetrahydrofuran-3-one.

their gas chromatogram in Figure 1. Due to the complexity of the boiled beef flavor, most of the peaks were not pure compounds. Therefore, a three-step procedure, described in the experimental section, was adopted to obtain homogeneous gas chromatographic fractions for identification. The gas chromatogram used to collect the 18 broad fractions is shown in Figure 2.

The volatile compounds evaporated from the boiled beef during cooking were collected. Their gas chromatogram was very similar to that of the volatiles isolated from the boiled beef. Only minor quantitative differences of some peaks were observed. They were combined with the volatile flavor compounds isolated by flash vaporization for further fractionation and characterization.

The volatile compounds isolated from boiled beef by the flash vaporation and continuous evaporation method, as used in this investigation, did have a characteristic pleasant boiled beef aroma. Furthermore, two of the broad fractions, numbers 6 and 10, appeared to have a characteristic boiled beef aroma. However, none of the 54 compounds identified in the isolated volatile compounds (Table 1) had an aroma which could be considered to be typical of that of boiled beef. The present data, therefore, indicate that boiled beef flavor was a complex mixture of many compounds in which the as yet unidentified compound or compounds with a characteristic boiled beef flavor might be present, but not yet identified. Implications by previous investigators that boiled beef flavor was a proper combination of the common, simple carbonyl and sulfur compounds, are probably an oversimplification of a complex, delicate problem.

Among the 54 compounds identified in boiled beef volatiles (Table 1), the following are of particular interest.

Alcohols

Unsaturated alcohols have not been reported previously as components of boiled beef flavor. Three such compounds have been identified in the present investigation. Hoffman (1962) isolated 1-octen-3-ol from oxidized linoleate and soybean oil. Since beef may contain a small amount of arachidonic acid, this compound might also be formed from hydroperoxidoarachidonate by the same mechanism as suggested by Stark and Forss (1964) for butter fat. 1-Penten-3-ol was reported previously as a component of strawberry volatiles (Winter and Willhalm, 1964). It can be easily formed through the autoxidation of linolenate by a mechanism analogous to that proposed by Stark and Forss (1964) for the formation of 1-octene-3-ol

The third unsaturated alcohol, 2-hexen-1-ol, has been detected by Takei et

Table 1—Volatile compounds identified in boiled beef

Class of compounds	Fraction No.[a]	Compounds identified	Peak size[b]
Hydrocarbons	N-8-2	n-Hexane	Small
	N-10-2-2	n-Dodecane	Small
	N-13-3	n-Pentadecane	Medium
	N-16-2	n-Hexadecane	Small
	N-13-4	n-Octadecane	Small
	N-12-2	1-n-Undecene	Large
	N-12-5	1-n-Pentadecene	Small
Alcohols	N-1-1	Ethanol	Large
	N-2-1	1-Propanol	Large
	N-3-4	2-Methyl-1-Propanol	Small
	N-4-6	1-n-Butanol	Small
	N-5-4	3-Methyl-1-Butanol	Extra small
	N-6-6	1-n-Pentanol	Medium
	N-9-6-2	1-n-Octanol	Medium
	N-4-7	1-Penten-3-ol	Small
	N-6-9	2-Hexen-1-ol*	Small
	N-8-8	1-Octen-3-ol	Extra large
Ester	N-3-1	Ethyl Acetate	Extra large
Ether	N-12-4	Dipentyl ether	Small
Lactone	N-8-11	γ-valerolactone	Extra small
Aldehydes	N-4-2	3-Methyl-1-butanal	Small
	N-5-2	1-n-Pentanal	Large
	N-6-3	1-n-Hexanal	Extra large
	N-7-3	1-n-Heptanal	Large
	N-8-5	1-n-Octanal	Large
	N-9-4-2	1-n-Nonanal	Large
	N-17-1	1-n-Hexadecanal	Medium
	N-9-5	2-n-Octen-1-al	Small
	N-8-6	6-Methyl-2-hepten-1-al*	Medium
Ketones	N-6-5-2	4-Octanone	Small
	N-9-4	3-Nonanone	Small
	N-12-3	3-Dodecanone	Extra small
	N-2-4	Diacetyl	Large
	N-5-5	Acetoin	Extra large
Acids	A-2-1	n-Propionic Acid	Medium
	A-2-3	n-Butanoic Acid	Medium
	A-7-2	n-Hexanoic Acid	Small
Sulfides	N-8-10	Methyl propyl sulfide*	Extra small
	N-9-2	Methyl allyl sulfide*	Extra small
	N-9-2-2	Dimethyl disulfide*	Extra small
	N-10-2	Diallyl sulfide*	Extra small
Aromatic Compounds	N-4-3	Benzene	Large
	N-6-2	Toluene	Small
	N-7-2	n-Propyl benzene	Small
	N-9-0	1,4-Dichlorobenzene	Medium
	N-8-10-2	Benzaldehyde	Extra large
	N-10-9	3-Methyl benzaldehyde*	Small
	A-9-1	2,6-Di-tert.-butyl-p-hydroxy toluene	Medium
Hetero-cyclic Compounds	N-6-7	2-Methyl tetrahydrofuran-3-one	Small
	N-7-7	5-Thiomethylfurfural*	Small
	N-8-4	2-Pentylfuran	Medium
	N-8-12	Thiophen-2-carboxaldehyde	Extra small
	N-10-7-2	2,5-Dimethyl-1,3,4-trithiolane	Large
	N-6-4-2	2,4,5-Trimethyl-3-oxazoline	Extra large

*Tentatively identified

[a]"N" designates nonacidic fraction and "A" acidic fraction. The first, second and third numerals indicate the number of gas chromatographic fractions collected during the first, second and third gas chromatography.

[b]Peak size was considered to be "Extra small" when the peak area was below 50, "Small" between 50 and 300, "Medium" between 300 and 1,000, "Large" between 1,000 and 2,000 and "Extra large" above 2,000.

al. (1938) in black tea oil. It can be produced from 1-hexen-3-ol by oxytropic rearrangement, according to Green and Hickingbottom (1957).

Carbonyl compounds

The number of aldehydes and ketones identified was not as large as expected. Many of the carbonyl compounds which are autoxidative decomposition products of lipids were not found in boiled beef. This is probably due to the fact that the beef used for this investigation was lean. Fatty tissues were carefully trimmed off from the meat before cooking.

Diacetyl and acetoin were frequently reported as components of meat flavor (Hornstein and Crowe, 1964; Landmann and Batzer, 1966; Yueh and Strong, 1960; Lineweaver and Pippen, 1961; Kami and Otaishi, 1967; Grey and Shrimpton, 1967). The relatively large amount of diacetyl could certainly contribute a buttery note to the cooked meat. Since acetoin could be easily converted to diacetyl under air, the extra large peak of acetoin could serve as an abundant supply of the diacetyl.

Heterocyclic compounds

The two heterocyclic compounds with sulfur and nitrogen respectively in the ring, 2,5-dimethyl-1,3,4-trithiolane and 2,4,5-trimethyl-3-oxazoline, were first reported as components of the volatiles of boiled beef by Chang et al. (1968). Since each of these two compounds was a sub-fraction of the two broad fractions with odor reminiscent of boiled beef, they were anticipated to have the typical boiled beef flavor. Unfortunately, when they were synthesized in the laboratory, it was found that their aroma was not characteristic of that of boiled beef. Nevertheless, these two interesting compounds were present in boiled beef in relatively large and extra large amounts, respectively.

Two furan compounds have been found in the volatiles of boiled beef. One of them, 2-methyltetrahydrofuran-3-one, has been previously reported in roasted coffee aroma isolates by Gianturco et al. (1964). Its infrared spectrum is shown in Figure 3 and its mass spectrum in Figure 4. The other furan compound, 2-pentyl furan, has been claimed by Chang et al. (1966) as predominantly responsible for the reversion flavor of soybean oil. They successfully isolated this compound from a reverted-but-not-yet rancid soybean oil with a peroxide number of only 4.3 meq/kg. This compound was identified by its infrared and mass spectra and the identification was confirmed by synthesis of the authentic compound.

The remaining two heterocyclic compounds identified contained sulfur. The extra small peak, with spicy meat aroma, was the sulfur compound analogus to furfural, namely, thiophen-2-carboxaldehyde. Its infrared, NMR and mass spectra have been published previously (Peron et al., 1967; Martin et al., 1968; Bowie et al., 1967). A thiophene compound, 2-methylthiophene, has been reported in chicken flavor by Nonaka et al. (1967). Thiophen compounds have also been reported in coffee aroma (Gianturco et al., 1964a, b; 1966; Gautschi et al., 1967). The other sulfur compound identified was 5-thiomethylfurfural. Its infrared and mass spectra are shown in Figures 5 and 6.

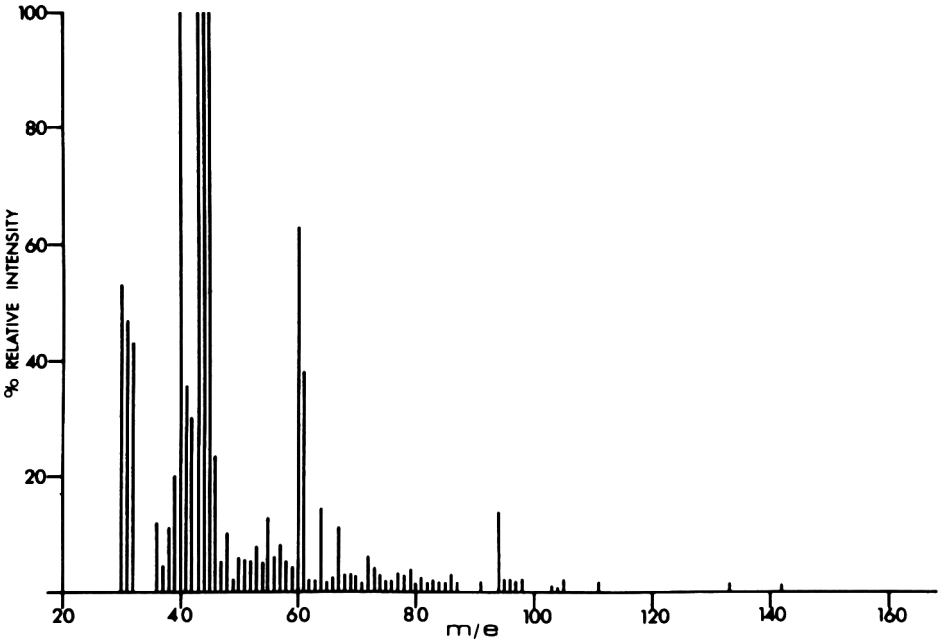

Fig. 4—Mass spectrum of the fraction identified as 2-methyltetrahydrofuran-3-one.

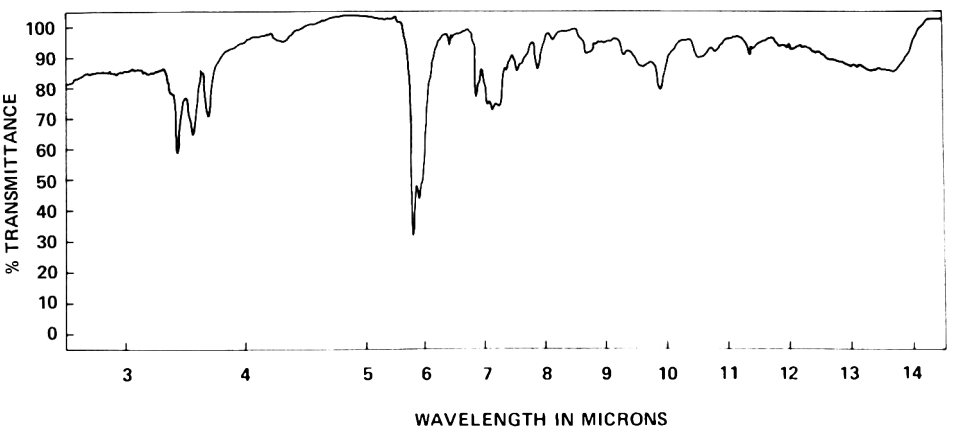

Fig. 5—Infrared spectrum of the compound identified as 5-thiomethylfurfural.

Contaminants

Two of the compounds identified in the volatiles isolated from boiled beef were most likely not responsible for its aroma, but were rather contaminants. 2,5-Di-tert-butyl-p-hydroxytoluene was the common antioxidant, BHT. It was probably a compound used to stabilize the feed. The other compound, 1,4-dichlorobenzene, was probably a metabolite or decomposition product of the pesticide used. The identification of these compounds clearly demonstrate that the gas chromatography–infrared–mass spectrometry combination used in this investigation is a powerful tool, indeed for the identification of trace constituents in foods.

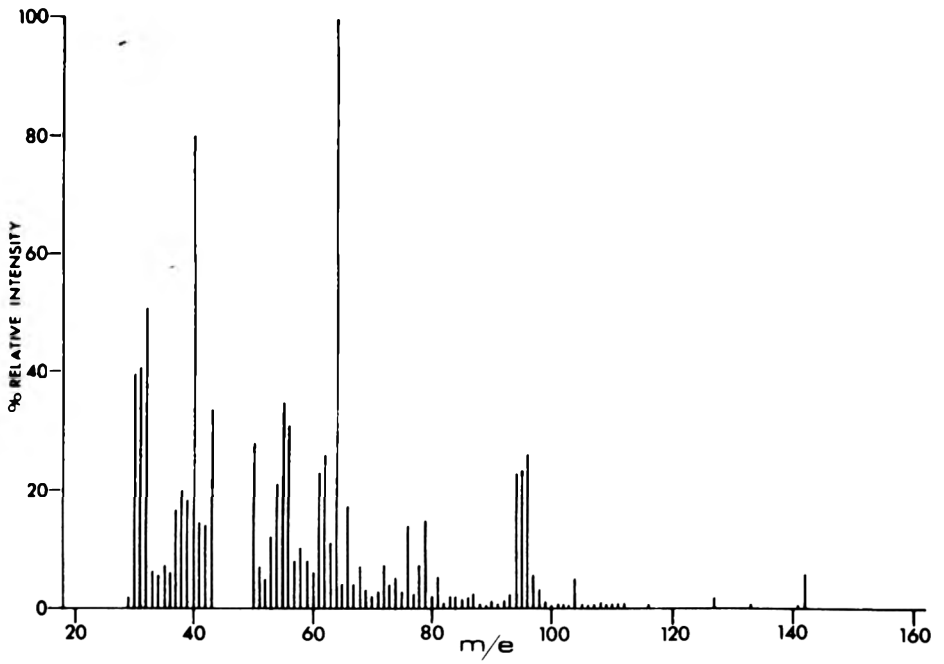

Fig. 6—Mass spectrum of the fraction identified as 5-thiomethylfurfural.

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Ms received 10/20/72; revised 12/23/72; accepted 12/28/72.

Paper of the Journal Series, New Jersey Agricultural Experiment Station, Rutgers, The State University of New Jersey, Dept. of Food Science, New Brunswick, NJ 08903.

Supported by the Agricultural Research Service, U.S. Dept. of Agriculture Grant No. 12-11-100-7669 (73), administered by the Eastern Research & Development Div., Philadelphia, Pa.

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THE INHIBITION OF WARMED-OVER FLAVOR IN COOKED MEATS

INTRODUCTION

IT IS WELL KNOWN that retorted uncured canned meats do not develop WOF. Zipser and Watts (1961) stated that the production of antioxidants in meat itself at high temperatures was responsible for stability against oxidative rancidity. However, the chemical nature of the antioxidant and its specific location in the meat were not determined.

Although numerous papers have been published on the use of antioxidants and chelating agents in meats, most have dealt with chemical substances such as propyl gallate, butylated hydroxyanisole, citric acid (Klose et al., 1952; Lineweaver et al., 1952; Hanley et al., 1953), polyphosphates (Tims and Watts, 1958; Greene, 1969) and sodium ascorbate (Greene et al., 1971).

The purpose of this investigation was: (1) to study the role of substances formed in retorted canned meats on the inhibition of WOF development of model systems; (2) to determine the relative stability toward WOF development of different types of meat subjected to high temperatures; and (3) to determine whether browning reaction products formed during interaction of sugars and amino acids during retorting could inhibit WOF development in cooked ground beef.

EXPERIMENTAL

Preparation of retorted meats

Beef top rounds were trimmed of subcutaneous adipose tissue. Hams were deboned and then defatted in a similar manner as beef. Light and dark turkey meat was obtained from Nicholas White turkeys which were frozen for 1 wk after slaughter. These meats were ground individually through a 1/8-in. plate and packed in 208 × 208 (approximately 150g) aluminum cans. All cans were sealed while maintaining at least 250 mm mercury. Thermocouple probes were inserted into at least three cans of each type of meat during several retorting runs. A portable potentiometer (Leeds and Northrup) was used to record temperatures. The various meats with an initial temperature of 15.5°C were processed for 75 min until an internal temperature of 115.5°C was reached.

Preparation of retorted extracted beef

Ground lean beef was extracted according to the method of Sato and Hegarty (1971). The extracted residue was placed in 208 × 208 aluminum cans and retorted in the same manner as the various ground meats to obtain retorted extracted beef (REB).

Preparation of concentrated broth and water extract

Retorted ground beef was removed from the can and ground through a 1/8-in. plate. 150g of this ground meat were extracted four times with three vol of water for 1 hr at 4°C. Constant stirring was provided with a propeller-type mixer. The retorted meat-water mixture was filtered through cheesecloth and the filtered extract was concentrated in a rotary evaporator to about 5–10 ml to give the concentrated extract of retorted beef. The broth which separated from meat during retorting was filtered through cheesecloth to remove fat and extraneous meat particles and also concentrated by rotary evaporation.

Preparation of extracted retorted beef

The residue obtained from water extraction of retorted beef (ERB) was packaged in 10-g samples, wrapped in polyethylene-coated paper and frozen until ready for use.

Preparation and treatment of model systems used to study the WOF reaction

The previously described water-extracted tissue residue or fresh ground beef was used in model systems to which iron, ascorbic acid, reducing compounds, or the various materials derived from retorted meat were added. Ascorbic acid and iron used were of analytical reagent grade. The reducing compounds used were: reductic acid (K and K Labs., Plainview, N.Y.) and dihydroxymaleic acid (Calbiochem, Los Angeles, Calif.). Other chemicals used were glutaric acid (Eastman Kodak Co., Rochester, N.Y.), maltol (Aldrich Chemical Co., Milwaukee, Wisc.) and pyrazine (K and K Labs., Plainview, N.Y.).

The tissue residue or meat containing the various substances was treated and incubated by the method described by Sato and Hegarty (1971).

Objective lipid oxidation measurement

The incubated samples were evaluated for the extent of lipid oxidation by the thiobarbituric acid test as described by Tarladgis et al. (1960). This method was also used successfully by several investigators (Younathan and Watts, 1960; Keskinel et al., 1964; Greene, 1969; Witte et al., 1970; and Greene et al., 1971) to measure lipid oxidation in raw or cooked meats.

Subjective evaluation

Subjective flavor and odor evaluations were conducted by several members of the laboratory familiar with WOF. In most cases, differences in WOF development in various samples were so obvious that formal taste panel evaluation was not carried out.

Preparation of freeze-dried diffusate and dialysate

The freeze-dried diffusate and dialysate were prepared by the previously described method of Sato and Hegarty (1971).

Preparation of model systems of sugars and amino acids

The model systems were prepared with dextrose or lactose as the carbohydrate, while either glycine, lysine, or leucine served as the source of amino groups. Solutions containing either 20% dextrose or 10% lactose and either 5% glycine, lysine, or leucine were sealed in 208 × 208 aluminum cans without vacuum. They were processed for 60 min until an internal temperature of 115.5°C was attained. Aliquots of these retorted solutions were added to fresh ground beef. Sugar-amino acid systems which were not retorted and sugar or amino acid solutions retorted alone served as controls.

Table 1—Effect of concentrated broth and water extract from retorted beef on WOF development of cooked ground beef stored 2 days at 4°C

Treatment	Subjective odor	TBA value[a]
Ground beef, control	Strong WOF	0.500
Ground beef + concentrated broth[b] of retorted beef	Meaty, pleasant	0.111
Ground beef + concentrated water extract[b] of retorted beef	Meaty, pleasant	0.101

[a]TBA value in absorbance
[b]0.5g per 10g ground beef

Preparation and treatment of ground meat loaves

To 1000g of lean ground beef were added the various commercially available vegetable protein products or dry milk powders. Deglanded cottonseed flour was obtained from Southern Regional Research Lab., New Orleans, La. Texgran was obtained from Swift & Co., Oak Brook, Ill. Textured vegetable protein was obtained from Archer-Daniels-Midland Co., Decatur, Ill. Wheat germ, Kretschmer Wheat Germ Products, Minneapolis, Minn., was purchased in a local market. Nonfat dry milk (NFDM) and spray-dried whey (SDW) were obtained from Land O'Lakes Creameries, Inc., Minneapolis, Minn.

The loaves were placed into aluminum pans, covered with foil, then placed in a pre-heated (177°C) oven and cooked to an internal temperature of 71°C (160°F). The heating time was approximately 80 min. A potentiometer was used to record internal temperatures. The meat loaves were then cooled to room temperature and allowed to incubate at 4°C for 2–5 days. These loaves were analyzed by the TBA test and also by subjective odor and flavor evaluation.

RESULTS & DISCUSSION

IT WAS FOUND initially that the concentrated broth or water extract obtained from retorted beef inhibited the development of warmed-over flavor (WOF) of cooked ground beef. The inhibition of this stale or rancid odor was shown to occur as indicated by a decrease in TBA value and also by subjective evaluation (Table 1). On the other hand, neither the water extract nor the heated water extract from raw beef (nonretorted) inhibited WOF development in water-extracted beef tissue (Table 2). These results indicate that water extract from raw beef did not contain precursors necessary to develop the WOF-inhibiting substance.

The diffusate fractions which were obtained from either the broth or the water extract by dialysis and then freeze dried were also found to retard development of WOF (Table 3). The dialysate, on the other hand, was found to be ineffective in preventing or retarding WOF.

These results indicate that an inhibitory or antioxidative-type substance was present in beef which has been retorted. Moreover, the substance was water soluble and of low molecular weight.

In order to confirm the presence of this antioxidative substance model systems consisting of either retorted beef (RB), extracted retorted beef (ERB) or retorted extracted beef (REB) were used. To these model systems were added either (1) ascorbic acid or (2) iron or (3) ascorbic acid plus iron.

The results in Table 4 clearly show that antioxidative materials are present in retorted beef. When iron was added to ERB, there was an increase in TBA value, an indication of the acceleration of WOF (Table 4, Exp. 1). Also, when iron or ascorbic acid was added to REB there was an increase in TBA value. But when iron, ascorbic acid, and iron plus ascorbic acid were added to RB, TBA values did not increase (absence of WOF development) despite the presence of these lipid oxidation catalysts. These results indicate that inhibition was not due to removal or inactivation of either catalytic substance. The data show that both the inhibitor and the precursors of the inhibitor are water soluble and readily extracted from the meat. If the meat was extracted prior to retorting or if the retorted meat was water extracted, no inhibition was observed. That is, there was acceleration of WOF (increase in TBA value).

Inclusion of iron and ascorbic acid with ground beef during the retorting process also did not affect the apparent production of the inhibitory substance (Table 5). It was found that when such meat is ground, exposed to air and allowed to incubate for 2 days at 4°C, WOF development does not occur. This evidence strongly supports the experimen-

Table 2—Effect of heated water extract from raw beef on the WOF development of extracted beef muscle stored 2 days at 4°C

Treatment	TBA value[a]
Control, extracted beef only	0.095
Extracted beef + concentrated water extract[b] of fresh beef	0.345
Extracted beef + heated concentrated water[c] extract of fresh beef	0.295
Extracted beef + concentrated water extract[c] of retorted beef	0.122

[a]TBA value in absorbance
[b]Amount equivalent to the extract from 12g of meat
[c]Amount equivalent to the extract from 24g of meat

Table 3—Effect of diffusate and dialysate from retorted beef on the development of WOF in cooked ground beef stored 2 days at 4°C[a]

Treatment	Subjective odor	TBA value[b]
Ground beef, control	Strong WOF	0.466
Ground beef + freeze-dried diffusate[c]	No WOF	0.045
Ground beef + freeze-dried dialysate[c]	WOF	0.430

[a]Data taken from three experiments
[b]TBA value in absorbance
[c]1g per 10g ground beef

Table 4—Effect of iron and ascorbic acid on extracted retorted, retorted extracted and retorted beef

Experiment no.	Treatment	TBA value[a]
1	ERB,[b] control	0.054
	ERB + 2 ppm Fe(II)[e]	0.234
2	REB,[c] control	0.140
	REB + 2 ppm Fe(II)	0.201
	REB + 10 ppm ascorbic acid	0.246
	REB + 2 ppm Fe(II) + 10 ppm ascorbic acid	0.296
3	RB,[d] control	0.025
	RB + 2 ppm Fe(II)	0.020
	RB + 10 ppm ascorbic acid	0.018
	RB + 2 ppm Fe(II) + 10 ppm ascorbic acid	0.015

[a]TBA value in absorbance
[b]ERB—extracted retorted beef
[c]REB—retorted extracted beef
[d]RB—retorted beef
[e]Iron was added in the form of $FeCl_2$ at a level necessary to provide 2 ppm Fe(II).

Table 5—Effect of retorting ground beef with added iron, ascorbic acid and iron plus ascorbic acid

Treatment	TBA value[a]
(Beef) retorted, control	0.029
[Beef + 2 ppm Fe(II)] retorted	0.031
(Beef + 10 ppm ascorbic acid) retorted	0.029
[Beef + 2 ppm Fe(II) + 10 ppm ascorbic acid] retorted	0.034

[a]TBA value in absorbance

Table 6—Effect of retorting treatment on TBA values of various meats

Sample	TBA value[a] Days incubation at 4°C 0	1	2	3	Odor after 3 days
Retorted beef	0.022	0.023	0.022	0.010	Meaty
Retorted pork	0.018	0.012	0.018	0.015	Meaty
Retorted light turkey meat	0.036	0.040	0.037	0.040	Good
Retorted dark turkey meat	0.045	0.092	0.145	0.272	WOF

[a]TBA value in absorbance

tal results obtained in Table 4, Exp. 3.

It is well-known that lipid oxidation is influenced considerably by iron and ascorbic acid. The peroxidation of mitochondrial and microsomal fractions was induced by iron (McKnight et al., 1965 and Wills, 1969). Nonheme iron was found to be an active catalyst of linoleoate oxidation in meats (Liu, 1970 and Liu and Watts, 1970). The prooxidative effect of ferrous iron in the oxidation of linoleic acid in emulsions was shown to occur by Marcuse and Fredriksson (1971). The prooxidative effect of ascorbic acid was demonstrated in rat tissue homogenate (Bernheim, 1963; Barber, 1966) and in microsomes and mitochondria (Ottolenghi, 1959; Wills, 1969). Iron plus ascorbate was found to catalyze lipid peroxidation (Bernheim, 1963; Robinson, 1965). In a previous publication (Sato and Hegarty, 1971), low amounts of ascorbic acid in the presence of Fe(II) accelerated the development of WOF in water-extracted meat tissue. The antioxidative nature of retorted meat in the presence of prooxidants such as ascorbic acid and iron indicates that inhibitors of WOF development were produced in meat during the retorting process.

Tests were made to determine whether antioxidative substances could be produced in retorted pork and turkey meats as well. Retorted pork and turkey meats were removed from cans, ground and incubated at 4°C in beakers which were loosely capped with foil in order to maximize exposure to oxygen. The TBA analyses were conducted on the samples after 0, 1, 2 and 3 days. The data in Table 6 indicate that antioxidative substances responsible for retardation of WOF development were produced in retorted pork and turkey meat as well as in retorted beef. However, in retorted dark turkey meat there was a substantial increase in TBA value after 3 days' incubation (0.272, Table 6). This increase in TBA value indicates that the antioxidative substance was produced in lesser amounts. It is also possible that larger amounts of phospholipid and total lipid content in dark turkey meat might be too great to be controlled by the antioxidative factor produced in normal quantities.

The exact reason for the instability of retorted dark turkey meat toward oxidation is unknown. However, Keskinel et al. (1964) found that the extent of lipid oxidation in raw ground dark turkey meat was greater than that in light meat. Marion and Forsythe (1964) stated that the rate of oxidation in raw ground dark turkey meat was greater than that in light turkey meat because of its higher total lipid content. Acosta et al. (1966) found that the phospholipid content as a percent of total lipid was higher in dark meat than in light meat. Therefore, dark meat was probably more susceptible to oxidation because of its higher phospholipid content.

It should be recognized that these turkey meats were frozen and thawed prior to their use and similar results may not necessarily be obtained with fresh turkey meats.

It is interesting to note that beef diffusate obtained from retorted beef was found to retard development of WOF in cooked ground pork (Table 7). On the other hand, WOF development was not completely inhibited in turkey meats, even though there was substantial reduction in TBA values as can be seen from the data in Table 7.

WOF development in cooked beef proceeds at an extremely rapid rate when exposed to air at refrigerated temperatures. Control of this unpleasant odor and taste can apparently be achieved with antioxidants derived from retorted beef (Tables 1, 3 and 7). Since these diffusates have a pleasant meaty odor, the implications of this investigation for prevention of WOF in precooked or partially cooked meat products for use, for instance, by fast-food service facilities are quite obvious. It is possible that these inhibitory substances can be prepared by retorting and extracting meats with inferior binding properties such as beef hearts, beef briskets, jowls and tongue muscle.

The inhibitory property of diffusates obtained from retorted meat may be due to the presence of certain reducing compounds produced as the result of interaction between sugars and either amino acids or protein in a browning-type reaction. A comprehensive review of the chemistry of browning reaction is given by Hodge (1953; 1967), Ellis (1959) and Reynolds (1965). The interaction of sugars and amino acids in beef extract or diffusate was studied by several investigators (Hornstein and Crowe, 1960; Macey et al., 1964; and Zaika et al., 1968). Sugar-amino acid interaction in heated diffusate from water extract of beef was studied by Wasserman and Spinelli (1970). However, all of these studies were concerned pri-

Table 7—Effect of freeze-dried diffusate from retorted beef on inhibition of WOF development of various meats stored 2 days at 4°C

Treatment	Subjective odor	TBA value[a]
Ground pork, control	WOF	0.278
Ground pork + beef diffusate[b]	Pleasant	0.068
Light turkey meat, control	Strong WOF	1.00
Light turkey meat + beef diffusate[c]	WOF	0.650
Dark turkey meat, control	Strong WOF	0.905
Dark turkey meat + beef diffusate[c]	WOF	0.395

[a]TBA value in absorbance
[b]600 mg per 10g ground pork
[c]500 mg per 10g turkey meat

marily with the flavor and aroma of meats.

The results in Table 8 show that browning reaction products obtained from the interaction of sugars and amino acids inhibit WOF development in cooked ground beef. Sugar-amino acid solutions which were not retorted and sugar or amino acid solutions retorted alone did not inhibit WOF development in ground beef (Table 9). Recent findings (Table 10) also indicate that certain reducing compounds produced during browning reactions might be responsible for inhibition of WOF development. For example, it was found that reductic acid (2,3-dihydroxy-2-cyclopenten-1-one), which is formed in browning reactions (Hodge, 1953; 1967), was a very powerful inhibitor of WOF development in cooked ground beef (Table 10). This inhibition appears to be due to an ascorbic acid type inhibition where there is an antioxidant effect at relatively high concentration; i.e., the inhibition may be due to oxygen scavenging property (Sato and Hegarty, 1971). This explanation seems to be consistent with the fact that reductic acid was found to retard fat oxidation in frozen minced red salmon and herring flesh (Tarr and Cooke, 1949) and to retard enzymic browning of fruits (Tarr and Cooke, 1950).

It is also speculated that the added inhibitor (reducing compound) retarded oxidation by acting either as a free radical acceptor or a hydrogen donor.

Maltol was found to be an effective inhibitor of WOF development in cooked ground beef (Table 10). Hodge et al. (1967) reported that maltol is produced in typical Maillard-type reactions in foods. Patton (1950a, b) reported the formation of maltol in lactose-glycine systems and in heated skim milk. The mechanism of the inhibition by maltol is not understood.

Dihydroxymaleic acid and pyrazine (Table 10) showed a definite but weaker inhibitory effect on WOF development than maltol. Dihydroxymaleic acid was found to retard enzymic browning of fruits (Tarr and Cooke, 1950). Pyrazine was included in the experiment because it was shown to be produced in a lactose-casein browning system (Ferretti et al., 1970).

Hodge (1953) and Hodge et al. (1963) reported that various reducing compounds called reductones were produced during browning reactions. Amino-hexose-reductones were found to be effective antioxidants in soybean, cottonseed and corn oils (Evans et al., 1958). The increased stability of sugar cookies was attributed to the formation of reducing substances during baking (Griffith and Johnson, 1957). These literature references seem to lend support to recent findings (Table 10) that certain reducing and other compounds produced during browning reactions might be responsible for WOF inhibition in cooked ground meat.

Supporting evidence implicating reducing-type substances as inhibitors of WOF was obtained when several different vegetable protein products and dry milk products containing large amounts of protein and sugar were incorporated into ground meat loaves. These experiments were designed to see whether cooked meat loaves contained reducing-type substances which would act as inhibitors of WOF development. The data in Table 11 show that meat loaves were found to be

Table 8—Effect of retorted solutions of sugars and amino acids on the inhibition of WOF development in ground beef stored 2 days at 4°C

Treatment	Concentration of additive ppm	Subjective odor	TBA value[a]
Ground beef, control	–	Strong WOF	0.400
Ground beef + (glucose + glycine)	5,000	No WOF	0.113
Ground beef + (glucose + lysine)	5,000	No WOF	0.051
Ground beef + (glucose + leucine)	5,000	No WOF	0.088
Ground beef + (lactose + glycine)	10,000	No WOF	0.092

[a]TBA value in absorbance

Table 9—Effect of various retorted and nonretorted sugar, amino acid or sugar-amino acid solutions on WOF development of cooked ground beef stored 2 days at 4°C

Experiment no.	Treatment	Concentration of additive %	TBA[a] value
1	Ground beef, control	–	0.239
	Ground beef + retorted glucose[b]	0.1	0.290
	Ground beef + retorted glycine[c]	0.1	0.215
	Ground beef + nonretorted (glucose + glycine)[d]	0.1	0.216
	Ground beef + retorted (glucose + glycine)[d]	0.1	0.145
2	Ground beef, control	–	0.292
	Ground beef + nonretorted (glucose + glycine)[d]	0.2	0.262
	Ground beef + retorted (glucose + glycine)[d]	0.2	0.160

[a]TBA value in absorbance
[b]10% glucose solution
[c]10% glycine solution
[d]5% glucose + 5% glycine

Table 10—Effect of various chemicals on the inhibition of WOF development in cooked ground beef stored 2 days at 4°C[a]

Treatment	Concentration of additive %	Subjective odor	TBA value[b]
Control	–	WOF	0.260
Reductic acid	0.1	No WOF	0.035
Dihydroxymaleic acid	0.1	WOF	0.140
Glutaric acid	0.1	WOF	0.192
Maltol	0.1	No WOF	0.034
Pyrazine	0.1	WOF	0.157

[a]Data taken from three experiments
[b]TBA value in absorbance

Table 11—Effect of various nonmeat protein products on the inhibition of WOF development in cooked ground meat loaves[a]

Treatment	Concentration of additive, %	2 Days at 4°C			5 Days at 4°C		
		Odor	Taste	TBA value[b]	Odor	Taste	TBA value[b]
Cottonseed flour	4	Meaty	Meaty	0.050	Meaty	Meaty	0.059
Nonfat dry milk heated to 125° C	2	Good	Bitter	0.047	No WOF	Bitter	0.032
Spray dried whey heated to 125°C	2	Good	Bitter	0.036	No WOF	Bitter	0.050
Nonfat dry milk, nonheated	4	Meaty	Meaty	0.029	Good	No WOF	0.054
Spray dried whey, nonheated	4	Meaty	Meaty	0.049	Good	No WOF	0.036
Texgran[c]	4	No WOF	No WOF	0.054	Meaty	Beany	0.254
Texgran[d]	4	Good	Good	0.092	No WOF	Beany	0.210
TVP[e]	2	Good	Meaty	0.115	Bland	Trace WOF	0.188
Wheat germ	4	Good	Good	0.074	Good	No WOF	0.094
Control	–	WOF	WOF	0.619	WOF	WOF	1.23

[a]Data taken from six experiments
[b]TBA value in absorbance
[c]Textured soy flour, caramel color added
[d]Textured soy flour, no caramel color
[e]Textured vegetable protein

extremely stable toward rancidity upon refrigerated storage.

Milk solids are known to contain appreciable amounts of protein and carbohydrates (Bell and Whittier, 1965). The Maillard-type interaction between lactose and milk protein is well documented (Coulter et al., 1951; Patton, 1955; and Ferretti et al., 1970). Vegetable soy protein products contain appreciable amounts of protein and carbohydrates (Rakosky, 1971). Glandless cottonseed flour contains large amounts of protein and a lesser but significant amount of carbohydrate (Martinez et al., 1970).

These literature references and the results of these experiments suggest that inhibition of WOF development in meat loaves was due presumably to the presence of antioxidative substances which were produced as the result of some kind of interaction between amino acids or proteins and carbohydrates upon heating. The stability of meat loaves containing soy protein products could also be due in part to the presence of antioxidants in soybean flour (Pratt, 1972).

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Ms received 10/1/72; revised 11/25/72; accepted 11/30/72.

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INFLUENCE OF pH AND FIBER CONTRACTION STATE UPON FACTORS AFFECTING THE TENDERNESS OF BOVINE MUSCLE

INTRODUCTION

WHEN CONSIDERING the relationships between the mechanical properties of bovine or ovine muscle and pH and water-holding capacity (WHC), few authors have considered how these relationships can be affected by fiber contraction state. Bouton et al., (1972a, b) studied the effect of pH on some properties of ovine muscle and showed that large increases in WHC can counteract the increased shear force values which are usually associated with cold-shortening. The relationship between shear force values and fiber contraction state has been demonstrated by many authors (including Herring et al., 1965, 1967a; Marsh and Leet, 1966; and Davey et al., 1967). However the influence of fiber contraction state on other important structural properties has received comparatively little attention. Herring et al., (1967b), Buck and Black (1967) and Kruggel and Field (1971) have suggested that stretching might increase collagen solubility but were not able to demonstrate a statistically significant effect. Bouton and Harris (1972a, b, c), using a direct mechanical measurement of adhesion between the meat fibers, showed that connective tissue strength could be significantly reduced by stretching.

This present work extends that work by determining the effect of pH on connective tissue strength, WHC and thermally induced fiber contraction, as well as shear force in both stretched and cold-shortened muscles.

MATERIALS & METHODS

Animals and pre-slaughter treatments

20 Hereford steers aged approximately 2.0 yr with a mean liveweight at slaughter of 408 kg (S.E. 9kg) were used. They were penned individually and fed both alfalfa and commercial cattle cubes for 3–10 wk prior to slaughter. Adrenaline injections pre-slaughter were used to produce muscles with different ultimate pH values. It was possible by systematic variation of dose strength, number of injections and times they were given before slaughter to obtain values close to predetermined values (Shorthose, 1972, private communication).

Measurement of pH

pH values of both raw and cooked meats were measured directly using a Phillips PW 9408 Digital pH Meter with a Phillips probe-type combined electrode (C64/1). Ten measurements were made on each sample and the mean used as the value for the sample.

Measurement of WHC

WHC of the raw meat was measured using the centrifugal method of Akroyd as modified by Bouton et al. (1971).

Measurement of sarcomere lengths

Sarcomere lengths were measured by a light diffraction method similar to that described by Rome (1967). A Helium-Neon laser, 3mW, of wavelength 632.8 nm, was used as the light source. Sarcomere lengths were measured on raw, unfixed slivers of muscle, held between glass microscope slides. Three measurements of the diffraction pattern were made on each of two pieces of muscle from each sample and the mean sarcomere length calculated.

Measurement of the mechanical properties of the cooked meat

Warner-Bratzler (W-B) shear force measurements were made with a modified version of the shear device described by Bratzler (1932). The samples of cooked meat, sheared at right angles to the fiber axis, were 4–8 cm long with a rectangular cross-sectional area of 1 cm^2 (1.5 × 0.67 cm) and with the fibers lying parallel to the long axis of the sample. The method for measuring connective tissue strength or the adhesion between the meat fibers has been described by Bouton and Harris (1972b).

Sample preparation and cooking method

The muscles used were the deep pectoral (DP) and semi-tendinosus (ST) muscles. Both DP muscles and one ST muscle were removed 25–30 min after slaughter from each carcass which was then transferred to a cold room at 0–1°C. The remaining ST muscle was removed from the carcass two days after slaughter. One DP muscle was stretched by about 50% of its excised length and nailed to a plastic covered board while the other was placed in a cold room at 0°C within 45 min of slaughter and allowed to cold shorten. The ST muscle excised pre-rigor, was split down its length, to increase the rate of cooling, and allowed to cold shorten at 0–1°C.

All these muscles, including the ST muscle excised 2 days post-slaughter, were wrapped in polyethylene film after removal and stored at 0°C. At 5 days post-slaughter they were removed from storage for water holding measurements on the raw meat and also for cooking.

The samples were cut into rectangular blocks weighing approximately 140–180g and cooked for 1½ hr, tightly wrapped in polyethylene bags and totally immersed in a water bath at 80 ± 0.5°C. The samples were cooled in their own juices for ½ hr by total immersion in cold running water. They were weighed and their lengths measured both before and after cooking to determine the weight lost in cooking and

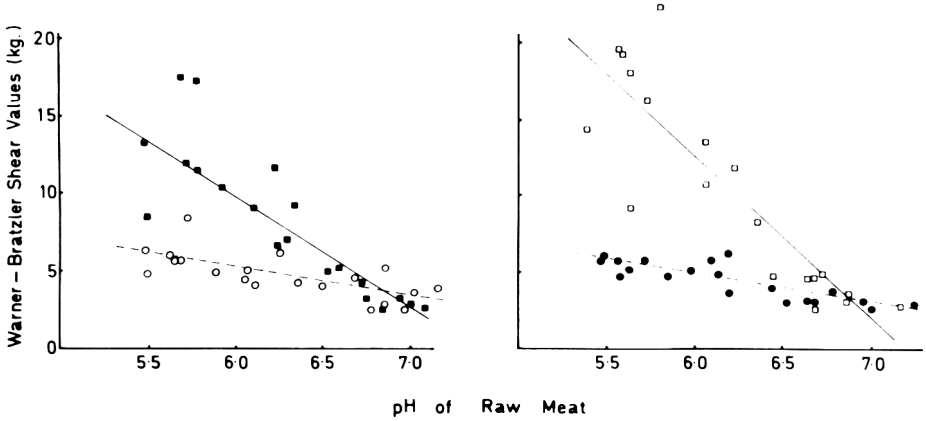

Fig. 1—The relationships between Warner-Bratzler shear force values (kg) and the ultimate pH of cold-shortened (■) and pre-rigor stretched DP muscles (○) and of cold-shortened (□) and control (●) ST muscles.

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meat fiber shrinkage. The solids content of the cooking juices was determined by evaporating 1g aliquots to dryness at 105°C for 18 hr.

The cooked samples, wrapped in polyethylene to prevent moisture loss, were stored overnight at 0–1°C and then cut into samples suitable for Warner-Bratzler shear measurements and adhesion measurements.

RESULTS

Fiber contraction states

The ST muscles which went into rigor on the carcass had sarcomere lengths with a mean value of 2.30 μm (S.E. 0.25 μm). The regression between sarcomere length and pH was significant ($P < 0.01$); sarcomere length decreased from about 2.5 μm when the pH was 5.5 to 2.0 μm when the pH was 7.0. The cold-shortened ST muscles had sarcomere lengths with a mean value of 1.55 (S.E. 0.19 μm). The means of the sarcomere lengths of the DP muscles removed pre-rigor were 1.57 μm (S.E. 0.20 μm) for the cold-shortened muscles and 3.00 μm (S.E. 0.53 μm) for the stretched muscles. The stretched muscles had been stretched to 155 ± 10% of their excised length. Although there was a trend to shorter sarcomeres at high pH values no significant relationship between pH and sarcomere length was found for the DP muscles. Even though there is this trend towards shorter sarcomeres in the stretched DP and in the control ST muscles, this is unlikely to influence shear force values which are relatively insensitive to such differences when the sarcomere lengths are greater than 2.0 μm (Bouton et al., private communication).

Effect of pH and fiber contraction state on W-B shear force values

The shear force values obtained for both muscles and for each contraction state are shown in Figure 1. The regression between shear force and pH was highly significant ($P < 0.001$) and linear for both contraction states of both muscles. In both muscles there was a highly significant difference ($P < 0.01$) between the slopes of the regression lines representing the two contraction states. Consequently the shear force results obtained for the cold-shortened and stretched muscles were significantly different at the pH of the control animals (about 5.6). This difference in shear force values attributable to differences in fiber contraction state decreased with increasing pH values to become nonsignificant around 7.0. This result illustrates that increasing WHC can counteract the effects attributable to cold or rigor shortening.

Connective tissue strength

This was assessed by measurements of adhesion between the muscle fibers. The results are shown in Figure 2. There was a significant ($P < 0.01$) negative linear relationship between ultimate pH and adhesion values in both muscles and for both contraction treatments. There was again a highly significant difference between the slopes of the regression lines representing the two contraction states in both muscles. Contraction of the muscle fibers significantly ($P < 0.01$) increased connective tissue strength. The significant difference between the two contraction states again decreased with increasing pH, showing that myofibrillar contraction had little or no effect on connective tissue strength at high pH or WHC values.

The raw stretched muscles with a high pH tended to have shorter sarcomeres than those with a low pH. This did not seem to affect the dependence of adhesion values (which were measured on cooked meat) on pH because, as discussed later on, the effect of thermal contraction on cooking could have compensated for this discrepancy.

When adhesion values of the two muscles were compared the DP muscles had significantly ($P < 0.01$) greater adhesion values than the ST control muscles and the cold shortened ST muscles yielded similar adhesion values to those obtained for the stretched DP muscles.

Water-holding capacity of raw meat

The water-holding capacity results are shown in Figure 3. The results for both muscles and each contraction state were significantly ($P < 0.01$) and linearly related to pH. For the DP muscles, where the differences in contraction state were greatest, the WHC of the stretched muscles was significantly greater ($P < 0.01$) than that of the contracted muscles. The difference in WHC due to contraction state in the ST is significant only at the $P < 0.05$ level. The difference between muscles is significant ($P < 0.01$) largely because the DP muscles in their stretched state have much longer sarcomeres than the ST muscles.

Water retained after cooking

The amounts of moisture lost from the two muscles during cooking are shown in Figure 4. These results paralleled the

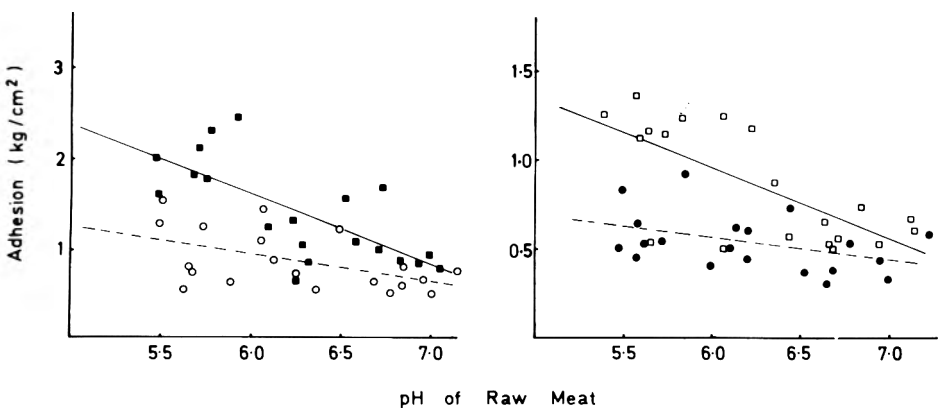

Fig. 2—The relationships between adhesion values (kg/cm²) and the ultimate pH for cold-shortened (■) and stretched (○) DP muscles and of cold-shortened (□) and control (●) ST muscles.

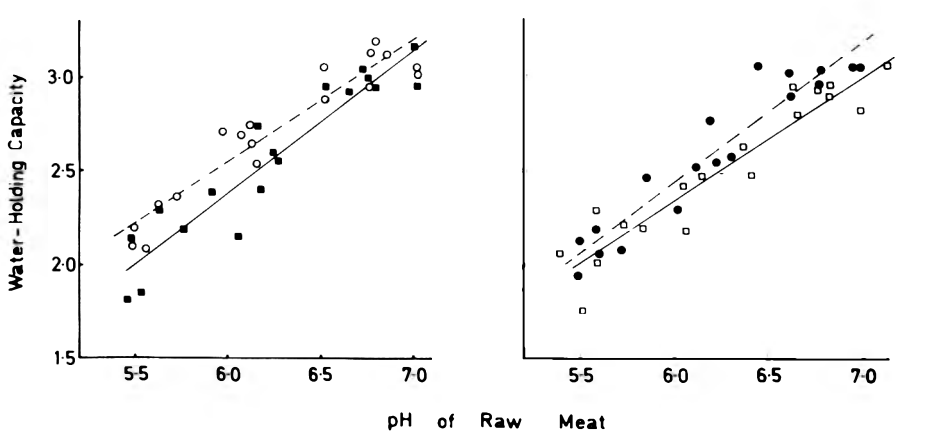

Fig. 3—The relationships between the WHC (g H_2O/g fat-free dried meat residue) of raw cold-shortened (■) and stretched (○) DP muscles and cold-shortened (□) and control (●) ST muscles and their ultimate pH.

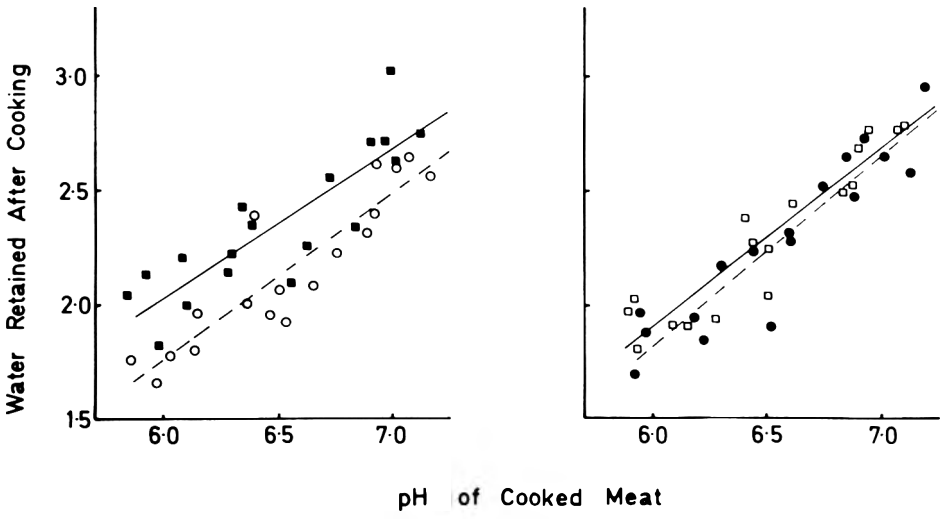

Fig. 4—The relationships between the water retained after cooking (g H_2O/g fat-free dried meat residue), at 80° C for 1½ hr, and the pH of the cooked meat for cold-shortened (○) and stretched (■) DP muscles and for cold-shortened (●) and control (□) ST muscles.

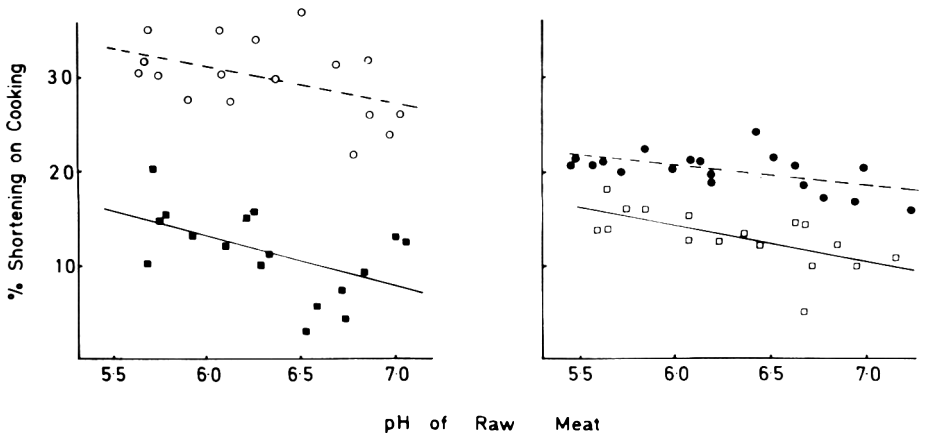

Fig. 5—The relationships between the percentage shortening during cooking and the ultimate pH of cold-shortened (■) and stretched (○) DP muscles and cold-shortened (□) and control (●) ST muscles, cooked for 1½ hr at 80° C.

WHC results obtained for the raw meat in that the water retained after cooking was significantly greater for the stretched than for the contracted DP muscles. No significant difference was found between the control and contracted ST muscles in water retained after cooking.

Length changes during cooking

Length changes during cooking (Fig. 5) were in all but one case significantly related to pH. The exception was for the stretched DP muscles where the relationship only approached significance ($P < 0.05$). Muscles with the longest sarcomeres shortened most during cooking. The mean percentage fiber length change due to cooking was 30.2 ± 4.1 for the stretched DP (mean sarcomere length 3.00 ± 0.56 μm) and 11.6 ± 4.5 for the contracted DP muscles (mean sarcomere length 1.57± 0.20 μm). Corresponding mean changes in fiber length due to cooking were 20.2 ± 2.2 for the control ST (mean sarcomere length 2.31 ± 0.25 μm) and 13.1 ± 3.1 for the contracted ST (mean sarcomere length 1.55 ± 0.19 μm). Shrinkage in length due to cooking was greater when the actin filaments were least interdigitated with the thicker myosin filaments. This suggests that the actin filaments, when not inside the A-band, could shorten more on heating than the myosin filaments of the A-band.

CONCLUSIONS

FOR MUSCLES of normal pH (5.4–5.6) shear force values were, as expected, highly dependent on muscle fiber contraction state. Connective tissue strength, assessed by adhesion measurements, was also significantly increased for samples which contained predominantly contracted fibers, compared to samples containing mainly stretched fibers. As pH, and hence WHC, increased so both shear force values and connective tissue strength became increasingly independent of fiber contraction state.

These results show that fiber contraction state affects connective tissue strength as well as shear force values so that sample presentation could affect organoleptically determined tenderness. Muscle fibers are longer in steaks than in slices of roasts which usually cut into thin sections across the fibers. Subjective appreciation of tenderness in steaks is thus more likely to be influenced by differences in fiber toughness than is likely with thin slices of roast meat. Conversely in roast slices with meat fibers about 2–3 mm long the connective tissue might be expected to be primarily responsible for maintaining sample integrity during chewing.

Although the contraction state of uncooked muscle has been related to the tenderness of the cooked meat, simple relationships between sarcomere length and tenderness cannot always be assumed since changes in fiber length produced by cooking varied with both ultimate pH and sarcomere length and were related to shear force and adhesion measurements.

At equivalent pH, differences in WHC attributable to fiber contraction state were small and unlikely to have much effect on tenderness.

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Ms received 10/5/72; revised 11/30/72; accepted 12/5/72.

This work was supported in part by funds from the Australian Meat Research Committee.

The authors thank Miss Denise Lehmann and Mr. R.F. Dickinson for their skilled technical assistance and Mr. S. Penna for care of the animals.

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FATTY ACID COMPOSITION OF BOVINE INTRAMUSCULAR AND SUBCUTANEOUS FAT AS RELATED TO BREED AND SEX

INTRODUCTION

THE QUANTITY and composition of bovine lipids, as well as that of other animal lipids, have been shown to contribute to the organoleptic qualities of the meat. Several researchers have investigated the influence of total lipid and fatty acid composition on muscle palatibility and correlations with such sensory parameters as juiciness, tenderness and flavor have been reported (Hornstein et al., 1961; Waldam et al., 1968; Dryden and Marchello, 1970; Thrall and Cramer, 1971).

The factors affecting the amount and type of lipid deposited by bovine (Terrell, 1967; Hornstein et al., 1967; O'Keefe et al., 1968; Hood and Allen, 1971), porcine (Allen et al., 1967a, b, c), and ovine (Cramer and Marchello, 1964) animals have been widely studied. These investigations have implicated a large number of factors including age, sex, liveweight, anatomical location and nutrition; all of which produce variations in the amount and composition of animal lipids.

Differences in lipid content among breeds within several species have been reported (Reid et al., 1968; Bramblett et al., 1971; Lohman, 1971). The fact that breed exerts an influence on many of the characteristics of the animal body indicates that a similar influence on lipid composition might be expected. While differences in iodine number reported in the literature (Callow and Searle, 1957; Callow, 1958) suggest such compositional variations, a more systematic study relative to particular fatty acids is needed.

Hormonal differences among animals have been implicated in variations in lipid composition due to sex. It has been established that castration results in a greater accumulation of lipids (Turton, 1962; Teague et al., 1964; Allen and Bray, 1964; Ray and Kromann, 1971; Field, 1971). Differences in the iodine number of the intramuscular lipid from normal and castrated porcine males (Lawrie et al., 1964) as well as in specific fatty acids, particularly C18:0 and C18:1 (Allen et al., 1967b), have been reported. In bovine animals, differences in C16:0, C18:0 and C18:1 (Terrell, 1967) between steers and heifers have been observed. Hood and Allen (1971), however, observed greater compositional differences in the intramuscular lipid when bulls were compared to steers and heifers than when steers and heifers were compared. These workers also reported differences between the triglyceride fraction of the intramuscular and subcutaneous lipids with respect to C16:1 and C18:1.

The present study investigated the influence of breed and sex on the fatty acid composition of the intramuscular and subcutaneous lipids from Limousin-sired and Simmental-sired bulls and steers.

EXPERIMENTAL

Sample description

36 Limousin-sired and Simmental-sired bulls and steers, representing three animals per treatment (crossbreed × sex), self-fed on a ration of 50% barley, 30% oats, 15% beet pulp, 2.5% molasses, 0.5% urea and a 2% salt, vitamin and mineral mixture were slaughtered at approximately 452 ± 20Kg (approximately 1-yr old). The carcasses were hung for 3 days; subsequently the section of the longissimus adjacent to the twelfth vertebra, and the biceps femoris were excised from the right side, placed in polyethylene bags and held overnight at 2°C. A portion of the subcutaneous fat from identical locations was similarly treated. These samples were then wrapped in polyethylene-coated freezer paper and stored at −37°C to −40°C until required for chemical analysis. Prior to analysis the meat samples were thawed for 15 hr at 23.5°C and aged for 7 days in a home-style refrigerator at 3.5°C.

A slice approximately 2.5 cm thick was removed from the proximal end of the longissimus and biceps femoris muscles. Three 2.54 cm cores were weighed together and dried on a Virtis Freeze-Mobile Freeze Dryer, model 10-140BA. After drying, the samples were reweighed and stored under nitrogen at −10°C. The subcutaneous fat from the longissimus was separated into external and internal layers while only a single layer was visible over the biceps femoris. The subcutaneous fat samples were then dried and stored under conditions similar to that described for the meat samples.

Extraction and methylation

Total intramuscular lipid was extracted using the method of Bligh and Dyer (1959). 5–10g of partially frozen muscle was homogenized with 95 ml of a monophasic solvent mixture of chloroform, methanol and water (1:2:0.8 v/v/v) for 2 min at full speed in a Virtis 23 homogenizer. The extraction mixture was filtered with slight suction through a Whatman No. 1 filter paper and the residue washed with 25 ml water and 25 ml of chloroform. The washed residue was rehomogenized and the filtrate from the two extractions transferred to a 500 ml separatory funnel. On separation, the chloroform layer was filtered through Whatman No. 2 filter paper and evaporated to dryness using a Buchler Portable Flash Evaporator, model PF-100N. The flasks were then flushed with nitrogen and placed in a desiccator over concentrated sulfuric acid to remove any remaining traces of moisture. Total extractable lipid was subsequently determined gravimetrically. Subcutaneous lipid was isolated in a similar manner to that described for the intramuscular lipid eliminating the second extraction procedure.

Methyl esters were prepared for gas liquid chromatographic analysis according to the method of Metcalfe et al. (1966).

Chromatographic conditions

The fatty acid methyl esters were separated on a dual column Aerograph, gas chromatograph model 1740-1, equipped with flame ionization detectors and helium as carrier gas. Samples were injected onto 2.7m × 3.2mm steel columns packed with 10% EGSS-Y organosilicone polyester on 100–120 mesh Gas CHROM Q. The flow rates were 30 ml/min for helium, 25 ml/min and 250 ml/min for hydrogen and air, respectively. The columns were operated isothermally at a temperature of 200°C with injector and detector temperatures maintained at 250°C and 230°C, respectively. The gas chromatograph was equipped with a Varian Aerograph, model 20, single pen recorder and a Varian Aerograph model 477 digital integrator. The individual methyl esters were identified by comparing the retention times with known fatty acid mixtures (Hormel Institute) and by logarithmic plots of retention time versus carbon number. The relative percent based on the total esters measured was determined by integration of individual peak areas.

Statistical analysis

An analysis of covariance was used to adjust for missing values for the intramuscular lipid samples while an analysis of variance was used to determine significant differences among subcutaneous fat samples. Duncan's multiple range test (1955) was used to compare treatment means.

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RESULTS & DISCUSSION

THE FATTY ACID composition of the total intramuscular and subcutaneous lipids from the six crossbreeds is presented in Table 1. A logarithmic plot of the unknown peaks C:X and C:Y indicated that they might be C15:1 and C17:1, respectively. This tentative identification is in agreement with that suggested by Terrell (1967) and O'Keefe et al. (1968). Terrell (1967) identified C:X as either C14:2 or C15:1 while O'Keefe et al. (1968) suggested that this peak represented a branched chain fatty acid. The fatty acids C14:0, C16:0, C16:1, C18:0, C18:1 and C18:2 accounted for over 90% of the 13 fatty acids measured. A statistical analysis was therefore carried out for these six major fatty acids only.

Effect of crossbreed

A significant ($P < 0.05$) crossbreed effect was observed for C14:0, C16:1 and C18:0 in both the intramuscular and subcutaneous lipids. A further crossbreed effect for C16:0 was found only in the subcutaneous fat depot. A division of the crossbreed effect into sire, cow and sire × cow effects revealed the following relationships (Table 2). Limousin crossbreeds contained significantly ($P < 0.05$) more C14:0 and C16:1 than Simmental crossbreeds in both intramuscular and subcutaneous lipids. In the latter fraction Limousin crossbreeds were significantly ($P < 0.05$) higher in C16:0 than Simmental crossbreeds while the reverse was true for C18:0. No other significant differences due to sire were observed. Angus crossbreeds were significantly ($P < 0.05$) higher in C16:1 than Hereford or Shorthorns in the intramuscular lipids while Angus and Hereford were significantly ($P < 0.05$) higher in this fatty acid than Shorthorn in the corresponding subcutaneous fat depots. The higher content of C18:0 in the intramuscular lipids of Shorthorn crossbreeds together with their tendency to have more C14:0 and C16:0 probably explains the slightly lower unsaturated/saturated ratio observed for these crossbreeds in Table 1. In the subcutaneous fat depots Hereford crossbreeds were significantly ($P < 0.05$) higher in C14:0 than either the Angus or Shorthorn crossbreeds. A significant ($P < 0.05$) sire × cow interaction was observed for C16:0 in the subcutaneous fat depots only. Figure 1 illustrates that the differences in the level of this fatty acid in the Angus and Shorthorn crossbreeds contributed to the interaction effect observed.

It has been suggested that an inverse relationship exists between the amount of lipid deposited and the degree of saturation (Callow, 1958). Although not significant, there appeared to be a trend among the Limousin crossbreeds toward a higher percent extractable lipid and a lower unsaturated/saturated ratio. Differences among breeds in relative growth rates in percent fat in the carcass have been demonstrated (Berg and Butterfield, 1968; Lohman, 1971). Link et al. (1970) reported differences in the relative proportions of fatty acids present at various stages of growth in bovine animals with the greatest difference occuring among the polyunsaturated fatty acids. It has also been suggested that compositional

Table 1—Fatty acid composition of the intramuscular and subcutaneous lipids from six bovine crossbreeds[a,b,c]

Fatty acid	Intramuscular lipid[d]						Subcutaneous lipid					
	S × A	S × H	S × SH	L × A	L × H	L × SH	S × A	S × H	S × SH	L × A	L × H	L × SH
C10:0	0.1	0.1	0.1	0.1	0.1	0.1	Tr	Tr	Tr	Tr	Tr	Tr
C12:0	0.1	0.1	0.1	0.1	0.1	0.1	Tr	0.1	0.1	Tr	0.1	0.1
C14:0	2.9	3.0	2.8	3.7	3.4	3.6	3.3	3.7	3.1	3.9	4.2	3.8
C14:1	0.8	0.7	0.7	1.2	0.9	0.9	1.3	1.1	1.2	1.5	1.5	1.3
C15:0	0.5	0.5	0.5	0.7	0.4	0.7	0.7	0.8	0.7	0.8	0.8	0.7
C : X	1.2	1.3	1.3	1.5	1.0	1.1	0.2	0.2	0.2	0.1	0.3	0.1
C16:0	23.4	24.5	24.0	25.0	24.6	25.1	23.3	24.2	23.4	24.6	24.0	25.5
C16:1	4.8	4.7	4.2	5.8	5.1	4.9	5.9	5.9	5.3	6.4	6.5	6.2
C17:0	1.6	1.4	1.7	1.7	1.3	1.9	2.2	2.2	2.1	1.9	2.1	2.0
C : Y	1.9	1.5	1.6	2.1	1.6	1.8	2.0	1.8	1.8	1.8	2.0	1.5
C18:0	14.2	15.0	16.2	12.7	13.9	15.4	13.8	14.3	14.1	12.2	13.1	13.3
C18:1	42.2	40.5	42.8	40.6	40.7	39.7	44.5	42.8	45.0	43.7	42.7	43.5
C18:2	5.8	5.4	5.0	5.0	5.1	4.5	2.7	3.0	2.5	2.6	2.6	2.1
Unsat./Sat.	1.3	1.2	1.2	1.3	1.2	1.1	1.3	1.2	1.2	1.3	1.3	1.2

[a]Means expressed as relative percent of 13 fatty acids measured
[b]L = Limousin; S = Simmental; A = Aberdeen Agnus; H = Hereford; SH = Shorthorn
[c]C:X and C:Y tentatively identified as C15:1 and C17:1, respectively
[d]Means adjusted for missing values

Table 2—Mean levels of fatty acids of the intramuscular and subcutaneous lipid from two sires and three cows[a,b]

Fatty acid	Intramuscular[c]					Subcutaneous				
	Sire		Cow			Sire		Cow		
	Limousin	Simmental	Angus	Hereford	Shorthorn	Limousin	Simmental	Angus	Hereford	Shorthorn
C14:0	3.6	2.9	3.3[b]	3.1[b]	3.1[b]	4.0	3.4	3.6[b]	3.9	3.5[b]
C16:0	24.9[b]	23.9[b]	24.2[b]	22.5[b]	24.5[b]	24.7	23.6	24.0[b]	24.1[b]	24.4[b]
C16:1	5.3	4.6	5.3	4.6[b]	4.6[b]	6.4	5.7	6.1[b]	6.2[b]	5.7
C18:0	14.0[b]	15.1[b]	13.5[b]	13.2[b]	15.8	12.9	14.1	13.0[b]	13.7[b]	13.7[b]

[a]Means expressed as relative per cent of 13 fatty acids measured
[b]Means bearing a common superscript letter are not significantly ($P < 0.05$) different.
[c]Means adjusted for missing values

Table 3—Mean levels of six fatty acids of the intramuscular and subcutaneous lipid from bulls and steers[a]

Fatty acid	Intramuscular[c]		Subcutaneous	
	Bull	Steer	Bull	Steer
C14:0	3.5	2.9	3.9	3.4
C16:0	24.5[b]	24.3[b]	24.8	23.5
C16:1	5.2	4.7	5.9[b]	6.1[b]
C18:0	14.2[b]	14.8[b]	13.8[b]	13.2[b]
C18:1	38.8	43.3	42.2	45.1
C18:2	6.1	3.6	2.9	2.2

[a]Means expressed as relative per cent of 13 fatty acids measured
[b]Means bearing a common superscript letter are not significantly ($P < 0.05$) different.
[c]Means adjusted for missing values

Table 4—Means of three fatty acids of bulls and steers within the longissimus and biceps femoris[a,b]

	Fatty acid					
	C16:0		C18:0		C18:2	
Anatomical location	Bull	Steer	Bull	Steer	Bull	Steer
Longissimus	24.5	25.1	14.9	16.4	6.5	3.3
Biceps femoris	24.6	23.5	13.5	13.3	5.7	3.9

[a]Means expressed as relative per cent of 13 fatty acids measured
[b]Means adjusted for missing values

differences in subcutaneous fat accompany increases in weight and fatness (Waldam et al., 1968). It is conceivable, therefore, that genetic influences produce variations in the physiological growth rate which result in variations in fatty acid composition.

Effect of sex

Significant ($P < 0.05$) sex effects were observed for C14:0, C16:1, C18:1 and C18:2 in the intramuscular lipid. Bulls were higher in C14:0, C16:1, C18:2 and lower in C18:1 than steers (Table 3). In the subcutaneous lipid significant ($P < 0.05$) sex effects were found for C14:0, C16:0, C18:1 and C18:2. Bulls were associated with higher levels of C14:0, C16:0, C:18:2 and lower levels of C18:1 than steers. The main difference between the intramuscular and subcutaneous lipids was the presence of significantly ($P < 0.05$) more C16:1 in bulls than steers in the former fraction compared to significantly ($P < 0.05$) more C16:0 in bulls than steers in the latter fraction (Table 3).

Few studies have investigated differences between bulls and steers. Hood and Allen (1971) reported higher levels of C18:0 and C18:2 and lower levels of C16:0 and C18:1 in the intramuscular lipid fractions of bulls than in those of steers or heifers. These compositional differences were attributed to the possible effect of sex hormones on enzyme systems such as desaturase (Marsh and James, 1962). This might account for the higher levels of C16:1 and C18:1 in the subcutaneous fat depots compared to that in the intramuscular lipid observed in this study. The latter observation is in agreement with that reported by Hood and Allen (1971) for the triglyceride fraction of the intramuscular and subcutaneous lipids. It is also possible that the androgens of the male stimulate fat catabolism and that intricate relationships between various hormones and enzymatic systems could account for compositional differences in the intramuscular lipid of bulls and steers. While compositional differences in the lipids of bulls and steers do exist more research with normal and castrated males is required to establish more fully the nature of these differences.

Interaction effects

Significant ($P < 0.05$) sex x location interactions were observed for C16:0, C18:0 and C18:2 in the intramuscular lipid. A comparison of the means in Table 4 indicates that for C16:0 and C18:0 there was a greater difference due to muscle in steers than in bulls. Higher levels of C18:2 in the longissimus of bulls than in the biceps femoris accompanied by the reverse situation in steers accounted for the interaction effect observed for this fatty acid. A significant ($P < 0.05$) crossbreed x sex x location interaction for C14:0 resulted from variations in the level of this fatty acid in the longissimus and biceps femoris of bulls and steers within the six crossbreeds. In the subcutaneous fat significant ($P < 0.05$) interactions were evident for C16:0 and C16:1 only. Significant ($P < 0.05$) crossbreed x sex and sex x location interactions occurred for C16:0 while crossbreed x sex and crossbreed x location effects were observed for C16:1. Figure 2 illustrates that in all crossbreeds with the exception of Limousin x Shorthorn, bulls had a higher level of C16:0 than steers. The relationship between the levels of C16:1 in bulls and steers is less well defined as illustrated in Figure 3. Steers from Simmental x Hereford and Simmental x Shorthorn as well as from Limousin x Angus and Limousin x Shorthorn crossbreeds were significantly ($P < 0.05$) high-

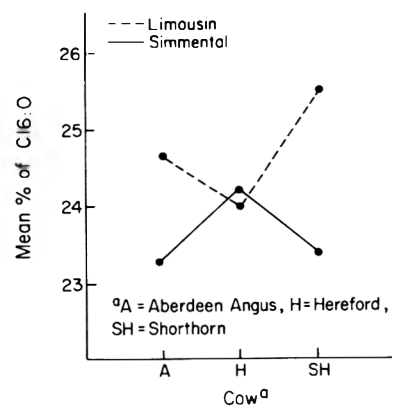

Fig. 1—Influence of sire and cow on the significant ($P < 0.05$) sire x cow interaction for C16:0 in the subcutaneous lipid.

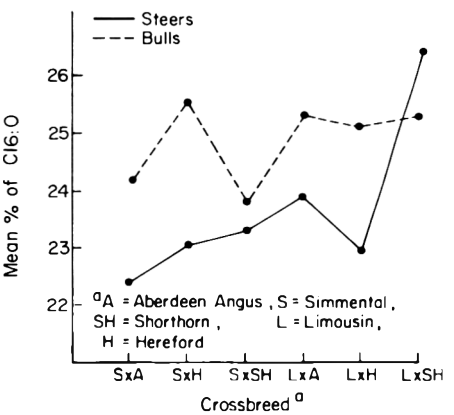

Fig. 2—Influence of crossbreed and sex on the significant ($P < 0.05$) crossbreed x sex interaction for C16:0 in the subcutaneous lipid.

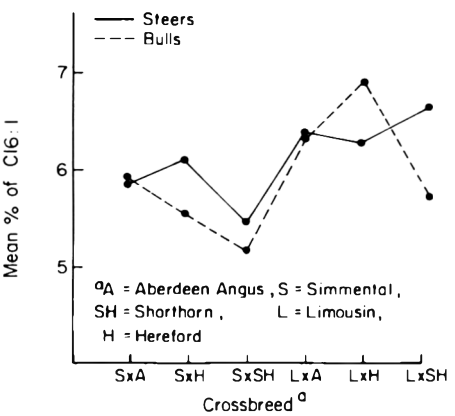

Fig. 3—Influence of crossbreed and sex on the significant ($P < 0.05$) crossbreed x sex interaction for C16:1 in the subcutaneous lipid.

Table 5–Palmitic acid (C16:0) levels in the subcutaneous fat of bulls and steers[a,b]

Sex	Anatomical location		
	Longissimus (external)	Longissimus (internal)	Biceps femoris
Bull	25.1	25.6	23.7
Steer	23.6	23.7	23.4

[a]Means expressed as relative per cent of 13 fatty acids measured
[b]Mean values of 18 observations

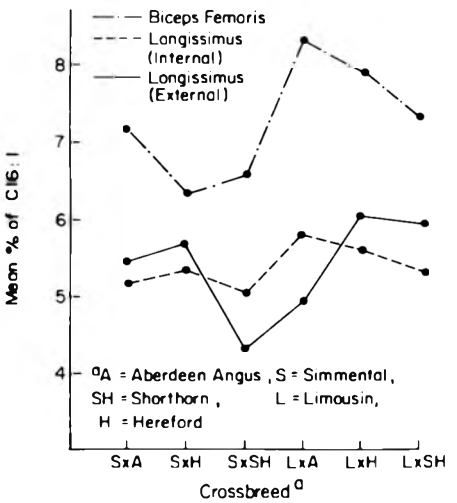

Fig. 4–Influence of crossbreed and location on the significant (P < 0.05) crossbreed x location interaction for C16:1 in the subcutaneous lipid.

er in C16:1 than bulls. The reverse situation was observed with respect to the Simmental x Angus and Limousin x Hereford crossbreeds. The sex x location interaction observed for C16:0 is due to greater differences in the level of this fatty acid between the subcutaneous fat over the biceps femoris and the external and internal layers over the longissimus in bulls than steers (Table 5). The significant ($P < 0.05$) crossbreed x location interaction observed for C16:1 was due to variations in the level of this fatty acid in the external and internal layers over the longissimus from the six crossbreeds. This fatty acid was consistently higher in the biceps femoris in all the crossbreeds and therefore did not contribute to the interaction effect (Fig. 4).

This study provides evidence for the influence of breed and sex on the fatty acid composition of bovine intramuscular and subcutaneous lipids.

The effect of sire was evident for C14:0 and C16:1 in both the intramuscular and subcutaneous lipids. Significant ($P < 0.05$) sire effects were also found in the subcutaneous lipid for C16:0 and C18:0. The effect of cow was observed for C16:1 and C18:0 in the intramuscular lipid compared to C14:0 and C16:1 in the subcutaneous lipid. Bulls were associated with significantly ($P < 0.05$) higher levels of C14:0 and C18:2 in both lipid fractions than steers in addition to significantly ($P < 0.05$) more C16:0 in the subcutaneous lipid. Steers were found to be significantly ($P < 0.05$) higher in both the subcutaneous and intramuscular lipids with respect to C18:1 only.

Although the fundamental biological mechanisms responsible for these differences have yet to be delineated, the differences observed might be in part responsible for some of the sensory differences reported on these same animals by McLandress (1972).

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Ms received 5/19/72; revised 11/30/72; accepted 12/5/72.

The authors thank Dr. G. Atkinson for assistance in the statistical analysis.

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RADIANT ENERGY-INDUCED CHANGES IN BOVINE MUSCLE PIGMENT

INTRODUCTION

CONSUMERS associate color more than any other quality with freshness of meat; the bright cherry red of oxymyoglobin (MbO_2) is more acceptable than the reddish brown color of metmyoglobin (MetMb). It is the concentration and chemical state of myoglobin (Mb) and hemoglobin (Hb) in the muscle that largely are responsible for the color of fresh meat. Oxidation of Mb to MetMb may be induced by factors such as light, bacteria, lipid oxidation, packaging films and storage temperature. If light is important in oxidizing Mb and Hb, a more fundamental knowledge of the range in wavelength of radiant energy that causes changes in Mb and Hb of fresh muscle would be important to the design of lighting for retail meat display cases.

The literature contains few reports of work directly related to the specific wavelengths of radiant energy that cause changes in muscle pigments. On the basis of work with colored filters, Townsend and Bratzler (1958) suggested that 560–630 mμ light was the region of the spectrum most damaging to frozen meat pigments. Solberg and Franke (1971) found no one wavelength among six selected wavelengths of the visible light spectrum resulted in greater damage to meat pigments than any other wavelength. However, any illuminated sample contained more oxidized heme pigments than the controls.

General effects of fluorescent and ultraviolet (UV) light on fresh meat color were reported by Ramsbottom et al. (1951), Kraft and Ayres (1954), Rikert et al. (1957) and Marriott et al. (1967). Kraft and Ayres (1954) and Marriott et al. (1967) observed a steady change from bright red to dull red in fresh beef exposed to fluorescent light. In contrast, Ramsbottom et al (1951) and Rikert et al. (1957) reported no deterioration of color in fresh meat exposed to fluorescent light. Both Ramsbottom et al. (1951) and Kraft and Ayres (1954) found UV light damaging to the color of oxygenated fresh meat.

This study was designed to determine effects of specific wavelength regions of the UV and visible light spectrum on color of fresh bovine semitendinosus (ST) muscle. Three ranges in wavelengths of the spectrum were studied: (1) 253.7 ± 5 nm; (2) 404.7 ± 5 nm; and (3) 577.0 ± 5 nm. Constant temperature (4.5° ± 1°C) with varying levels of oxygen (100, 20 and 0%) constituted the environmental conditions. Both reflectance and absorption spectrophotometry were employed to measure changes in Mb occurring during exposure and/or extraction processes.

MATERIALS & METHODS

ONE BOVINE semitendinosus (ST) muscle from each of four carcasses was procured as needed for the experiment. Steaks, 2 cm thick, numbered 1 to 10 from the distal to the proximal end of the muscle, were cut immediately before being exposed to a given treatment. A sample, 3.5 cm in diameter, was removed from the dorsal side or superficial portion of each steak. The dorsal side of the muscle, which was lighter (more white fibers) than the ventral side, arbitrarily was selected for treatment to avoid introducing large variations in the red-white fiber ratio. After each steak was removed from the ST muscle, the remainder of the muscle was vacuum packaged, sealed in a Saran bag, and stored in a refrigerator at −1°C until the next sample was needed.

Exposure and spectral reflectance

Each sample was placed with its interior surface (proximal side) up and allowed to "bloom" in air 5 min before the sample surface was covered with Cryovac L-300 gas permeable film and placed in a metal sample holder (Fig. 1). After the sample was prepared at approximately 22°C in approximately 10 min, it was flushed for 30 min in the chamber of the experimental system (Fig. 1) at 4.5° ± 1°C with 100% oxygen, 20% oxygen (compressed air) or 0% oxygen (nitrogen). Those conditions also were used during a 3 hr period of exposure to radiant energy in which spectral reflectance was read every 30 min. Preliminary studies indicated color changes were no greater after 34 hr than after 3 hr of exposure to radiant energy. The temperature at the surface of the meat during flushing and exposure was maintained at 4.5° ± 1°C by passing the O_2, air or N_2 through copper coils in an isopropyl alcohol-dry ice bath. The compressed gas and glass traps retarded moisture condensation and freezing of the condensation in the copper tubing. Following the 30 min flushing process, the sample was exposed to radiant energy from a 500-watt medium-pressure mercury lamp fitted with standard mercury line interference filters of 254, 405 or 577 nm. Intensities at the meat surface, measured with a Weston illumination meter, Model 56, were 92, 1000 and 3000 footcandles, respectively, for the three wavelengths. This work was planned to determine the effect on bovine muscle pigments of each of those wavelengths at the highest intensity attainable with the 500-watt mercury lamp. Preliminary work with neutral density filters indicated that intensity had little effect on changes in the pigments.

To minimize external influences, control samples were placed in the same sample holder as treated samples, and held for 3 hr in the 20% oxygen atmosphere with a solid metal plate to block the radiant energy. All samples were exposed and measured spectrophotometrically at the same surface location. A Bausch and Lomb 600 recording spectrophotometer equipped with a reflectance attachment and magnesium carbonate as a reference standard was used to measure reflectance. Reflectance of radiant energy was read on the transmission scale (R_T) rather than on the absorbancy scale (R_A). Reflectance values, recorded across the entire visible spectrum, were read at 474, 525, 571, 600, 630, 650 and 685 nm. Data for 474, 525, 571 and 630 nm were converted to K/S values (Judd and Wyszecki, 1963). Ratios of 474/525 and 571/525 were calculated from both raw data and K/S values. A compensating polar planimeter was used to measure the total area (in sq cm) under the curve, and to measure the area under the curve of the red portion (630–700 nm) of each spectrophotometric curve.

Samples also were scored subjectively for color on a 6-point scale, 1 being designated very bright red and 6 very grey-brown. After exposure, the samples were packaged in Whirlpak freezer bags, frozen at −26°C and held at −26°C until a group of four to six samples had been treated.

Extraction, absorption spectra and polyacrylamide gel electrophoresis

After the last sample in each group of four to six was exposed to radiant energy and frozen, all samples in the group were held approximately 18 hr at −26°C before being thawed 1 hr at 21°C. Mb derivatives and other water soluble components were extracted by homogenizing the sample in a Waring Blendor with an equal volume of phosphate buffer (pH 6.0) for 2 min and centrifuging at 4.5°C for 20 min at 15,000 rpm. After filtering twice, the extracts were stored at 4.5°C until used (no longer than 24 hr).

Absorption spectra on portions of sample supernatants were measured by personnel in the Kansas State University Dept. of Biochemistry with a Carey 14 recording spectrophotometer standardized with 0.005M phosphate buffer.

Polyacrylamide gel electrophoresis was conducted on muscle extracts at pH 8.4, according to a modification of the method of Satterlee et al. (1971). Gels were flush with the bottom of the tubes; whereas, Satterlee et al. (1971) allowed space at the bottom of each gel. Gels

were stained with benzidine, and banding patterns of the Mb derivatives observed.

pH, moisture and lipid analyses

After the sample to be exposed was removed from each steak, the remaining muscle was placed in Whirlpak bags, frozen, and held at -26°C for 4–8 wk. Frozen muscle from all samples of each carcass was thawed at 5°C for approximately 18 hr, mixed well and aliquots analyzed for pH (Rogers et al, 1967), total moisture and ether extract (Bowers et al, 1968).

Statistical analyses

Data for all reflectance and absorbance measurements were analyzed by analysis of variance for a randomized complete block design. Sums of squares for treatment combinations were subdivided to ascertain the effect of each atmosphere as compared with the control, and to determine differences among atmospheres and among wavelengths. Also, the interaction between atmosphere and wavelength and between treatment combinations and exposure period was studied.

RESULTS

Reflectance spectra

Mean values for percentage reflectance of control (20% oxygen, dark) samples and of samples exposed to radiant energy at 254, 405 and 577 nm in three atmospheres, as measured at seven wavelengths, are shown in Figures 2, 3, and 4. Mean values for reflectance of control samples during the 3 hr exposure period were between 49 and 60% in the red area (630, 650 and 685 nm) of the spectrum, were less than 20% at 474, 525 and 571 nm and were 30% at 600 nm. A "high" percentage reflectance at 630, 650 or 685 nm indicates a sample that appears "redder" than samples with a lower percentage reflectance at those wavelengths. Therefore, as measured by percentage reflectance, control samples were "red" and contained considerable MbO_2. The mean visual scores (Table 1) also indicated that the control samples appeared bright red.

The effect on pigments of intact bovine muscle exposed to radiant energy (254, 405 and 577 nm) in 20% oxygen is indicated by the small (< 1 to 12%, Fig. 3) but statistically significant ($P < 0.05$ and $P < 0.001$, Table 2) differences in percentage reflectance between control samples and samples exposed to the three wavelengths of radiant energy and measured at seven wavelengths. The smallest difference in percentage reflectance between control and exposed samples occurred in 254 nm of UV light measured at 474 nm; whereas, the greatest difference occurred in 254 nm of UV light measured at 630 nm. Irrespective of wavelength of exposure, differences between control and exposed samples averaged 1 to 2% when measured at 474, 525 and 571 nm. At 600 nm differences between control and exposed samples averaged 5%; at 630 and 650 nm differences averaged 9%, and at 685 nm they were 4.5%.

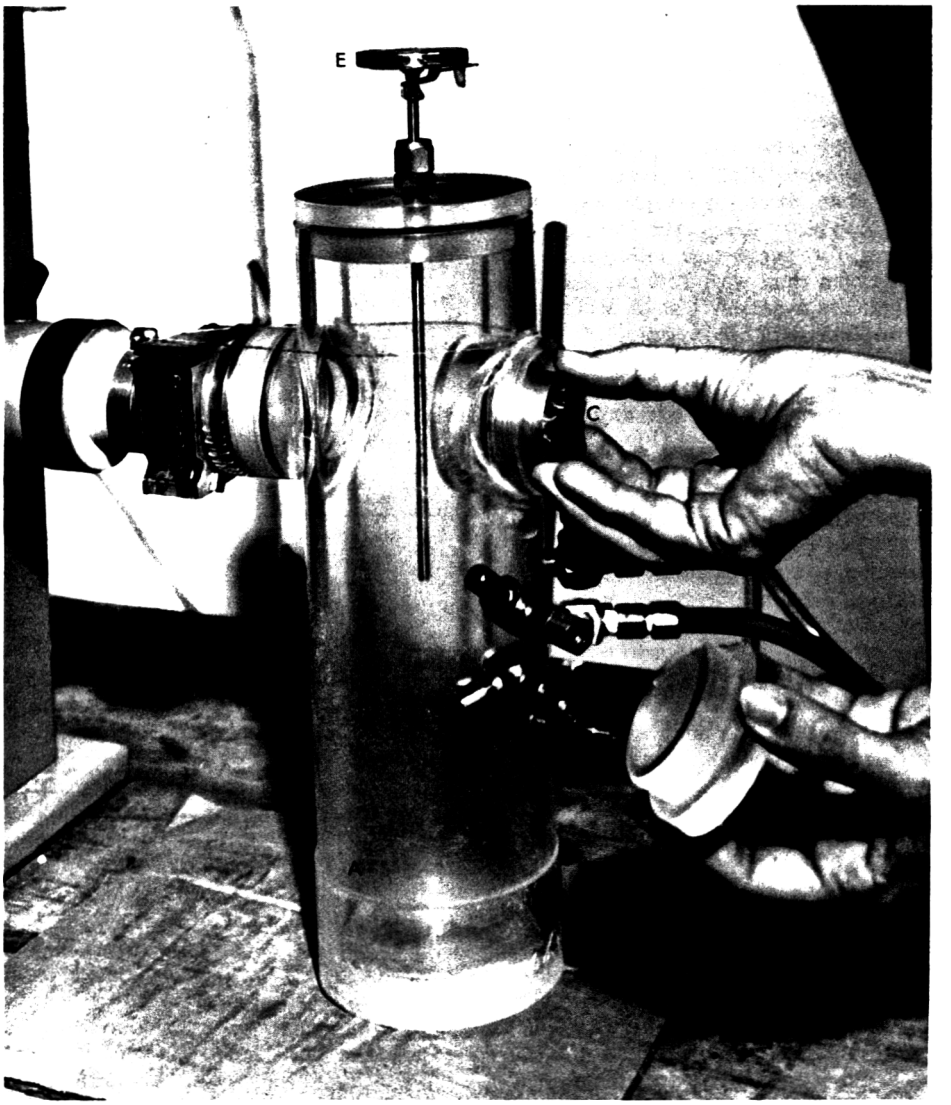

Fig. 1—Sample chamber (A) showing placement of sample holder (B) and sample (C) so that interference filter (D) can provide selected wavelengths of radiant energy and thermometer (E) can record temperature.

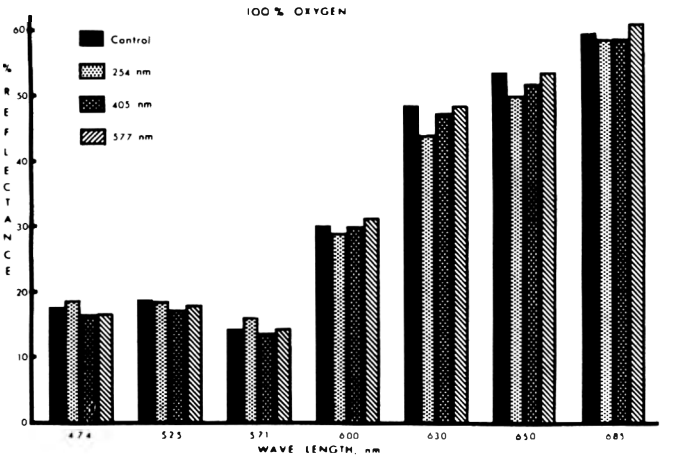

Fig. 2—Effects of three wavelengths of radiant energy in 100% oxygen on bovine muscle on mean percentage reflectance at selected points on the spectrophotometric curve.

Percentage reflectance of samples exposed to radiant energy in 0% oxygen (Fig. 4) followed a trend similar to percentage reflectance of samples exposed in 20% oxygen, but as would be expected, differences between control and exposed samples were slightly greater (< 1 to 4.5%) than those for samples exposed in 20% oxygen. Also, the larger mean squares for percentage reflectance with 0% oxygen than with 20% oxygen (Table 2) indicate a greater loss of MbO_2 in an atmosphere with no oxygen.

In general, when samples were exposed to radiant energy in 100% oxygen, differences between control and exposed samples were not significant (Fig. 2, Table 2).

Irrespective of wavelength used for exposure (254, 405, 577 nm), differences among atmospheres were significant ($P < 0.001$) at all wavelengths for which reflectance was measured from 474 to 685 nm. Likewise, irrespective of atmosphere, differences among wavelengths of exposure (254, 405, 577 nm) were significant ($P < 0.05$ or 0.001) at each wavelength for which reflectance was measured between 474 and 650 nm. The interaction between atmospheres and wavelengths used for exposure was significant ($P < 0.001$) at each wavelength for which reflectance was measured (Table 2).

Selected ratios (calculated from raw data for reflectance) are presented in Table 3. The ratio of the area of the red portion (630 to 700 nm) to the total area under the spectrophometric curve was not affected significantly by atmospheric conditions, wavelength (254, 405, 577 nm), exposure period or interaction between atmosphere and wavelength and between treatment combinations and exposure period. In general, ratios of 571/525 nm were affected ($P < 0.05$, 0.01 or 0.001) by atmospheric conditions, wavelength (254, 405, 577 nm), exposure period and the interactions studied.

Table 1—Mean data for selected measurements of bovine muscle samples exposed to radiant energy for 3 hr

Treatment combinations	Visual color score[a]	474/525 nm[b]	571/525 nm[b]	Soret band wavelength nm
Control (20% oxygen, dark)	1.0	1.10	1.45	415
100% oxygen				
254 nm	4.3	1.03	1.23	413
405 nm	1.3	1.01	1.26	415
577 nm	1.5	1.18	1.38	414
20% oxygen				
254 nm	4.0	0.89	1.34	413
405 nm	2.5	1.14	1.44	413
577 nm	2.8	1.06	1.34	413
0% oxygen				
254 nm	4.0	0.80	1.34	413
405 nm	4.5	0.82	1.32	414
577 nm	4.8	0.78	1.37	413

[a]Based on 6-point scale (1, very bright red . . . 6, very greyish-brown)
[b]Calculated from means of K/S values

Differences in reflectance data attributable to replications (carcasses) were significant ($P < 0.001$) at all wavelengths measured (Table 2) and significant for the ratios 474/525 ($P < 0.001$) and 571/525 ($P < 0.05$), Table 3.

Reflectance data ratios for 474/525 nm and 571/525 nm, calculated from mean K/S values, are given in Table 1. In 100% oxygen, 474/525 ratios for samples exposed at each of the three wavelengths (254, 405, 577 nm) and 474/525 ratios for samples exposed at 405 and 577 nm in 20% oxygen were similar to the 474/525 ratio for control samples. However, at all three wavelengths in 0% oxygen and at 254 nm in 20% oxygen, the 474/525 ratios were lower than the 474/525 ratio for control samples. Since the K/S ratio of 474/525 falls when Mb is formed (Snyder and Armstrong, 1967) the lower values with 0% oxygen indicate increased Mb formation, as would be expected, for oxygenated samples in a 0% oxygen atmosphere.

Ratios for 571/525 nm, calculated from mean K/S values, were highest for the control samples (1.45) and those exposed at 405 nm in 20% oxygen (1.44). Ratios for all other treatments were similar (1.32–1.38), except for samples exposed to 254 and 405 nm in 100% oxygen (1.23 and 1.26, respectively). The K/S ratio for 571/525 nm falls when MetMb is formed (Snyder and Armstrong, 1967); thus, ratios slightly lower than

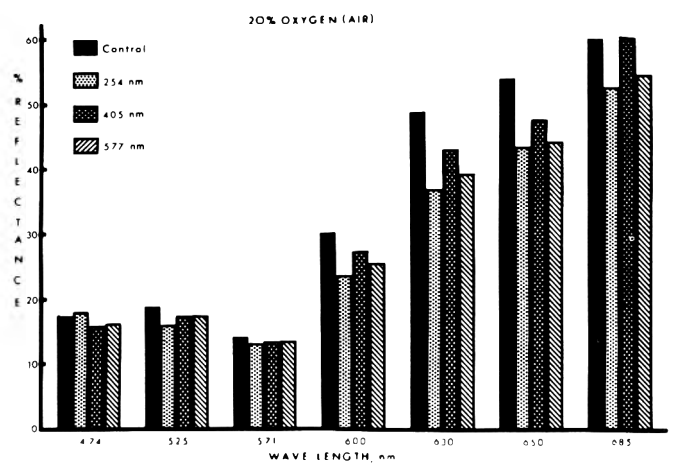

Fig. 3—Effects of three wavelengths of radiant energy in 20% oxygen (air) on bovine muscle on mean percentage reflectance at selected points of the spectrophotometric curve.

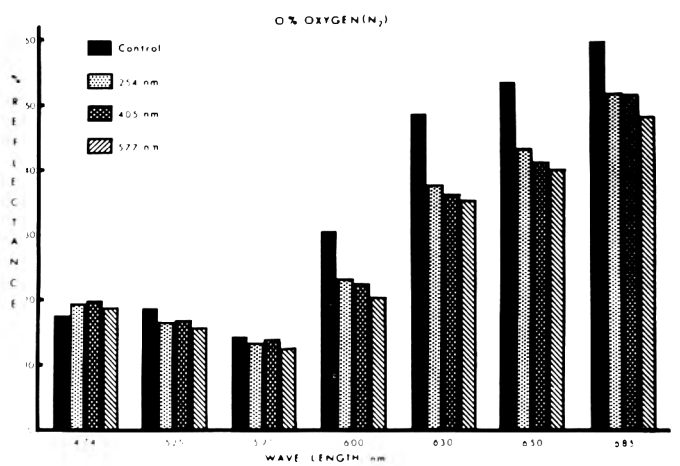

Fig. 4—Effect of three wavelengths of radiant energy in 0% oxygen (nitrogen) on bovine muscle on mean percentage reflectance at selected points on the spectrophotometric curve.

Table 2—Mean squares and F values[a] for percentage reflectance at seven wavelengths

Source	df[b]	Wavelength, nm 474	525	571	600	630	650	685
Replications (carcasses)	3	109.91*** (36.24)	97.96*** (40.61)	74.72*** (38.80)	803.35*** (124.27)	1035.30*** (84.60)	1169.04*** (85.09)	1632.43*** (103.93)
Treatment combinations	9							
Control vs. radiant energy in 100% oxygen	1	5.51 (1.82)	12.91* (5.36)	1.69 (0.88)	0.42 (0.07)	73.85 (1.86)	36.38 (2.65)	0.32 (0.02)
Control vs. radiant energy in 20% oxygen	1	18.59* (6.14)	82.70*** (34.32)	25.32*** (13.12)	530.39*** (82.10)	1888.60*** (47.53)	1649.21*** (120.03)	727.37*** (46.30)
Control vs. radiant energy in 0% oxygen	1	86.45*** (28.53)	119.93*** (49.76)	33.31*** (17.26)	1543.61*** (238.95)	3396.26*** (85.47)	3138.45*** (228.42)	1803.96*** (114.83)
Among atmospheres	2	216.47*** (71.44)	58.57*** (24.30)	60.45*** (31.32)	1613.31*** (249.74)	2602.41*** (212.62)	2621.46*** (190.79)	1900.66*** (120.98)
Among wavelengths (254, 405, 577)	2	52.36*** (17.28)	10.41* (4.32)	20.78*** (10.77)	55.82*** (8.64)	160.46*** (13.33)	55.03* (4.01)	22.08 (1.41)
Atmosphere × wavelength	4	14.19*** (4.68)	24.71*** (10.25)	37.22*** (19.28)	91.38*** (14 .15)	163.16*** (13.33)	138.02*** (10.05)	159.34*** (10.14)
Periods exposed	7	7.53* (2.48)	0.42 (0.17)	2.33 (1.21)	5.27 (0.81)	13.35 (1.09)	8.87 (0.65)	32.17 (2.05)
Treatment × periods Exposed	63	4.05* (1.34)	3.17* (1.32)	2.19 (1.13)	21.00*** (3.25)	38.69*** (1.16)	36.47*** (2.65)	31.98*** (2.04)
Error	210	3.03	2.41	1.93	6.46	12.24	13.74	15.71

[a]Values in parenthesis are F values
* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

[b]Levels of significance, $\nu_2 = 00$ (210)

df, ν_1	0.05	0.01	0.001	df, ν_2	0.05	0.01	0.001
1	3.84	6.63	10.8	4	2.37	3.32	4.62
2	3.00	4.61	6.91	7	2.01	2.64	3.47
3	2.60	3.78	5.42	63	1.32	1.47	1.66

ratios for control samples such as those for 254 and 577 nm in 20% oxygen, for 577 in 100% oxygen, for each of the three wavelengths in 0% oxygen, and the even lower ratios for 254 and 405 nm in 100% oxygen may indicate some MetMb formation.

Visual scores (Table 1) generally agreed with the trends noted for percentage reflectance in the red region (630 to 685 nm) of the spectrophotometric curves. Scores indicated that 405 and 577 nm visible light in 0% oxygen and 254 nm UV light in 0, 20 or 100% oxygen produced the most greyish brown samples. All other treatments resulted in very bright to bright red muscle color after 3 hr of exposure.

Absorbance spectra

Absorbance spectra were used to detect denaturation, if any, of the muscle pigments. Phosphate buffer extraction prior to measuring absorbance indicated that the pigment in all samples was soluble, and thus, still in the native form. Examination of the absorbance spectra for Soret band position substantiated this finding. The Soret band for samples from all treatment combinations was between 413 and 415 nm (Table 1), which, according to generally accepted data (Brown et al., 1962, 1967; Saterlee, 1971, personal communication) indicated no irreversible Mb denaturation. However, a strong "wet dog" odor, similar to the odor accompanying denaturation caused by gamma ray irradiation, was noted after samples were exposed to 254 nm UV light.

Differences in absorbance data were not significant among treatments; differences attributable to carcasses (replications) were significant ($P < 0.001$). Nonsignificant differences among treatments for absorbance spectra of phosphate buffer extracts would be expected. During extraction, oxygenation of reduced pigments and MetMb reducing activity would produce primarily reduced oxygenated pigments in the extracts.

Polyacrylamide gel electrophoretic patterns

Gel electrophoresis patterns indicated no major qualitative differences in the Mb derivatives that were attributable to treatments. Gels prepared from unexposed samples had one major band, whereas gels from all samples exposed to radiant energy contained three major bands and two less prominent bands. Relative amounts of the three major bands were not different among the treatments. No free heme band, an indication of irreversible denaturation, was noted in any of the gels.

pH, total moisture (TM) and ether extract

The pH of ST muscle from carcass 3 was higher (5.75 vs. 5.60) than the pH of muscle from the other carcasses. Percentage moisture and percentage ether extract were similar for muscle from carcasses 2, 3 and 4; both TM and ether extract were slightly higher for muscle from carcass 1 than from the other three carcasses. Differences in pH, TM and ether extract could have accounted for some of the differences in the reflectance and absorbance spectra attributable to carcass.

DISCUSSION

RESULTS of this experiment indicate some MetMb was formed after exposure of intact bovine ST muscle to radiant energy. Statistically significant differences in percentage reflectance at 630, 650 and 685 nm for control samples vs. samples exposed to 254, 405 and 577 nm radiant energy in 20% oxygen are indicative of less MbO_2 after exposure.

In this experiment, control samples were exposed to 20% oxygen in the dark under the identical conditions used for exposure to 20% oxygen with 254, 405

Table 3—Mean squares and F values[a] for ratios calculated from raw data for reflectance

Source	df	Area red/ total area	474/525 nm	571/525 nm
Replications (carcasses)	3	0.074 (1.41)	0.074*** (12.05)	0.011* (3.71)
Treatment combinations	9			
Control vs. radiant energy in 100% oxygen	1	0.000 (0.0)	0.034** (11.33)	0.000 (0.0)
Control vs. radiant energy in 20% oxygen	1	0.000 (0.0)	0.018* (6.00)	0.071*** (11.83)
Control vs. radiant energy in 0% oxygen	1	0.024 (0.46)	0.028** (9.33)	1.431*** (238.50)
Among atmospheres	2	0.030 (0.58)	0.003 (1.00)	1.599*** (266.50)
Among wavelengths (254, 405, 577)	2	0.036 (0.69)	0.053*** (17.67)	0.195*** (32.50)
Atmosphere x wavelength	4	0.075 (1.44)	0.029*** (9.67)	0.085*** (14.17)
Periods exposed	7	0.051 (0.97)	0.013*** (4.65)	0.047*** (7.68)
Treatment x periods exposed	63	0.052 (0.99)	0.003 (0.90)	0.013*** (2.11)
Error	210	0.052	0.003	0.006

[a]Values in parenthesis are F values
*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$

and 577 nm radiant energy. Only 10 experimental units were available from each ST muscle of a carcass. Nine units were needed to study the effects of 254, 405 and 577 nm of radiant energy in 0, 20 and 100% oxygen. Thus, the available units did not allow for separate control samples in 0 and 100% oxygen in the dark.

A significant ($P < 0.05$ at 474 nm; $P < 0.01$ at 525, 571, 600, 630, 650 and 685 nm) effect of radiant energy was shown for control samples vs. exposed samples (254, 405, 577 nm) in 20% oxygen (Table 2). Therefore, the effect of radiant energy (254, 405, 577 nm) at other oxygen levels (0, 100%) can be compared.

A protective effect of oxygen on MbO_2 is indicated, which agrees with the findings of Daun et al. (1971). They found an enriched oxygen atmosphere prolonged acceptable meat color, independent of microbial growth, when compared with samples stored in an air atmosphere.

A significant ($P < 0.001$) interaction occurred between environmental atmosphere (oxygen level) and wavelength (254, 405, 577), probably because with 0% oxygen, 577 nm radiant energy resulted in the greatest loss of MbO_2; whereas, with 20 and 100% oxygen, 254 nm radiant energy resulted in the greatest loss of MbO_2.

Visual scores generally were in agreement with the results from reflectance spectrophotometry. The changes in muscle color, as measured by reflectance data, appeared similar to, but not identical to, the generally recognized color changes observed in the retail display case. The exposed muscle often appeared more grey than brown, but no treatment resulted in enough discoloration to be of practical importance. Others (Broumand et al., 1958 and Brody, 1970) have reported that at least 60% MetMb in the tissue is necessary before a grey or brown color is observed visually. The interaction between oxygen level and wavelength in MetMb formation in fresh bovine muscle tissue may be of academic interest.

The K/S values calculated from the data also indicate some Mb and MetMb formation when compared to results in the literature (Stewart et al., 1965; Snyder and Armstrong, 1967; Zimmerman and Snyder, 1969). In this experiment K/S ratios for 571/525 nm indicated that 254 and 405 nm of radiant energy in 100% oxygen resulted in slightly greater loss of MbO_2 than 577 nm light. In the absence of oxygen, the loss of MbO_2 indicated by spectral reflectance may result from a change to Mb, as would be expected, and as was shown by the 474/525 K/S ratio, as well as from the loss of some MbO_2 as shown by the 571/525 K/S ratio. Progressively larger mean squares (Table 2) for 100, 20 and 0% oxygen, respectively, for percentage reflectance at 630, 650 and 685 nm indicate less loss of MbO_2 with light as the oxygen level is increased. This may be a result of the cumulative effect of MetMb and Mb formation from both light exposure and environmental atmosphere.

The possibility exists of an intermediate photochemical reaction that causes the oxidation of MbO_2 and Mb and is initiated by light. Solberg and Franke (1971) stated that oxidation of oxygenated heme pigments may be a result of photosensitive molecules other than the pigments themselves. In their work oxygenated heme pigments did not exhibit an increased response in their region of peak absorption, as would be expected, if those pigments were directly involved in a photochemical reaction. They hypothesize the involvement of riboflavin or flavin mononucleotide in this intermediate photochemical reaction.

The involvement of lipids in such intermediate reactions also seems possible, because UV light such as at 254 nm, even at a low intensity, is known to catalyze the oxidation of lipids. In 20 and 100% oxygen, exposure at 254 nm increased loss of MbO_2 more than exposure at 405 and 577 nm. Watts (1954) and Greene (1969) reported the co-catalytic effect of heme compounds and lipids. Greene (1969) found that free radical intermediates from lipid oxidation decomposed the hemes, and that the fat antioxidants, butylated hydroxyanisole and propyl gallate, offered substantial protection against formation of MetMb. Investigation of the effect of antioxidants on muscle pigments in the presence of radiant energy seems warranted.

Also, more information is needed on the effect of intensity of the radiant energy, environmental temperature and exposure time at specified wavelengths with certain environmental atmospheres on muscle pigment changes induced by radiant energy. Differences between or among selected muscles of the carcass in their response to exposure to the various wavelengths of the spectrum should be studied.

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Ms received 7/28/72; revised 10/24/72; accepted 10/25/72.

Contribution No. 270, Dept. of Foods & Nutrition; No. 420, Dept. of Animal Science & Industry; and No. 53, Dept. of Statistics.

Data are from the Ph.D. dissertation of the senior author.

The authors gratefully acknowledge the technical assistance of Mrs. Lois Anderson.

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EFFECT OF LIGHT, pH AND BUFFER STRENGTH ON THE AUTOXIDATION OF PORCINE, OVINE AND BOVINE MYOGLOBINS AT FREEZING TEMPERATURES

INTRODUCTION

A GREAT DEAL of work has been reported on the color of fresh meat, much of it dealing with the oxymyoglobin content of the meat. The bright red color of fresh meat is due to the predominance of oxymyoglobin (MbO_2) on the surface of the meat. The conversion of MbO_2 to brown metmyoglobin (MetMb) is of importance to the food scientist and the mechanism has been described by Brown and Tappel (1958) and Brown and Mebine (1969).

The effect of display conditions is important to food scientists, as they affect autoxidation of the MbO_2 of fresh meat. Ramsbottom et al. (1951) indicated that fading and subsequent flavor losses in fresh meat were dependent upon the display lamps used, the intensity of the lamps and the exposure time. Kraft and Ayres (1954) showed that ultraviolet light caused the rapid autoxidation of MbO_2 in fresh meat, thereby causing the meat to turn dark brown very rapidly. To obtain similar degrees of discoloration under fluorescent light required much longer times. Lentz (1971) indicated that frozen beef would discolor in 1–3 days when held in a lighted display case, whereas storage at the same temperature in the absence of light caused no discoloration during the same period of storage. Solberg and Franke (1971) studied the effect of visible light (from 420–632.8 nm) on the autoxidation of beef MbO_2 in intact meat held at either 1.1 or 5°C. They concluded that visible light had a small, but significant, effect on the autoxidation of the MbO_2 in intact beef. Satterlee et al. (1972) indicated that soft white fluorescent light did affect the autoxidation of purified bovine MbO_2 and that the effect seemed to be less at pH 5.5 than at 6.0.

The temperature of frozen storage is also a factor which affects the rate of autoxidation of oxymyoglobin. Brown and Dolev (1963) observed that the autoxidation rates for both bovine and tuna MbO_2 were greater at freezing temperatures, when compared to temperatures above freezing. Satterlee and Zachariah (1972) observed that the same phenomenon occurred when porcine and ovine MbO_2 were frozen.

It is very evident that the color stability of the red meat pigment oxymyoglobin is affected by factors such as light, temperature and pH. The purpose of this study is to investigate the autoxidation of MbO_2 isolated from bovine, ovine and porcine meat sources at freezing temperatures with varying light conditions, pH and buffer strength.

EXPERIMENTAL

Isolation of myoglobins

Myoglobins from ovine, porcine and bovine muscle were extracted and purified on DEAE cellulose (ovine and porcine) or CMC (bovine) column chromatography according to the procedure of Satterlee and Zachariah (1972). The purity of the myoglobins was confirmed by polyacrylamide gel electrophoresis.

Preparation of oxymyoglobins

The oxymyoglobins were prepared according to the procedure of Brown and Mebine (1969). The MbO_2 from each of the three sources was dialyzed against pH 7.0 phosphate buffer of the desired molarity and stored in that buffer at 5°C until needed.

Light sources

All fluorescent lights used in this study were 40w bulbs of the following types: soft white, warm white, pink and red. The fluorescent bulbs were used in pairs in order that a light intensity of 250–300 ft-c could be obtained. The intensity of the red fluorescent bulbs was so low that only 80–100 ft-c could be obtained from as many as four bulbs.

The incandescent bulbs used were 100w standard white bulb and a 150w cool white flood. The intensity of incandescent light was also maintained at 250–300 ft-c.

All light intensities were measured with a General Electric type 213 light meter contained in a leak-proof glass container submerged 1/2 cm below the surface of the bath.

Illuminated cold bath

To keep the various myoglobin and oxymyoglobin samples at the desired temperature between −5 to −29°C, a methanol-water bath was used. To maintain the temperature of the bath, a Lauda model IC-6 refrigeration unit was used. The various light sources were suspended above the bath and were used to illuminate the individual samples contained in pyrex screw cap tubes suspended horizontally 1/2 cm below the surface of the bath. The temperature of the bath was maintained to within ± 1/2°C.

Along with each illuminated myoglobin sample a control sample was run in a light-tight foil covered container. These samples are defined as the controls in the light studies.

Determining autoxidation rates

The pH of oxymyoglobin solutions was adjusted using 0.1N HCl and immediately the initial absorbance was measured at 580 nm. The samples were then frozen in a dry ice-methanol mixture. In each experiment described, samples were thawed at regular intervals, absorbance measured and immediately refrozen. Upon completion of the experiment a few crystals of $K_3Fe(CN)_6$ were added to the sample and the absorbance measured at 580 nm in order to determine the value for zero % MbO_2.

Buffers used

The various buffers used in this study were: 10 mM, 50 mM, 100 mM, 500 mM and 1.0M phosphate, pH 6.0.

Experiments performed

The following experiments were performed to determine the effect of buffer strength, light, storage temperature and freezing-thawing on the stability of bovine, ovine and porcine MbO_2.

Buffer strength. To determine the effect of buffer strength on the autoxidation of bovine, ovine and porcine MbO_2, the autoxidation rates of each were determined at pH 6.0, and −19°C under soft-white fluorescent light at each buffer strength previously given.

Light. In order that the effect of light could be determined on the autoxidation rates of each of the three MbO_2 studied, each of the three MbO_2 sources were placed in 10 mM phosphate buffer, pH 6.0 at −19°C and illuminated with each light source at an intensity of 250–300 ft-c (red light, 80–100 ft-c), to ascertain the effect of light on autoxidation rate.

Storage temperature. The effect of storage temperature on the autoxidation rate of each MbO_2 (bovine, ovine and porcine) was determined by placing the MbO_2 in 10 mM phosphate buffer, pH 6.0 and storing the sample at the desired temperature under soft-white fluorescent illumination (250–300 ft-c).

Since porcine MbO_2 was found to precipitate upon frozen storage, an additional experiment was performed to determine the effect of storage temperature on the stability of total porcine myoglobin. This was performed by determining the amount of total procine myoglobin left in solution after thawing the samples stored at various freezing temperatures. Myoglobin concentration was determined as the

cyanomet derivative by its absorbance at 540 nm.

Freezing-thawing. It was felt that the freezing and thawing of the various MbO_2 samples during the experiments described above could affect the stability of the three MbO_2 an experiment was performed where MbO_2 samples from each of the three sources were thawed and refrozen six times during the experiment, with a second experiment consisting of only three freeze-thaw cycles. The autoxidation rates obtained were analyzed by the Student's t test (Steel and Torrie, 1960) to see if one rate was significantly different from the other.

RESULTS & DISCUSSION

TABLE 1 indicates that buffer strength does definitely affect the autoxidation of bovine, ovine and porcine MbO_2 at −19°C. The autoxidation rate constant (K) is larger for each MbO_2 in 10 mM phosphate buffer, pH 6.0 than in any other buffer. As the buffer increases to 100 mM, the rate constants decrease. The rate constants seem to increase slightly for bovine and ovine, as the buffer strength is increased to 1M. A major increase in autoxidation rate was observed for porcine oxymyoglobin in 1M buffer.

To determine if the rapid autoxidation which took place in 10 mM buffer was due to pH changes in the weak buffer system, the pH of the solution was measured each time the samples were thawed. The pH maintained itself at 6.0 ± 0.05 units throughout the entire experiment. Therefore, it seems that it is a buffer strength effect which causes the increase in the autoxidation rates at −19°C.

It was felt that the freezing and thawing of the MbO_2 samples during the determination of the autoxidation rates may affect the stability of the MbO_2 and subsequently the rate. When the autoxidation rates of MbO_2 from each of the three sources were compared after either frequent freezing and thawing (6 within a 24-hr period) or infrequent freezing and thawing (3 within a 24-hr period), it was found that the frequent freezing and thawing resulted in a slightly larger autoxidation rate constant. When the rates were compared statistically by the Student's t test, no significant difference could be shown to be present between the rates.

Table 2 indicates that the type of light does have an effect on the autoxidation rate of all three MbO_2 studied, at −19°C. Those bulbs emitting low wavelength light (soft white and warm white) yielded the largest rate constants. Bulbs emitting primarily long wavelength light (pink, red and incandescent) gave the lowest autoxidation rate constants for all three MbO_2 studied. Both soft white and warm white fluorescent light bulbs emit light primarily in the 400–590 nm region of the spectrum, precisely where MbO_2 is capable of absorbing light energy. The pink, red and incandescent bulbs emit longer wavelength light (from 625 nm region of the spectrum) which cannot be easily absorbed by the MbO_2 molecule. The light emittance spectra for each bulb used is given in the ISCOTABLES handbook (Instrumentation Specialties Company Inc., 1967, Lincoln, NE 68507).

Short wavelength fluorescent light causes a small increase in the autoxidation of bovine, ovine and porcine MbO_2 when stored at −19°C, the long wavelength red fluorescent and incandescent lights seem to have no effect on any of the autoxidation rates. The pink fluorescent light, which emits most of its light at 625 nm, caused a small increase in all three autoxidation rates. Porcine MbO_2 stability was affected by red fluorescent light, whereas bovine and ovine MbO_2 was unaffected.

When the autoxidation rates were measured at varying temperatures from −5 to −27°C, it was found that the temperature range of −11 to −12°C caused a rapid increase in the rate. This occurred for all three myoglobins studied and is shown on Figure 1. As was previously seen by Satterlee and Zachariah (1972), porcine had the fastest autoxidation rate and ovine and bovine exhibited the slowest rates. Brown and Dolev (1963) also noticed the acceleration of beef and tuna MbO_2 autoxidation upon freezing. The concentration of both buffer salts and MbO_2 outside of the ice crystals during freezing may account for the enhanced autoxidation rate for the various MbO_2 solutions at freezing temperatures. In Table 1 it was shown that autoxidation rates are slightly enhanced at high buffer

Table 1–Effect of buffer strength on the autoxidation rate constants for bovine, ovine and porcine oxymyoglobin at −19°C. Illumination with soft white fluorescent light (250–300 ft-c)

	Autoxidation rate (hr^{-1}) × 10^3		
Phosphate buffer strength (pH 6.0)	Bovine	Ovine	Porcine
10 mM	6.2	7.5	19.0
50 mM	3.4	3.0	6.4
100 mM	3.0	2.0	0.4
500 mM	3.1	2.5	0.4
1 M	3.1	2.8	4.4

Table 2–Effect of various lights on the autoxidation rate constants for bovine, ovine and porcine oxymyoglobin at −19°C and in 10 mM phosphate buffer, pH 6.0

	Autoxidation rate (hr^{-1}) × 10^3		
Light[a]	Bovine	Ovine	Porcine
Fluorescent			
Soft white	6.2	7.5	19.0
Warm white	5.7	6.6	18.4
Pink	4.4	5.5	16.5
Red	4.1	5.0	15.0
Incandescent			
150w cool flood	4.1	5.0	13.1
100w	4.0	4.8	10.3
No light (Control)	4.1	5.0	11.2

[a]The light intensity was from 250–300 ft-c for all lights except red which had an intensity of 80–100 ft-c.

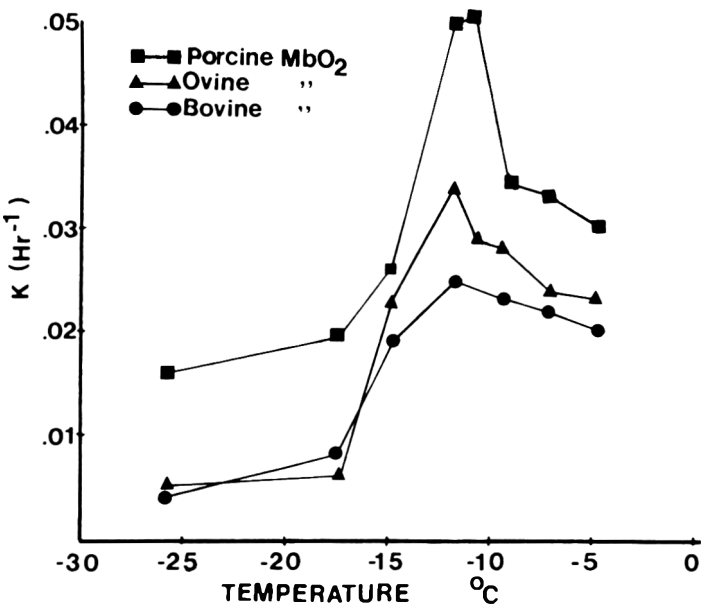

Fig. 1–Effect of freezing temperature on the autoxidation rate constant (K) of bovine, ovine and porcine MbO_2 in 10 mM phosphate buffer, pH 6.0 and under soft white fluorescent light.

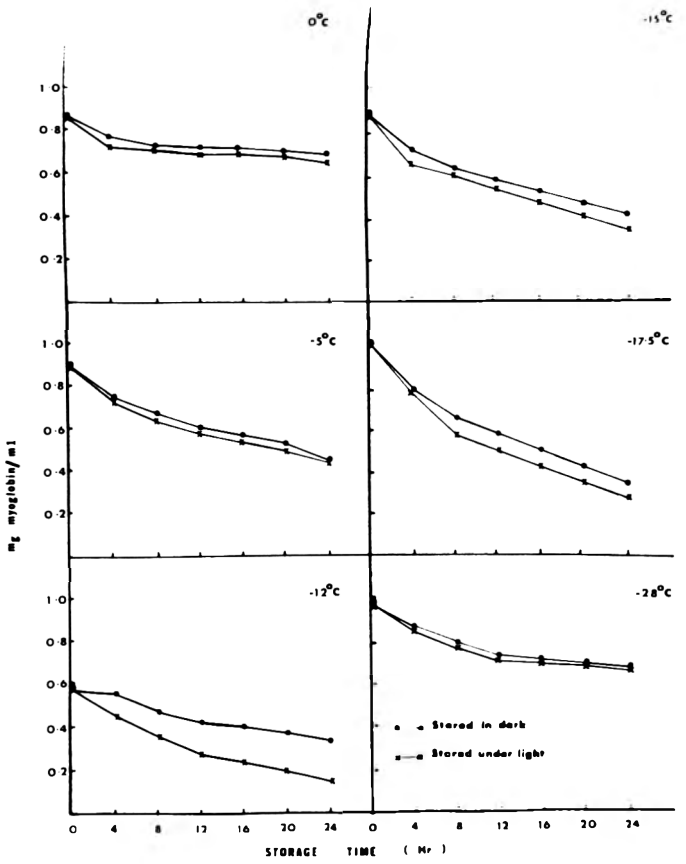

Fig. 2—The solubility (stability) of porcine MbO_2 stored at various freezing temperatures under soft white fluorescent light as well as in the dark. All samples were in 10 mM phosphate buffer, pH 6.0.

strengths. But the increase in buffer strength outside of the ice crystals can only partially explain the large increase in autoxidation rate upon freezing to −11 to −12°C.

Another factor which perhaps contributed to the apparent rapid autoxidation of porcine MbO_2 at freezing temperatures was the instability of the entire myoglobin molecule at these temperatures. Figure 2 shows the effect of temperature and soft white fluorescent light on the solubility of total porcine myoglobin. The solubility or stability of the porcine myoglobin was affected at all temperatures studied. The temperatures from −5 to −17.5°C caused the greatest destruction of the porcine myoglobin. The effect of temperature was less above −5°C or below −17°C. The fact that freezing temperatures will destroy or precipitate both porcine MbO_2 and MetMb, leads to the conclusion that the rapid autoxidation rates previously seen for porcine MbO_2 are a combination of MbO_2 autoxidation and precipitation during frozen storage.

Another fact that can be obtained from Figure 2 is that at −12, −15 and −17.5°C, light (soft white fluorescent) enhances the precipitation of the MbO_2 in solution.

CONCLUSION

AT FREEZING temperatures, both buffer strength and short wavelength fluorescent light affect the autoxidation of bovine, ovine and porcine MbO_2. Storage temperature is another factor which can affect the stability of the red meat pigment protein, MbO_2. At storage temperatures of −11 to −12°C the three MbO_2 studied are least stable.

The information described in this study indicates that the preservation of the red color in frozen beef, pork or lamb can be enhanced by storage in the absence of fluorescent light and at temperatures below −17 to −18°C.

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Ms received 10/10/72; revised 12/15/72; accepted 12/19/72.

Published as Paper Number 3483, Journal Series, Nebraska Agricultural Experiment Station. Research reported was conducted under Project No. 16-21.

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EFFECT OF POSTMORTEM MUSCLE CHANGES ON POULTRY MEAT LOAF PROPERTIES

INTRODUCTION

INCREASED MARKETING usage of bound poultry meat products such as loaves, roasts, rolls and similar items has created a need to evaluate the effect of processing techniques on finished product quality. Chicken muscle rapidly undergoes rigor mortis, starting within 3 hr and reaching completion between 12–24 hr (deFremery and Pool, 1960). Many chemical and physiochemical changes occur within this time interval.

Most postmortem changes in nitrogen extractability from breast muscle are due to changes in the myofibrillar proteins (Khan and van den Berg, 1964; Sayre, 1968; Weinberg and Rose, 1960). Myosin extractability rapidly decreases during the first 3 hr postmortem while actomyosin extractability maximizes within 24–36 hr of aging (Sayre, 1968; Khan and van den Berg, 1964). Postrigor tenderization occurs rapidly during the first 24 hr of aging with little tenderization on further aging (Khan and van den Berg, 1964; Koonz et al., 1954).

No significant changes of physiochemical properties have been observed in actomyosin extracted from prerigor and postrigor muscle (Hay et al., 1972), although greater hydration of myofibrillar proteins occurs with an increase of aging time (Sayre, 1968). Froning and Norman (1966) found that light muscle tissue from poultry possesses a significantly larger water-holding capacity than dark tissue.

Prerigor poultry or beef muscle tissue has a significantly higher emulsifying capacity and possesses greater emulsion stabilizing ability than postrigor muscle (Acton and Saffle, 1969; Froning and Neelakantan, 1971). Muscle pH and emulsifying characteristics are highly correlated (Froning and Neelakantan, 1971) and adjustment of meat pH from 5.0 to 8.0 significantly increases the tensile strength of meat loaf slices (Maesso et al., 1970a). No study has reported the influence of muscle postmortem state on poultry meat loaf properties.

Vadehra and Baker (1970) stated that the binding mechanism important to fabricated meat products is a heat mediated reaction. Acton (1972) found that binding strength of poultry meat loaves significantly increased as the internal temperature increased from 35°C to 82°C. The loss of water-holding capacity of meat during heating (Hamm and Deatherage, 1960) and its possible role in binding was reviewed by Vadehra and Baker (1970). Schnell et al. (1970) reported that muscle tissue disruption and addition of salt, polyphosphates or ribonucleic acid which reduce cooking loss of poultry loaves also result in an increase of binding strength, irrespective of the chemical or mechanical actions involved.

This study was conducted to determine the effect of postmortem aging of broiler meat on the pH, water-holding capacity and extractability of myofibrillar proteins and to relate these tissue parameters to the cooking loss, binding strength and shearing force of poultry meat loaves. The effect of freezing 24 hr postrigor meat was included as an additional treatment.

EXPERIMENTAL

Source of meat

Broilers of 8-wk and 10-wk age groups were obtained from a Clemson University flock. The broilers were slaughtered, bled and scalded at 55°C for 1 min. For a prerigor muscle treatment (0 hr postmortem), the pectoralis muscles were immediately excised, coarse ground through an 8 mm plate and reground through a 5 mm plate with an Osterizer Model 480 Food Grinder. The prerigor meat was used for all analyses within 30 min after death.

Other treatments of meat included placing the carcasses in an ice tank for 3, 6, 12 and 24 hr before excising the pectoralis muscles, grinding and evaluation. An additional treatment involved freezing the pectoralis muscles from 24 hr postmortem carcasses in Cryovac bags for 2 wk at −20°C. Thawing in the bags was conducted at room temperature (22°C) for 10–12 hr followed by grinding. Drip or fluid exudate from the thawed muscles was blended into the ground meat prior to analysis.

Extraction of muscle tissue for nitrogen fractions

The extraction and fractionation procedure was modified from the method of Sayre (1968). 20g of finely ground muscle from each postmortem interval were mixed with 10 vol of phosphate buffer (pH 7.0, ionic strength 1.0) and blended for 45 min at 4°C. The slurry was centrifuged at 30,900 × G for 30 min and the supernatant filtered through Whatman #1 paper. The residue was resuspended in 10 vol of the phosphate buffer. The extraction was repeated and the second supernatant combined with the first. Actomyosin in the supernatant was fractionated at an ionic strength of 0.25 by diluting with 3 vol of distilled water and centrifuging for 30 min at 30,900 × G. Myosin was fractionated from the supernatant by further dilution to attain an ionic strength of 0.04 and centrifuged as described above. Proteins remaining soluble at an ionic strength of 0.04 were designated the sarcoplasmic fraction.

Muscle tissue and extract fractions were analyzed for protein nitrogen by the Kjeldahl method (AOAC, 1970). Total nitrogen in the fractions was corrected for nonprotein nitrogen by preparing and analyzing 5% trichloracetic acid filtrates. Protein concentrations were expressed as mg protein nitrogen per g wet tissue.

pH and water-holding capacity

The pH of meat samples was measured using tissue slurries. 10g of meat were blended with 100 ml of distilled water for 1 min in an Osterizer. The pH from duplicate samples was recorded.

Water-holding capacity was determined using a centrifugation technique modified from Hamm (1960). Triplicate 15g samples of meat were placed in 2.8 × 11 cm centrifuge tubes, 22.5 ml of 0.6 M NaCl solution added and the contents stirred for 1 min with a glass rod. After holding for 15 min at 4°C, the meat slurry was again stirred for 1 min and immediately centrifuged at 12,000 × G for 15 min. The supernatant layer was decanted and the volume recorded. The amount of added solution retained by the meat is reported as the water-holding capacity in ml per 100 g meat.

Loaf preparation and evaluation

Loaves of 150–160g of the ground meat containing 1.5% NaCl (w/w) were prepared by blending the meat with the salt for 1 min in a Kitchen Aid 3-C Mixer, weighing and pressing into loaf form. Aluminum pans 11 cm × 8.5 cm × 3.5 cm with aluminum-lined board tops were used. The loaves were cooked from an initial internal temperature of 4°C to a final internal temperature of 82–84°C in a 176°C oven. After removal from the oven and cooling to approximately 50°C, one end of the pan lid was opened. The weight of fluid exudate (condensate and juices) drained from the loaf is reported as the percent cooking loss based on the initial meat weight.

Binding strength of loaf slices was determined as previously reported by Acton (1972). A trained panel of nine members evaluated the strength of tissue bind using a 9-point hedonic scale (1 = extremely poor bind; 9 = extremely good bind). Meat slices approximately 3 cm × 3 cm × 1.2 cm were used for evaluation.

Two samples measuring 7 cm × 3 cm × 1.5

cm were cut from each loaf for shearing force determinations. An Allo-Kramer Shear Press equipped with a 3000 lb ring was used with a 30 sec downstroke at a range of 300. Shearing force was calculated as kg per g meat.

Statistical analysis

Results were subjected to analysis of variance and the significance of means tested by Duncan's method (Steel and Torrie, 1960). Two replications were conducted (with duplicate or triplicate samples) for each parameter with broilers of each age group for an overall four replications within the study. Linear correlation analysis was conducted to test for significant relationships between variables.

RESULTS & DISCUSSION

EXTRACTABILITY of the nitrogen fractions from muscle tissue as a function of postmortem aging time is given in Table 1. Myosin extractability significantly ($P < 0.05$) decreased from the initial 0–3 hr aging through 12 hr of postmortem aging. The extractable quantity of the actomyosin fraction was initially (0–3 hr) at a low level, 1.5–2.1 mg protein N/g tissue, but significantly increased between 3 hr and 12 hr of aging to 6.6 mg protein N/g tissue. Myosin and actomyosin extractability remained constant from 12 hr to 24 hr postmortem. The sarcoplasmic protein fraction did not significantly change in extractable quantity during the 24 hr aging period or in the frozen 24 hr tissue sample. These data are consistent with the findings reported by Sayre (1968) and Weinberg and Rose (1960). No significant changes were observed for nonprotein nitrogen content (Table 1) during the aging period. Wierbicki et al. (1956) also reported no increase of nonprotein nitrogen in bovine muscle during an aging period of several days.

The myosin fraction from 24 hr tissue frozen for 2 wk at −20°C was considerably lower than the 24 hr nonfrozen tissue (Table 1). However, there was a significant ($P < 0.01$) increase in the quantity of extractable actomyosin from the frozen tissue. Actomyosin nitrogen increased approximately 83% to 12.6 mg N/g tissue whereas the myosin content decreased approximately 86% to 1.4 mg N/g tissue. It is evident that the freezing and thawing of the muscle tissue promoted a greater ease of extraction of actomyosin while extraction of the remaining myosin decreased. The formation of high molecular weight aggregates which can be centrifuged out of solution at low centrifugal fields has been reported by Buttkus (1970) to occur on freezing of rabbit and trout myosins.

During the first 6 hr postmortem, muscle pH significantly dropped from pH 6.3 to pH 5.9 (Table 1). Little change in pH occurred during the remainder of the aging period. A decreasing pH during the time course of rigor mortis has been used previously as a criterion for distinguishing the prerigor state from the postrigor state (Bate-Smith and Bendall, 1956; Acton and Saffle, 1969; Froning and Neelakantan, 1971).

The water-holding capacity of the ground meat significantly ($P < 0.05$) increased at each interval of aging from 3 hr to 24 hr postmortem (Table 1). There appeared to be no direct correlation between muscle pH and the water-holding capacity in this study ($r = 0.44$). Hydration accompanied the formation of actomyosin as noted by Sayre (1968). Hamm (1960) stated that the minimum water-holding capacity coincides with the maximum of muscle rigidity. The water-holding capacity and pH values of the frozen 24 hr aged muscle tissue were not significantly ($P < 0.05$) different from those found for the 24 hr unfrozen muscle.

The percent cooking loss, tissue binding strength and shear force for loaves prepared with meat during postmortem aging are given in Table 2. No significant ($P < 0.05$) changes in cooking loss or binding strength were observed when loaves were prepared with tissue excised at any interval during the 24 hr aging period. However, when loaves were prepared with the 24 hr frozen tissue, there was a significant decrease of cooking loss when compared to the cooking loss of loaves of meat from the 0 to 6 hr periods. Binding strength of the loaves of the 24 hr frozen tissue was also significantly higher when compared to some of the earlier intervals. Maesso et al. (1970b) found no significant difference in average tensile strength of fresh loaves and once frozen loaves, but they reported a significant decrease in tensile strength in loaves frozen and thawed twice versus fresh and once frozen. The effect of freezing as a physical and physiochemical treatment warrants further study due to the current marketing of poultry loaves in the frozen uncooked and precooked state.

There was a significant ($P < 0.05$) correlation between water-holding capacity of the meat tissue and the cooking loss observed from the loaves ($r = -0.71$). Schnell et al. (1970) reported a significant reduction in the amount of cookout when NaCl, polyphosphate or ribonucleic acid were used in loaf preparation. The interaction of NaCl with the soluble muscle proteins is known to increase water-holding capacity (Hamm, 1960). No significant correlation was observed between

Table 1—Extractable nitrogen fractions, pH and water-holding capacity of muscle tissue as a function of time postmortem[a]

Hours postmortem	Nitrogen fraction (mg N/g muscle)				pH	Water-holding capacity[b]
	Myosin	Actomyosin	Sarcoplasmic	Nonprotein		
0[c]	9.33[a]	2.07[ab]	11.45[a]	3.4[a]	6.3[a]	9.2[a]
3	10.80[a]	1.50[a]	10.62[a]	3.4[a]	6.1[b]	0.4[b]
6	4.42[b]	4.38[bc]	12.06[a]	3.8[a]	5.9[c]	7.2[a]
12	2.08[c]	6.60[cd]	12.11[a]	3.7[a]	5.9[c]	15.8[c]
24	2.61[bc]	6.87[d]	11.48[a]	3.7[a]	5.8[c]	25.3[d]
24 F[d]	1.40[c]	12.60[e]	10.98[a]	3.6[a]	5.8[c]	29.4[d]

[a]Any two means within a column having the same or one of the same letters are not significantly different at $P < 0.05$.
[b]ml of 0.6M NaCl solution retained per 100g muscle tissue
[c]Tissue excised and analysis begun within 30 min postmortem
[d]Tissue excised at 24 hr postmortem, frozen at −20°C for 2 wk, thawed and analyzed

Table 2—Percent cooking loss, tissue binding strength and shear force values for poultry loaves prepared with meat at 0 to 24 hr postmortem[a]

Hours postmortem	% Cooking loss	Binding strength	Shear force, kg/g		
			8 wk	10 wk	(Mean)
0[b]	14.2[a]	7.5[ab]	2.58[a]	2.84[a]	(2.71[a])
3	15.2[a]	7.4[ab]	2.18[b]	2.92[a]	(2.55[b])
6	15.3[a]	6.4[a]	1.82[c]	2.15[b]	(1.98[c])
12	13.5[ab]	6.6[a]	1.79[c]	1.97[b]	(1.88[c])
24	11.8[ab]	7.6[ab]	1.63[c]	2.05[b]	(1.84[c])
24 F[c]	9.7[b]	8.1[b]	1.78[c]	2.11[b]	(1.94[c])

[a]Any two means within a column having the same or one of the same letters are not significantly different at $P < 0.05$.
[b]Tissue excised and processing begun within 30 min postmortem
[c]Tissue excised at 24 hr postmortem, frozen at −20°C for 2 wk, thawed and further processed

water-holding capacity and binding strength, r = 0.34. However, there was a significant relationship ($P < 0.01$) between binding strength and cooking loss (r = 0.67) which is in agreement with the reports of Schnell et al. (1970) and Vadehra and Baker (1970).

No significant broiler age effect (8 wk versus 10 wk) was observed for any response except shear force values for meat loaf slices. In general, muscle tissue of the 10 wk old group of broilers produced loaves of higher shear resistance when compared to loaves from meat of the 8 wk old broilers. The overall means of shear resistance compared by time postmortem (Table 2) shows that loaves prepared with prerigor tissue, 0–3 hr postmortem are significantly ($P < 0.05$) less tender than loaves prepared with postrigor tissue, 6–24 hr postmortem. Loaves from frozen 24 hr meat gave shear responses similar to loaves of the postrigor intervals.

Although prerigor meat of broilers and of beef and pork, in comparison to postrigor meat, has been shown to possess significantly better emulsification properties for comminuted meat products (Froning and Neelakatan, 1971; Acton and Saffle, 1969; Trautman, 1964), no significant industrial advantage appears to exist for the use of prerigor meat in poultry loaf production. The rapid onset of rigor in poultry muscle is the most obvious disadvantage to normal processing procedures involved in the usage of prerigor meat. In addition, slightly higher cooking losses occur when loaves are prepared with prerigor tissue. Further research is needed to evaluate the effect of freezing treatments on poultry loaf quality.

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Ms received 10/1/72; revised 12/11/72; accepted 12/12/72.

Technical Contribution No. 1045 of the South Carolina Agricultural Experiment Station, Clemson University, Clemson, SC 29631.

The authors thank the Poultry Science Dept., Clemson University, for use of their facilities and materials.

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EFFECT OF BINDING TO MUSCLE PARTICULATE FRACTIONS ON GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE ACTIVITY

INTRODUCTION

IT IS OFTEN desirable to study enzymic reactions or sequences in preparations of homogenized tissue. A crude homogenate is a better representation of the intact cell than a particulate-free or purified system and at the same time allows certain manipulations which are not possible with intact cells, such as a rapid and even distribution of added substrates and cofactors. However, there are certain disadvantages attendant on such experiments. One of the major ones is the possibility that certain components may interact which are not capable of doing so in the intact cell. If one of these components is an enzyme, the interaction may modify the kinetic properties of the enzyme leading to erroneous conclusions. Examples of modifications of enzymic properties by interaction with subcellular particulate fractions have been reported (Mendicino et al., 1968; Arnold and Pette, 1970). The interactions are often sensitive to pH, ionic strength and metabolite concentrations (Arnold and Pette, 1968, 1970; Roodyn, 1957; Hultin and Westort, 1966; Rose and Warms, 1967). A significant problem which might be caused by modification of enzymic properties due to enzyme-particulate interactions would be an error in estimation of enzyme concentration in a crude homogenate.

In this report, we demonstrate a significant effect on the activity of chicken muscle glyceraldehyde-3-phosphate dehydrogenase by binding to the particulate fraction of the homogenized tissue.

MATERIALS & METHODS

Preparation of bound and soluble glyceraldehyde-3-phosphate dehydrogenase

Breast muscle (Pectoralis major) of domestic female chickens was used throughout this study. The birds were killed by decapitation. The muscle was excised as rapidly as possible and immediately placed in distilled water at 0–4°C. After cooling, the muscle was removed, blotted dry, and the connective and adipose tissue removed. It was then sliced into small cubes and a 10% homogenate (weight/volume) prepared by blending intermittently for 1 min in deionized distilled water at 0–4°C in an Osterizer blender at full speed. All further operations were performed at 0–4°C. The homogenate was filtered through a double layer of cheese cloth and the filtrate was centrifuged at 40,000 rpm for 30 min in the No. 40 rotor in a Spinco Model L ultracentrifuge. The supernatant fraction was discarded; this fraction contained substrates and cofactors as well as α-glycerophosphate dehydrogenase and triosephosphate isomerase which are essentially completely soluble. Removal of these factors simplified interpretation of the glyceraldehyde-3-phosphate dehydrogenase results. The sedimented fraction contained approximately 85% of the glyceraldehyde-3-phosphaste dehydrogenase of the cell and was used as the particulate enzyme.

Soluble enzyme was prepared by treatment of the particulate enzyme with 0.1M NaCl, centrifuging at 40,000 rpm for 30 min in the No. 40 rotor of a Spinco preparative ultracentrifuge, and dialysis of the supernatant fraction for 18 hr at 4°C against a solution containing 1 mM imidazole, pH 6.5; 0.1 mM dithiothreitol; and 0.1 mM NAD^+. The dialysate was centrifuged at 1500 rpm for 15 min in the SS-34 rotor of a Sorvall RC-2 centrifuge, and the supernatant fraction served as the source of soluble glyceraldehyde-3-phosphate dehydrogenase.

Assay for glyceraldehyde-3-phosphate dehydrogenase

Glyceraldehyde-3-phosphate dehydrogenase was assayed in two ways. The first employed the method of Rassner (1965) at pH 7.6. The assay medium of this method solubilized all the enzyme of the particulate preparation; thus, the data obtained for both the soluble and bound preparations were on the basis of soluble enzyme and could be directly compared. The results of this determination were used to adjust the concentrations of glyceraldehyde-3-phosphate dehydrogenase in the bound and soluble preparations to equal values for the stopped-flow assays. A turnover number of

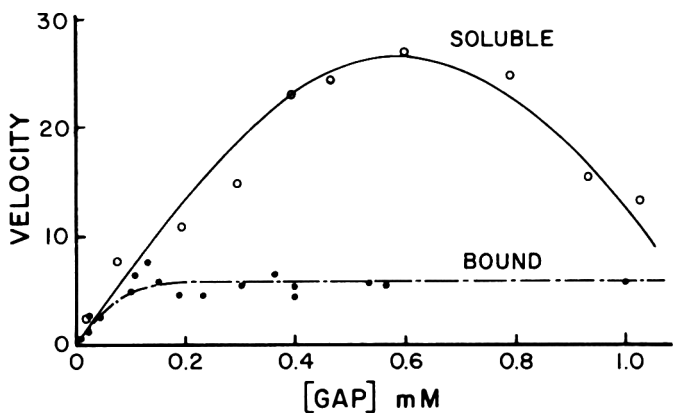

Fig. 1—Activities of soluble and bound glyceraldehyde-3-phosphate dehydrogenase as a function of glyceraldehyde-3-phosphate concentration. The soluble (Sol GAPDH) and bound (Bd GAPDH) preparations were adjusted to a concentration of 5 μM glyceraldehyde-3-phosphate dehydrogenase and assayed by a stopped-flow technique. One drive syringe contained imidazole buffer, pH 6.5, 10 mM; NAD^+, 1.2 mM; dithiothreitol, 2 mM; sodium arsenate, 2 mM and varying concentrations of glyceraldehyde-3-phosphate at twice the desired final concentration. The other syringe contained enzyme (either soluble or particulate) at a concentration of 10 μM adjusted to pH 6.5. The concentration of particles in the preparation of bound enzyme was 20 mg of insoluble protein per ml. All components were present in the assay in quantities equal to one-half that described above since equal volumes from each drive syringe were mixed during the assay. Changes in transmission at 340 nm were recorded and converted to nmoles of NADH with the use of standard curves. The velocity of the reaction is given in nmoles NAD^+ reduced per sec.

12.000 (Cori et al., 1948; Velick, 1955; Kochman and Rutter, 1968) was used to calculate the concentration of the enzyme.

The glyceraldehyde-3-phosphate dehydrogenase activities of the bound and soluble preparations were then compared at a concentration of 5 μM using a stopped-flow technique with the Aminco-Morrow apparatus. We wished to study the enzyme at this relatively high concentration to duplicate as much as possible conditions which might exist in a tissue homogenate. One drive syringe contained imidazole buffer, pH 6.5, 10 mM; NAD^+, 1.2 mM; dithiothreitol, 2 mM; sodium arsenate, 2 mM; and various concentrations of glyceraldehyde-3-phosphate at twice the desired final concentration. The other syringe contained enzyme (either soluble or particulate) at a concentration of 10 μM adjusted to pH 6.5. In the particulate preparation, the concentration of insoluble protein was approximately 20 mg/ml. All components were present in the assay in quantities equal to one half that described above since equal volumes from each drive syringe were mixed during the assay. Changes in transmission at 340 nm were monitored to follow the reaction. Standard curves were prepared by adding known quantities of NADH in place of the glyceraldehyde-3-phosphate for every series of experiments.

The conditions for the stopped-flow assay were designed to keep all the enzyme bound and were based on the solubility characteristics of the enzyme (Dagher, 1971). The amount of solubilization brought about by the assay medium was determined by suspending the particulate enzyme in the assay medium, minus the arsenate, centrifuging at 40,000 rpm for 30 min in the No. 40 rotor and determining the enzymic activity in the supernatant and sedimented fractions. The arsenate was left out of the medium because it inactivated the glyceraldehyde-3-phosphate dehydrogenase with time. The inactivation rate was much slower than the rate of reaction catalyzed by the enzyme, and thus posed no problem in the assay. The solubilization procedure, however, required a time period sufficiently long for the inactivation by arsenate to be a problem. An equivalent amount of phosphate under these conditions had no solubilizing effect on the enzyme. Less than 2% of the total glyceraldehyde-3-phosphate dehydrogenase was solubilized by the medium. Although pH 6.5 is below what is usually considered the normal physiological value, it was chosen because muscle homogenates reach this pH or lower very quickly if not strongly buffered, and this pH may be achieved in living muscle (Carter et al., 1967; McLoughlin, 1970). Also, at this pH essentially all the enzyme remained bound, and this made interpretation of the results simpler. At pH 7 under the same conditions, about 30% of the enzyme would be solubilized (Dagher, 1971).

RESULTS & DISCUSSION

RESULTS OBTAINED in the stopped-flow assays using equal quantities of glyceraldehyde-3-phosphate dehydrogenase (5 μM) both as soluble enzyme and with greater than 98% of the enzyme bound to the particulate fraction are shown in Figure 1. Under these conditions of assay binding of glyceraldehyde-3-phosphate dehydrogenase significantly modifies its activity.

The maximal velocity (V_{Max}) and Michaelis constant (K_M) for each form of the enzyme was calculated from the reciprocal plots of the data in Figure 1. Data at high substrate concentrations were ignored in the case of the soluble enzyme where there was obviously considerable substrate inhibition. V_{Max} was 29 nmoles of NAD^+ reduced per sec for the soluble enzyme while the value for bound glyceraldehyde-3-phosphate dehydrogenase was 7 nmoles per sec. The K_M (glyceraldehyde-3-phosphate) for the soluble enzyme was 0.2 mM, and for the bound it was 0.07 mM. Thus, while glyceraldehyde-3-phosphate dehydrogenase in the soluble phase was more active than bound enzyme at all substrate concentrations, bound glyceraldehyde-3-phosphate dehydrogenase retained a greater percentage of its maximal activity at low substrate concentrations than did the soluble enzyme. In addition, there was a marked inhibition by substrate of soluble glyceraldehyde-3-phosphate dehydrogenase while there was no evidence for this with the bound enzyme over the same range of substrate concentrations.

The state of glyceraldehyde-3-phosphate dehydrogenase of chicken muscle is obviously an important determinant of its kinetic behavior. Conditions leading to partial binding of this enzyme could easily occur in homogenized muscle (Newbold and Lee, 1965; Cook, 1968). Such binding could have an important effect on studies of glycolysis through this change in activity. This would be especially true under conditions where glyceraldehyde-3-phosphate dehydrogenase is the rate-limiting enzyme (Newbold and Scopes, 1971). Maintaining a sufficiently high ionic strength may relieve the problem, but the ionic strength required may be a function of the pH (Hultin and Westort, 1966). At high protein concentrations, the soluble enzymes of muscle bind to the structure proteins, myosin and delta protein, even at high ionic strength (Amberson and Bauer, 1971). That reversible binding-solubilization of glyceraldehyde-3-phosphate dehydrogenase may be a physiological phenomenon (Arnold and Pette, 1968) would not negate the fact that homogenization leads to major changes in distribution of ions and metabolites; thus changes in distribution of glyceraldehyde-3-phosphate dehydrogenase between bound and soluble phases may take place which do not occur in vivo. Such interactions between enzymes and subcellular particulate structures may possibly account for some of the differences observed in rates of glycolysis between whole and minced muscles (Newbold and Scopes, 1971).

Enzymic reactions or sequences are often studied in tissue homogenates, and it is usually assumed that the enzyme behaves in the homogenate the same as in situ. Glyceraldehyde-3-phosphate dehydrogenase of chicken breast muscle binds to the particulate fraction of the homogenized tissue under some conditions. In this study, we demonstrate that such binding modifies the activity of the enzyme.

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Ms received 9/19/72; revised 12/11/72; accepted 12/12/72.

This work was supported in part by U. S. Public Health Service Grant GM-12064 from the National Institute of General Medical Sciences.

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CONTROL OF Staphylococcus aureus IN SAUSAGE BY STARTER CULTURES AND CHEMICAL ACIDULATION

INTRODUCTION

ALTHOUGH the manufacture of fermented sausage products represents a large and valuable industry in the United States and other countries, there are few reported studies on the microbiological aspects of the process involved. Successful manufacture of dried and semi-dried fermented sausage products such as thuringer, cervelat, summer sausage, Lebanon bologna and pepperoni requires a strong fermentation to yield the desired characteristics, including the typical tangy flavor and chewy texture. The characteristic tang is considered to be due to lactic acid and other products of bacterial fermentation (Kramlich, 1971).

Starter cultures of suitable lactic acid bacteria are finding increasing use by some manufacturers to overcome problems associated with the production of fermented sausage. Jensen and Paddock (1940) described a process employing *Lactobacillus* species. More recently, *Pediococcus cerevisiae* was introduced by Niven et al. (1958) and this culture was made available to the industry in lyophilized form (Everson, 1971). The same culture is now available in a more suitable frozen form and is being widely accepted by the industry because it improves sausage uniformity and shortens production cycles (Everson et al., 1970; Rothchild and Olsen, 1971).

Not all manufacturers, however, use starter cultures to assist in the fermentation process. Chance inoculation, if relied upon, requires a long holding time. For some sausages, the practice of "backslopping" is used extensively. In this case part of a previously fermented batch is held at 38°C for 24 hr and then added to the next batch as a starter. This system is potentially dangerous if there are food-poisoning organisms in the environment which may contaminate the meat and grow out in the starter meat. Chemical acidulation has also been used to achieve the desired fermentation in the manufacture of some sausage products. Glucono-delta-lactone (GDL), alone or in combination with citric acid, has been most frequently used. GDL is hydrolyzed to gluconic acid in the presence of water. This hydrolysis yields an immediate drop in pH but causes problems by an excessively rapid reduction of water content which is difficult to control. The amounts of chemicals that may be added to fermented meat products are controlled by Federal legislation (Bailey, 1970).

A successful fermentation is required not only to yield the desired flavor and texture but also to prevent the growth of undesirable organisms, particularly food-borne pathogens. While every precaution must be taken in the selection and handling of raw ingredients and sanitation of equipment, it is generally conceded that some food-poisoning organisms, especially *Staphylococcus aureus* may be present and a rapid, controlled fermentation is needed to guarantee the safety of the final product.

As part of research studies on the inhibitory properties of various lactic acid bacteria against food-borne pathogens and spoilage organisms (Daly et al., 1972), it was decided to examine the effect of commercially available fermented meat starter cultures on the growth and survival of *S. aureus* in simulated beaker-sausage incubated at 21°C, 30°C and 37°C. Some comparative studies employing GDL and citric acid as chemical acidulants were also included. The fact that *S. aureus* can be a serious problem in fermented sausage products has been demonstrated by the recent occurrence of several outbreaks of staphylococcal food-poisonings associated with Genoa sausage produced by two different major manufacturing companies (NCDC, 1971a, b). Some of the sausage samples tested contained more than 1 million coagulase-positive staphylococci per gram.

MATERIALS & METHODS

Bacterial cultures

S. aureus, American Type Culture Collection (ATCC) 13565 (type A toxin producer) and *S. aureus* ATCC 14458 (type B toxin producer) were used in this study. Stock cultures were maintained on Liver Infusion agar slants (Difco) at 5°C. Working cultures were transferred daily in Brain Heart Infusion (BHI) broth (Difco).

The starter cultures used in this study were obtained as frozen concentrates from Microlife Technics, Sarasota, Florida and stored at -20°C until used. The culture designated *P. cerevisiae* (NRRL B-5627 - Lactacel) is the strain widely used in industry (Everson, 1971; Everson et al., 1970). *Lactobacillus plantarum* (NRRL B-5632 - Lactacel DS) and a mixed culture (Lactacel MC) containing both *P. cerevisiae* and *L. plantarum* were also used.

Laboratory scale sausage production

Beaker-sausage was made by the method described by Deibel et al. (1961). The following ingredients were added to cold (5°C) ground beef base: glucose 1.5%, sodium chloride 3.0% and sodium nitrate 0.01%. The meat and curing salts were thoroughly mixed and, in studies employing starter cultures, the sausage emulsion was then divided into 250-g aliquots. Test samples were inoculated with 1 ml of an appropriate dilution of an 18–24-hr culture of *S. aureus* grown on a New Brunswick Controlled Environment Shaker (150 rpm) at 30°C. After thawing by immersion for 15–20 min in 37°C water, the contents of a can of starter culture (6 oz) was added to 500 ml of tap water. Starter cultures were added at the rate of 2.5 ml of this diluted concentrate per 250-g of sausage emulsion. 40-g aliquots of the inoculated product (temperature was about 20°C) were then placed in 50 ml beakers, covered with aluminum foil and stored at 21°C, 30°C or 37°C; temperature equilibration occurred within about an hour or less because of the small volume used.

When chemical acidulation was used, 0.75% GDL (Matheson, Coleman and Bell, Division of Matheson Co., Inc., Norwood, Co.) was added to the sausage emulsion prior to inoculation with *S. aureus*. The levels employed here were similar to those allowed for these chemicals (0.5% to 1.0% for GDL and approximately 0.02% for citric acid, the latter allowed in dry sausage only in conjunction with antioxidants) (Bailey, 1970; Federal Register, 1970). In experiments combining chemical acidulation and inoculation with starter cultures, additions were the same as when each method was used alone.

Enumeration of staphylococci in meat emulsions

Meat samples were blended with 9 vol of sterile distilled water at low speed in a Waring Blendor for 2 min. Subsequent dilutions were made in Butterfield's phosphate buffer (FDA, 1969). Surface plating on three laboratory media was carried out to enumerate the staphylococci: Tryptone-pyruvate-plasma-fibrinogen (TPPF) agar, recently developed by Chugg (1971) and which allows a direct count of coagulase-positive staphylococci on the agar plate, Vogel-Johnson Agar (Difco) and modified lactic agar (Elliker et al., 1956) with 0.5% sodium pyruvate and 7.0% sodium chloride.

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Table 1–Effect of three different starter cultures on the growth of S. aureus in fermented sausage incubated at 37° C

Time	S. aureus alone		S. Aureus + Lactacel[a]		S. aureus + Lactacel MC[b]		S. aureus + Lactacel DS[c]	
(hr)	Staph./g	pH	Staph./g	pH	Staph./g	pH	Staph./g	pH
0	30,000	5.95	30,000	6.0	30,000	5.95	30,000	5.95
5	150,000	6.0	100,000	5.5	100,000	5.7	150,000	5.9
10	13,000,000	5.85	120,000	5.25	280,000	5.45	1,400,000	5.5
25	98,000,000	5.7	60,000	4.6	210,000	4.55	410,000	4.4
50	63,000,000	5.2	90,000	4.3	40,000	4.2	40,000	4.25

[a] 16×10^7 cfu added/g of meat
[b] 7×10^7 cfu added/g of meat
[c] 14×10^7 cfu added/g of meat

Table 2–Effect of three different starter cultures on the growth of S. aureus in fermented sausage incubated at 37° C

Time	S. aureus alone		S. aureus + Lactacel[a]		S. aureus + Lactacel MC[b]		S. aureus + Lactacel DS[c]	
(hr)	Staph./g	pH	Staph./g	pH	Staph./g	pH	Staph./g	pH
0	190,000	5.9	180,000	5.9	190,000	5.9	180,000	5.9
5	1,200,000	5.95	900,000	5.7	1,000,000	5.75	1,500,000	5.85
10	13,000,000	5.85	720,000	5.05	4,100,000	5.20	9,500,000	5.6
25	95,000,000	5.8	430,000	4.35	2,300,000	4.4	17,000,000	4.6
50	38,000,000	5.1	1,100,000	4.3	2,400,000	4.25	2,900,000	4.2

[a] 21×10^7 cfu added/g of meat
[b] 8×10^7 cfu added/g of meat
[c] 16×10^7 cfu added/g of meat

Suspect colonies from all three media were confirmed as coagulase-positive staphylococci by isolation in BHI broth (Difco), followed by subsequent testing for coagulase. The counts of *S. aureus* recorded in this report are those on TPPF agar because this medium yielded consistently higher counts than Vogel-Johnson agar and was more selective for coagulase-positive staphylococci than the modified lactic agar; plates with 30–300 colonies were selected for counting and data in tables.

Viable counts of the starter culture bacteria in the diluted concentrate were determined on the agar of Rogosa et al. (1951). The pH values of the meat homogenates in 9 vol of distilled water were recorded with a Corning model 12 research pH meter.

RESULTS & DISCUSSION

Control of *S. aureus* by starter cultures

Table 1 shows the viable counts of *S. aureus* ATCC 13565 (type A toxin-producer) resulting from a relatively low inoculum (30,000/g) in simulated sausage mixes at 37°C in the presence and absence of added starter bacteria; these are typical data from an experiment which gave essentially the same results when repeated several times. The pH changes are also recorded. In the absence of starter, *S. aureus* proliferated rapidly and reached very high levels at 10, 25 and 50 hr. The initial inoculum levels used in this experiment were higher than normally would be expected in a carefully controlled industrial operation. However, greater than 1×10^6 coagulase-positive staphylococci per gram were found in sausage products incriminated in food-poisoning outbreaks (NCDC, 1971a, b) and high levels, similar to those reached here, were reported by Metcalf and Deibel (1969) also using a beaker-sausage process. Lactacel gave the most rapid pH drop of the three cultures used here and also better control of the *S. aureus* population. This active starter culture allowed only a slight initial increase and then successfully prevented subsequent growth of *S. aureus*. There was approximately 99.9% inhibition of *S. aureus* by the Lactacel culture (*P. cerevisiae*) at 25 hr, i.e., greater than 3 log cycles reduction compared to *S. aureus* growing alone. Culture Lactacel MC (*P. cerevisiae* plus *L. plantarum*) showed greater than 99% inhibition of *S. aureus* at 25 hr. The reduced inhibition and slower initial rate of acid production, as indicated by the pH changes, may, in part, be due to the lower level of inoculum of this culture. Starter culture Lactacel DS (*L. plantarum*), although used at a concentration almost equivalent to Lactacel, showed a much reduced initial rate of acid production and less inhibition at 25 hr.

When a higher initial population (190,000/g) of *S. aureus* was used, the degree of inhibition due to the starter cultures was reduced, despite a more rapid fermentation by Lactacel and Lactacel MC in this trial (Table 2). However, the Lactacel culture, in particular, did give greater than 99% inhibition at 25 hr, even at this high *S. aureus* concentration. Thus, results presented in Tables 1 and 2 show the ability of physiologically active starter cultures to prevent the growth of *S. aureus* when the food-poisoning bacterium is present in raw product over a wide population range; it is suggested that even greater control would be achieved with lower initial *S. aureus* populations. However, reduced inhibition in the presence of higher *S. aureus* inocula cautions against carelessness in the overall sausage manufacturing process, even when starter cultures are used to aid fermentation. In this regard, thorough mixing of added starter is important to prevent occurrence of pockets in the product with high *Staphylococcus* counts and reduced numbers of starter bacteria. In a report now in preparation it will be shown that the degree of inhibition of *S, aureus* found here (99%) is sufficient to prevent toxin production in food products.

Substantial inhibition of *S. aureus* was also achieved by the starter cultures when the temperature of fermentation was 30°C (Table 3). In this case, also, less inhibition was observed with Lactacel DS. Acton et al. (1972) showed that fermentation temperatures within the range 22°C to 37°C did not significantly affect the flavor of summer sausage made with the same *P. cerevisiae* strain used in this study. These workers, however, indicated the need for research to ascertain the potential safety of a product prepared at the lower fermentation temperature, 22°C. Table 4 shows the results on the ability of the three starter cultures to control the growth of *S. aureus* at 21°C.

Table 3—Effect of three different starter cultures on the growth of S. aureus in fermented sausage at 30° C

Time	S. aureus alone		S. aureus + Lactacel[a]		S. aureus + Lactacel MC[b]		S. aureus + Lactacel DS[c]	
(hr)	Staph./g	pH	Staph./g	pH	Staph./g	pH	Staph./g	pH
0	30,000	5.9	30,000	5.9	30,000	5.9	30,000	5.9
5	130,000	5.8	60,000	5.7	60,000	5.75	90,000	5.9
10	1,300,000	5.8	180,000	5.35	290,000	5.5	340,000	5.65
25	79,000,000	5.5	140,000	4.7	470,000	4.75	1,900,000	4.9
50	68,000,000	5.3	220,000	4.45	590,000	4.45	530,000	4.4

[a] 20×10^7 cfu added/g of meat
[b] 12×10^7 cfu added/g of meat
[c] 18×10^7 cfu added/g of meat

Table 4—Effect of three different starter cultures on the growth of S. aureus in fermented sausage at 21° C

Time	S. aureus alone		S. aureus + Lactacel[a]		S. aureus + Lactacel MC[b]		S. aureus + Lactacel DS[c]	
(hr)	Staph./g	pH	Staph./g	pH	Staph./g	pH	Staph./g	pH
0	30,000	6.0	30,000	5.95	30,000	6.0	30,000	6.0
5	35,000	6.0	35,000	5.95	40,000	5.95	30,000	5.95
10	260,000	6.0	80,000	5.7	80,000	5.85	62,000	5.85
25	15,000,000	5.8	1,400,000	5.15	1,900,000	5.2	2,100,000	5.15
50	94,000,000	5.65	2,000,000	4.8	3,100,000	4.65	1,800,000	4.50

[a] 17×10^7 cfu added/g of meat
[b] 9×10^7 cfu added/g of meat
[c] 14×10^7 cfu added/g of meat

The degree of inhibition was not as dramatic as that observed at 30°C and 37°C. This is most likely due to the slower rate of acid production at 21°C. There was little difference between the three cultures at this fermentation temperature. The effects of fermentation temperature on the degree of inhibition of *S. aureus* observed in this study would suggest that 30 or 37°C would be more desirable from a food-safety aspect, at least with the *S. aureus* levels of inoculum used here. A search for a suitable starter organism with a lower temperature optimum might be worthwhile if a lower fermentation temperature was more suitable, considering all aspects of the manufacturing process.

Control of *S. aureus* by chemical acidulation

Direct acidification by the addition of certain chemicals is used in some cases instead of biological acidification by the end-products of microbial metabolism, mainly lactic acid. GDL is used because it yields gluconic acid in the presence of water and a decrease in pH results. Citric acid possesses useful inhibitory properties against *S. aureus*, probably by chelation of essential metal ions, although other mechanisms may be involved.

The viable counts of *S. aureus* 13565 in simulated fermented sausage made with and without glucono-delta-lactone (0.75%) and citric acid (0.1%) and fermented at 37°C are shown in Table 5. In the chemically acidified sausage mix the initial pH was 4.9 as compared to 5.8 in the control. There was substantial inhibition of *S. aureus* in the early stages of the fermentation but at 25 hr there was a large, significant increase in viable staphylococci. This increase was maintained at 50 hr so that at this time the *S. aureus* population was essentially the same as that in the control. Similar findings were observed when the incubation temperature was 30°C and 21°C. The recovery of *S. aureus*, however, was not as great at 21°C so that significant inhibition was still evident at 25 hr (See summary Table 7).

Control of *S. aureus* by combined starter culture addition and chemical acidulation

In the presence of high *S. aureus* numbers some initial multiplication of this food-borne pathogen occurred, especially in the presence of the initially less-active starter culture, Lactacel DS (Tables 1 and 2). While early multiplication was prevented by the chemical acidulation technique, a large increase in viable *S. aureus* population took place later in the fermentation period, rendering this process very suspect in the control of this potentially dangerous food-poisoning organism. It was decided, therefore, to combine the two processes and the results are outlined in Table 6 for test samples incubated at 37°C. The data show that the initial increase in *S. aureus* population was prevented, as previously shown in Table 5. However, in this case, the pH continued to drop substantially, due to the ability of the starter organisms to initiate their fermentation at the relatively low pH. The continued acid production by the starter organisms prevented any increase in viable *S. aureus* and made this the most adequate system used in this study for controlling *S. aureus*. Similar findings were observed at 30°C and 21°C.

Table 5—Effect of GDL (0.75%) and citric acid (0.1%) on growth of S. aureus in fermented sausage at 37° C

Time	Control[a]		Experimental	
(hr)	Staph./g	pH	Staph./g	pH
0	30,000	5.8	30,000	4.9
4	70,000	5.8	15,000	4.7
10	7,200,000	5.75	40,000	4.7
25	96,000,000	5.7	1,400,000	4.85
50	32,000,000	5.4	39,000,000	4.9

[a] No GDL or citric acid added

Table 6–Effect of combination of starter culture and added chemicals on the growth of S. aureus in fermented sausage incubated at 37° C

Time	S. aureus alone		S. aureus + L[a] + Chemicals[d]		S. Aureus + MC[b] + Chemicals[d]		S. aureus + DS[c] + Chemicals[d]	
(hr)	Staph./g	ph	Staph./g	pH	Staph./g	pH	Staph./g	pH
0	30,000	5.95	45,000	4.8	38,000	4.8	41,000	4.8
5	150,000	6.0	18,000	–	21,000	–	32,000	–
10	13,000,000	5.85	6,800	4.4	16,000	4.45	20,400	4.55
25	98,000,000	5.7	<3,000	4.05	<3,000	3.95	13,000	4.00
50	63,000,000	5.2	<3,000	3.95	<3,000	3.80	4,400	3.80

[a]Lactacel added 19×10^7 cfu/g of meat
[b]Lactacel MC added 9×10^7 cfu/g of meat
[c]Lactacel DS added 15×10^7 cfu/g of meat
[d]Chemicals added were GDL (0.75%) and citric acid (0.1%)

The results showing percentage inhibition values, are summarized in Table 7. Shown are values for only one strain since results with the other *S. aureus* strains were essentially the same.

Application to industrial processes

While definite conclusions from these experiments are limited to the factors actually compared in this simulated sausage procedure, it is hoped that the results help to give a more clear picture of the growth patterns of *S. aureus* in fermented meat products. The ability of physiologically active starter cultures to successfully inhibit *S. aureus* has been demonstrated, even at relatively high levels of the pathogen. This beneficial effect of the lactic acid bacteria has previously been demonstrated, using other suspending media (Daly et al., 1972; Gilliland and Speck, 1972; Reddy et al., 1970). Goepfert and Chung (1970) attributed the failure of *Salmonella* to grow in fermented sausage to the low pH and high salt concentration present. The beaker-sausage method (Deibel et al., 1961) used in this study could be used as a type of activity test and by appropriate manipulations of ingredients and incubation conditions, the potential usefulness of new and existing starter cultures in a particular sausage-making procedure could be ascertained. Such practical activity tests are widely used in the dairy industry to assist in the selection of starters for cheese making.

Chemical acidulation as used here gave useful initial control of *S. aureus* but good growth of the pathogen occurred later in the fermentation period. This growth occurred at relatively low pH levels 4.8–4.9. Other workers have reported the ability of *S. aureus* to grow at low pH, especially in meat environments which were more conducive to growth of this organism than BHI broth (Genigeorgis et al., 1971); at similar brine concentrations and pH levels, it took fewer cells to initiate growth in meats than in the broth. This was especially true in meats with high brine concentration and low pH. Furthermore, Metcalf and Deibel (1969) reported the development of a large *S. aureus* population in the acid environment of sausage (final pH 4.3). Recently, Reimann et al. (1972) indicated that 0.6% GDL was effective in maintaining the pH below 5.0 for at least 2 months in beef and pork stored at 2–5°C; this pH in combination with added salt apparently inhibits growth of staphylococci and *Clostridium botulinum* in meat held at refrigeration temperatures.

The growth behavior of *S. aureus* in an industrially prepared product is difficult to predict because of the interaction of so many factors, e.g., nature of the meat and other ingredients, available oxygen, water-activity, temperature, pH and the effects of other organisms, particularly lactic acid bacteria. However, the results of this study, and those of other workers, would caution against the adoption of a certain pH attainment, such as 5.0–5.2, as a criterion of safety of a fermented product where *S. aureus* is concerned. Regular line sampling for *S. aureus* throughout the process would be a useful indication of possible danger but in the absence of this, analysis of the final product for *Staphylococcus* toxin is the only sure way to determine the safety of a product.

From the limited data reported in this study, it would appear that a combination of chemical acidulation and starter cultures could be used successfully in fermented meats, at least from a food-safety standpoint. Since only one concentration each of GDL and citric acid (and these in excess of the legal limits) was used, more research is needed to see if lower levels of these chemicals have a role in meat fermentations in producing the desired flavor and texture, as well as in aspects of food-safety. The combination of the two methods certainly allows the achievement of a wide range of pH values.

Properly selected, physiologically active starter cultures will ensure the required pH decrease and safety of a fermented meat product. Such starters have been shown to improve sausage uniformity and shorten production cycles (Everson et al., 1970). With this knowledge in mind and in view of the recent public outcry against the addition of chemicals to food products, perhaps more natural biological acidification should be the method of choice in meat fermentations.

Table 7–Summary of the inhibition of S. aureus in fermented sausage by the control processes used in this study

	Inhibition[a]					
	% @ 37°C		% @ 30°C		% @ 21°C	
Condition	25 hr	50 hr	25 hr	50 hr	25 hr	50 hr
Lactacel DS	99.58	99.43	97.57	99.22	86.09	98.10
Lactacel MC	99.78	99.38	99.41	99.13	87.88	96.64
Lactacel	99.94	99.86	99.82	99.68	90.46	97.21
Chemical acidulation[b]	98.54	none	98.80	34.33	98.82	97.21
Chemical Acidulation						
+ Lactacel DS	99.99	>99.99	99.98	>99.99	99.57	99.97
+ Lactacel MC	>99.99	>99.99	>99.99	>99.99	99.75	99.99
+ Lactacel	>99.99	>99.99	>99.99	>99.99	99.75	99.99

[a]% inhibition compared to S. aureus control
[b]Chemicals added were GDL (0.75%) and citric acid (0.1%)

Constant use of a particular culture will ensure uniformity of product but research workers should also examine the possibility of using new cultures to give variety to existing products and develop new ones. Finally, industrial users need not fear the requirement of an elaborate starter maintenance program when using these cultures. Commercially available cultures do not require special attention and may be treated like any other additive. Recent advances in the techniques for storing these cultures may make them even more attractive for future use.

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Ms received 10/29/72; revised 12/11/72; accepted 12/12/72.

Technical Paper No. 3452, Oregon Agricultural Experiment Station.

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BIOCHEMICAL AND MICROBIAL STUDIES ON SHRIMP: VOLATILE NITROGEN AND AMINO NITROGEN ANALYSIS

INTRODUCTION

DURING the postmortem storage period on ice, the shrimp tail (abdomen) is subjected to a number of external and internal factors which can cause changes in the levels and types of non-protein nitrogenous components. Increases in total volatile nitrogen content (TVN) have been reported by several investigators (Campbell and Williams, 1952; Fieger and Friloux, 1954; Iyengar et al., 1960; Montgomery et al., 1970; Cobb and Vanderzant, 1971). Trimethylamine (TMN) formation has been noted, especially during the period when spoilage odors appear (Bethea and Ambrose, 1961; Campbell and Williams, 1952; Iyengar et al., 1960; Montgomery et al., 1970). Recent reports (Shewan et al., 1971; Tozawa et al., 1971), however, have indicated that analysis of TMN by the Dyer procedure (Dyer, 1945) employed by some of these investigators may actually have resulted in other amines being measured as TMN. Both amino nitrogen increases (Campbell and Williams, 1952) and decreases (Fieger and Friloux, 1954; Velankar and Govindan, 1958) have been observed during the ice-storage period. In a recent study (Cobb and Vanderzant, 1971) changes in the nitrogenous compounds in shrimp were indicated to occur as a result of both endogenous enzymic (proteolytic and arginase) activity and the activity of specific bacteria.

This investigation was the result of the following observations: (1) when analyzing TVN and TMN levels in shrimp by the microdiffusion technique (Conway, 1958), TMN values appeared to parallel (at much lower levels) TVN values, suggesting that ammonia might be distilling and being measured as TMN; (2) when shrimp were stored at refrigeration temperatures in petri dishes (Cobb and Vanderzant, 1971), TVN levels appeared to increase with time, but when shrimp were stored directly on ice, TVN levels sometimes remained constant or decreased.

This is a report of improvement of the microdiffusion procedure, to allow accurate TMN analysis in the presence of high levels of ammonia and dimethylamine, and of a method of adjusting TVN levels for drip and the washing action of water from melting ice.

EXPERIMENTAL

Stock solutions

Trimethylamine-hydrochloride (TMN), dimethylamine-hydrochloride (DMN), and NH_4Cl were dissolved in trichloracetic acid (TCA) solutions (200 ml of 7% TCA – 80 ml of H_2O) to correspond to tissue extracts. Each stock solution contained approximately 0.5 mg N/ml.

Shrimp extracts

Shrimp extracts were made by blending in a Waring Blendor at least five shrimp at a ratio of 1g shrimp to 2 ml 7% TCA. The mixture was centrifuged and the supernatant preserved at 5°C until used. For amino acid analysis, the supernatant was frozen and recentrifuged to remove additional protein.

Microdiffusion analysis of volatile nitrogen

The modified Conway microdiffusion dish (Obrink, 1955) was utilized for microdiffusion analysis. Prior to use, dishes were washed in a nonionic detergent and rinsed with distilled H_2O followed by an ethanol rinse. Releasing agents (RA) employed in this study were saturated K_2CO_3 (RA-1), saturated Na_3PO_4 (RA-2), saturated Na_3PO_4 to which solid KOH had been added until a slight turbidity persisted (RA-3), and 10N NaOH (RA-4). RA-3 was designated Na_3PO_4 · KOH. The trapping agent was 3.1% H_3BO_3 containing 1 ml mixed indicator (Conway, 1958) to 50 ml solution. For TMN analysis 0.5 ml 40% formaldehyde (HCHO) was added to the sample prior to reaction with the releasing agent (RA-3 for analysis of shrimp extracts). After diffusion for the alloted time (1.5 hr for analysis of extracts), the trapping agent was titrated to the original color with 0.02N HCl. A Metrohm Herisau Dosimat (Brinkman Instruments) with a 1 ml pipet was employed for titrations. All analyses were conducted in triplicate or greater.

Steam distillation for rapid total volatile nitrogen (TVN) analysis

For rapid TVN analysis, a micro-Kjeldahl distillation apparatus was used. 5 ml RA-2 were added to 5 ml of sample. Volatile N was trapped in 5 ml of boric acid solution (50 ml 3.1% H_3BO_3 solution, 1 ml mixed indicator).

Table 1—The effects of various releasing agents on the Conway distillation (3.5 hr) of dimethylamine (DMN), NH_3 and trimethylamine (TMN) in the presence of HCHO

Releasing Agent	DMN[a] %	NH_3[b] %	TMN[a] %
Saturated K_2CO_3	67.5[c]	0.7	98
10N NaOH	29	6	100
Saturated Na_3PO_4	9.5	<0.1	77
Saturated Na_3PO_4 · KOH	15	<0.1	98

[a]0.48 mg N/ml
[b]0.45 mg N/ml
[c]Mean of three or more determinations with three measurements each determination

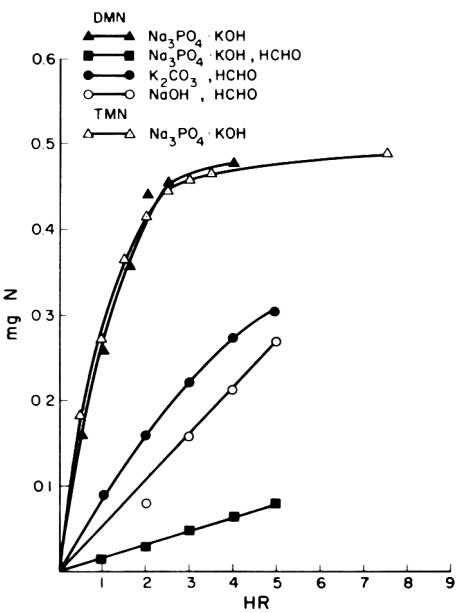

Fig. 1 – Microdiffusion analysis of trimethylamine (TMN) and dimethylamine (DMN) vs. time with different releasing agents and HCHO.

Distillation was continued until 10 ml of distillate had been collected.

Amino acid analysis

A fully automated Spinco model 121 amino acid analyzer was employed for amino acid analysis.

Amino nitrogen analysis

Amino nitrogen was analyzed by a modification of the copper procedure of Spies and Chambers (1951). The copper procedure was modified as follows. Cupric chloride (0.16M) and borate buffer (57.21g of Na_3BO_3, 100 ml of N HCl made to 2 liters) were made according to Pope and Stevens (1939). Trisodium phosphate solution was made by dissolving 136.86g $Na_3PO_4 \cdot 12H_2O$ (0.36M) per liter H_2O. Cupric phosphate suspension was made by adding 1 vol of cupric chloride to 2 vol of trisodium phosphate with rapid stirring, followed by 2 vol of borate buffer. Cupric phosphate suspension (7.5 ml, freshly stirred) was added to 5.0 ml of shrimp extract (7% TCA). The TCA extract and cupric phosphate suspension were carefully mixed, allowed to stand 5–10 min and centrifuged. The supernatant was removed, checked for turbidity (to avoid, do not remove all of the liquid in centrifuge tube) and the absorbance was read at 630 nm in a Beckman DB spectrophotometer. A standard curve employing appropriate amounts ($0.25 - 3.5 \times 10^{-4}$ mole) of a mixture of 18 ml of 0.1M arginine and 82 ml 0.1M glycine (to correspond with amino acid composition of shrimp extracts) was made. Standards were dissolved in TCA (200 ml of 7% TCA – 80 ml H_2O) and dilutions were made with TCA.

Amino nitrogen values were also calculated from amino acid analyzer data.

Organoleptic evaluation

Organoleptic evaluations were made by trained quality control personnel from a large shrimp processing plant. Samples were classified as fresh (less than 2 days out of water), good, poor (acceptability questionable) and spoiled.

Expression of results

TVN, TMN, DMN and NH_3 analyses were expressed as milligrams nitrogen (mg N) or for shrimp extracts as mg N/100g shrimp. Amino nitrogen values were expressed as millimoles/100g shrimp (mM/100g shrimp).

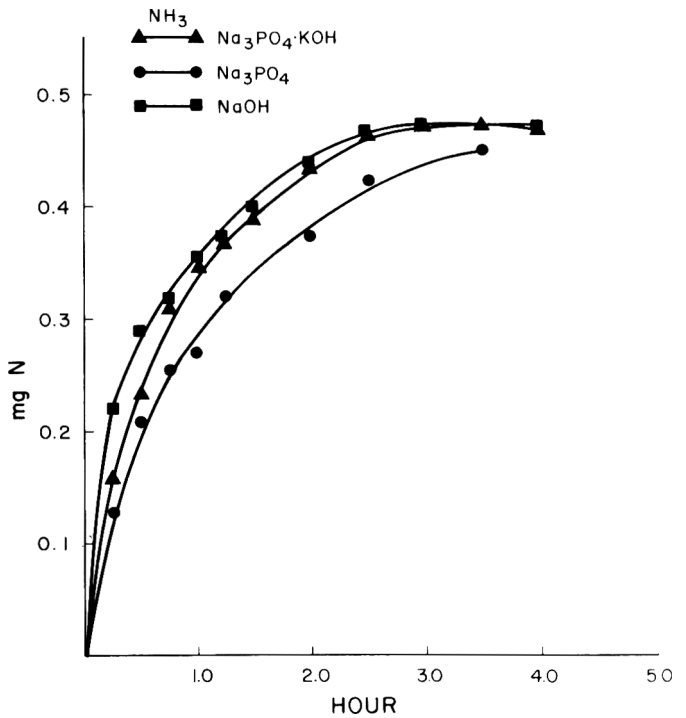

Fig. 2—Distillation of NH_3 vs. time with various releasing agents.

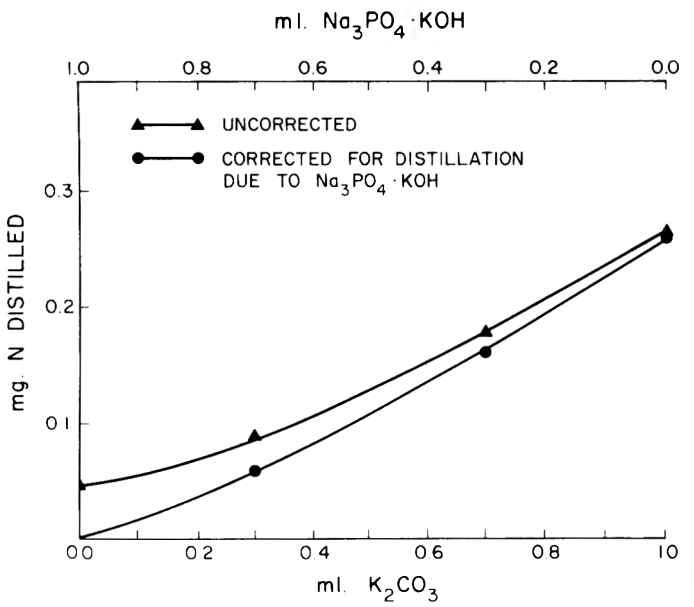

Fig. 3—The effect of different levels of K_2CO_3 on the distillation of dimethylamine in the presence of HCHO.

RESULTS & DISCUSSION

Measurement of trimethylamine (TMN), dimethylamine (DMN) and ammonia

TMN analysis by the microdiffusion procedure (Beatty and Gibbons, 1937) depends upon separation by distillation and trapping of TMN while preventing distillation of NH_3, primary amines and secondary amines by reaction with formaldehyde (HCHO). For distillation of total volatile nitrogen (TVN) or TMN, the pH of the solution is adjusted with a suitable base (releasing agent). Table 1 presents the effects of various releasing agents and HCHO on the distillation of NH_3, DMN and TMN in the microdiffusion procedure at room temperature (approximately 23°C). Distillation of DMN was evident with all of the releasing agents employed. With saturated K_2CO_3 which has generally been employed as a releasing agent in microdiffusion analysis (Shewan et al., 1971), DMN distillation was especially noticeable, averaging 67.5%. The use of saturated Na_3PO_4 solution as the releasing agent reduced the interference of DMN and NH_3 in TMN analysis. Addition of KOH to the saturated Na_3PO_4 solution (designated $Na_3PO_4 \cdot KOH$) was necessary for complete distillation of TMN. With $Na_3PO_4 \cdot KOH$ as the releasing agent, distillation of DMN was less than with NaOH as the releasing agent, and distillation of NH_3 was insignificant. With $Na_3PO_4 \cdot KOH$ as releasing agent, splattering (because of evolution of CO_2) during the neutralization process was not evident as it fre-

quently was when K_2CO_3 was used as the releasing agent.

Distillation of TMN and DMN vs. time is presented in Figure 1 and of NH_3 vs. time in Figure 2. With Na_3PO_4 · KOH as the releasing agent and HCHO omitted, TMN and DMN curves appeared to level off with slight increase after about 3 hr. It is evident from Figure 1 that HCHO retarded but did not prevent distillation of DMN. From these curves, it was concluded that about 3.5 hr distillation at 23°C would be the best time for measuring TMN in shrimp extracts. NH_3 distillation was complete after 2.5 hr when 10N NaOH and Na_3PO_4 · KOH were used as releasing agents. However, when saturated Na_3PO_4 was used as the releasing agent, NH_3 distillation was not complete at 3.5 hr. Despite the problem of incomplete distillation of TMN and NH_3, as will be subsequently demonstrated, saturated Na_3PO_4 is a useful reagent in the measurement of TVN by steam distillation. Also as will be discussed in subsequent communications, saturated Na_3PO_4 may be uniquely useful in analyzing shrimp extracts.

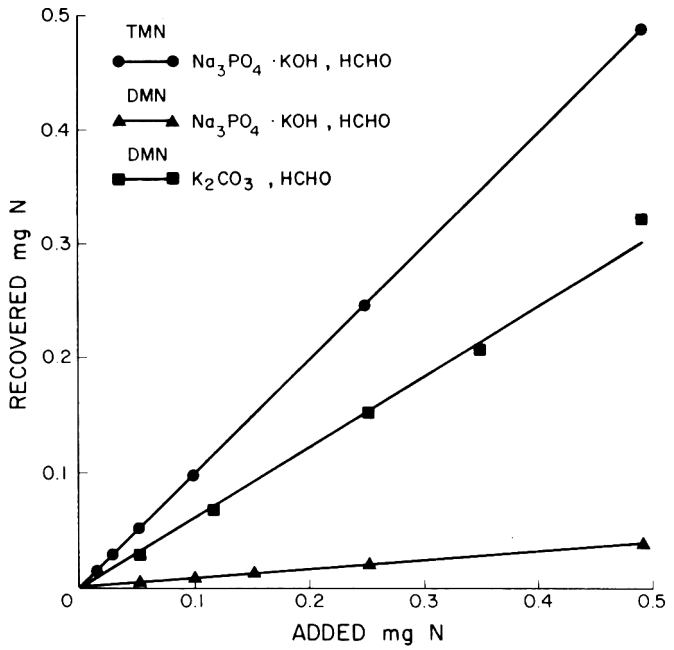

Fig. 4—Microdiffusion analysis (5 hr) of trimethylamine (TMN) and dimethylamine (DMN) vs. concentration with different releasing agents and HCHO.

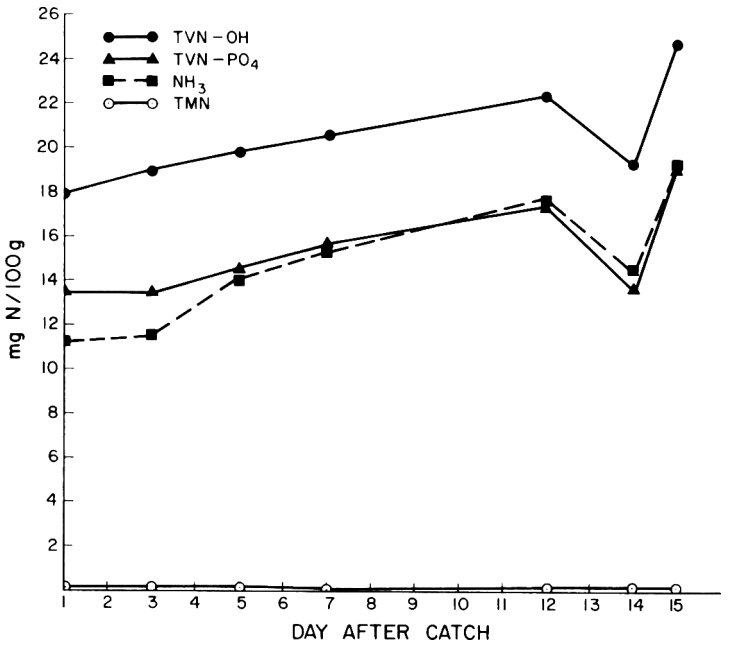

Fig. 5—Total volatile nitrogen (TVN) and NH_3 analysis of extracts from ice-stored shrimp. TVN-OH = TVN analysis with NaOH as releasing agent. TVN-PO_4 = TVN analysis with Na_3PO_4 · KOH as releasing agent.

It appeared that K_2CO_3 was interfering with the DMN-HCHO reaction. Further evidence for the interference of K_2CO_3 in the DMN-HCHO reaction was obtained by substituting varing amounts of the Na_3PO_4 · KOH releasing agent with saturated K_2CO_3 (Fig. 3). As the amount of K_2CO_3 increased, the distillation of DMN increased.

Using Na_3PO_4 · KOH as the releasing agent, TMN was measured accurately to levels as low as 0.05 mg N/ml (Fig. 4). Distillation of DMN in the presence of HCHO was linear with concentration and different with different releasing agents. This provided a method of estimating DMN content. DMN standards, after the addition of HCHO, were reacted with Na_3PO_4 · KOH and with K_2CO_3 and allowed to distill for 5 hr. Mixtures of TMN and DMN (simulating unknowns) were allowed to react in the same manner. From the distillation of the standards the constants A and B were calculated as follows:

$$A = \frac{(ml_1 - ml_2)}{ml_t}$$

$$B = \frac{ml_2}{ml_t}$$

where, ml_1 = ml to titrate distilled DMN, K_2CO_3 as the releasing agent; ml_2 = ml to titrate distilled DMN, Na_3PO_4 · KOH as the releasing agent; and ml_t = ml to titrate total amount of DMN. DMN and TMN concentrations of unknowns were established by the following calculations:

$$C_{DMN} = \frac{14.01N\ (ml_3 - ml_4)}{A}$$

where, C_{DMN} = mg nitrogen/ml; N = normality of acid; ml_3 = ml to titrate unknown with K_2CO_3 as the releasing agent; ml_4 = ml to titrate unknown with Na_3PO_4 · KOH as the releasing agent; and $C_{TMN} = 14.01N\ (ml_4 - BC_{DMN})$ (where C_{TMN} = mg nitrogen/ml). Employing this method of simultaneous analysis, recoveries of TMN and DMN from a solution containing 0.239 mg TMN/ml and 0.242 mg DMN/ml averaged 97.3 and 101.1% (6 analyses), respectively.

Table 2—The analysis of NH_3, trimethylamine (TMN) and total volatile nitrogen (TVN) in trichloroacetic acid extracts from shrimp

Sample no.	NH_3[a] mgN/100g	TMN mgN/100g	TVN – % Variation from NH_3 + TMN					Log[c] Bacteria/g
			Conway – 1.5 hr		Conway – 4 hr		Steam distill.	
			Na_3PO_4 · KOH[b]	10N NaOH	Na_3PO_4 · KOH	10N NaOH	Na_3PO_4	
1	20.4	Tr.	–13.7	3.9	4.4	6.9	6.8	7.0
2	21.8	Tr.	–19.3	–8.7	–10.1	–6.4	–1.4	5.8
3	19.3	1.3	11.7	33.0	31.5	44.1	25.2	7.5
4	27.7	1.2	–4.0	13.8	7.9	21.8	13.8	6.4
5	17.4	0	22.4	65.5	62.6	73.6	40.0	5.9
6	21.6	1.0	4.2	26.5	27.4	33.6	25.2	5.9
7[d]	13.2	1.5	10.2	35.4	37.4	48.2	31.3	–
8	11.8	0	–2.5	21.1	4.3	11.0	16.1	–
Mean			1.13	23.81	20.68	29.10	19.98	
SD			12.91	20.99	21.92	24.30	12.59	

[a]Determined from amino acid chromatograms
[b]Releasing agent
[c]Plate counts supplied by industry
[d]Samples 7 and 8 probably spoiled

The procedure of Dyer (1945) for TMN analysis utilizes reaction with HCHO in the presence of saturated K_2CO_3 to prevent extraction of NH_3, primary amines and secondary amines with toluene. Tozawa et al., (1971) have suggested that saturated K_2CO_3 be replaced with 25% KOH to prevent DMN interference. Since the reaction to prevent diffusion or extraction of DMN is the same, results of this study suggest that Na_3PO_4 · KOH should be investigated for use in Dyer's procedure.

Total volatile nitrogen (TVN) analysis in shrimp extracts

TVN analysis in shrimp extracts is usually a measure of NH_3 and to a lesser extent volatile amines. From the NH_3 distillation curves (Fig. 2), it appeared that at least 2.5 hr should elapse before the chambers were opened. However, in practice this was not the case. In Table 2 are presented results of the analysis of eight extracts from commercial shrimp samples. When Na_3PO_4 · KOH was used as the releasing agent and samples were titrated after 1.5 hr distillation, TVN (TVN-PO_4) values were similar to combined NH_3-TMN values. With other releasing agents and with longer times, TVN values differed greatly from combined NH_3-TMN values. Steam distillation as described by Gagnon and Fellers (1958a) with NaOH or K_2CO_3 as releasing agents continued to produce basic material, even when 50 ml or more of distillate had been collected from a 5-ml sample. Steam distillation employing saturated Na_3PO_4 as the releasing agent gave values similar to the 1.5-hr TVN-OH values. Excessive foaming, which was not satisfactorily controlled by antifoaming agents, prevented the employment of Na_3PO_4 · KOH as a releasing agent during steam distillation.

In Figure 5 is presented the analysis of extracts taken from shrimp during a 15-day storage period. During the initial period, TVN-PO_4 values were slightly higher than NH_3 values, but corresponded very well after the 3 day sample. TVN (TVN-OH) values with NaOH as releasing agent paralleled TVN-PO_4 values but were considerably higher. It appeared that at basic pH some nitrogenous component was being degraded to produce volatile nitrogen or else amines other than TMN were present. Distillation experiments, particularly those employing NaOH as a releasing agent, also tend to support the hypothesis that some nitrogenous component was being hydrolyzed by NaOH to yield ammonia or volatile amines. Gagnon and Fellers (1958a, b) employing NaOH for steam distillation of volatile base (TVN) from breaded shrimp found NH_3 contents of approximately 50 times those which can be calculated from the data in this communication. Since high levels of TVN from the breading were highly unlikely, hydrolysis of nitrogenous components was probable. Production of TVN during distillation of volatile nitrogen in beef and salmon muscle has been reported by Pearson and Muslemuddin (1968).

In an effort to establish the identity of the additional volatile nitrogen obtained by the use of NaOH as releasing agent, shrimp extracts were treated in the following manner. The shrimp extract was analyzed by the Conway procedure (Na_3PO_4 · KOH as releasing agent) and with the amino acid analyzer. 1 ml of extract was placed in a 10-ml sample bottle and 1 ml of 7N NaOH was added. Then the bottle was sealed and incubated for 4 hr at room temperature. The bottle was cooled to 0°C to prevent escape of volatile nitrogen and 8 ml of N HCl were added. An aliquot of the treated sample was analyzed with the amino acid analyzer. For example, an extract from fresh shrimp gave the following analyses: TVN (1.5 hr Conway, Na_3PO_4 · KOH as releasing agent), 19.76 mg N/100g; TVN (4 hr Conway, Na_3PO_4 · KOH as releasing agent), 29.80 mg N/100g; NH_3 before treatment, 14.71 mg N/100g; and NH_3 after treatment, 34.05 mg N/100g. This indicated that the additional volatile nitrogen was NH_3. Confirmation was obtained by running 4 hr Conway analyses, then quantitatively removing the trapping agent and analyzing it for TVN, DMN and TMN. Tests for both DMN and TMN were negative while TVN was 28.60 mg N/100g, proving that the additional TVN was NH_3.

Both TMN and TVN (Montgomery et al., 1970) have been used to assess spoilage in shrimp. The TVN limit for acceptable shrimp has been set as 30 mg N in Australia and Japan. A limit of 5 mg TMN has also been set. The results of the present studies suggest that standardization of analytical techniques is needed. The techniques employed in this study (1) reduce interference from DMN and NH_3 in TMN analysis and (2) provide method of TVN analysis of shrimp extracts which is reflective of the NH_3-TMN content. A rapid method of distillation which will give TVN values similar to those observed for the Conway procedure is also presented.

Because much of the world's shrimp catch is stored in ice prior to being processed, TVN and TMN contents may not always be good indicators of shrimp quality. Much of the volatile nitrogen may be

Table 3–The analysis of amino nitrogen (AA-N) and total volatile nitrogen (TVN) in trichloroacetic acid extracts of shrimp

Sample	Quality[a]	Amino nitrogen (AA-N) mM/100g		TVN[c] mg/100g	TVN/AA-N mg/mM[d]
		Analyzer[a]	Copper[b]		
1[e]	fresh	16.53	20.61	14.35	0.69
2	fresh	23.27	24.62	7.57	0.31
3	good-(14 day)	21.88	23.12	28.00[f]	1.21
4	good-(14 day)	17.27	21.87	23.60[f]	1.08
5	poor	12.09	13.25	16.80	1.22
6	poor	19.85	17.80	25.20	1.42
7	poor	23.06	18.58	22.96	1.38
8	poor	8.82	9.95	18.76	1.88
9	spoiled	11.86	13.33	21.40	1.61
10	good		16.30	21.20	1.30
11	good		14.56	21.20	1.45
12	good		17.18	35.79	2.08
13	poor		23.30	40.23	1.73
14[g]	poor		3.06	5.48	1.79
15[g]	spoiled		1.46	12.42	8.51
16	spoiled		11.06	32.50	2.94

[a]Amino acid analyzer–value includes α-amino nitrogen, proline nitrogen and NH_3

[b]Copper procedure

[c]Total volatile nitrogen measured by 1.5 hr Conway procedure with Na_3PO_4 · KOH as releasing agent (samples 1–9) and by Kjeldahl distillation with saturated Na_3PO_4 as releasing agent (samples 10–16).

[d]Copper procedure used to obtain AA-N values

[e]Samples 1–10 were from the Gulf of Mexico, sample 12 was from the Carribean Sea and remainder were from the Indian Ocean.

[f]NH_3

[g]Peeled-deveined shrimp

lost through the washing action of ice or drip (Iyengar et al., 1960). One source of NH_3 is from free amino acids (Cobb and Vanderzant, 1971) which have generally been shown to decrease in iced shrimp as the quality decreases (Fieger and Frilous, 1954; Velankar and Govindan, 1958). Consequently, adjustment of TVN values on the basis of amino acid content might improve the reliability of TVN data.

Amino nitrogen (AA-N) determination

The copper procedure of Pope and Stevens (1939) employed by Campbell and Williams (1952) and Fieger and Frilous (1954) for AA-N measurement in shrimp extracts is too laborious for routine use. Spies and Chambers (1951) have adapted the copper procedure to optical methods. During this study the latter procedure was simplified for use with TCA extracts. AA-N values for fresh shrimp obtained by the copper procedure were frequently higher than those obtained by use of the amino acid analyzer (Table 3). AA-N values by the copper procedure for poor quality or spoiled shrimp were frequently less than analyzer values. These apparent differences were probably due to the difficulty of quantifying some of the ninhydrin positive material on the chromatograms. Also the copper procedure does not measure NH_3.

In Table 3 are listed the AA-N, TVN levels and AA-N/TVN ratios for 16 samples of shrimp. TVN analyses for samples 1–9 were by the 1.5 hr microdiffusion procedure and for samples 10–16 by the distillation procedure. The latter values, as previously demonstrated (Table 2), are approximately 20% higher than if they had been obtained by the 1.5 hr microdiffusion procedure.

The shrimp used for the analyses in Table 3 came from the Gulf of Mexico, Carribean Sea and Indian Ocean. Both intact and peeled-deveined tails were analyzed. TVN and AA-N analyses were variable and appeared to have little relation to quality. Samples 12, 13, and 16 had TVN levels above 30 mg N/100g. Sample 12, which was from a freezer boat operating in the Carribean area, was of good quality with a bacterial count of 88,000/g while the acceptability of sample 13 was questionable and sample 16 was spoiled. Five additional samples from the Carribean area and of good quality also had TVN levels greater than 30 mg N/100g. TVN/AA-N ratios appeared to be more reflective of the quality of the shrimp than either TVN or AA-N alone. Because of the production of NH_3 during distillation, the method of TVN analysis affected the magnitude of the ratio (Table 2). From the samples in Table 3 and numerous other analyses, it appears that TVN/AA-N ratios (1.5 hr Conway analysis) of fresh shrimp from the Gulf of Mexico are < 1.0 mg N/mM; ratios of poor quality shrimp of questionable acceptability are > 1.2 mg N/mM; and shrimp with ratios > 1.5 mg N/mM are usually spoiled.

A number of factors can affect the levels of TVN and AA-N in shrimp. The effects of extensive washing are evident in the analyses of the peeled-deveined samples 14 and 15 in Table 3. TVN and AA-N are low, but the ratio still appears to be applicable. From the recalculation of previous data obtained with sterile shrimp extracts (Cobb and Vanderzant, 1971) the TVN/AA-N ratio increases at the rate of approximately 0.032 mg N/mM/day as the result of NH_3 production due to endogenous enzymic activity. The same study indicated that TVN production is also a function of the type and numbers of bacteria on the shrimp.

Amino nitrogen determination has previously been a slow or unwieldy procedure. The modification of the procedure of Spies and Chambers (1951), described in this communication allows rapid AA-N determination. Analysis of TVN using the Conway procedure or steam distillation with Na_3PO_4 as releasing agent and of AA-N using the copper procedure are simple, and relatively inexpensive to perform. Future communications will show the value of these determinations in relation to bacterial indicators as they pertain to quality of shrimp.

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Ms received 10/21/72; revised 12/21/72; accepted 12/28/72.

Journal Paper No. 10085 of the Texas Agricultural Experiment Station.

This project was partially funded by the National Oceanic and Atmospheric Administration's Office of Sea Grants, Dept. of Commerce, through institutional grant 2-35213 to Texas A&M University.

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SENSITIVITY OF Vibrio parahaemolyticus TO COLD IN OYSTERS, FISH FILLETS AND CRABMEAT

INTRODUCTION

THERE IS increasing interest in *Vibrio parahaemolyticus* among microbiologists working in the field of food technology and public health. The organism, which until comparatively recently was recognized as a cause of human food poisoning only in Japan (Sakazaki, 1965), has now been incriminated in food poisoning outbreaks in other countries (Battey et al., 1970; Barker et al., 1970; Anon., 1971; Sakazaki, 1971) and has been implicated in such diverse pathological situations as cholera-like gastroenteritis (Sakazaki, 1971), human wound infections (Twedt et al., 1969; Roland, 1970), and diseases of marine invertebrates (Krantz et al, 1969; Vanderzant and Nickelson, 1970). In its best known pathological role as a food-borne pathogen, *V. parahaemolyticus* has characteristically caused food poisoning in individuals consuming uncooked or lightly cooked seafoods during warm periods of the year. This points up two characteristics which are peculiar to this organism and which have been cited (Sakazaki, 1971) as reasons for the apparent low incidence of *V. parahaemolyticus* food poisoning in North America and Western Europe. These are halophilism and sensitivity to both heat and cold.

The organism requires some salt for growth and survival, but this is an osmotic requirement principally and can be satisfied by other systems, so that *V. parahaemolyticus* will grow on blood agar and certain other low-salt media. The organism is very sensitive to heat, being killed at 60°C for 5–15 min (Temmyo, 1966; Vanderzant and Nickelson, 1972). It has also been reported to be sensitive to cold, failing to grow, and, indeed, dying out at temperatures below 6–8°C. Freezing has been reported to be lethal to the organism (Sakazaki, 1966; Liston et al., 1971; Matches et al., 1971; Vanderzant and Nickelson, 1972; Covert and Woodburn, 1972). However the isolation of *V. parahaemolyticus* has been reported from refrigerated and frozen market food samples (Fishbein et al., 1970; Vanderzant and Nickelson, 1970; Thomson and Trenholm, 1971) and this suggests that the organism may survive freezing when present naturally in sea food materials The investigation discussed here was carried out to try to elucidate this point. Since *V. parahaemolyticus* has been isolated most frequently from oysters in the Pacific Northwest (Baross and Liston, 1970), and these animals are frequently eaten raw or only lightly cooked, most work was done with this food. However, some experiments were conducted using crabmeat and fish fillets. In conjunction with these experiments, a media comparison was conducted but will be the subject of a separate report.

MATERIALS & METHODS

Organisms

The organisms used in these studies were obtained from J. Baross and included *V. parahaemolyticus* ATCC 17802 and strain 6-421 (isolated by J. Baross from oysters as 6-1BOH6 -421) and *V. alginolyticus* strain V-374 (originally isolated by H. Zen-Yoji). Additionally, streptomycin-resistant mutants (*V. parahaemolyticus* strains 17802SM and K4SM3 and *V. alginolyticus* strain V-374SM) were used in order that a comparison could be made of the recovery efficiency of various selective media with "nonselective" medium containing streptomycin to inhibt the growth of contaminating organisms. The results of the media comparison experiment will be reported elsewhere, but certain of the data which relate to the cold sensitivity of *V. parahaemolyticus* are reported here.

Preparation of bacterial inocula

The organisms were grown in trypticase soy broth (Baltimore Biological Lab–BBL) with 2.5% added NaCl, adjusted to pH 7.5 (TSBS) at room temperature (21–22°C) for 18–20 hr as a shake culture. Dilutions were made with salt peptone water containing 0.1% Bacto-peptone, and 3% NaCl adjusted to pH 7.5. Streptomycin-resistant mutants were grown in TSBS to which 500 μg streptomycin per ml was aseptically added after sterilization.

Food materials

Live Pacific oysters (*Crassostrea gigas*) obtained from a commercial shucking plant at Purdy, Wash., were used in all experiments. They were shucked aseptically (APHA, 1970) and packaged in Polymylar pouches. Fish fillets of English sole (*Parophrys vetulos*) and canned picked crabmeat (*Cancer magister*) were obtained from local fish processing plants and used in a fresh condition.

Experimental procedure

Oysters were contaminated either by allowing thoroughly depurated oysters to feed in filtered aged seawater containing approximately 10^6 organisms per ml or by injecting an appropriate dilution of the organisms into the region of the digestive tract of freshly shucked oysters, using a syringe and needle, to obtain approximately 10^6 organisms per gram. Fish fillets and crabmeat were contaminated within Polymylar pouches by pipetting 1 ml of an appropriate dilution of organisms onto the surface of the materials, then gently massaging the outside of the pouch to approach even distribution of the inoculum. In various experiments the inoculated oysters were refrigerated or frozen at 11°, 8°, 5°, 1°, −15° and −30°C and subsequently stored for varying periods of time at the same respective temperature. Fish and crabmeat were treated in a similar manner, except that only 1°, −15° and −30°C were utilized. Samples were removed from storage at intervals, thawed at room temperature and the content of *V. parahaemolyticus* or *V. alginolyticus* determined by a surface plate count on at least three of the following four media:

Salt-starch agar (VPS) – a modification suggested by J. Baross of the seawater starch medium (Baross and Liston, 1970) as follows: 0.7% Bacto-peptone, 0.3% yeast extract, 0.5% soluble starch, 0.05% KH_2PO_4, 5.0% NaCl, 1.5% agar, made with distilled water and adjusted to give a final pH of 7.8. After autoclaving, the medium was cooled in a water bath to 45–50°C, and penicillin was added to give a concentration of 6 units penicillin per ml.

Brom Thymol Blue Teepol Agar (BTBT) (Eiken, 1969).

Thiosulfate Citrate Bile Sucrose Agar (TCBS) (Eiken, 1969).

Trypticase Soy Agar (BBL) with 2.5% added NaCl, adjusted to give a final pH 0f 7.8. After autoclaving, the medium was cooled in a water bath to 45–50°C, and streptomycin was added to give a concentration of 500 μg streptomycin per ml (TSASS).

Duplicate plates for each appropriate dilution were incubated at 37°C and/or 43°C for 18–20 hr, and counts of *V. parahaemolyticus* obtained on plates containing 30–300 colonies. Counts were confirmed after an additional 18–20 hr incubation at the appropriate temperature to allow for growth of "sub-lethally" damaged cells that might not have appeared after the original 18–20 hr of incubation. In instances where less than 10 organisms per g were expected, the counts obtained on 10 plates were added together. BTBT, TCBS and TSASS were incubated aerobically and VPS was incubated anaerobically in Gas-Pack jars (BBL). Only those colonies which conformed to the characteristics of *V. parahaemolyticus* (or *V.*

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alginolyticus) on the appropriate medium (BTBT, TCBS, or VPS, given in the above references) were counted. No *V. parahaemolyticus* or *V. alginolyticus* could be detected in the uninoculated test samples. Under the conditions of this experiment, no growth was observed on TSASS when plated with uninoculated test samples; therefore, all colonies which appeared on TSASS plates were considered to be the inoculated streptomycin mutant. Representative colonies from BTBT, TCBS, VPS and TSASS were periodically tested biochemically for growth characteristics in 1% trypticase broth with 0, 3, 7 and 10% NaCl, Voges-Proskauer reaction, and sucrose fermentation (Baross and Liston, 1970), and cultures were observed under a phase microscope for motility and Gram-stained. All representative colonies so tested conformed to the accepted characteristics (Sakazaki, 1966). The same procedure was followed for *V. alginolyticus*, except that counts made on VPS and TSASS incubated at 37°C were read after 15–16 hr only, due to the swarming phenomenon exhibited by *V. alginolyticus* on these two media when incubated at 37° (but not at 43°C).

Linear regression analyses and combined regression and analysis of co-variance were conducted using the CDC 6400 computer at the University of Washington Computer Center.

RESULTS

THE SURVIVAL of *V. parahaemolyticus* strains ATCC 17802 and 6-421 and *V. alginolyticus* strain V-374, injected via needle and syringe into shucked oysters which were then treated at 1°, −15° and −30°C is shown in Figures 1, 2 and 3, respectively. The initial counts were 6.9 × 10^6, 1.5 × 10^6 and 6.8 × 10^6 organisms per gram, respectively. These graphs were derived as follows: the first section, representing the mortality associated with the chilling/freezing process itself, connects the pre-treatment count with the calculated origin of the second section which is the line of best fit by least squares for all storage data at a particular temperature. After an initial reduction of approximately one to two logs, there is a linear (log) reduction in the number of surviving organisms with time. The slope of the line representing storage mortality is steeper for 1°C than for −15°C, which is steeper than for −30°C. These data thus suggest a two-stage mortality with an initial cold shock mortality due to the chilling/freezing of the samples to the respective holding temperatures, followed by a mortality related to the storage conditions themselves.

Table 1 shows the results of duplicate experiments using *V. parahaemolyticus* 17802, designed to explore the pattern of mortality in relation to the freezing process itself. While the data do confirm an approximate one log sharp mortality associated with the overall freezing process with little difference between −15° and −30°C, they indicate that the killing effect is most obvious after the end of the period of arrest (i.e., during the period of falling temperature in the frozen state).

To simulate more realistically natural conditions than were obtained by contamination via needle and syringe injection and to provide information over a wider range of temperatures, oysters were allowed to become naturally contaminated with the streptomycin-resistant mutant *V. parahaemolyticus* 17802SM, then shucked aseptically, packaged and treated at 11°, 8°, 5°, 1°, −15° and −30°C. The survival of the organism under these conditions is illustrated by the data in Table 2. The organisms tended to die out slowly, even in oysters held at 11°, 8° and 5°C, and the general two-stage pattern of decline at 1°, −15° and −30°C was similar to that obtained using ATCC 17802, 6-421 and V-374 injected into shucked oysters.

The survival of the two streptomycin-resistant mutant strains of *V. parahaemolyticus* in fish fillets at 1°, −15° and −30°C is illustrated by the data in Table 3. The same general pattern of an initial sharp mortality, followed by a storage mortality, was shown using fish fillets. An apparent strain difference was observed, in that K4SM3 appeared to survive longer at −15°C, but further investigation would be required to establish this point.

Results of similar experiments using

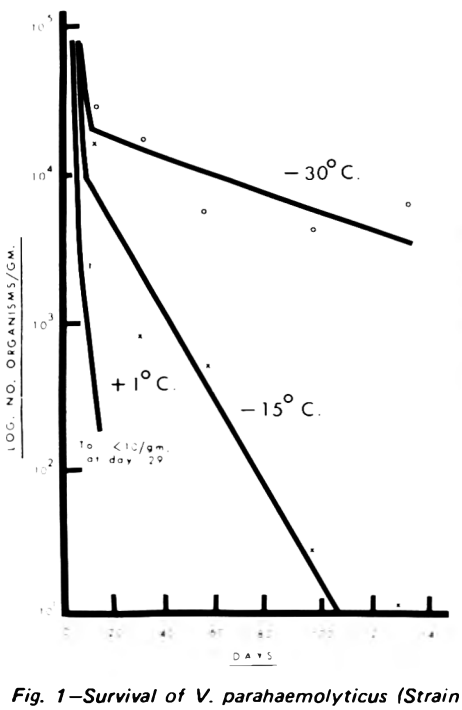

Fig. 1—Survival of V. parahaemolyticus (Strain ATCC 17802) in oysters exposed to +1°, −15° and −30°C. (Initial count was 6.9 × 10^6 organisms/g. Data points represent the mean of count averages obtained on BTBT and VPS at 37° and 43°C.)

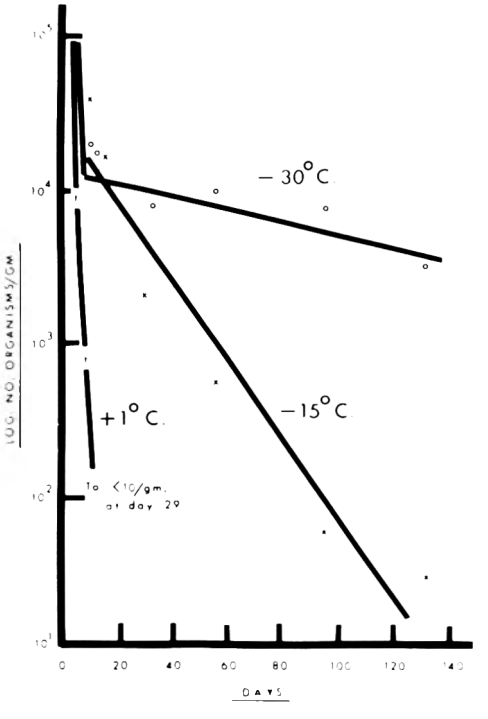

Fig. 2—Survival of V. parahaemolyticus (Strain 6-1BOH6-421) in oysters exposed to +1°, −15° and −30°C. (Initial count was 1.5 × 10^6 organisms/g. Data points represent the mean of count averages obtained on BTBT and VPS at 37° and 43°C.)

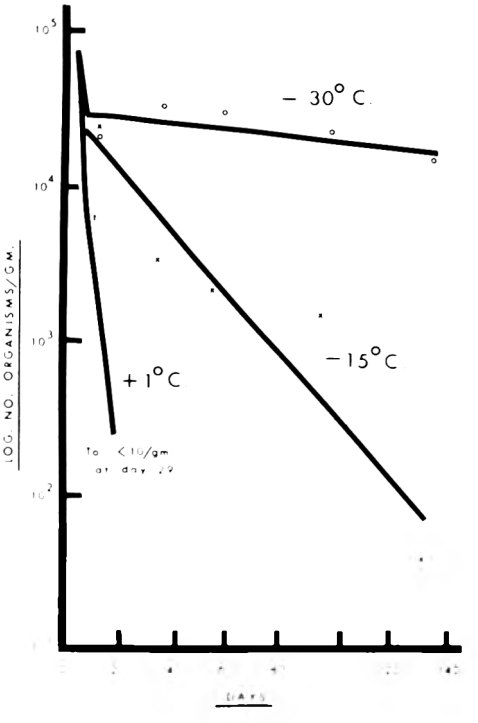

Fig. 3—Survival of V. alginolyticus (Strain V-374) in oysters exposed to +1°, −15° and −30°C. (Initial count was 6.8 × 10^6 organisms/g. Data points represent the mean of count averages obtained on BTBT and VPS at 37° and 43°C.)

crabmeat are shown in Table 4. The pattern of death observed in crabmeat appears to be different from that observed in oysters and fish, in that death at 1°C and −15°C was essentially linear with little evidence of the two-stage mortality seen in oysters and fish fillets. The two strains of *V. parahaemolyticus* did show a marked two-stage mortality pattern at −30°C, although the extent of the first-stage and second-stage death varied; the *V. alginolyticus* strain yielded an indication of two-stage mortality at −30°C. Crabmeat appeared to offer less protection against chilling and freezing lethality to vibrios than either fish or oyster tissue, but further invesigations would be required to elucidate this point.

DISCUSSION

THE SHARP REDUCTION of counts observed in the first 24 hr of the chilling and freezing experiments in this study was previously reported by Asakawa (1967) for the vibrios in tuna meat frozen at −10°C and −15°C. The same kind of effect was noted by DiGirolamo et al. (1970) for salmonellae in Pacific oysters frozen at −18°, −23° and −34°C. More recently, Vanderzant and Nickelson (1972) reported a sharp decrease in viable cells during the first 2 days of storage at 3° and −18°C in whole shrimp and in shrimp homogenate, and Covert and Woodburn (1972) noted similar results in fish homogenate frozen at −5 ± 1° and −18 ± 1°C.

The rate of freezing is known to affect lethality of the freezing process for bacteria. Thus Mazur (1966) has noted that a fast constant rate of heat removal, i.e., 75–100°C decrease per minute, is less lethal than a slower constant rate of 0.75–1°C per minute. However, these refer to constant rates of temperature decrease for the period between achievement of freezing point and achievement of ambient temperature. The freezing rates were not constant in the experiments reported here because the size of the samples used was so small that there was, in fact, little difference in the freezing rate in each case. This would explain why in most cases the extent of the initial (24 hr) mortality was the same regardless of ambient temperature.

It is perhaps not surprising that storage lethality should be related inversely to the temperature of storage. This type of result was reported by Asakawa (1967) for the tuna meat held at 0° and −10°C. However, Matches et al. (1971) have reported that in fish homogenate the lethal rate during storage was highest at −34°C and lowest at 0.6°C. It has been suggested (Raj and Liston, 1961) that protection against the lethal effects of freezing might be related to the influence of animal protein on rates of water crystallization due to the protein's water binding ability. This effect would be reduced in cases where the protein is denatured. Matches et al. (1971) used an autoclaved fish homogenate in their studies and this might explain the difference between their findings and those of Asakawa (1967) and the present investigation. It is interesting to note that Vanderzant and Nickelson (1972) observed no significant difference in the behavior of *V. parahaemolyticus* in refrigerated whole shrimp and shrimp homogenate which was not autoclaved. The reduction of a protein's water-binding ability due to heat denaturation might also provide an explanation for the apparent different results obtained with crab as compared to fish and oysters. Picked crabmeat is taken from heat-processed crabs and is, moreover exposed to high salt concentrations in brine flotation procedures. The protein of crabmeat might be expected to be quite thoroughly denatured, thus providing little or no protection.

While the results of these experiments have been presented primarily in terms of variations in lethality with temperature, the more important practical observation may be that *V. parahaemolyticus* and *V. alginolyticus* survive freezing in seafoods at least for some time. Thus, viable organisms were obtained from oysters at 130 days at −15° and −30°C (the end of the testing period) and up to approximately 40 days at 1°C. In fish fillets they were detectable after approximately 60 days at −15° and −30°C and after 15 days at 1°C. In crabmeat they could be recovered at least up to 30 days at 1°, −15° and −30°C. These experiments with oysters, fish fillets and crabmeat, coupled with

Table 1—Survival of V. parahaemolyticus (Strain ATCC 17802) in injected oysters sampled at selected stages of freezing at −15° and −30° C

Freezing time (hr)	−15°C[a]		−30°C[a]		Stage of freezing
0	290.0[b]	190.0	290.0	190.0	Pre-freeze
2	300.0	200.0	280.0	250.0	Start of period of arrest
4	–[c]	–	260.0	230.0	End of −30°C period of arrest
7	365.0	100.0	–	–	End of −15°C period of arrest
24	33.0	15.0	26.0	30.0	End of freezing process
72	11.0	9.0	29.0	25.0	Post freezing process

[a]Duplicate experiments
[b]Counts given in thousand organisms/g and represent the mean of two count averages obtained on BTBT at 37°C and VPS at 43°C
[c]Not done

Table 2—Survival of V. parahaemolyticus (Strain 17802SM) in refrigerated and frozen oysters[a]

Days of storage	Temperature of storage +11°C	+8°C	+5°C	+1°C[d]		−15°C[d]		−30°C[d]	
0	58.0[b]	58.0	58.0	58.0	320.0	58.0	320.0	58.0	320.0
1	–[c]	–	–	–	190.0	–	54.0	–	57.0
2.5	9.7	6.9	5.3	6.8	–	16.0	–	8.7	–
8	10.0	4.2	4.4	1.6	19.0	3.5	21.0	9.5	–
14	–	0.18	1.6	–	16.0	–	9.1	–	49.0
	+11°, +8° and +5°C Completed								
17				2.2	–	2.0	–	2.8	–
21				0.39	–	2.2	–	2.4	–
24				–	5.6	–	13.0	–	85.0
27				0.05	–	1.3	–	6.7	–
30				–	0.03	–	19.0	–	75.0
42				0.002	0.005	0.45	2.7	4.1	28.0
59				Completed		0.73	–	5.1	–
66						–	0.75	–	29.0
82						–	1.7	–	13.0

[a]Depurated oysters contaminated by natural uptake
[b]Counts given in thousand organisms/g as obtained on TSASS at 37°C
[c]Not done
[d]Duplicate experiments

Table 3—Survival of V. parahaemolyticus (Strains 17802SM and K4SM3) on refrigerated and frozen fish fillets[a]

Days of storage	Temperature of storage: +1°C				−15°C				−30°C			
	17802SM		K4SM3		17802SM		K4SM3		17802SM		K4SM3	
0	210.0	130.0	140.0	87.0	210.0	130.0	140.0	87.0	210.0	130.0	140.0	87.0
2	17.0	1.9	42.0	7.0	1.5	5.8	47.0	11.0	60.0	21.0	17.0	6.1
10	1.5	<0.01	0.3	<0.01	–[b]	–	–	–	–	–	–	–
12	–	–	–	–	0.83	0.37	10.0	2.1	24.0	12.0	13.0	5.5
19	<0.01	–	<0.01	–	–	0.01	15.0	0.51	–	5.7	–	–
	+1°C Storage completed											
21					1.6	–	–	–	10.0	–	9.4	1.4
25					0.59	0.02	2.3	0.47	23.0	0.47	13.0	0.85
31					0.54	<0.01	4.1	0.10	13.0	0.86	10.0	1.30
47					0.04	–	0.29	0.01	12.0	0.58	3.8	0.51
63					<0.01	–	0.21	–	3.6	–	2.4	0.21

[a]Counts for duplicate experiments for each organism at each temperature of storage are given in thousand organisms/g as obtained on TSASS at 37°C.
[b]Not done

Table 4—Survival of V. parahaemolyticus (Strains 17802SM and K4SM3) and V. alginolyticus (Strain V-374SM) on refrigerated and frozen picked crabmeat

Days of storage	Temperature of storage: +1°C			−15°C			−30°C		
	17802SM	K4SM3	V-374SM	17802SM	K4SM3	V-374SM	17802SM	K4SM3	V-374SM
0	27.00.0[a]	280.0	31000.0	2700.0	280.0	31000.0	2700.0	280.0	31000.0
1.5	1100.0	42.0	11000.0	260.0	160.0	12000.0	12.0	160.0	35.0
4	120.0	49.0	200.0	11.0	42.0	170.0	–[b]	130.0	19.0
9.5	4.6	24.0	3.9	0.2	30.0	0.25	–	69.0	16.0
21	0.05	0.43	0.03	<0.01	3.7	0.03	1.2	58.0	1.2
28	<0.01	0.01	<0.01	–	0.14	<0.01	0.72	35.0	<0.01
	+1°C Storage completed								
35				–	0.21	–	2.0	32.0	–
41				–	–	–	–	12.0	–

[a]Counts given in thousand organisms/g as obtained on TSASS at 37°C. Plates with V. alginolyticus were counted after 13–15 hr, prior to swarming colonies becoming confluent.
[b]Not done

the results reported by Vanderzant and Nickelson (1972) for shrimp, could explain the recovery of *V. parahaemolyticus* from frozen seafoods. If the initial level of contamination of the raw product is high enough to permit survival of more than a few hundred vibrio cells per gram, then the relatively low lethal rate at freezer storage temperatues would ensure that viable cells would persist for a month or more – much more in the case of oysters.

In view of the reported heat sensitivity of *V. parahaemolyticus*, the recovery of the organism from market samples of crab and precooked shrimp suggests contamination of these products after processing (Anon., 1971) though on a practical basis the results obtained in this study would suggest that such contamination would persist for a shorter period on precooked products than on raw products. Monitoring programs should be instituted for high-risk seafoods and also for shellfish and shellfish growing areas during warm periods of the year. In this regard, the similarity between the cold sensitivity of *V. parahaemolyticus* and *V. alginolyticus* may be particularly useful, since there appears to be a relationship between the incidence of these two biotypes, at least in oysters (Baross and Liston, unpublished) such that *V. alginolyticus* might be used as an indicator organism for *V. parahaemolyticus*. Of course, this would require that tolerance levels be set for *V. alginolyticus* which would relate directly to the *V. parahaemolyticus* hazard. The results of this study indicate that chilling, freezing, or frozen storage temperatures per se cannot be relied upon to successfully eliminate the organism from seafoods or prevent the health hazard that is presented by *V. parahaemolyticus*.

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Ms received 10/5/72; revised 12/23/72; accepted 12/28/72.

Contribution No. 373, College of Fisheries, University of Washington. This project was supported, in part, by the Washington Sea Grant Program under Grant No. 1-35320 from the National Oceanic and Atmospheric Administration.

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VITAMIN E CONTENT OF SELECTED BABY FOODS

INTRODUCTION

AMERICAN BABIES in the first year of life receive most of their nutrients from synthetic formulas or mother's milk, precooked cereals and canned strained foods. Baby food sales amounted to $344,700,000 in 1970 (Anonymous, 1971). Now that vitamin E has been added to the list of recommended dietary allowances by the National Research Council (1968), it is important to know how much of this vitamin is supplied by the various baby foods. The RDA for infants to 1 yr of age is 5 IU, with a ratio of 0.6 mg alpha-tocopherol/g polyunsaturated fatty acid (E/PUFA) being desired.

At present the information on baby foods is limited to the vitamin E content of milk, milk products, simulated milks for infant nutrition (Herting and Drury, 1969a), and infant formulas and cereals (Dicks-Bushnell and Davis, 1967). These references suggest that the amount of vitamin E present in the products evaluated would not be adequate, either in terms of the total amount of vitamin E present, or the low E/PUFA ratio. Various references in the bulletin compiled from the literature in 1965 by Dicks included values for human and cow's milk and some simulated formulas. The review by Dicks (1965) also contained information on foods for the general population. Bunnel et al. (1965) presented data on an extensive list of fresh, frozen and canned foods. Slover et al. (1969a) assayed wheat and wheat products, and Herting and Drury (1969b) reported on the alpha-tocopherol content of cereal grains and processed cereals. The reports by Dicks-Bushnell and Davis (1967), Slover et al. (1969a), and Herting and Drury (1969b) indicated that processing of cereals usually resulted in extensive loss of vitamin E.

No information is currently readily available on the vitamin E content of the strained fruits, meats, vegetables, cereals, breakfasts and mixed dinners obtained in the grocery stores for infant feeding.

This study was undertaken to determine the values of vitamin E in baby foods, and to compare these values for baby foods with values for similar fresh, frozen, or canned foods for the general population which have been assayed by other investigators.

The dry matter content and total lipid content of these foods were not determined; however, the approximate values as listed in other literature sources are included.

For a complete review of the importance of vitamin E in infant diets and an evaluation of vitamin E in a standard baby soft diet for infants to 1 yr of age, see Davis (1972).

EXPERIMENTAL

BABY FOODS of one national commercial brand and selected products of a second brand were obtained from two local supermarkets during the years 1969-1971. They were pur-

Table 1—Vitamin E content of strained fruits and desserts

Product, Brand G	% Moisture[a]	% Fat[a]	Tocopherols: Alpha Mean[b] mg/100g	Alpha Range mg/100g	Alpha IU/100g[c]	Beta-plus-gamma Mean mg/100g	Beta-plus-gamma Range mg/100g	Delta Mean mg/100g	Delta Range mg/100g	Total Mean mg/100g
Applesauce (2)[d]	80.8	0.2	0.52 ± 0.22	0.10–1.15	0.77	t[e]	t	t	t	0.52
Apricots (2)	76.7	0.1	0.70 ± 0.23	0.36–1.08	1.04	0.13 ± 0.03	0–0.17	nd[f]	0	0.83
Bananas (2)	77.5	0.2	0.23 ± 0.06	0.16–0.35	0.34	nd	0	nd	0	0.23
Chocolate custard (2) pudding	76.5	1.8	0.23 ± 0.05	0.13–0.33	0.34	nd	0	nd	0	0.23
Cottage cheese (2) and pineapple			0.17 ± 0.03	0.14–0.24	0.25	nd	0	nd	0	0.17
Fruit dessert (2)	77.6	0.3	0.31 ± 0.04	0.27–0.39	0.46	nd	0	nd	0	0.31
Orange pudding (2)	77.6	0.3	0.19 ± 0.03	0.16–0.25	0.28	nd	0	nd	0	0.19
Pears (2)	82.2	0.1	0.55 ± 0.21	0.35–1.18	0.82	0.07 ± 0.01	0.05–0.10	0.07 ± 0.02	0.00–0.08	0.69
Peaches (2)	78.1	0.2	1.33 ± 0.37	0.44–2.05	1.98	0.07 ± 0.03	0.04–0.15	nd	0	1.40
Plums (2)	74.8	0.2	0.38 ± 0.12	0.12–0.52	0.57	0.05 ± 0.03	0.00–0.06	0.20 ± 0.14	0.00–0.40	0.63
Prunes (2)	76.7	0.2	0.38 ± 0.14	0.20–0.63	0.57	0.07 ± 0.04	0.00–0.16	nd	0	0.45

[a]Moisture and fat values taken from Bowes and Church (1963); Handbook 8 (1963); or Heinz (1970).
[b]Mean ± 1 standard deviation, as served
[c]IU = mg alpha × 1.49
[d]The number in parenthesis is the number of jars evaluated.
[e]t = trace
[f]nd = none detected

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chased periodically over the time period, just as the homemaker would purchase them for use in the home. An effort was made to purchase samples with different lot numbers. By purchasing samples from different lot numbers it was hoped to obtain samples of varying storage ages. The length of time a product had been stored would be expected to have an influence on the amount of vitamin E left in the food.

Duplicate 50-g samples were weighed out of each 128-g jar of fruit, vegetables, mixed dinners, breakfasts and desserts. 40-g samples were obtained from 99-g jars of meat and 15-g samples from egg yolk. From two to four jars of each specific food were assayed according to the method of Dicks-Bushnell and Davis (1967) with the modifications indicated below. Some products were also assayed using thin-layer chromatography (TLC) on Eastman Chromagram 6062 or Bakerflex 1-B prepared plates (Eastman Kodak, 1969).

The weighed samples were extracted for 10 min with 200 ml absolute ethanol, and saponified for 10 min with 25 ml concentrated potassium hydroxide solution in the presence of 35g ascorbic acid. The nonsaponifiables were re-extracted with 100 ml of hexane.

Of the hexane extract, 50 ml were evaporated to about 5 ml and used for chromatography on a column of Florisil. The Florisil-filled column was saturated with hexane before the extract was added. Hexane alone was used to elute some interfering substances and hexane plus 6% ethyl ether was used to elute the tocopherols. The eluates were evaporated to dryness and dissolved in 0.5 ml benzene. Aliquots of the benzene solution were used for 2-dimensional paper chromatography.

Another 25 ml of the hexane extract were evaporated to dryness and dissolved in benzene. Aliquots of this benzene solution were used for TLC. Benzene was used as the mobile phase for TLC.

For both paper chromatography and TLC, the spots were located under ultraviolet light, cut out and eluted in 4.5 ml 0.56% ethanolic 2,2'-bipyridine solution. To the eluates were added 0.5 ml of 0.2% ferric chloride. In exactly 2 min the OD was read at 520 nm with the Beckman DU. Results were calculated from standard curves run with alpha-, beta-, and delta-tocopherols.

With the paper chromatographic procedure and pure tocopherol, the correlation coefficients (r^2) for the regression of concentration on absorbance for alpha-tocopherol was 0.92, for beta-tocopherol was 0.91, and for delta-tocopherol was 0.87. All r^2 values were highly significant (1% level). Recovery of pure tocopherol was 96.30% for alpha-, 65.80% for beta- and 54.50% for delta-tocopherol. Thus measurements for alpha-tocopherol were more reliable than measurements of beta- and delta-tocopherols.

RESULTS & DISCUSSION

SINCE alpha-tocopherol is the most biologically active form of vitamin E, its determination was the primary concern of this study. Since other tocopherols can also contribute some vitamin E activity an attempt was made to isolate and identify them. No effort was made to determine whether these tocopherols were tocopherols or tocotrienols (Slover et al.,

Table 2—Vitamin E content of strained vegetables

Product	Brand No. /reps	% Moisture[a]	% Fat[a]	Tocopherol							
				Alpha			Beta-plus-gamma		Delta		Total
				Mean[b] mg/100g	Range mg/100g	IU/100g[c]	mg/100g	mg/100g	Mean mg/100g	Range mg/100g	Mean mg/100g
Beets (2)[d]	G	89.2	0.1	0.12 ± 0.03	0.08–0.18	0.18	t[e]	t	nd[f]	nd	0.12
Carrots (2)	G	93.1	0.1	0.84 ± 0.17	0.56–1.19	1.25	0.23 ± 0.03	0.00–0.26	nd	nd	1.07
Carrots (3)	H	95.9	0.3	0.40 ± 0.20	0.17–0.72	0.60	0.09 ± 0.04	0.00–0.16	t	t	0.49
Corn, creamed (3)	G			0.16 ± 0.05	0.09–0.32	0.24	0.09 ± 0.03	0.00–0.12	0.11 ± 0.02	0.00–0.14	0.36
Green beans (2)	G	92.5	0.1	0.16 ± 0.03	0.00–0.22	0.24	0.09 ± 0.02	0.06–0.12	nd	nd	0.25
Garden vegetables (3)	G	88.8	0.3	0.61 ± 0.09	0.00–0.75	0.91	0.41 ± 0.22	0.15–0.96	0.18 ± 0.09	0.00–0.35	1.20
Mixed vegetables (2)	G	89.8	0.1	0.22 ± 0.03	0.18–0.27	0.33	0.05 ± 0.01	0.00–0.08	0.11 ± 0.03	0.00–0.13	0.38
Peas (3)	G	85.5	0.2	0.15 ± 0.03	0.00–0.20	0.22	0.62 ± 0.12	0.42–0.76	0.10 ± 0.06	0.00–0.22	0.87
Peas, creamed (4)[g]	H	81.5	2.1	t	t	t	0.52 ± 0.15	0.14–0.74	0.22 ± 0.10	0.10–0.54	0.74
Spinach, creamed (4)	G	93.1	0.1	1.55 ± 0.31	1.11–1.99	2.31	0.08 ± 0.02	0.00–0.10	t	t	1.63
Squash (2)	G	93.2	0.1	0.36 ± 0.04	0.32–0.43	0.54	0.48 ± 0.14	0.29–0.67	nd	nd	0.84
Squash (3)	H	93.0	0.1	0.35 ± 0.07	0.26–0.52	0.52	0.13 ± 0.05	0.08–0.24	nd	nd	0.48
Sweet potato (4)	G	81.8	0.1	0.50 ± 0.08	0.30–0.62	0.74	t	t	nd	nd	0.50
Sweet potato (3)	H	86.2	0.2	0.38 ± 0.08	0.21–0.51	0.57	0.24 ± 0.12	0.00–0.40	0.09 ± 0.03	0.00–0.14	0.71

[a]Moisture and fat values taken from Bowes and Church (1963); Handbook 8 (1963); or Heinz (1970).
[b]Mean ± 1 standard deviation, as served
[c]IU = mg alpha × 1.49
[d]The number in parenthesis is the number of jars evaluated.
[e]t = trace
[f]nd = none detected
[g]Contained soy oil

Table 3—Vitamin E content of strained meats, high meat dinners and egg yolks

Product, Brand G	% Moisture[a]	% Fat[a]	Tocopherol									
			Alpha			Beta-plus-gamma		Delta		Total		
			Mean[b] mg/100g	Range mg/100g	IU/100g[c]	Mean mg/100g	Range mg/100g	Mean mg/100g	Range mg/100g	Mean mg/100g
Beef (2)[d]	89.1	1.0	0.73 ± 0.16	0.50–1.01	1.09	nd[e]	nd	nd	nd	0.73
Beef, egg noodles (2) and vegetables	89.1	1.0	0.33 ± 0.04	0.27–0.40	0.49	0.06 ± 0.02	0.00–0.10	nd	nd	0.39
Beef liver (2)	79.4	3.3	0.32 ± 0.10	0.18–0.52	0.48	nd	nd	nd	nd	0.32
Chicken (2)	76.4	9.7	0.30 ± 0.05	0.23–0.36	0.45	0.14 ± 0.03	0.00–0.19	0.09 ± 0.04	0.00–0.18	0.53
Chicken noodle dinner (3)	89.0	0.8	0.15 ± 0.04	0.11–0.22	0.22	0.06 ± 0.02	0.00–0.08	0.06 ± 0.01	0.00–0.08	0.27
Ham (2)			0.45 ± 0.06	0.30–0.52	0.67	t[f]	t	nd	nd	0.45
Turkey (4)			0.34 ± 0.06	0.00–0.41	0.51	nd	nd	nd	nd	0.34
Turkey and rice with (3) vegetables	81.3	3.2	0.13 ± 0.03	0.00–0.18	0.19	t	t	nd	nd	0.13
Veal (2)	80.4	3.9	0.22 ± 0.04	0.18–0.30	0.33	t	t	nd	nd	0.22
Egg yolk (3)	70.0	18.4	0.60 ± 0.21	0.30–1.01	0.89	0.59 ± 0.37	0.00–1.09	0.47 ± 0.48	0.00–1.44	1.66
High meat, beef with (2) vegetables	81.6	3.7	0.52 ± 0.13	0.28–0.67	0.77	0.06 ± 0.03	0.00–0.13	nd	nd	0.58
High meat, chicken with (3) vegetables	79.6	4.6	0.23 ± 0.06	0.12–0.30	0.32	0.08 ± 0.03	0.00–0.15	0.16 ± 0.04	0.00–0.21	0.47
High meat, ham with (2) vegetables			0.22 ± 0.05	0.14–0.33	0.33	0.11 ± 0.03	0.00–0.16	nd	nd	0.33
High meat, turkey with (2) vegetables	3.2	3.2	0.14 ± 0.03	0.00–0.17	0.21	nd	nd	nd	nd	0.14
High meat, veal with (2) vegetables	1.6	1.6	0.13 ± 0.03	0.09–0.20	0.19	t	t	t	t	0.13

[a]Moisture and fat values taken from Bowes and Church (1963); Handbook 8 (1963); or Heinz (1970).
[b]Mean ± 1 standard deviation, as served
[c]IU = mg alpha × 1.49
[d]The number in parenthesis is the number of jars evaluated.
[e]nd = none detected
[f]t = trace

1967, 1969a, 1969b; Pennock et al., 1964).

Vitamin E values for fruits and desserts are presented in Table 1; vegetables in Table 2; meats, high-meat dinners and egg yolks in Table 3; and breakfasts, mixed dinners and soups in Table 4. All values are reported as mg tocopherol/100g of baby food, as served. One jar of vegetables, fruits, dinner and desserts contains 128g. One jar of meats, or egg yolks contains 99g. Alpha-tocopherol is also given as international units/100g (IU). For purposes of comparison if a value found in the literature for a product was within ± 1 standard deviation of the observed mean, it was considered to be not different from the observed mean. This criterion was selected because many literature values either were measurements made on only one sample, or had no statement of reliability, range or number of observations.

Fruits and desserts, which were all Brand G products, contained 0.17–1.33 mg alpha-tocopherol/100g product. Applesauce with 0.52 was similar to raw apples with 0.50 reported by Booth and Bradford (1963a), 0.72 reported by Harris et al. (1950), and 0.31 reported by Bunnell et al. (1965). Bananas with 0.23 mg/100g were similar to raw bananas with values of 0.37 and 0.22 reported by Harris et al. (1950) and Bunnel et al. (1965). Apricots contained 0.70 mg alpha-tocopherol/100g, and were similar to 0.50 total tocopherols in raw apricots reported by Kramer and Tarján (1959). Pears with 0.64 mg/100g had more alpha-tocopherol than raw pears with skin with 0.50 and raw pear flesh with 0.10 (Booth and Bradford, 1963a). Plums contained 0.38, which was less than 0.70 reported by Booth and Bradford (1963a) for raw plums. Prunes contained 0.38 mg alpha- and 0.45 mg total tocopherols, and were much less than the 4.10 mg total tocopherols reported for stewed prunes by Strohecker et al. (1960). This difference could be due in part to the difference in water content of pureed baby foods and stewed prunes. Harris et al. (1950) reported 0.69 mg alpha-tocopherol/100g dry powdered chocolate pudding. This cannot validly be compared to the 0.23 mg that was found in the cooked infant pudding. Likewise, suitable values are not available for comparison with the values presented for cottage cheese and pineapple, fruit dessert, orange pudding and peaches.

Some vegetables were found to contain an amount of alpha-tocopherol similar to that reported in the literature. Brand H carrots with 0.40 mg were similar to cooked carrots with 0.46 (Booth and Bradford, 1963b) and raw carrots with 0.45 (Harris et al., 1950).

Brand H peas, which were creamed and which listed soy oil as an ingredient,

contained 0.48 mg alpha-tocopherol/100g of product in one of four samples. No alpha-tocopherol was found in the other samples.

Products containing less alpha-tocopherol than similar products reported in the literature were Brands G and H squash with 0.36 and 0.35 mg respectively, Brand G sweet potatoes with 0.50 mg, Brand H sweet potatoes with 0.38 mg and Brand G corn with 0.16 mg. The literature value for raw pumpkin squash was 1.30 mg total tocopherols (Chufat, 1946) for raw sweet potatoes was 4.00 mg alpha-tocopherol (Emmerie and Engel, 1938), and for raw corn was 1.10–1.40 mg tocopherol (Green, 1963). Bunnell et al. (1965) however, reported only 0.05 mg alpha-tocopherol/100g for canned corn. The value of 0.12 mg for beets is less than 0.20 reported by Emmerie and Engel (1938) for raw beets.

Brand G green beans with 0.16 mg alpha-tocopherol contained more than the 0.03 mg reported by Bunnell et al. (1965) for canned green beans. Brand G spinach, which was creamed, was the richest vegetable source of alpha-tocopherol with 1.55 mg/100g. This was higher than the 0.02 mg found in canned spinach by Bunnell et al. (1965) and was probably due to the ingredients used to cream the spinach.

Brand G garden vegetables contained 0.61 mg alpha-tocopherol and Brand G mixed vegetables contained 0.22 mg. No suitable values were found in the literature for comparison.

Table 3 presents the tocopherol content of meats, high-meat dinners and egg yolks. These were all Brand G products. Chicken with 0.30 was less than the reported value for broiled chicken breast of 0.37 mg (Bunnell et al., 1965). Less alpha-tocopherol than was reported in the literature was found for beef liver with 0.32 mg found and 0.63 reported for broiled liver (Bunnell et al., 1965), and for egg yolk with 0.60 found and 1.16 reported for whole raw eggs (Harris et al., 1950). Beef with 0.73 mg was higher than values reported for raw beef steak, 0.47 (Booth and Bradford, 1963a) and broiled T-bone steak, 0.13 (Bunnell et al., 1965). Values for ham, 0.45; turkey, 0.34; and veal, 0.22 were higher than the reported values of 0.28 mg for fried hamsteak (Bunnell et al., 1965); 0.06 mg for raw turkey (Diplock et al., 1962); and 0.05 mg for pan fried veal cutlet (Bunnell et al., 1965).

Table 4 presents the values of alpha-tocopherol in Brand G mixed foods. These values ranged from 0.13–0.36 mg/100g. It is not possible to make reliable comparisons with other information sources for these products. The strained cereals with applesauce and bananas might be compared with the infant cereals assayed by Dicks-Bushnell and Davis

Table 4—Vitamin E content of strained breakfasts, vegetable dinners and soups

Product, Brand G	% Moisture[a]	% Fat[a]	Tocopherol								
			Alpha			Beta-plus-gamma		Delta		Total	
			Mean[b] mg/100g	Range mg/100g	IU/100g[c]	Mean mg/100g	Range mg/100g	Mean mg/100g	Range mg/100g	Mean mg/100g
Breakfasts											
Cereal, egg yolks (2)[d] and bacon	84.7	4.9	0.20 ± 0.02	0.16–0.23	0.30	nd[e]	nd	nd	nd	0.20
Mixed cereal with (2) applesauce & bananas			0.20 ± 0.03	0.17–0.26	0.48	0.11 ± 0.02	0.07–0.15	0.10 ± 0.03	0.00–0.13	0.53
Oatmeal with apple- (2) sauce and bananas			0.36 ± 0.12	0.19–0.55	0.54	0.19 ± 0.03	0.14–0.24	nd	nd	0.55
Rice with applesauce (2) and bananas			0.22 ± 0.02	0.11–0.41	0.33	nd	nd	0.05 ± 0.01	0.00–0.07	0.27
Dinners and soups											
Creamed chicken soup (2)	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Macaroni, tomato and (2) bacon	87.2	2.3	0.34 ± 0.04	0.26–0.42	0.51	0.09 ± 0.02	0.00–0.12	0.06 ± 0.02	0.00–0.10	0.49
Vegetables and chicken (2)	89.3	0.9	0.12 ± 0.02	0.00–0.14	0.18	0.07 ± 0.00	0.05–0.08	nd	nd	0.19
Vegetables and lamb (2)	89.5	2.4	0.25 ± 0.04	0.20–0.32	0.37	0.08 ± 0.01	0.06–0.11	0.11 ± 0.02	0.10–0.13	0.44
Vegetables and liver (2)	88.2	2.5	0.25 ± 0.02	0.21–0.29	0.37	0.07 ± 0.01	0.00–0.10	t[f]	t	0.32
Vegetables and turkey (2)	88.6	0.4	0.13 ± 0.03	0.00–0.18	0.19	nd	nd	nd	nd	0.13

[a]Moisture and fat values taken from Bowes and Church (1963); Handbook 8 (1963); or Heinz (1970).
[b]Mean ± 1 standard deviation, as served
[c]IU = mg alpha X 1.49
[d]The number in parenthesis is the number of jars evaluated.
[e]nd = none detected
[f]t = trace

(1967) on the basis of 100g. Mixed cereal with 0.32 mg/100g was more than Brand A with 0.23 and Brand B with 0.09. Oatmeal with 0.36 was similar to Brand A with 0.29 and more than Brand B with 0.08. Rice with 0.22 had less than 0.49 for Brand A but more than 0.03 for Brand B. However, it should be pointed out that the cereals assayed by Dicks-Bushnell and Davis were the dry instant cereals, not prepared for consumption. 100g of the canned cereals equals 7 tablespoons while 100g of the dry cereals equals more than 40 tablespoons. In terms of quantities which an infant would really consume, the dry cereals would contain much less vitamin E than the canned.

In Brand G garden vegetables, spinach and turkey, the results from one lot to another were so different that additional samplings were made. Egg yolks and the puddings contained large quantities of lipids that interfered with column, paper and TLC separations.

The requirement for vitamin E is thought to be related to the amount of PUFA in the diet. It was not possible to assay the PUFA content of these foods. However, the approximate fat content for these foods has been listed as reported by Heinz (1970), Watt and Merrill (1963) and Bowes et al. (1963). Linoleic acid is usually used as the critical fatty acid in determining vitamin E requirements. It has been suggested that 0.6 mg alpha-tocopherol is required to balance 1g linoleic acid (NRC-NAS, 1968). With the exceptions of seeds, nuts and vegetable oils, a few products listed in Handbook 8 (Watt and Merrill, 1963) have more than 50% of the fat as linoleic acid. For the fruits the ratio of mg alpha-tocopherol to g fat (E/fat) was 0.5 to 7.0. If they had 50% of the fat as linoleic acid the ratio would be 1.0 to 14.0, which is more than adequate. Vegetables had E/fat ratios of 0.8 to 15.5. If they had 50% of the fat as linoleic acid, the ratios would be 1.6 to 31.0. Beef, with an E/fat ratio of 0.7 and beef and noodles and vegetables and turkey, with 0.3 would be 1.4 and 0.6 if half of the fat were linoleic acid. The other items on Table 3 and 4 had ratios of 0.2 or less, and would be inadequate if half of the fat were linoleic. However, they might have much less than 50% of the fat as linoleic acid and still have adequate E/PUFA ratios.

One product, Brand G creamed chicken soup, contained no discernible vitamin E. This product was in a jar having a closure that the company had stopped using more than a year prior to the time the food was purchased. In a private communication the manufacturer reported that this product had been processed in January, 1968. It was assayed in August, 1971. The same manufacturer also reported that cereals which were 6 months old contained up to 100% more vitamin E than those that were 2 yr old and that age of sample must be considered in any complete evaluation of vitamin E. The recent trend for product dating will assist in determining the age of a product.

Harris (1962) reviewed the literature regarding the effect of processing and storage on vitamin E in foods. He concluded, "The available data indicate that the tocopherol content of foods depends upon genetic factors, season, agronomic factors, storage conditions and processing factors. Tocopherols are sensitive to oxygen and oxidation reactions, especially in the presence of catalysts, heat, alkalies and certain radiations." In some cases there was as much as a two-fold difference in alpha-tocopherol between samples with different lot numbers. Since samples were purchased from different sources at different times and with different lot numbers, it is probable that storage conditions and length of storage were sources of some of the differences.

When considering vitamin E requirements for infants, 5 IU per day are recommended and a ratio of 0.6 mg vitamin E/g linoleic acid is desired. Fruits and vegetables are good sources in terms of the E/PUFA ratio but not in terms of the absolute content of vitamin E. However peaches, apricots, squash, sweet potatoes and spinach are good sources in terms of both the absolute content of vitamin E and the E/PUFA ratio.

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Ms received 10/5/72; revised 12/8/72; accepted 12/12/72.

Research Paper No. 888 of the Idaho Agricultural Experiment Station.

The author gratefully acknowledges the technical assistance of Mrs. Julie Rolland. Gifts of alpha-tocopherol from Hofmann-La Roche Inc., Nutley, N.J. and mixed tocopherol concentrate from Distillation Products Industries, Kingsport, Tenn. were gratefully received. Appreciation is also expressed to Dr. Mary Zaehringer for guidance and editorial assistance.

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FLUOROMETRIC DETERMINATION OF VITAMIN A IN FOODS

INTRODUCTION

THE OFFICIAL AOAC method for vitamin A analysis is the colorimetric Carr-Price reaction. This method has been termed insensitive and nonspecific by Maynard and Loosli (1969) and De Ritter (1967). The use of other colorimetric reagents have been suggested, such as trifluoroacetic acid (Neeld and Pearson, 1963), trichloroacetic acid (Bayfield, 1971) and other Lewis acids (Dugan et al., 1964) but all the colorimetric reactions generally add large errors because of the difficulty in the detection of the end point, and insensitivity. Derse (1971) further maintains that sterols interfere with the colorimetric procedures.

Sobotka et al. (1943) first reported a procedure utilizing vitamin A's fluorescent characteristics for the analysis of fish liver oils. Fujita and Aoyama (1951), De (1955) and others followed with other fluorometric procedures, but these methods have not been commonly used, probably due to interference from carotenes and vitamin D (Gyorgy and Pearson, 1967), and from phytofluene (Thompson et al. 1971). The work of Thompson et al. with blood and liver vitamin A has shown that when the fluorescence of phytofluene is corrected for, the fluorometric assay is superior to the Carr-Price and other procedures in convenience, sensitivity, specificity and accuracy. Garry et al. (1970) also reported the presence of an interfering compound in plasma and serum extracts which they had not identified, but which they say may be the compound phytofluene, identified by Thompson's group.

We have adapted and modified the Thompson et al. (1971) procedure to the analysis of vitamin A in food.

MATERIALS & METHODS

THIS PROCEDURE for vitamin A analysis utilizes the characteristic fluorescence of vitamin A at 480 nm for quantitative measurement of the vitamin in different foods. A correction formula is applied to the raw fluorescent readings to correct for the phytofluene. Pure retinol and phytofluene are needed to obtain constants for this formula. Retinol was obtained from Hoffmann-LaRoche, Inc., Nutley, N.J., and the phytofluene was extracted from tomato paste.

Extraction of phytofluene

Fancy tomato paste (46% solids, West Coast Canners, Inc., courtesy of the Campbell Soup Company) was extracted following the procedure based on Koe and Zechmeister (1952) and revised by Thompson. 2 kg of tomato paste were adjusted to pH 8 with KOH and stirred with an equal volume of methanol. After standing overnight, the mixture was filtered through several layers of cheesecloth and squeezed dry. The remaining solid was stirred with 1.5 liters of a 1:1 mixture of carbon tetrachloride and methanol, and again filtered with cheesecloth. This extraction was repeated with another liter of the solvent mixture, and the filtrates were combined and washed several times with water. The deep red filtrate was dried with sodium sulfate and evaporated under reduced pressure. A portion of the oily residue was dissolved in petroleum ether and chromatographed on a column of 100g alumina (2% water). The principle fluorescent band was detected with an ultraviolet lamp and this band was eluted with 2% diethyl ether in petrol. (Caution must be taken to use an all glass apparatus and to shield the column from light and the excessive use of the ultraviolet lamp.) The elutant can be further purified by placing an aliquot into a silicic acid column. Phytofluene can be separated from two yellow pigments with petroleum ether. The phytofluene band is colorless, but highly fluorescent, and it elutes between the two yellow layers.

Vitamin A correction formula

Thompson's group developed a formula for calculating the fluorescence due to vitamin A in a blood or liver extract. This correction formula is based on the difference between the excitation spectrum of vitamin A and phytofluene. By taking two readings on each sample at different excitation wavelengths, one can calculate the proportion of fluorescence due to vitamin A.

This correction formula is shown below. The fluorescence at 330 nm excitation due to vitamin A is calculated by:

$$\alpha = \frac{P}{P-A}\left[\frac{Q_1}{q_1}(x - B_1)\right] - \frac{1}{P-A}\left[\frac{Q_2}{q_2}(y - B_2)\right]$$

where A and P are constants obtained from measurements of the ratio of the fluorescence of pure retinol and phytofluene, at 360 and 330 nm excitation and 480 nm emission, respectively, x and y are sample fluorescences at 330 nm and 360 nm, and Q_1 and Q_2 are readings of quinine sulfate in 0.1N H_2SO_4 at 330 nm and 360 nm. The quinine solution is used as a standard to correct for daily variations in sensitivity of the instrument if a spectrophotofluorometer is used for analysis. A, P, Q_1 and Q_2 were all calculated at the same time, being cautious not to change any instrument adjustments. q_1, q_2 and B_1, B_2 are quinine sulfate and blank readings at each subsequent analysis at 330 and 360 nm.

Having determined α, then the concentration of vitamin A in I.U. per gram food is obtained as follows:

$$\text{I.U. Vitamin A/g food} = \left(\frac{\alpha}{\text{g of food tested}}\right)\left(\frac{\text{I.U. retinol}}{\text{Fluorescence unit at 330 nm excitation}}\right)(\text{dilution factor})$$

The term "I.U. retinol/fluorescent unit at 330 nm excitation" is the same as the slope of the standard curve for retinol at 330 nm, and it is obtained, after conversion of μg to International Units, from Figure 1. The dilution factor is determined by the volume of the hexane extract vs. the volume of the hexane aliquot tested.

The standard curves of fluorescence of retinol in hexane and quinine sulfate in 0.1N H_2SO_4 at 330 nm and 360 nm are shown in Figure 1.

The fluorescent spectra of hexane extracts of saponified instant breakfast powder (both fortified with retinyl palmitate, and nonfortified) at 330 nm and 360 nm excitation are shown in Figures 2 and 3.

An Amico Keirs (Silver Springs, Md.) spectrophosphorimeter was used to obtain these spectra. The spectra show flattened peaks at

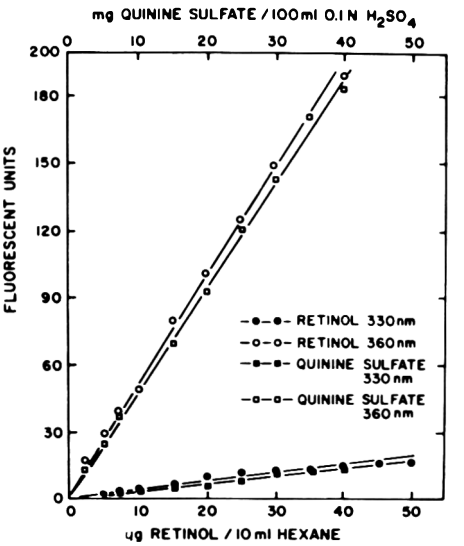

Fig. 1—Standard curves of fluorescence of retinol and quinine sulfate.

480 nm emission that are not interrupted by any other fluorescent maximum. It can be seen from our model system of instant breakfast extract that vitamin A can be quantified without interferences that would add error to the fluorescent analysis.

For the food analysis, reported in this paper, a Coleman Electronic Photofluorometer Model 12C with primary and secondary filters, manufactured by Perkin-Elmer, Coleman Instrument Div., Maywood, Ill., was used.

Extraction of vitamin A from foods

All vitamin A determinations are performed upon the unsaponifiable fraction of food samples. Varied saponification and separation steps are utilized for different food types because some foods require modified saponification procedure and/or further separation before fluorometric analysis. Three different extraction procedures are discussed below.

Table 1—Fluorometric determinations of vitamin A in fortified foods

Type of food	Vitamin claim μg/g	Average % of claim
Instant breakfast powder	30.23	116
Dry cereal (puffed wheat)	47.10	124
Dry cereal (puffed corn)	47.10	120
Fortified donut	15.06	137
Sponge cake	11.25	129
Devil's food cake	11.25	136
Peanut butter pastry	11.75	147
Chocolate bar	16.80	105
Freeze dried beef casserole	41.70	164
Margarine	33.20	102
Butter	33.20	131

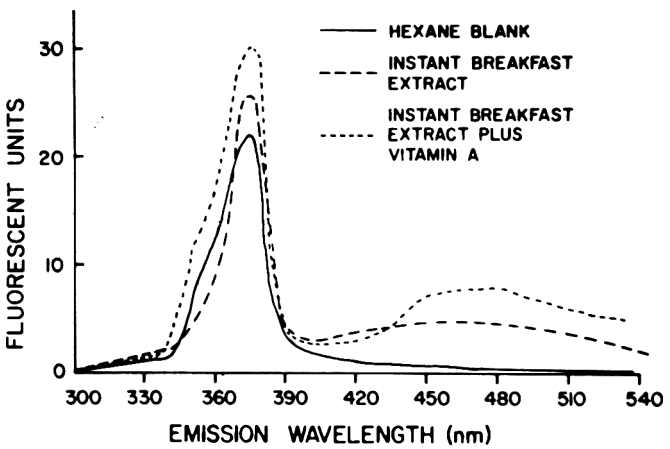

Fig. 2—Fluorescent scans at 330 nm excitation wavelength.

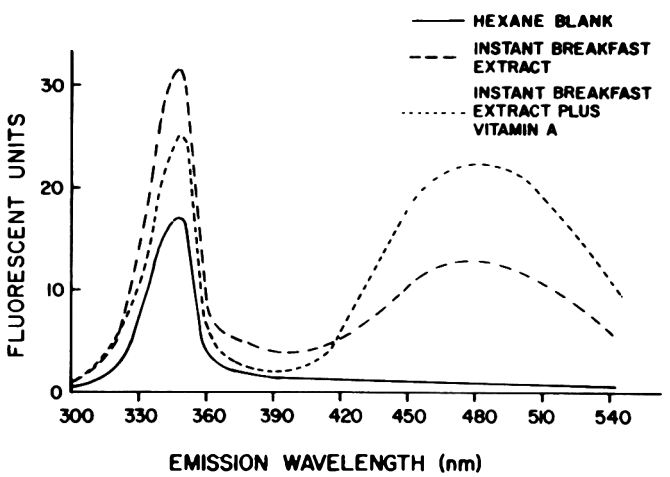

Fig. 3—Fluorescent scans at 360 nm excitation wavelength.

Low-fat, low-carotenoid foods

Convenience foods such as instant breakfast, most cereals and most prepared foods can be assayed for vitamin A content by saponifying 5g of homogenized sample (cereals should be finely ground) in 8 ml of 1% ethanolic pyrogallol and 4 ml of 60% aqueous KOH at a temperature between 85–90°C for 30 min. 5 ml of water is added to each sample at the 15 min mark. The resultant saponified material is homogenized and extracted twice with a 50 ml hexane using a tissue homogenizer (Inframo #8-111, Wayne, N.J.). 10 ml of this hexane extract is used directly for fluorometric measurements.

Care must be taken during saponification to avoid excessive boiling, exposure of the food sample to air, or exposure to light. Adjustment of water bath temperature, sample size and the covering of glassware with aluminum foil will limit the degradation of vitamin A.

High-fat foods

Foods with high fat content such as butter, margarine and ice cream, may not be completely saponified. Therefore, one should limit the sample size so that no more than 1g of fat is present. For example, samples of 1.5g or less of margarine can be saponified following the method previously described for low-fat foods.

High carotenoid foods

Fortified vegetable entrees, some margarines and other foods containing large amount of carotenoids, give very high fluorescent readings at the 360 nm excitation (y) wavelength, occasionally leading to negative result for (α). This is due to interference from carotenoids (other than phytofluene). In these cases the hexane extract can be further purified by a procedure described by Olson (1961). 10 ml of hexane extract is placed on a 1 × 15 cm column of 10g of deactivated alumina. The carotenoids are eluted first with 40 ml of hexane; then 80 ml of hexane with 2% benzene, 65 ml hexane with 2% acetone, and 40 ml of 10% acetone in hexane are eluted through the column. Retinol is eluted in the last 10% acetone portion. 10 ml of this extract is read directly in the photofluorometer, and the necessary dilution adjustments are made in the formula.

The final ethanol-water balance in the saponified material is crucial for complete hexane extraction. If improper separation occurs when hexane is added, the addition of a few ml of ethanol can be used to break the emulsion (Freed, 1966).

RESULTS & DISCUSSION

THE FLUORESCENCE of vitamin A is sufficiently intense to permit detection of quantities well below the microgram level (Udenfriend, 1962). As with all fluorometric assays, however, the researcher must work in ranges where Beer's Law applies. We have found in our laboratory that the best results with our instruments are obtained with foods containing 25–85 I.U. vitamin A per gram of sample. (This is equivalent to 12.5–42.5 I.U. per 10 ml extract.)

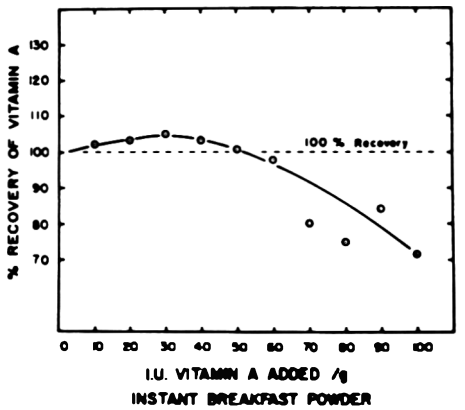

Fig. 4—Percent recovery of vitamin A palmitate when incremental additions are made to instant breakfast powder.

Figure 4 shows the % recovery of vitamin A palmitate when incremental additions are made to instant breakfast powder. The retinyl palmitate was added dry before saponification and a % recovery was observed for additions, as high as 60 I.U. per gram of powder. The results indicate a recovery of 100% ± 5% up to 60 I.U. per gram of powder for a total linear recovery range of 35–95 I.U. per gram food.

Utilizing the procedures described in this paper, vitamin A in several types of foods were investigated. Table 1 includes a list of fortified foods along with their respective claimed fortification and the % recovery of that claim. Most commercial operations add additional quantities of vitamin A (above what is claimed of the label) as an "overage" to assure compliance with the label claim and to allow for losses during storage and cooking. In all cases, a recovery greater than the claim (taken at 100%) was found indicating the presence of such "overage."

Six determinations of butter and seven determinations of wheat cereal were taken in order to demonstrate the precision which may be obtained with this analytical method. The results indicate that butter had an average content of 43.5 μg of vitamin A with a standard deviation of 1.2 μg, and the wheat cereal averaged 57.5 ± 2.8 μg. Much higher standard deviations are found in products such as the freeze dried casserole, where there is inherent vitamin A variability due to variations in product formulation and quality control thereof.

It is very difficult to satisfactorily compare the results of the fluorescent method to those from the Carr-Price method, particularly because of inherent errors in the latter method (Thompson et al. 1971). The accuracy of the fluorescent method is clearly demonstrated, however, in Figure 4 as 100 ± 5% of vitamin A is recovered from standard additions of the vitamin to instant breakfast powder.

Since the presention and submission of this manuscript, Thompson's group has published a paper describing a rapid fluorometric method for the determination of vitamin A in milk, butter, cheese and ice cream (Thompson et al., 1972). Our work, therefore confirms and extends the applicability of the method to food systems.

CONCLUSIONS

WHEN CARE is taken to shield all extraction steps from light, the fluorometric method for food analysis is accurate. For best results, investigators should use a spectrophotofluorometer with narrow band widths. Since this method is simple to perform and very rapid, we suggest that this fluorometric procedure be considered in lieu of the Carr-Price and other colorimetric methods for the analysis of vitamin A in food.

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Ms received 8/19/72; revised 10/21/72; accepted 10/25/72.

Presented at the 32nd Annual Meeting of the Institute of Food Technologists in Minneapolis.

Research supported by Office of Economic Opportunity Grant CG 2305 A/o and U.S. Dept. of Agriculture, Food & Nutrition Service Contract No. 12-35-600-8.

The authors acknowledge the assistance and advice of Dr. David H. Strumeyer and Dr. Stanley E. Katz from the Dept. of Biochemistry & Microbiology, Rutgers University, and Miss Eunyong Chung from the Dept. of Nutrition, Rutgers University.

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SOME MINOR VOLATILE COMPONENTS FROM THERMALLY DEGRADED THIAMINE

INTRODUCTION

TEMPERATURE, pH and time of heating or processing are the most important factors contributing to loss of thiamine in foods. Thiamine retention during cooking, processing and storage of food products has been extensively studied (Rice and Beuk, 1945; Farrer, 1950, 1953; Bendix et al., 1951; Sabry and Tannous, 1961; and Herrmann and Tunger, 1966). However, comparatively few studies concerned with the identity of reaction products from thermally degraded thiamine have been reported.

Obermeyer and Chen (1945) reported initial cleavage of thiamine to its pyrimidine and thiazole moieties, together with some unknown degradation products under the conditions encountered in bread baking. Matsukawa et al. (1951) tentatively identified 4-methyl-5-(β-hydroxyethyl) thiazole, formic acid, 2-methyl-4-amino-5-aminomethyl pyrimidine, 2-methyl-4-amino-5-hydroxymethyl pyrimidine and a thioketone alcohol (CH_3-C(=O)-CH(SH)-CH_2CH_2OH) from an aqueous thiamine solution which was refluxed for 30 hr. Gaudiano et al. (1966) studied the decomposition products of thiamine in injectable solutions by comparing R_f values of degradation products with authentic samples by paper chromatography. These workers reported the presence of 2-methyl-4-amino-5-aminomethyl pyrimidine, 2-methyl-4-amino-5-hydroxymethyl pyrimidine, thiochrome, 4-methyl-5-(β-hydroxyethyl) thiazole and several unidentified spots from autoclaved thiamine hydrochloride samples (pH 6.5). Arnold et al. (1969) identified hydrogen sulfide, 2-methyl thiophene, 2-methyl furan and 4,5-dihydro-2-methyl thiophene as volatile products produced during boiling of phosphate-buffered thiamine solutions at pH 6.7. Morfee and Liska (1972) studied the distribution of degradation products of thiamine in simulated milk systems, and identified elemental sulfur as a major degradation product in buffered, slightly acidic or basic thiamine solutions heated at 121°C for 50 min. Recently, Dwivedi et al. (1972) positively identified 4-methyl-5-(β-hydroxyethyl) thiazole as a major breakdown product from heated thiamine solutions (pH 5.0–7.0). The purpose of this study was to identify volatile compounds produced during thermal degradation of thiamine.

EXPERIMENTAL

THIAMINE SOLUTIONS of 10 mg/ml USP grade thiamine hydrochloride were adjusted to pH 5.0, 6.0, 7.0 and 8.4 with 1N NaOH. Solutions were allowed to stand at room temperature for 2 hr to establish equilibrium between different forms of thiamine at the adjusted pH. 50 ml of equilibrated thiamine solutions from each sample were transferred into screw-cap bottles and sealed with Teflon-lined caps. Duplicate samples were autoclaved for 1 hr at 121°C.

Heated thiamine solutions were cooled with tap water and extracted twice with 100 ml diethyl ether. Ether extracts were dried with 40g anhydrous sodium sulfate and concentrated to dryness on a rotary evaporator. During concentration, the temperature of the water bath was maintained at 20°C. Dried ether extracts were redissolved in 0.5 ml diethyl ether. Control samples were treated similarly except for the heat treatment step.

Volatile constituents of the ether extracts were analyzed by gas-liquid chromatography

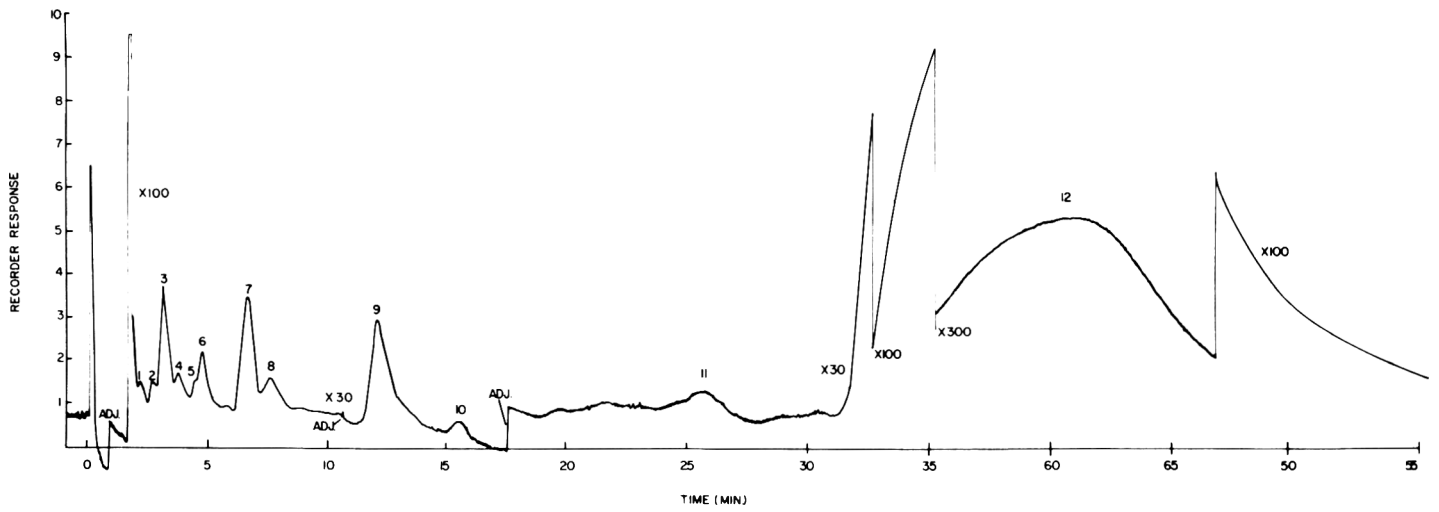


Fig. 1–Gas-liquid chromatogram of ether soluble, volatile components from thermally degraded thiamine at pH 6.0, 5% Dexil 300 GS on Chromosorb G 80/100 mesh column, 128°C.

(GLC) in conjunction with rapid scan mass spectrometry (MS). The effluent from the column was directed via a single-stage Llewellyn helium separator to an Atlas CH-4 mass spectrometer equipped with a double ion source. Mass spectra were recorded with a Honeywell 1508 visicorder. A 6-ft × 1/16-in ID stainless steel column packed with 5% Dexil 300 GS on Chromosorb G 80/100 mesh, was used to separate the components of the concentrate. The injector temperature was 228°C, flame ionization detector temperature 330°C and column temperature 128°C. The inlet pressure of helium (carrier gas) was maintained at 25 psi. Similar gas chromatographic conditions were employed for ether extract of heated thiamine solutions at pH 8.4 except that the column temperature was programmed from 90–230°C at 2°C/min. The injector temperature for this sample was 235°C initially and 247°C at the end of program. For GLC-MS analysis, 20 eV ion source served as gas chromatograph detector, while the 70 eV source provided the usual mass spectral fragmentation patterns. Scans were made from m/e 25 to m/e 250 in 2.5 sec. The MS operating conditions were: filament current 20 μa, electron voltage 70 eV, accelerating voltage 3.0 kV, analyzer pressure 7×10^{-7} mm and multiplier voltage 1.6 kV.

RESULTS & DISCUSSION

GAS CHROMATOGRAPHIC separation with 20 eV detection of volatile compounds from ether extracts of thiamine solutions adjusted to pH 6.0 and 8.4 are shown in Figures 1 and 2, respectively. Ether extracts of heated thiamine solutions adjusted to pH 5.0 and 7.0 gave chromatograms similar to the one obtained for the pH 6.0 sample, but the chromatogram of the pH 8.4 thiamine sample was quite different. Differences in the volatile compounds at neutral or acidic vs. alkaline conditions may be attributed to opening of the thiazole ring under alkaline conditions. Ether extracts of unheated controls did not give any sizable peaks under aforementioned GLC conditions.

Identifications of volatile compounds obtained from ether extracts were determined on the basis of mass spectral fragmentation patterns and GLC retention time. Sample compounds reported in this study were not available for direct comparison, and these identifications should therefore be considered tentative.

Tentative identifications of volatile ether-soluble compounds produced by heating neutral or slightly acidic thiamine solutions are presented in Table 1.

The mass spectrum of peak 7 could not be interpreted as due to any one compound of molecular weight 130. The apparent "loss" of 14 mass units in the spectrum could be explained by assuming two compounds, both tetrahydrothiophenes. The postulated structures are given in Table 1. The mass spectrum of peak 7 is in agreement with the reported mass spectrum of 2-methyl-3-oxy tetrahydrothiophene (Stoll et al., 1967), if m/e 130, 43 and 87 are assumed to arise from the 2-acetyl tetrahydrothiophene (Table 1).

Peak 8 was probably due to a dehydration product of 4-methyl-5-(β-hydroxyethyl) thiazole and may have the structure tentatively assigned to peak 8 in Table 1. Since no standard mass spectrum is available, the exact structure of this compound is still a matter of speculation.

The compound responsible for peak 9 could not be identified from its mass spectrum. This compound has a molecular weight of 132 and a base peak at m/e 43. The ion at m/e 35 (SH_3) may indicate a sulfide.

The very large peak 12 in Figure 1 was due to 4-methyl-5-(β-hydroxy-ethyl) thiazole; positive structural proof of this compound has been obtained earlier (Dwivedi et al., 1972). The size of the peak indicates it to be a major ether-soluble thermal degradation product of acidic and neutral thiamine solutions.

Relatively few ether-soluble, volatile compounds from heated thiamine solution pH 8.4 were detected by GLC (Fig. 2) compared to acidic and neutral thiamine samples (Fig. 1).

The compound responsible for peak 2 in Figure 2 could not be identified, but it appeared to have a molecular ion at m/e 84. Peak 3 in this chromatogram gave a mass spectrum identical to peak 6 of Figure 1. Peak 4 was due to 2-methyl-3-oxy tetrahydrothiophene, as its mass spectrum matched the mass spectrum of peak 7 in Figure 1. Peak 5 was due to 4-methyl-5-(β-hydroxyethyl) thiazole. Mass spectra of the tailing portions of this peak showed ions at m/e 157 and 173. These

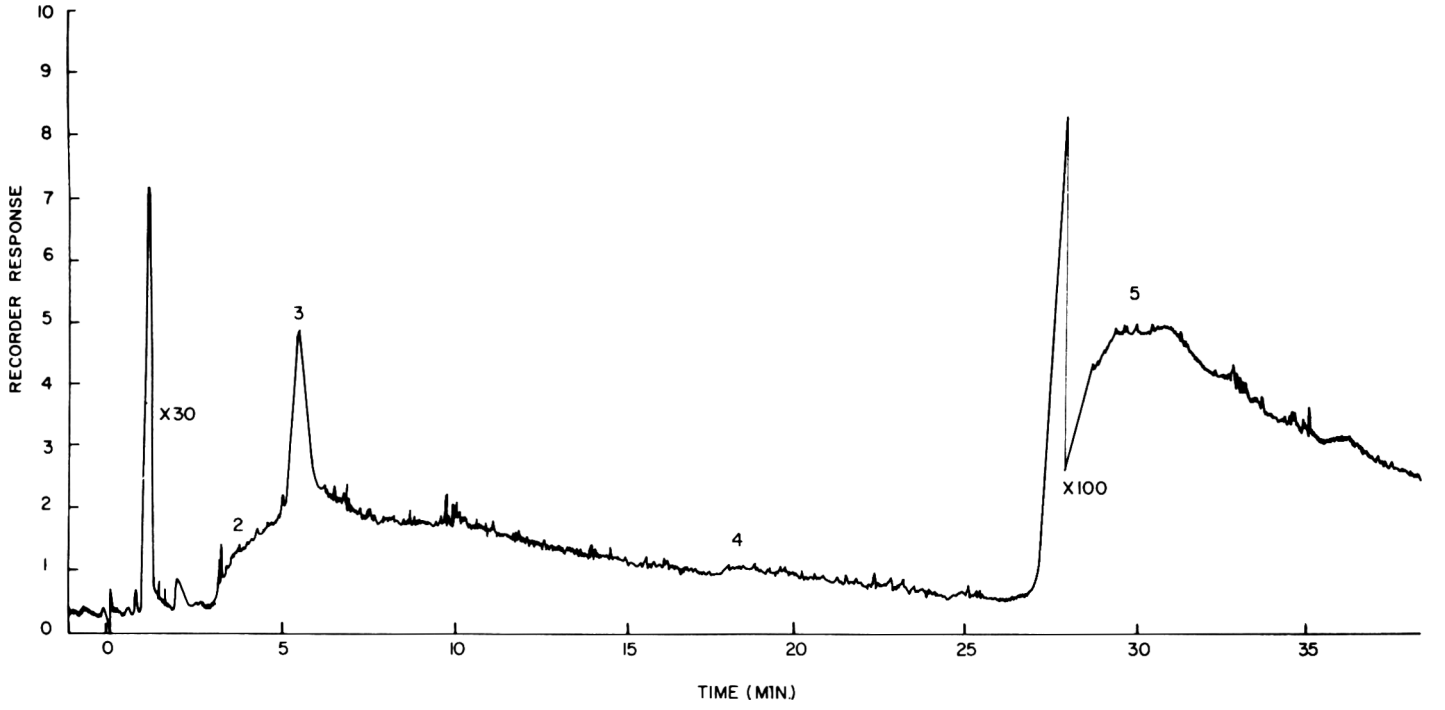
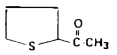
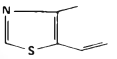
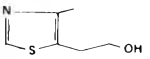


Fig. 2–Gas-liquid chromatogram of ether soluble, volatile components from thermally degraded thiamine at pH 8.4, 5% Dexil 300 GS on Chromosorb G 80/100 mesh column, 90–230°C at 2°C/min.

Table 1—Ether-soluble, volatile compounds from heated thiamine solution at pH 6.0

Peak	Name	Structure	M^+/principal ions	Mass spectrum reference
1	2-Methyl thiophene		98/97,98,45,39,53,99	Budzikiewicz et al., 1964 Arnold et al., 1969
3	4,5-Dihydro-2-methyl thiophene		100/85,59,100,99,39,45	Budzikiewicz et al., 1964 Arnold et al., 1969
5	2-Methyl sulfide-5-methyl furan		128/128,113,85	Stoll et al., 1967
6	4,5-Dimethyl thiazole		113/113,85,84,45,86,71	Shima et al., 1969
7	2-Methyl-3-oxy tetrahydrothiophene		116/60,116,45,59,27,88	Stoll et al., 1967
	2-Acetyl tetrahydrothiophene		130/43,130,87	Present study
8	2-Vinyl-3-methyl thiazole (?)		125/	
12	4-Methyl-5-(β-hydroxyethyl) thiazole		143/112,45,113,85,143,59	Dwivedi et al., 1972

may be the parent ions of the oxidation products of 4-methyl-5-(β-hydroxyethyl) thiazole, namely, 4-methyl thiazole-5-acetic acid and 2-oxy-4-methyl thiazole-5-acetic acid.

The flavor significance of volatile components arising from thermal degradation of thiamine is of interest. In the last few years a number of patents using thiamine as one of the ingredients for inducing or simulating meat and chicken flavor have been granted in the United States and Britain (Bidmead et al., 1968; Giacino, 1968; Giacino, 1969). A number of thiazole and thiophene related compounds have been identified from coffee and boiling chicken meat (Nonaka et al., 1967; Gianturco, 1967; Stoll et al., 1967). Some of these compounds likely originate from thiamine present in these products. Thiamine degradation products and/or intermediates may also react with other constituents in a food medium to produce an assortment of compounds.

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Ms received 7/6/72; revised 12/3/72; accepted 12/5/72.

Published as Paper No. 3426, Journal Series, Nebraska Agricultural Experiment Station. Research reported was conducted under Project No. 16—19.

This investigation was supported in part by Nutrition Foundation, Inc., research grant No. 427.

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SOLANUM ALKALOIDS: BIOSYNTHESIS AND INHIBITION BY CHEMICALS

INTRODUCTION

POTATO TUBERS turn green when exposed to light as a result of the formation of chlorophyll. Such tubers develop a bitter taste, cause health hazards and, in some instances, death because of high contents of solanine(s) which is currently known as a mixture of two glycoalkaloids, solanine (I) and chaconine (II) (Fig. 1). Hence, these potatoes are considered unfit for human consumption.

Solanine concentrations in normal whole potatoes are between 0.01–0.1% of the dry weight. Greatest accumulations are found in the node (eye) region with the periderm and cortex containing higher concentrations than the pith. The eyes ultimately develop into sprouts under favorable conditions. Following exposure to UV light, sprouts have been found to contain as high as 1.7% solanine.

The biogenesis of these two alkaloids indicated that potato sprouts or seedlings utilize acetate and mevalonate as precursors (Guseva and Paseshnichenko, 1958, 1961; Guseva et al., 1960). Subsequently, Guseva and Paseshnichenko (1962) established that the distribution of labeled carbon atoms in solasodine, synthesized from acetate or MVA, agrees with that expected on the basis of the known biosynthetic and cyclization scheme of squalene.

Studies on the formation and control of chlorophyll and solanine in potatoes have been carried out in our laboratories by treatments with chemicals, ionizing radiation, cultivar character and tuber maturity, controlled atmospheres and the intensity, quality and duration of light (Patil et al., 1971a, b; Salunkhe et al., 1972; Wu and Salunkhe, 1972a, b, c). The present paper deals with the incorporation of structurally appropriate precursors such as HMG, leucine, alanine and glucose into the glycoalkaloids of potato sprouts and the inhibition by chemicals.

MATERIALS & METHODS

ALAR®, ETHREL® AND TELONE® were obtained from uniRoyal Chemical, Division of uniRoyal Inc., Bethany, Conn.; Amchem Products, Ambler, Pa.; and Dow Chemical Company, Midland, Mich., respectively. L-alanine-U-^{14}C (100 mC/mM), L-leucine-U-^{14}C (248 mC/mM), HMG-3-^{14}C (7.1 mC/mM), D-glucose-U-^{14}C (4.92 mC/mM) and DL-MVA-2-^{14}C (DBED salt, 12.66 mC/mM) were purchased from New England Nuclear, Boston, Mass.

Russet Burbank potatoes of uniform size were selected from a local market and stored in a dark room at 16°C and 60% R.H. to develop sprouts void of chlorophyll. These sprouts (1.5–2 in.) because of their high metabolic activity and ability to synthesize a maximum amount of solanine, were used for biosynthetic precursor incorporation studies according to the procedure outlined by Guseva and Paseshnichenko (1958). 10-g sprouts were incubated (25°C) in a 50 ml nutrient solution (pH 6.7) containing 10 μC of the respective substrate for 2 days under 100 ft-c light. 20 μC of DL-mevalonic acid (MVA) were added to the incubation medium since only the L isomer is utilized. The sprouts were extracted with a hot ethyl alcohol (95%), evaporated to dryness, dissolved in 25 ml of 5% sulphuric acid, filtered and precipitated by ammonium hydroxide. The alkaloid fraction was again acidified, reprecipitated and washed with 1% ammonium hydroxide followed by ether and used in 0.05% HCl for measurement of radioactivity according to the procedure described in the previous publication (Jadhav et al., 1972). The glycoalkaloids were hydrolyzed by the method of Guseva et al. (1960).

The feeding experiments were arranged in a completely randomized block design each with two replications and two determinations per replication. The analysis of variance was computed and the means were compared by Tukey's ω-procedure (Steel and Torrie, 1960).

RESULTS

IT IS EVIDENT from the results in Table 1 that sprouts utilize HMG-3-^{14}C, L-leu-

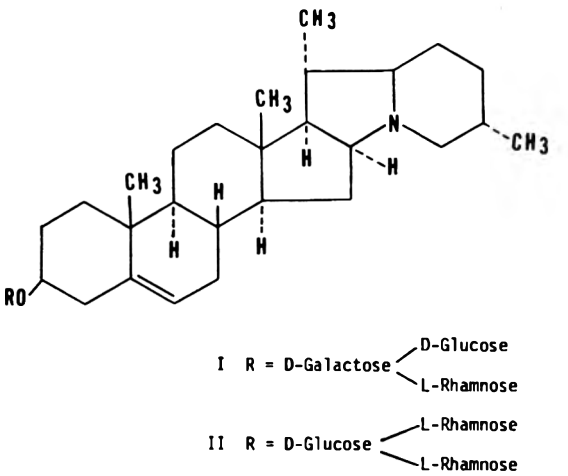

Fig. 1—Molecular structures of solanine (I) and chaconine (II).

Table 1—Utilization of precursors by potato sprouts[a]

	Precursors				
	HMG	Leucine	Alanine	Glucose	MVA
Alcohol extract (dpm)	719,525	967,300	575,575	842,200	8,259,625
Alkaloid fraction (dpm)	27,477	16,541	10,497	12,393	252,177
% Incorporation of added label into alkaloid fraction	0.1238	0.0745	0.0473	0.0558	1.135
Efficiency ratio[b]	1/9	1/15	1/24	1/20	1/1

[a]10g sprouts were incubated (25°C) in a 50 ml Hogland solution (pH 6.7) containing the respective precursor (10 μC) for 2 days under 100 ft-c light.
[b]Incorporation of each precursor into the alkaloid fraction/that of MVA

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cine-U-^{14}C, L-alanine-U-^{14}C and D-glucose-U-^{14}C for the synthesis of solanum alkaloids under the experimental conditions mentioned. The formation of solanines from these substrates, expressed as % incorporation of added label or efficiency ratio, was 9, 15, 24 and 20 times less than that of MVA-2-^{14}C.

The alkaloid fraction of the sprouts incubated with D-glucose-U-^{14}C was hydrolyzed and the radioactivity in the aglycone and the sugar moieties measured. The incorporation of the label into the sugar fraction was nearly 9 times higher than that into the aglycone.

As shown in Table 2, Alar, Ethrel and Telone significantly reduced the synthesis of alkaloids in comparison to the control by 49.4, 75.5 and 58.2%, respectively. However, neither Alar nor Telone reduced the amount of ^{14}C found in the alcohol extract; whereas, the alcohol extract from Ethrel treated sprouts contained nearly twice the amount of radioactivity as that of control sprouts.

DISCUSSION

IN HIGHER PLANTS, the role of HMG as a precursor of isoprenoid compounds such as carotenoids, steroids and steroidal alkaloids, terpenes, or intermediate metabolites has not been clearly established. However, in the light of the evidence related to the biogenesis of isoprenoids, the mechanism of incorporation of HMG can be explained in two possible ways. First, plants are able to synthesize an HMG-activating enzyme or HMG-CoA reductase (EC.1.1.1.34) which is responsible for the utilization of HMG (Hepper and Audley, 1969; Berry, 1971). Consequently, the rate of incorporation of HMG lies between those of acetate and MVA. The second hypothesis assumes that HMG is degraded to acetate or acetoacetate which in turn enters the isoprene units (Steele and Gurin, 1960; Potty, 1969). As a result, the incorporation of HMG becomes less efficient as compared to acetate or acetoacetate.

The incorporation of acetate into alkaloids of potato seedlings (Guseva and Paseshnichenko, 1961) has been shown to be nearly four times less than the active form of DL-MVA; while in our experiment, HMG was found to incorporate at about one-ninth of the efficiency of MVA. Thus, a comparison of the rates of incorporation of HMG, acetate and MVA indicates that the second pathway is applicable to the synthesis of alkaloids and the possibility of HMG going directly to MVA is eliminated. The incorporation of HMG would be more than acetate if it followed the first pathway.

The amount of label from L-leucine which appeared in the alkaloid fraction indicates an incorporation pathway similar to that for HMG. According to Davies et al. (1964), enzymatic degradation of leucine in plants occurs as follows: Leucine → α-ketoisocaproate → isovaleryl-CoA → 3-dimethylacrylyl-CoA → 3-methylglutaconyl-CoA → HMG-CoA → acetoacetate and acetyl-CoA. However, attempts to demonstrate the possibility of HMG-CoA →MVA were unsuccessful (Davies et al., 1964).

Alanine is known to be metabolized through its corresponding keto acid. However, a very low rate of incorporation may be due to its transformation into various plant metabolites. The efficiency ratio and the nature of distribution of labelled D-glucose between the aglycone and the sugar fraction raise some interesting points for future investigations on enzymatic glycosylation of solanidine in cell-free extracts of potato sprouts or seedlings. If glucose were incorporated after glycolytic breakdown via acetate, the efficiency would be smaller than 1/20 and more incorporation into aglycone would be expected. However, the results indicate predominant glycosylation. Schematically, the proposed role of various precursors in the alkaloid biogenesis is presented in Figure 2.

It appears from the inhibition studies that ethylene released from Ethrel affects plant metabolism and effectively reduces alkaloid synthesis in sprouts. Elmer (1932, 1936) discovered that germinating potatoes were inhibited in their sprout development if ripening apples and pears were stored in close proximity to them, an effect which he attributed to the action of ethylene produced with fruit volatiles. The effect of Alar is based on the fact that Alar and related substances are involved as inhibitors in the pathways leading to isoprenoids (Ryugo and Sachs, 1969). Telone, a mixture of 1,3-dichloropropene and related chlorinated hydrocarbons, reduced incorporation (dpm) into the alkaloid fraction more than Alar. Berry (1971) reported the effect of Telone on carotenogenesis and predicted that the inhibition of carotene synthesis could be attributed to the formation of a chlorine free radical from Telone.

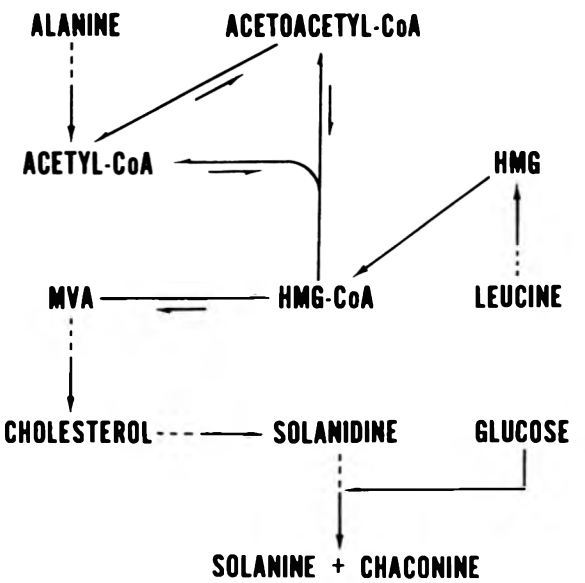

Fig. 2—Outline of the biosynthesis of solanine and chaconine.

Table 2—Effect of inhibitors on utilization of HMG-3-^{14}C by potato sprouts[a]

	Inhibitor, 10^{-3}M			
	Control	Alar	Ethrel	Telone
Alcohol extract (dpm)	719,525	732,825	1,602,275**	840,875
Alkaloid fraction (dpm)	27,477	13,780**	6,732**	11,490**
% Inhibition of alkaloids	0.0	49.4	75.5	58.2

[a]10g sprouts were incubated (25°C) in a 50 ml Hogland solution (zero or 10^{-3}M inhibitor) containing HMG-3-^{14}C (10 μC) for 2 days under 100 ft-c light.

**Significantly different from control at 0.01 level.

Although Alar and Telone decreased the rate of incorporation of label into the alkaloids of potato sprouts, the uptake of the label in the alcohol soluble fraction of the tissue was not affected (Table 2). It may be possible, therefore, that these chemicals somehow alter or affect physiological responses and metabolic activities to counteract the physiological role of solanum alkaloids in growing sprouts. The apparent increase in total radioactivity in the alcohol extract of sprouts treated with Ethrel may result from change in the shape, size and permeability of cells in contact with the radioactive material. According to the report of Amchem Products (1967-70), potato plants sprayed with Ethrel showed swelling of terminal buds.

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Ms received 9/21/72; revised 12/1/72; accepted 12/5/72.

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PHYSICAL, RHEOLOGICAL AND CHEMICAL PROPERTIES OF BANANAS DURING RIPENING

INTRODUCTION

NORMAL RIPENING of bananas can be controlled within limits by temperature regulation, ethylene application and modification of oxygen and carbon dioxide concentrations in the storage environment. In commercial practice ethylene is used to "trigger" ripening while the rate of ripening is controlled largely by temperature regulation. Major companies involved in the banana trade provide customers with ripening schedules that enable ripening periods to be varied from 4 to 8 days (Standard Fruit and Steamship Co. 1964; United Fruit Sales Corp. 1970).

Prolonged exposure to temperature extremes during ripening may lead to chilling injury or boiled fruit (Hall, 1967). However, there are reports (Dalal et al. 1969; Sanchez Nieva et al. 1969; Murata, 1970) of differences in fruit quality obtained within the range of normal ripening procedures. Assessment of banana quality has been limited largely to subjective evaluation and determination of major chemical constituents.

Biochemical and compositional changes associated with ripening bananas have been reviewed extensively (von Loesecke, 1950; Simmonds, 1966; Palmer, 1971). Rheological properties of the fruit have received little attention although texture is recognized as a major quality attribute in all fruits (Bourne, 1967). Finney et al. (1967) reported that firmness in bananas was closely related to reducing sugar and starch content during ripening at 60°F (15.6°C).

In the present study ripening temperatures representative of ripening under controlled atmosphere and tropical ambient conditions were selected and some rheological and chemical properties of bananas were examined during the ripening period. The aim was to obtain a better understanding of the relationships among such properties at different ripening temperatures.

EXPERIMENTAL

Materials

Hard green Valery bananas (Chiquita brand) were obtained from a local wholesaler. Upon arrival from the docks at Seattle, Wash., the fruit were treated with ethylene (approximately 1 ft^3/1000 ft^3 storage space) for 24 hr.

A 40-lb carton of fruit was used for each ripening trial which was carried out in a closed-cycle air dryer at 16 ± 1°C and 25 ± 1°C with a relative humidity of 85–95%. Each of the two ripening treatments was replicated three times.

Eight fruit were sampled every 2–3 days during ripening at 16 ± 1°C and every day during ripening at 25 ± 1°C. Five of these fruit were used individually in the measurement of peel color, pulp-to-peel ratio and force-deformation behavior. The pulp from the other three fruit was blended and used in the determination of total and reducing sugars and starch.

Procedures

Peel color was evaluated subjectively by comparing the fruit with color plates on a banana ripening chart (United Fruit Sales Corp. 1964). The color index for a given day represented the mean value for the eight fruit sampled. Using a Unicam SP 800 recording spectrophotometer the diffuse reflectance spectrum of two 35 mm diameter peel discs from each fruit was obtained. Each spectrum was recorded within the visible region (450 to 800 nm) at a scan rate of 200 nm/min. The Index of Variance Reflectance (IVR) proposed by Powers et al. (1953) as a criterion of color measurement

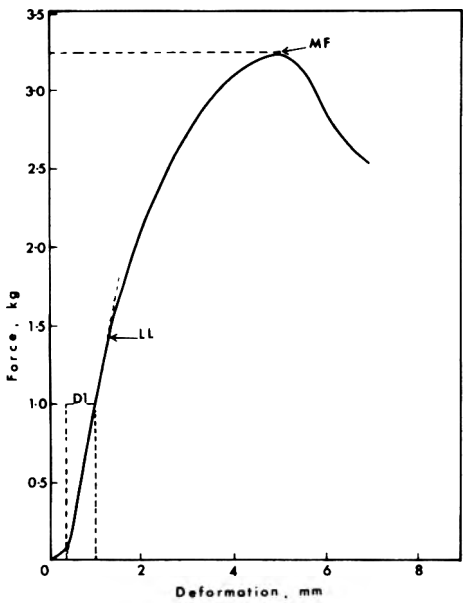

Fig. 1—Typical force-deformation curve for banana pulp tissue. MF, maximum force; LL, linear limit; D1, deformation due to 1 kg force.

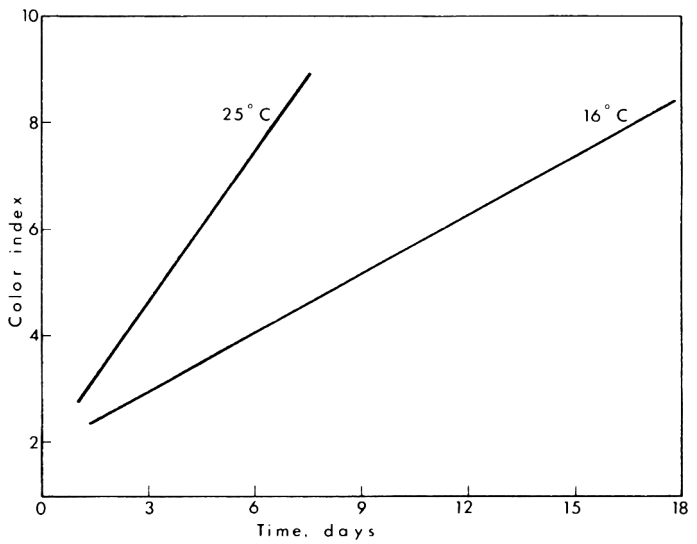

Fig. 2—Changes in peel color index with time for bananas ripened at 16 ± 1 and 25 ± 1°C.

in fruits was calculated using the following formula:

$$IVR = \frac{R_{730} - R_{672}}{R_{672}}$$

where R is the reflectance at the subscripted wavelength.

Pulp-to-peel ratio was calculated from the weights of whole and peeled fruit.

Rheological properties were derived from force-deformation tests obtained with an Instron model TMM universal testing machine. Cylindrical segments of pulp 2.5 cm long were cut from the middle region of peeled fruit and subjected to parallel plate compression at a loading rate of 0.5 cm/min. From the force-deformation curves (Fig. 1) values were obtained for maximum force, linear limit and deformation under 1 kg force. To compensate for differences in fruit diameter, maximum force and linear limit were divided by the mean cross sectional area of the sample, while deformation under 1 kg force was multiplied by that area to give bulk deformation.

Total and reducing sugars were determined from an 80% ethanol extract using the method of Ting (1956). Starch was determined from the reducing sugar equivalent of a hydrolyzed sample of alcohol insoluble solids. Hydrolysis was carried out by the AOAC (1965) method for starch.

RESULTS & DISCUSSION

THE RATE OF peel color change during ripening at 16°C was roughly half that at 25°C (Fig. 2). Coefficients of determination were 0.77 and 0.90 for the 110 and 85 data at 16 and 25°C respectively. The reflectance curves indicated that loss of chlorophyll, associated with the change in color from green to yellow (von Loesecke, 1950), appeared to follow changes in reflectance at 672 nm (Fig. 3). Finney et al. (1967) found a similar relation between the green to yellow trans-

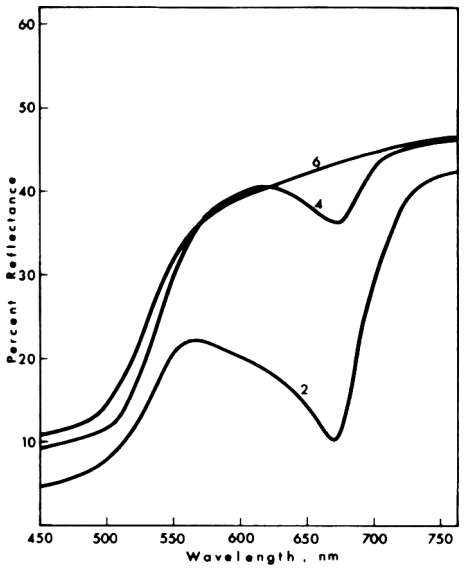

Fig. 3–Reflectance curves for the peel of bananas ripened at 16 ± 1°C. Numbers on the curves indicate peel color index.

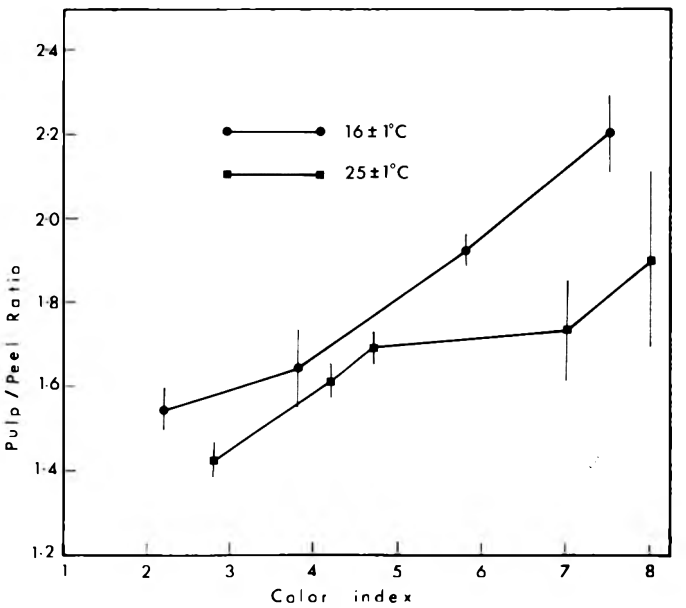

Fig. 5–Effect of ripening temperature on pulp-to-peel ratio of bananas. Vertical bars represent ± one standard deviation.

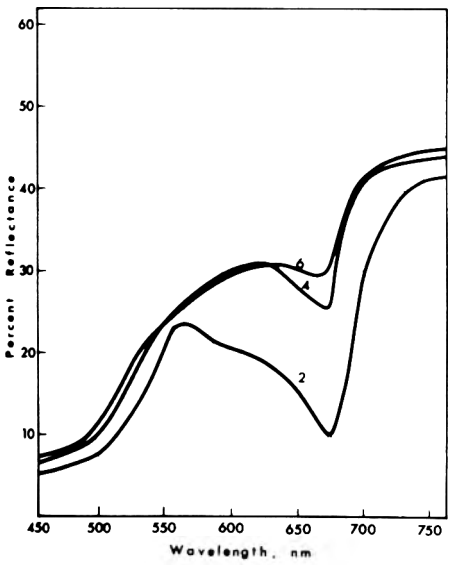

Fig. 4–Reflectance curves for the peel of bananas ripened at 25 ± 1°C. Numbers on the curves indicate peel color index.

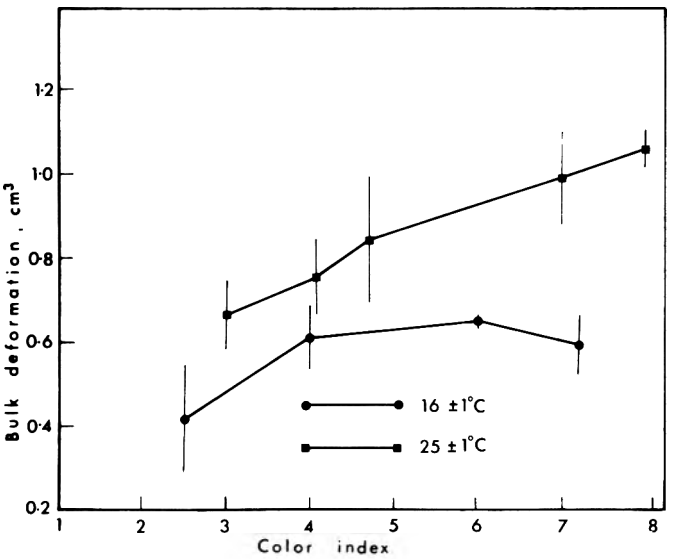

Fig. 6–Effect of ripening temperature on bulk deformation of banana pulp tissue under 1 kg force. Vertical bars represent ± one standard deviation.

formation and reflectance at 675 nm. Fruit ripened at 25°C tended to be greenish yellow in color. Chlorophyll retention in the peel of such fruit resulted in the persistence of a peak at 672 nm (Fig. 4). Incomplete destruction of chlorophyll in the peel of ripe bananas is characteristic of "boiled" fruit (Wilkinson, 1970) and fruit ripened at sub-optimal relative humidity (Haard and Hultin, 1969).

Earlier reports of an increase in pulp-to-peel ratio during ripening (von Loesecke, 1950; Simmonds, 1966) were confirmed in this study (Fig. 5). Values ranged from 1.35 in green fruit to 2.14 in over-ripe fruit. Results are presented as a function of color index, the most widely accepted index of ripeness, to provide a common basis for comparison of ripening treatments. Differences between curves indicate discrepancies in pulp-to-peel ratio for fruits similar in appearance, yet subjected to unlike ripening temperatures. Increasing pulp-to-peel ratio is related to accumulation in the pulp moisture derived from carbohydr breakdown and osmotic transfer fr peel to pulp (Palmer, 1971). During l temperature (16°C) ripening, pulp-to-p ratio increased linearly. At 25°C the ra did not increase steadily during ripeni and was generally lower in spite of slightly higher pulp moisture conter The lower pulp-to-peel ratios in the hi temperature treatment may be due to lower pulp starch content and possibly a increased hydrolysis of cellulosic or l brous constituents.

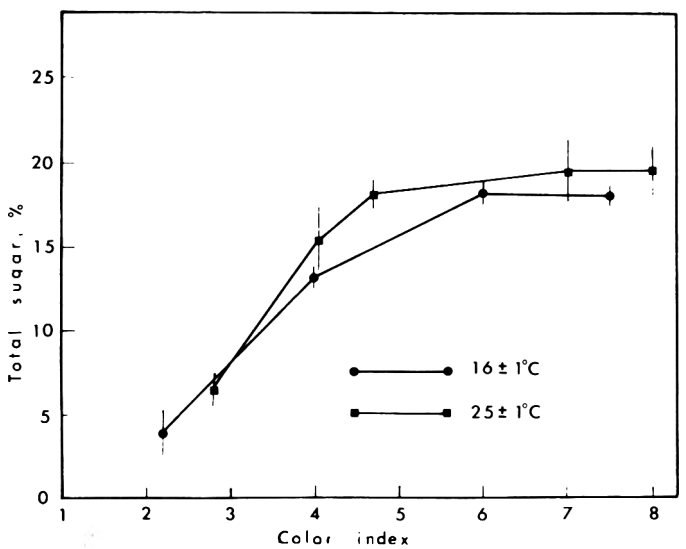

Fig. 7—Effect of ripening temperature on total sugar content of banana pulp tissue. Vertical bars represent ± one standard deviation.

Fruits ripened at 16°C were firm than those ripened at 25°C. Linear lim of ripe fruit tissue tended to be high during ripening at the lower temperatu but the patterns of change were the sam in each treatment. Bulk deformatio however, showed very different patter at the two ripening temperatures (Fig. 6 It remained relatively constant in rip fruits (beyond color index 4) at 16°C but increased linearly during ripening a 25°C. This indicates that the pulp be comes progressively softer with ripening The results support the observations o Sanchez Nieva et al. (1969) and Dalal e al. (1969) that bananas ripened at hig temperature are more susceptible t mechanical injury and exhibit a reduce shelf life.

The pattern of total sugar increase ir pulp tissue was the same at both ripenin temperatures, but sugar content wa slightly higher in fruit ripened at 25°C (Fig. 7). Ripening temperature affected the level as well as the pattern of increase in reducing sugars (Fig. 8). Throughout ripening at 25°C reducing sugars increased steadily while at 16°C there was a slight levelling off at advanced stages of ripeness. Ripe fruit from the 16°C treatment contained slightly more starch than those ripened at 25°C (Fig. 9). Increased starch hydrolysis results in a higher sugar/starch ratio and this suggests that there may be organoleptic differences between fruits ripened at different temperatures.

Correlation coefficients among selected variables in fruits ripened at the two temperatures (Tables 1 and 2) were highly significant ($P \leq 0.01$). Rheological parameters were closely related to sugar and starch content. The correlation coefficient between bulk deformation and reducing sugars was roughly equal at both treatments but its relationship with total sugars was markedly lower at 25°C. Color index and IVR of the peel were closely related to banana pulp rheological and chemical properties and, on the average, exhibited comparable accuracy as predictors of interior quality. Visual estimation of banana ripeness by color index would be preferred to the IVR method because of greater speed and simplicity. Furthermore, the effectiveness of spectral reflectance in the measurement of peel

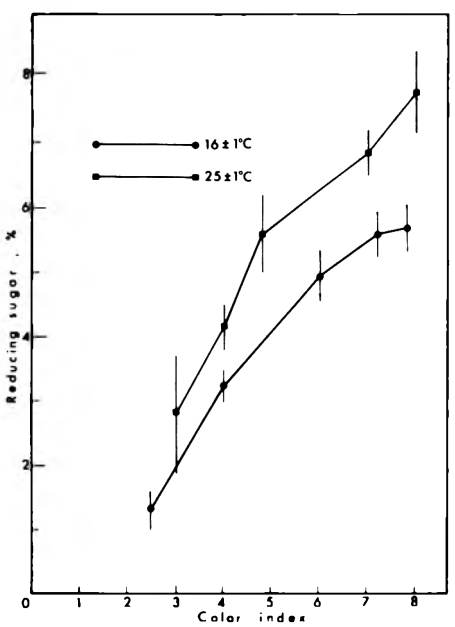

Fig. 8—Effect of ripening temperature on reducing sugar content of banana pulp tissue. Vertical bars represent ± one standard deviation.

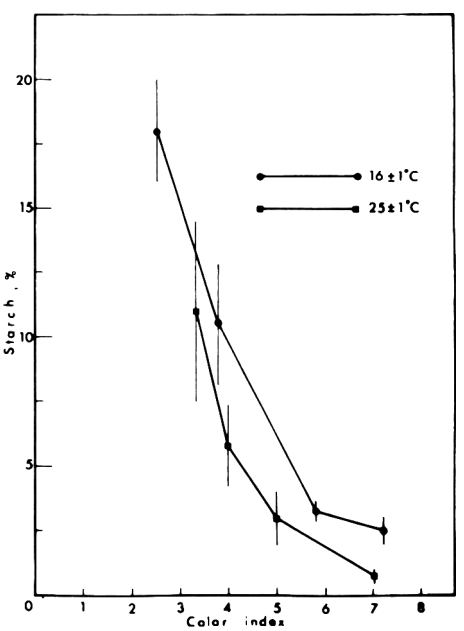

Fig. 9—Effect of ripening temperature on starch content of banana pulp tissue. Vertical bars represent ± one standard deviation.

Table 1—Simple correlations among selected physical, rheological and chemical properties of bananas ripened at 16 ± 1°C[a]

	Color index	Pulp/peel	IVR	Linear limit	Max force	Deformation	Total sugar	Reducing sugar
Pulp/peel	0.810							
IVR	−0.754	−0.672						
Linear limit	−0.452	−0.311	0.606					
Maximum force	−0.474	−0.332	0.628	0.996				
Deformation	0.645	0.532	−0.716	−0.645	−0.654			
Total sugar	0.881	0.746	−0.904	−0.587	−0.607	0.781		
Reducing sugar	0.947	0.814	−0.814	−0.516	−0.535	0.729	0.939	
Starch	−0.896	−0.744	0.904	0.632	0.632	−0.778	−0.993	−0.943

[a] $r_{.01} = 0.245$; n = 110

Table 2—Simple correlations among selected physical, rheological and chemical properties of bananas ripened at 25 ± 1°C[a]

	Color index	Pulp/peel	IVR	Linear limit	Max force	Deformation	Total sugar	Reducing sugar
Pulp/peel	0.661							
IVR	−0.734	−0.629						
Linear limit	−0.427	−0.483	0.645					
Maximum force	−0.432	−0.520	0.630	0.982				
Deformation	0.726	0.690	−0.583	−0.464	−0.477			
Total sugar	0.761	0.538	−0.814	−0.549	−0.510	0.492		
Reducing sugar	0.934	0.674	−0.818	−0.521	−0.507	0.725	0.838	
Starch	−0.826	−0.599	0.849	0.533	0.503	−0.604	−0.919	−0.925

[a] $r_{.01} = 0.275$; n = 85

color is limited by the uneven distribution of chlorophyll in the peel at various stages of ripeness. Even fully ripe fruits tend to have green tips. In view of the persistence of chlorophyll in the peel of bananas ripened at the higher temperature, it is possible that the accuracy of peel color as an index of ripeness may be affected by ripening temperature.

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Ms received 11/9/72; revised 1/6/73; accepted 1/8/73.

This research was supported by the Canadian International Development Agency.

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EFFECT OF METAL IONS ON THE COLOR OF STRAWBERRY PUREE

INTRODUCTION

SISTRUNK AND CASH (1970) studied the effects of a number of chemicals on the color of strawberry puree. They examined the effect of ascorbic acid, pH and holding time as well as the addition of certain metallic salts and EDTA on color difference meter (CDM) *a* and *a/b* values and the optical density of extracted pigments. An important finding was that stannic, stannous and aluminum chloride stabilized color as determined by the Hunter CDM. In their study, the optical density of extracts of the metal treated samples did not differ greatly from that of the control; whether or not the metal salts affected color stability by stabilizing the anthocyanins could not conclusively be determined.

We were particularly interested in resolving this question concerning the possibility of stabilization of anthocyanins with metallic salts. Our purpose was to study the interaction of metal ions with strawberry puree and further characterize the phenomenon relating to color effects.

EXPERIMENTAL

Procedure

Strawberries (*Fragaria ananassa* Duch. variety Northwest) were obtained from Oregon State University's North Willamette Experiment Station. Puree was prepared by thawing individually quick frozen (IQF) fruit and blending in a Waring Blendor. The puree was divided into six 200-g portions and stored under nitrogen at 2°C until needed. Aqueous solutions of metal salts, $SnCl_2 \cdot 2\,H_2O$, $SnCl_4 \cdot 5\,H_2O$ and $AlCl_3 \cdot 6\,H_2O$, were prepared in such a way that when 10 ml of these solutions were added to the 200-g portions of puree, the concentration of metal ion would be 0.2%. 10 ml of distilled water were added to another puree sample as a control.

Test tubes containing 20-g portions of sample were covered with Parafilm and placed in a 50°C water bath. Samples were periodically withdrawn for color and pigment analyses.

Strawberry juice utilized in some experiments was prepared by mixing 350g of puree with 350 ml of pH 3.40 citrate buffer and filtering through four layers of cheese cloth. The filtrate was clarified by filtering through Whatman No. 1 filter paper coated with a 1/8-in. layer of Celite.

200 ml of juice was made 0.2% in Sn^{++} by adding 10 ml of an aqueous $SnCl_2$ solution; the resulting turbid material was clarified by centrifuging and filtering through Whatman No. 1 paper. The control sample was prepared by diluting juice with an appropriate amount of distilled water.

Bleaching experiments were carried out by adding a solution of 400 ppm HSO_3^- in pH 3.4 citrate buffer to strawberry juice and strawberry puree to give samples 133 ppm in HSO_3^-. An aqueous 35% H_2O_2 solution was added to give samples 6% in H_2O_2.

Color and pigment determinations

Anthocyanins were assayed using the procedure of Swain and Hillis (1959). Modifications included using 0.1% HCl in methanol as the extracting solvent and measuring the absorbance at 510 nm. Concentration was expressed as μM of pelargonidin-3-glucoside per g fruit. Calculations were based on a molar absorbtivity of 36,600 (Wrolstad et al., 1970).

A Beckman Model DB-G recording double beam spectrophotometer was utilized to record the visible spectra of strawberry juice samples.

Color specifications were determined on a Gardner automatic color difference meter, Model AC-2A Series 200 instrument which was standardized against a red tile standard CSR 0093 (L = 26.8; *a* = +45.4; *b* = +15.1).

Thin-layer chromatography (TLC)

Pigments present in the acidic methanol extract used in the total anthocyanin assay were further purified using Polyclar AT (Wrolstad and Putnam, 1969; Wrolstad et al., 1970), concentrated and subjected to cellulose TLC. Plates 0.25 mm thick were developed ascendingly in glacial acetic acid-water-HCl (15:82:3).

RESULTS & DISCUSSION

THE STANNOUS, stannic and aluminum chloride treated samples remained red in color throughout the experiments whereas the control sample became a dull red-brown color. The visual appearance is documented by the Hunter color difference meter (CDM) *a* values (Figures 1, 2, 3, 4). Figure 1 shows the relationship between CDM *a* value and anthocyanin pig-

Table 1—CDM a values for strawberry puree and stannous chloride containing puree treated with bleaching agents

Sample	Time, days 0	1	5
Strawberry puree	21.8	17.0	12.1
Strawberry puree (Diluted with buffer)	–	–	10.8
Strawberry puree + HSO_3^-	–	–	9.4
Strawberry puree + H_2O_2	–	–	3.9
$SnCl_2$ treated puree	21.4	21.8	20.0
$SnCl_2$ treated puree (Diluted with buffer)	–	–	21.1
$SnCl_2$ treated puree + HSO_3^-	–	–	21.1
$SnCl_2$ treated puree + H_2O_2	–	–	14.0

Table 2—Anthocyanin content of strawberry juice and stannous chloride treated juice held at 50° C

Time days	Strawberry juice μg anthocyanin pigment/g juice	$SnCl_2$ treated juice μg anthocyanin pigment/g juice
0	183	173
1	131	128
5	34	20

[1] Present address: Checkerboard Foods, Ralston Purina Co., Checkerboard Square, St. Louis, MO 63199. Reprint requests should be sent to Oregon State University.

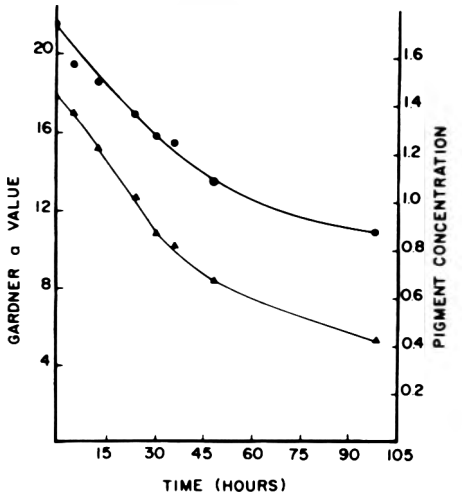

Fig. 1–Effect of storage at 50° C on the CDM a value (-●-) and anthocyanin pigment concentration (-▲-; μM pigment/g) of strawberry puree.

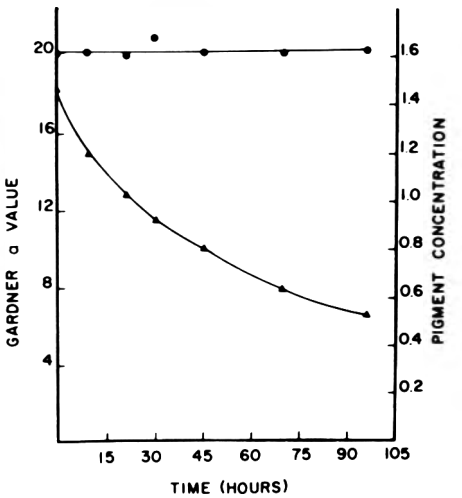

Fig. 2–Effect of stannous ion on the CDM a value (-●-) and pigment concentration (-▲-; μM pigment/g) of strawberry puree at 50° C.

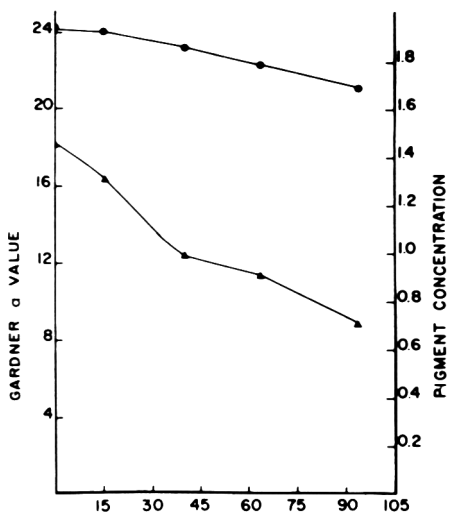

Fig. 3–Effect of stannic ion on the CDM a value (-●-) and pigment concentration (-▲-; μM pigment/g) of strawberry puree at 50° C.

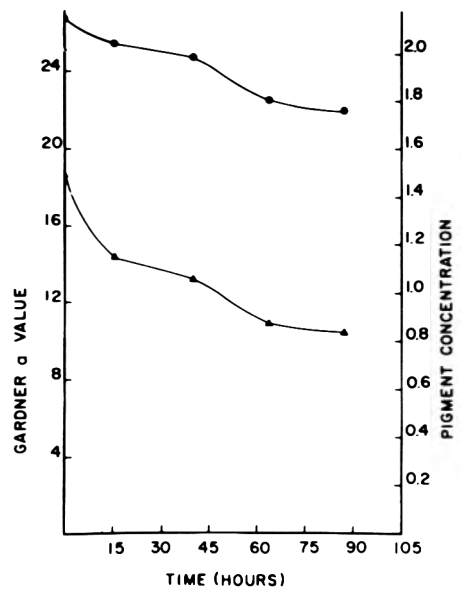

Fig. 4–Effect of aluminum ion on the CDM a value (-●-) and pigment concentration (-▲-; μM pigment/g) of strawberry puree at 50° C.

ment concentration in the control sample. The loss in redness which occurred with time is evidenced by the decrease in CDM *a* readings; degradation of anthocyanin concentration parallels this decrease. The CDM *a* value and pigment concentration for the stannous chloride treated sample are shown in Figure 2. The CDM *a* value does not change with time while anthocyanin degradation occurs similar to the control sample. This suggests that color stabilization is not due to anthocyanin stabilization. The results for stannic and aluminum treated samples are shown in Figures 3 and 4, respectively. All samples showed anthocyanins to degrade in a similar manner to the control. Stannic and aluminum chloride treated samples remained red in color and had CDM *a* values greater than the control.

In carrying out the total anthocyanin determination, the residue which remained after extracting the control sample with acidic methanol was a pale orange-pink color; the residue from the stannous, stannic and aluminum chloride treated samples, however, was reddish-blue. Repeated extractions with acidic methanol could not solubilize this material. These observations suggested that formation of an insoluble colored complex might be responsible for the pronounced color effects induced by the metal salts.

Sodium bisulfite would bleach strawberry puree but not stannous chloride treated puree. Table 1 gives the CDM *a* values for control and stannous chloride treated puree which was treated with sodium bisulfite and hydrogen peroxide. Only peroxide was effective in bleaching the stannous chloride treated sample; bisulfite had no effect. Bisulfite is a fairly specific bleaching agent for anthocyanins, its mechanism being the simple addition of bisulfite to the anthocyanin carbonium ion to form a colorless sulfonic acid addition compound (Jurd, 1964). Our findings would suggest that the color of the stannous chloride treated puree was not due to a compound which was an oxonium salt.

We were interested in seeing what effect stannous chloride would have on strawberry juice subjected to the same experimental conditions as the puree samples. Addition of stannous chloride to the juice precipitated red-colored material which could be separated by centrifuging. The stannous precipitate suspended in pH 3.4 citrate buffer was not bleached by HSO_3^- but could be bleached with H_2O_2. The visible absorption spectrum of the stannous filtrate was indistinguishable from the spectrum of the control strawberry juice sample. Figure 5 gives the visible absorption spectrum of freshly prepared strawberry juice and juice containing 133 ppm HSO_3^- and 6% H_2O_2. The stannous-treated filtrate was also bleached by both bisulfite and H_2O_2, the spectra being identical to that of the juice samples. The spectra of strawberry juice and the stannous filtrate after 5 days at 50°C are shown in Figure 6. The juice was browner in color than the stannous-treated sample; this is evidenced by the greater absorption in the region of 350–500 nm in the juice sample. Precipitation with stannous chloride apparently removed potent browning compounds but left the anthocyanins in solution. Table 2 lists the total anthocyanin content of the control juice and the $SnCl_2$ treated juice throughout the experiment. Differences between the two treatments are of a small order of magnitude. The data reported are for one experiment; however, additional experiments carried out under slightly different conditions gave similar results.

Strawberry anthocyanins reportedly do not contain a complex-forming group (Heintze, 1960). Cyanidin-3-glucoside, the minor constituent of strawberries, contains ortho phenolic groups and will chelate metal ions; however, its presence may not be great enough to affect the color of a product. In a previous study (Wrolstad et al., 1970) we found the main pigment (pelargonidin-3-glucoside) to vary from 72–95% in analyses of five varieties and 13 selections, Northwest containing 5% or less of the minor pig-

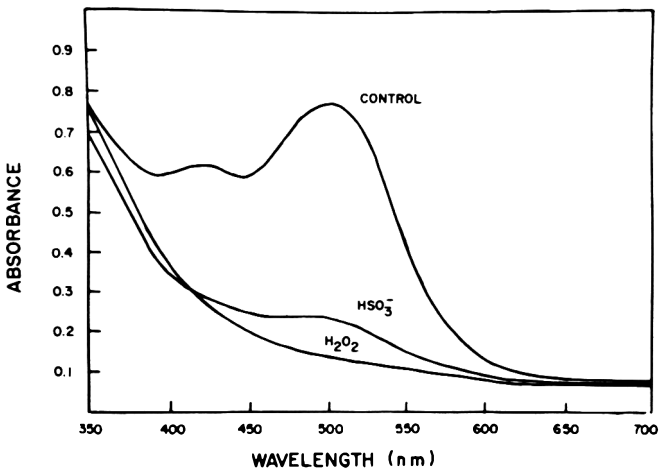

Fig. 5—Visible absorption spectra of freshly prepared strawberry juice and juice containing 133 ppm $NaHSO_3$ and 6% H_2O_2.

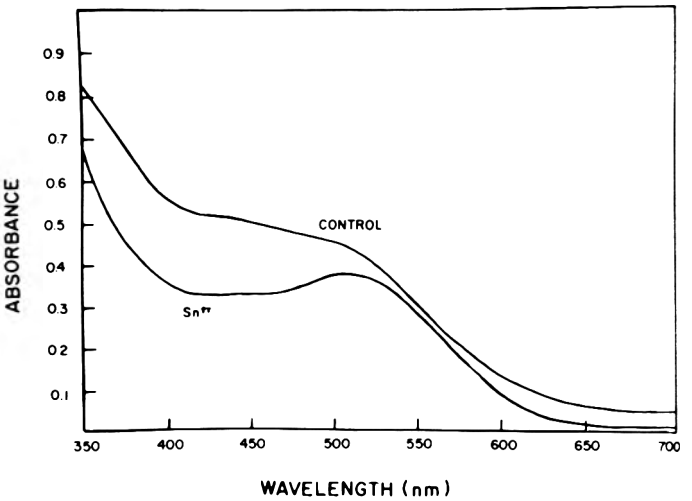

Fig. 6—Visible absorption spectra of strawberry juice and stannous chloride treated juice after 5 days at 50° C.

ment, cyanidin-3-glucoside. We prepared juice using OSU Agricultural Experiment Station selections, numbers 3109 and 3137, which contain substantial quantities of cyanidin-3-glucoside (20–28%). The thin-layer chromatogram (Figure 7) shows that cyanidin-3-glucoside (the spot with the low Rf in Sample 4) was present in extracts of this juice but not in the stannous chloride treated sample (Sample 3). A new red-colored compound with an Rf value slightly lower than pelargonidin-3-glucoside (the spot with the higher Rf in both samples) was present in the stannous chloride sample. Examination of this chromatogram under ultraviolet light showed the juice sample contained a blue fluorescent compound with an Rf value identical to the "new" red-colored compound present in the stannous treated juice. Spraying the plate with saturated stannous chloride did not convert the fluorescent spot to a visible, red-colored spot, however. Further characterization of this compound was not carried out.

Chandler and Clegg (1970a, b, c) in studying the pink discoloration in canned pears characterized a purple-pink insoluble complex which was heat-stable and could be bleached by hydrogen peroxide, but not by sodium bisulfite. The pigment was identified as a tin-cyanidin complex. They proposed that a conjugated anhydrobase which was formed from oxidation of leucoanthocyanin was the entity which formed the purple-pink chelate with tin. The phenomena occurring in our experiments might well be similar to that described by Chandler and Clegg. The two complexes are similar in regards to color, solubility, heat stability and reaction with hydrogen peroxide and SO_2. Co and Markakis (1968) have reported the presence of leucoanthocyanins of varying degrees of polymerization in strawberry fruit. These findings would suggest that strawberry leucoanthocyanins should be investigated regarding their ability to form colored complexes with metal ions.

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Chandler, B.V. and Clegg, K.M. 1970b. Pink

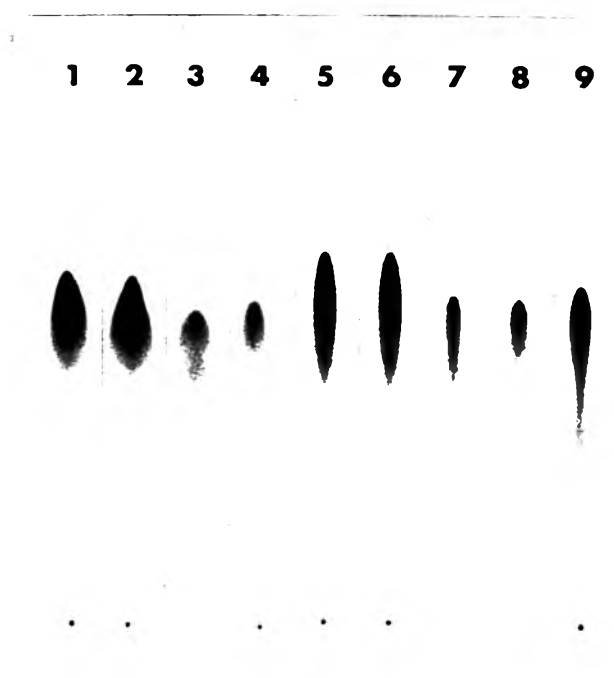

Fig. 7—Thin-layer chromatogram of anthocyanin extracts of strawberry juice and $SnCl_2$ treated juice. Sample identification from left to right: $SnCl_2$ treated juice (Northwest variety), Control juice (Northwest variety), $SnCl_2$ treated juice (Selections 3109 and 3137), control juice (Selections 3109 and 3137). Samples 5, 6, 7, and 8 are identical to Samples 1, 2, 3, and 4, respectively. Sample 9 is a heavier application of Sample 8.

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Ms received 7/20/72; revised 10/5/72; accepted 11/23/72.

Technical Paper No. 3387, Oregon Agricultural Experiment Station.

This research was supported by a Future Leader Grant from the Nutrition Foundation, Inc.

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ION EXCHANGE PURIFIED ANTHOCYANIN PIGMENTS AS A COLORANT FOR CRANBERRY JUICE COCKTAIL

INTRODUCTION

A PORTION of the annual crop of cranberries tends to be pale in color, producing a light colored cocktail which is not as appealing as full colored cocktail. In the past, pale berries were used for production of cranberry sauce and other products where color was not critical. As cranberry cocktail captures a larger share of the market for cranberry products, it may become necessary to utilize some of these light berries for cocktail. If it be necessary to add pigment to pale cranberry cocktail, the most acceptable material to use would be a natural pigment extracted from cranberries since in this case nothing is being added to the product which was not present originally. Such materials, of proven safety, could be used in many other products in addition to cranberries. The use of synthetic colors in foods is beginning to be questioned, and in the years to come, use of these materials may be restricted. It is important therefore, to develop alternative types of coloring agents suitable for addition to food products. The primary objective of this work was to study some properties of an anthocyanin pigment preparation derived from cranberries, and its effect on shelf life when added to cranberry cocktail.

Staples and Francis (1968) found that approximately 40% of the red anthocyanin pigments of cranberries remains in the press cake despite the double pressing currently used in the industry for juice extraction, which involves an initial pressing, a water leaching of the resulting press cake and a second pressing to remove additional juice. A method was developed (Chiriboga and Francis, 1970) for recovering this pigment by extracting with alcohol, transferring to an aqueous solution, adsorption of pigments on an ion exchange resin column, removal of water-soluble impurities by rinsing the column with distilled water and finally eluting the recovered pigments using ethanol. The pigment extract may be freeze dried to a powdered concentrate.

A number of workers have studied the toxicity of anthocyanins and related compounds and have concluded that these materials are harmless to health in the quantities ordinarily consumed. Horowitz and Gentili (1969) stated that the flavonoids are metabolized by the intestinal flora to carbon dioxide (from the A ring) and various aromatic acids (from the B ring), none of which can be considered harmful.

A number of other plant constituents may be extracted from pomace by alcohol, along with the desired pigments. Fuleki (1967) singled out sugars as the most plentiful and, for pigment analysis, the most troublesome impurity in pigment extracts. Tinsley and Bockian (1960) found that sugars tended to increase the degradation rate of the anthocyanin perlargonidin-3-glucoside. The presence of amino acids further accelerated the rate of pigment degradation. Sastry and Tisher (1952) observed that a tannin mixture extracted from the stems of grapes had a protective effect on anthocyanin pigments. Ough (1969) found that extractives from grape seeds may contain excessive quantities of tannin, resulting in off-aromas and flavors. Timberlake (1957) noted that while fruit juices normally contain small quantities of copper, zinc and iron, excessive quantities of certain metals may cause discoloration, oxidation, rancidity, turbidity, taste and odor changes, and impairment of nutritional values. The compounds, extractable by alcohol from cranberry press cake, which are likely to be of most concern for feedback to cranberry cocktail, are those which would contribute to off-flavors. This would include excessive quantities of tannins, phenolic acids and possibly proanthocyanins.

MATERIALS & METHODS

A SYSTEM was assembled for extracting and purifying a relatively large quantity of pigment from cranberry press cake for use in taste tests and other experimental work. The press cake was obtained from a commercial cranberry juice line (Ocean Spray Inc., Hanson, Mass). 250-ml aliquots of methanol containing 0.03% HCl were percolated through 600-g portions of press cake. The solvent was recirculated through the press cake for 20-min periods. At the end of each cycle the pigment-containing solvent was removed, fresh solvent was added and the process was repeated. During each extraction period, the rate of extraction was monitored by analyzing for total anthocyanin pigment at intervals of 1, 2, 3, 4, 5, 10, 15 and 20 min. Analyses were done using the simplified method developed by Fuleki and Francis (1968), involving measurement of optical density (O.D.) at pH 1.0 only, which required the removal of only 2 ml of solvent from the system for each analysis. The pigment solution was purified and concentrated using the method developed by Chiriboga and Francis (1970). The resulting purified pigment concentrate in ethanol was freeze dried to an easily handled powdered concentrate using a pilot plant model Repp freeze dryer. (Repp Industries, Inc., Gardiner, New York.). In order to freeze the alcoholic solution, it was necessary to bring the moisture content to 65% by adding water. When drying was complete, the vacuum was broken with nitrogen, to minimize oxidation of the pigment.

The total pigment from a single 600-g portion of press cake was extracted and purified,

Table 1—Anthocyanin content of experimental batches of cranberry juice cocktail prior to pasteurization

Sample (% light juice)	Initial anthocyanin content (mg/100 ml)	Crude pigment added (g/17 liters)	Final anthocyanin content (mg/100 ml)
0	13.8	–	13.8
5	9.8	1.0	10.5
10	8.9	2.0	10.3
15	12.0	3.1	13.7
30	8.6	6.45	13.8
60	6.4	8.7	13.7

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and total pigment determinations were performed at each step of the process, to determine losses.

A 6-month storage study was undertaken to test the stability of cranberry cocktail containing five levels of added anthocyanin pigment. Cranberry juice was extracted from 400 lb of dark and pale cranberries, to produce correspondingly dark and pale juices. Each batch comprised 17 liters made up of 4.42 liters of first press juice, 1.70 liters of second press juice and 10.88 liters of 15° Brix sugar-water solution. The second press juice was made by soaking the press cake from the first press in water, equivalent in volume to half the first press juice, for 30 min prior to pressing. The first press juice component was made by combining first press juice from the dark and light berries in varying proportions. The second press juice component was varied similarly in order to be able to make final juices by the above batch formula, yet containing varying proportions of light press juice. Batches of cranberry cocktail were prepared using 0, 5, 10, 15, 30 and 60% of light juice, to simulate the range of color likely to be encountered commercially. For example, the sample with 15% light juice contained 3.76 liters of first press dark juice, 0.66 liters of first press light juice, 1.45 liters of second press dark juice and 0.25 liters of second press light juice. The press juices were obtained from different lots of berries hence the initial pigment content is different (Table 1). Pigment was then added to the pale batches to bring the pigment content of each to the level of the control samples. Samples were flash pasteurized to a temperature of 190°F, filled hot into pint bottles, capped under a stream of nitrogen and cooled immediately. Samples were analyzed initially, and then weekly for 6 months.

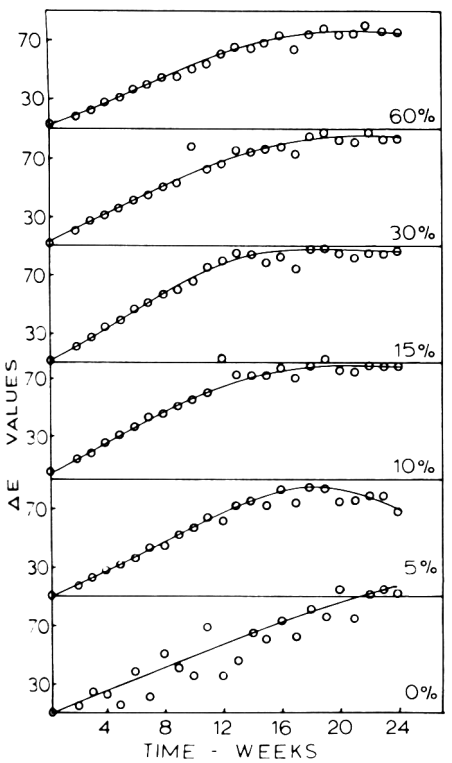

Fig. 1–Color changes, expressed as Δ E values, in pasteurized cranberry cocktail containing varying quantities of added pigment during a 6-month study. The percentages indicate the proportion of light colored juice. The curves in Figures 1, 2 and 3 represent the best fit by visual judgment.

Table 2–Extraction of pigment from 600g of cranberry pomace during successive extractions using methanol containing 0.03% HCl

Extraction no.	Pigment extracted (mg)	Pigment (% of total)
1	43.3	9.3
2	68.7	14.8
3	79.0	17.2
4	61.9	13.3
5	64.5	13.9
6	44.7	9.6
7	32.4	6.9
8	29.8	6.4
9-12	38.9	8.6

A General Electric Recording Spectrophotometer equipped with a Davidson and Hemmendinger Tristimulus Integrator was used to obtain transmission curves and XYZ values for each sample. These values were used to determine lightness (Y), a function of hue (X/Z), dominant wavelength (d), purity (Pe) and Δ E, a measure of color difference. We have found the X/Z ratio, actually the ratio of redness to blueness, to be a very convenient function of hue even though it is not related to Munsell hue or sanctioned by the C.I.E. The E values were calculated by the Friele, MacAdam, Chickering method (Chickering, 1967) on a CDC 3600 computer.

The total anthocyanin content and Degradation Index (D.I.) for pigments were determined using methods developed by Fuleki and Francis (1968a), while total flavonols were measured using methods developed by Lees and Francis (1971). The flavonol/anthocyanin ratio for each week was calculated by dividing the total flavonol content by the total anthocyanins.

The ion exchange resin (CG-50) used in this investigation tends to adsorb phenolic compounds other than anthocyanins. Samples of dry pigment powder were weighed out, dissolved in 50 ml of distilled water and analyzed for anthocyanins by the method of Fuleki and Francis (1968a), for flavonols by the method for tannins (AOAC 9.081 to 9.083, 1970) in wine and other beverages.

In order to determine whether addition of purified anthocyanin pigments have any adverse effects on the taste of cranberry cocktail, several sensory tests were conducted. A series of samples containing five different levels of added pigment, corresponding to the levels used in the storage study, plus a control sample, was prepared. Subjects were asked to rank the samples from "like best" to "like least" to see whether there was any consistent preference or dislike for samples containing added pigment. A simple preference test was also run, where the panelist was presented with two samples (a control and a sample containing a detectable level of added pigment) and asked to pick out the sample he preferred. Finally, the taste threshold for added pigment in cocktail was determined, using the method of Guadagni et al. (1971).

RESULTS & DISCUSSION

Extraction and purification of pigment

Measurement of cumulative pigment extraction for 12 extractions gave results as shown by Table 2. It was found that the greatest quantity of pigment was removed during the third extraction, with yields falling off sharply after the fifth. Extraction rates were calculated by dividing the total quantity of pigment extracted during each time interval by that time interval. It was found that, for each extraction period, rates declined until the third minute, after which the rates became constant. These results indicate that for maximum efficiency using this type of system, five extraction periods might be used, each approximately 3 min in length.

600g of cranberry press cake were processed to determine the quantity of dry pigment which could be produced. After the final freeze drying step 1221 mg of powdered pigment was recovered. This was sufficient to color slightly over 3 liters of colorless liquid to the color of cranberry cocktail, or approximately 30 liters of pale cocktail to full color.

At each step in the ion exchange purification process, total pigment analyses were run to determine the extent of pigment losses occurring at each stage of the process. A total of 26% of the pigment originally extracted was lost. Of this, 13% was lost during flash evaporation of methanol from the initial extract, 8.2% during the ion exchange purification step and 4.7% during the final freeze drying step. These losses could undoubtedly be reduced by using a full scale, more efficient system.

Analyses of the dried pigment powder showed 17% anthocyanins and 18% flavonols. The "tannin" content by the Folin-Denis reagent was 36%. This reagent is nonspecific and depends on reactions with phenolic groups. The anthocyanin and flavonol compounds would be included as tannins but probably slightly underestimated due to the calibration with tannic acid. At any rate, there was probably very little tannin other than the pigments in the crude powder. The remaining components of the crude extract were probably moisture, organic acids, carbohydrates and sugar. The pigment powder is very hygroscopic and unless protected, the moisture level will increase rapidly.

The extraction process was designed to partially purify and concentrate the anthocyanin pigments but the ion exchange process is not specific for anthocyanins. It also absorbs other phenolic compounds. To test the degree of purification, the pigments from a 500-g sam-

ple of press cake were extracted and passed through the ion exchange column. The eluted pigment solution from a fully loaded column, containing approximately 92% (1.7 mg) of the anthocyanins and 70% (1.0 mg) of the flavonols. Some of the flavonols were lost in the washing procedure. The eluate from the column was also analyzed for tannin content. The solution applied to the column contained approximately 6.7 mg of tannin of which 2.4 were found in the eluate. If we subtract 1.7 for anthocyanins and 1.0 for flavonols, the tannin content of the eluate other than pigments, is essentially zero. The ion exchange process effectively removed tannins, other than pigments, from the crude extract.

Storage study

Transmittance curves drawn by a G.E. Recording Spectrophotometer were substantially the same at equivalent times for the six samples throughout the 6-month storage study, with a minimum absorption peak at 420 nm and a maximum absorption peak at 520 nm. Over the 6-month storage period the minimum absorption peak shifted from 420 to about 443 nm for all samples, while the 520 nm peaks remained at the same position. The uniformity of the transmittance curves for the six experimental samples indicated that the added pigment was not causing different types of color changes in the product.

ΔE is a measure of the difference between two samples, computed from the X Y Z values for the samples. The control values for each set were the initial X Y Z values for each pigment's concentration at the start of the study, while ΔE values for each week indicate the extent of change from the initial color for each set, to determine whether samples with added pigment changed color at a different rate than the controls. It was found that the rate of color change was the same for all samples for the first 12 wk. After this time, the rate of color change seemed to level off slightly for the samples to which pigment had been added, as shown by Figure 1.

The Y values serve as a measure of lightness, increasing as samples become lighter. As shown by Figure 2, Y values appeared to increase at approximately the same rate for all six experimental packs indicating that, in any case, added pigment did not increase the rate of fading of color in the product.

The X/Z values, a function of hue, may be used as a measure of the tendency of a sample to become redder or bluer. As indicated by Figure 3, all samples tended to become bluer, as shown by decreasing X/Z values, over the 6-month test period. The rate and extent of this color shift appeared to be the same for all samples including the control, indicating that pigment addition did not measurably increase the shift toward a bluer product. This shift, while measurable, was not very obvious because at the end of the storage study all of the samples still had the characteristic bright red color of cranberry cocktail.

The total anthocyanin pigment content of cranberry cocktail to which pigment had been added was checked weekly for 6 months. The anthocyanin pigments were found to degrade markedly with time, as shown by Table 3. The samples containing added pigment retained a larger percentage of their total pigment content at the end of the 6-month storage period. This increase in stability appeared to be related to the quantity of added pigment in the sample which may, due to the presence of flavonols or other materials, have a beneficial effect on the anthocyanin pigments.

The Degradation Index, a measure of the percentage of degraded pigment in a sample, rose in all samples with time, as would be expected, but it was observed that the D.I. for the sample with the largest quantity of added pigment rose only 18% while that of the control sample rose 28%, which further indicates that the addition of pigment may tend to retard the rate of anthocyanin degradation.

The flavonol pigments of cranberries are important as they may have a protective effect on the anthocyanins, and do make a slight contribution to the total color. Analysis for flavonols over the stor-

Table 3—Anthocyanin and flavonol content of cranberry juice cocktail with added pigment

Storage time (wk)	Sample (% light juice) 0		5		10		15		30		60	
	Anthocyanin (A) or flavonol (F) content (mg/100 ml)											
	A	F	A	F	A	F	A	F	A	F	A	F
0	11.2	7.0	10.0	7.6	9.3	7.8	12.0	8.6	12.4	9.8	12.5	10.6
4	9.3	6.2	7.8	6.8	8.0	7.8	9.4	8.1	10.0	9.3	10.6	10.5
8	9.0	6.3	6.5	6.8	6.0	7.6	7.2	7.6	8.0	8.4	8.5	9.3
16	6.0	6.0	4.5	6.6	4.3	7.3	5.1	7.3	5.8	8.3	6.5	9.0
24	3.4	6.2	3.4	7.1	3.2	7.8	3.9	7.9	4.1	8.8	5.0	9.2

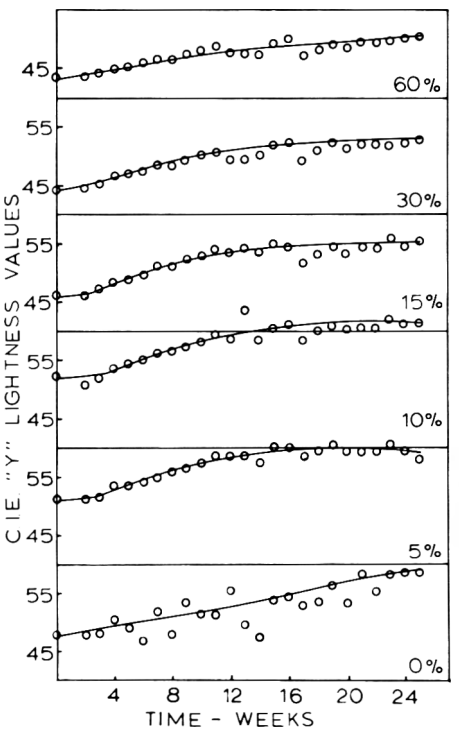

Fig. 2—Lightness, expressed as C.I.E. Y, of pasteurized cranberry cocktail containing varying quantities of added pigment during a 6-month study.

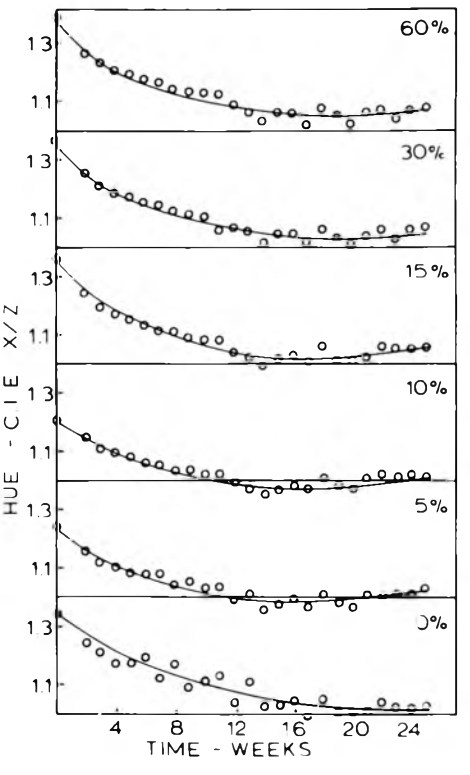

Fig. 3—Changes in hue (C.I.E. X/Z) of pasteurized cranberry cocktail with varying quantities of added pigment during a 6-month storage study.

Table 4–Responses to varying levels of added anthocyanin in cranberry cocktail using the triangle test

Pigment added (mg/100 ml)	Correct responses (%)
12	37.5
24	45.8
48	70.8

age period showed no marked decrease as was the case with the anthocyanins. There was no appreciable difference between cocktail to which pigment had been added and the control samples, but in all cases the flavonol levels declined an average of 9% from their initial values over the storage period. It was concluded that the flavonols appear to be more stable than the anthocyanins under the conditions of the experiment, and that the addition of anthocyanins to cranberry cocktail does not appear to affect the rate or extent of degradation of flavonols.

Flavonol/anthocyanin ratios were found to increase markedly during the storage period, reflecting the relative stability of the flavonols compared to the marked reduction in anthocyanin levels. An average increase of 195% was found and the rate of increase was nearly the same for the six experimental lots, except that the increase was slightly less in samples containing higher levels of added pigment, because of the increased stability of the anthocyanins.

Taste studies

Taste studies were run to determine whether addition of pigments had a detectable effect on flavor and if so, whether the flavor was objectionable. In order to test whether people tended to like or dislike the pigment flavor, six samples were presented to each panelist, including a control and five different levels of added pigment which were the same as used in the storage study. The 23 panelists were asked to rank the samples from like best to like least. Nearly all the panelists stated that they found it very difficult to distinguish between the samples. Statistical analysis indicated that the panelists as a group were not able to distinguish between any of the samples. A preference test was run involving two samples, a control and a sample with a detectable level of pigment added. 42 persons were tested and 22, or 52%, preferred the sample with added pigment. The taste was described as richer or fruitier. This experiment indicated that, while added cranberry pigment may impart a slight taste to cocktail, depending on the level used, this taste may be beneficial, improving the acceptability of the product, and, in any case, does not appear to be objectionable when used at low levels.

Taste threshold tests were conducted using a panel of six relatively expert tasters who had scored consistently well in previous tests with this material. Pigment levels of 100, 200 and 400 mg crude pigment per 30 oz of cranberry cocktail were used. At each level, 24 Triangle tests were run, consisting of four tests by each of the six panelists. The results are shown in Table 4. Using the method of Guadagni (1971) the taste threshold for the preparation of crude cranberry pigments was calculated as 29 mg crude pigment per 100 ml. This is a higher level than is likely to be used commercially.

CONCLUSIONS

IT WAS FOUND that addition of recovered anthocyanins to cranberry cocktail did not significantly affect the rate or extent of degradation of color. At moderate use levels, the flavor of cocktail was not adversely affected by pigment additions. It was observed that the rate of sediment formation in the bottled cocktail samples was slightly increased by addition of pigments, but this was not readily noticeable until about 6 months after processing, by which time the product would normally have been consumed. This problem may require further investigation by any potential user. In general, anthocyanin pigments recovered by the ion exchange process appear to be suitable for coloring cranberry products, and may have potential value for coloring a wide variety of other food products where a bright red color is required. More details of this work may be found in a thesis by the senior author (Chiriboga, 1972).

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Ms received 10/14/72; revised 1/9/73; accepted 1/15/73.

This research was supported by a grant from the Glass Container Manufacturers Inst., Inc., Wash., D.C. and Ocean Spray Cranberries, Hanson, Mass.

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CONTINUOUS DIFFUSION OF CHLOROGENIC ACID FROM SUNFLOWER KERNELS

INTRODUCTION

ALTHOUGH SUNFLOWER ranks second in importance as a world source of vegetable oil, the defatted meal has not been utilized as a protein source for human nutrition. Sunflower proteins are deficient in lysine but contain adequate levels of other essential amino acids and there are no toxic substances in the meal (Clandinin, 1958).

Sunflower proteins complex readily with chlorogenic acid in the meal to develop dark green or brown colors (Smith and Johnsen, 1948). Reflux extraction of sunflower meals with organic solvents will remove most of the chlorogenic acid but the extraction requires several hours and the proteins are severely denatured (Pomenta and Burns, 1971). A colorless protein isolate was prepared by treating the soluble sunflower proteins with sodium sulfite and isopropanol (Gheyasuddin et al., 1970). Improved procedures for the separation of chlorogenic acid from the proteins are essential if sunflower isolates, concentrates and flours are to have widespread applications in foods.

Recently, a process for the aqueous diffusion of chlorogenic, caffeic and quinic acids, and simple sugars, from whole kernels of dehulled sunflower has been developed (Sosulski et al., 1972). The process was based on the principle that low molecular weight substances such as polyphenolic acids, simple sugars, minerals and nonprotein nitrogen compounds will passively diffuse through the semipermeable membranes in the plant cells while large molecules such as triglycerides, protein, starch and crude fiber are retained in the kernel. After drying the diffused kernels and oil extraction, the defatted meal was found to contain 70% protein on a fat-free dry basis. At this protein level, the chlorogenic acid-free meal might compete favorably with soybean protein concentrate in processed foods since the product was entirely bland and colorless.

Because the diffusion process was very temperature dependent, Sosulski et al. (1972) employed six 1-hr extractions at 80°C for the nearly complete removal of chlorogenic acid from sunflower kernels. Due to the long extraction periods, the large volumes of water and high temperatures, the operational costs of the batch diffusion method would be prohibitive. In addition, the 80°C temperature caused excessive protein denaturation which was not desirable for some food applications. The objective of the present study was to develop a low temperature method for the continuous extraction of chlorogenic acid from sunflower kernels, utilizing a relatively low volume of water. During the course of this investigation, the extraction efficiency of testa-free kernels was found to be markedly greater than the intact kernel. A split kernel technique is proposed for rapid diffusion under low or high temperature conditions.

EXPERIMENTAL

THE PROCEDURE for the batch diffusion extraction of dehulled sunflower seeds, referred to as kernels in this report, has been described previously (Sosulski et al., 1972). 20-g samples of intact kernels (Commander variety) were subjected to six 1-hr extractions with 0.001N HCl at a solvent-to-kernel ratio of 20:1 in each extraction. The total volume of fresh solvent used for the six extractions was 2.4 liters. After each change of solvent, the extraction bottles were closed and placed in a shaking water bath operated at 60° or 80°C. The solvents were removed by filtration through cheesecloth and, after the last diffusion period, the extracted kernels were air dried in a 50°C oven for 2 hr, ground and defatted with Skelly F. The defatted meals were desolventized in a vacuum oven at 50°C for 3 hr.

Continuous diffusions were conducted in a 30 liter open vessel with an overflow outlet near the top and a tap water inlet near the base. The solvent-to-kernel ratio in the vessel was 20:1 and solvent flow rates were adjusted to give final ratios of 600:1 to 80:1. The pH of the solvent was maintained at 4.5 by additions of 1N HCl. Glass-coated immersion heaters maintained temperatures at 60°C or 80°C. Continuous stirring was employed during the extraction periods which varied between 1 and 6 hr. In the split kernel experiments, the testa could be skimmed from the solvent surface or later removed from the surfaces of the dried kernels by air aspiration.

Initially the testa on the dehulled seeds were removed by hand rubbing the kernels during the first 30 min of diffusion. A barley pearler was also employed to grind off the testa and outer portions of the kernels. In later experiments, the kernels were cut in two pieces manually and the testa floated free of the split kernels during circulation in the extraction vessel.

The chlorogenic acid content of the control and extracted meals were determined by refluxing the samples with absolute methanol for 5 hr (Smith and Johnsen, 1948). The phenolic acids in the methanol extracts were estimated as chlorogenic acid, according to the spectrophotometric method of Moores et al. (1948) using a standard curve of pure chlorogenic acid in methanol at the absorption maximum of 328 nm.

The proximate composition of the meals were determined by the AOAC (1970) procedures. The color and nitrogen solubility of control and diffused meals were determined at pH 10.

Table 1—Residual chlorogenic acid in sunflower meal (g/100g meal) after each hour of diffusion extraction of whole kernels by batch and continuous processes

Extraction method	Temp °C	Solvent: seed ratio	Duration of extraction, hr 0	1	2	3	4	5	6	Color of meal at pH 10
Batch – 6 solvent changes	80°	120:1	3.1	2.5	1.8	1.3	0.9	0.6	0.4	white
Continuous solvent flow	80°	400:1	3.1	2.3	1.5	1.0	0.5	–	–	light green
Batch – 6 solvent changes	60°	120:1	3.7	3.3	2.8	2.5	2.1	1.8	1.5	green
Continuous solvent flow	60°	120:1	3.7	2.9	2.2	1.9	1.6	1.4	1.3	green
Continuous solvent flow	60°	240:1	3.7	–	1.9	1.3	0.8	0.7	0.6	light green

RESULTS & DISCUSSION

Diffusion extraction of whole kernels

The batch method of diffusion extraction at 80°C for 6 hr with a solvent-to-kernel ratio of 120:1 was successful in reducing the chlorogenic acid level to 0.4g/100g meal (Table 1). At this level, the defatted meal was light in color and remained white when a meal slurry was adjusted to pH 10. In the continuous diffuser, the solvent flow rate was increased to 400:1 and, after only 4 hr at 80°C, the chlorogenic acid content of the meal was reduced to about the same level as in the batch method. While the defatted meal was again quite satisfactory in appearance, a greenish hue was evident when the meal was slurried and brought to pH 10. Repeated trials with even higher solvent flow rates indicated that the diffusion gradient between kernel and solvent was not the principal factor limiting the diffusion rate. It appeared that at least 4 hr of continuous diffusion was required to produce a stable light-colored meal.

At 60°C, the diffusion of chlorogenic acid from the intact sunflower kernels was even slower by batch and continuous extraction processes (Table 1). The sunflower kernels used in the 60°C experiment contained a slightly higher level of chlorogenic acid than the earlier sample but repeated trials with other sunflower samples confirmed that at least 6–8 hr of continuous diffusion was required to produce a satisfactory product.

By the end of the 6-hr treatment at 80°C, about one-half of the testa were floating free of the kernels. A color test on the testa-free kernels showed the presence of no color-forming substances while the kernels enclosed in the testa gave a typical green reaction at pH 10. This indicated that the testa were probably hindering the diffusion of chlorogenic acid from sunflower kernels. The testa did not detach from the kernel in the 60°C experiment and a portion was rubbed off by hand during the first 30 min of diffusion. After 4 hr, the testa-free kernels were found to contain only 0.2g chlorogenic acid/g meal.

Diffusion extraction of testa-free kernels

Quantitative analyses of the testa indicated that nearly all of the chlorogenic acid was present within the testa-free kernel. Treatment of cut kernels with alkali revealed that most of the pigment-forming compounds were concentrated in the outer portions of the kernel, just under the testa. A barley pearler was employed to scrape off the testa and 10–20% of the outer kernel parts. The testa-free pieces of inner kernel were found to contain only 2.0–2.2g of chlorogenic acid as compared to the original 3.1g/100g meal. Continuous extraction of the pearled kernels at 80°C and a high solvent flow rate gave a low chlorogenic acid product within 60–90 min (Table 2). The extraction was also made at 60°C but only one-half of the sample of chlorogenic acid diffused into the solvent during the first 90 min. While a satisfactory product could be obtained quite rapidly by the 80°C procedure, the solids losses were too high for commercial use.

Kernels were then cut into two pieces before continuous diffusion at 80°C and 60°C with 120 volumes of solvent (Table 2). On contact with water, the testa separated immediately from the cut kernels and floated to the surface where a large proportion of the seed coat could be skimmed off. Diffusion from these smaller kernel pieces appeared to be quite rapid and very low chlorogenic acid levels were obtained within 1 hr of diffusion at 80°C or 2 hr at 60°C. Reduced rates of solvent flow have been used without serious reduction in diffusion rates. Apparently, testa removal and temperature were more critical factors for rapid diffusion of chlorogenic acid than the rate of solvent flow.

While the present procedure for splitting the kernels involved cutting by hand, there are several commercial types of grain cutters on the market which could be modified for sunflower kernels. A grain cutter which produces little or no fines is essential because these would be lost during diffusion extraction.

Composition of extracted kernels and meals

In the control samples, the yields of oil and meal from the dehulled kernels averaged 53 and 45g/100g kernel, respectively, with invisible losses being about 2g (Table 3). While the yields of oil remained about the same, the extracted meal yields were reduced in proportion to the diffused solids. Losses of diffused solids from intact kernels were less than from cut kernels and the yields of meal in the latter experiments were reduced fur-

Table 2–Residual chlorogenic acid in sunflower meal (g/100g meal) after each 30 min of continuous diffusion of testa-free kernels

Kernel treatment	Temp °C	Solvent: seed ratio	Chlorogenic acid in meal	Residual chlorogenic acid after diffusion for				Color of meal at pH 10
				30	60	90	120 min	
Pearled kernel	80°	400:1	2.0	1.3	0.6	0.3	–	white
Pearled kernel	60°	400:1	2.2	2.0	1.9	1.0	–	green
Cut kernel	80°	120:1	3.1	–	0.3	–	–	white
Cut kernel	60°	120:1	3.1	–	–	–	0.4	white
Cut kernel	80°	80:1	3.1	–	0.3	–	–	white

Table 3–Effect of diffusion extraction procedures on the yield of products and composition of the meal, dry basis

Extraction method	Temperature °C	Duration of extraction (hr)	Yield (g/100g kernel)			Composition of the meal, %			Solubility of meal proteins, %
			Oil	Meal	Losses	Protein	Fiber	Ash	
Intact kernel experiments									
Nondiffused kernels	–	–	53	45	2	56.7	5.7	8.4	87
Batch	80°C	6	52	34	13	65.4	8.2	6.3	38
Batch	60°C	6	53	36	11	63.4	6.5	6.6	61
Cut kernel experiments									
Continuous	80°C	1	52	30	14	71.1	5.1	5.4	48
Continuous	60°C	2	53	30	14	69.7	5.2	5.4	86
Testa	–	–	–	3	–	16.9	28.0	2.8	–

ther by the removal of testa during or after diffusion. The testa constituted about 3% of the kernel weight.

Since the diffused solids were primarily nonprotein meal constituents (Sosulski et al., 1972), the protein contents of the extracted meals were enhanced while ash content decreased (Table 3). Unfortunately, the crude fiber levels in the extracted meals were also enriched in the intact kernel experiments. The separation of testa in the cut kernel experiments served to improve the composition of the extracted meals. The testa contained 9% ether extract, 17% protein and 28% fiber. The protein contents of these extracted meals increased to 70% or higher while fiber and ash levels were reduced to 5%.

During the longer batch extraction procedures the 60° and 80°C temperatures reduced the protein solubility from 87 to 61 and 38%, respectively (Table 3). The shorter continuous extraction periods for the cut kernels caused less denaturation of the meal proteins. The 60°C treated meal gave protein dispersibilities which were similar to the control meal.

It appeared that the separation of the testa from the kernel was essential for the rapid diffusion of chlorogenic acid from sunflower. Fortunately, the testa separated completely from the cut kernels when immersed in the aqueous solvent. Although the testa constituted only 3% of the kernel weight, its removal enhanced the protein content and reduced the fiber level in the meal.

Temperatures of 80°C or higher are required for rapid diffusion of chlorogenic acid from the cut kernels. Although the proteins are partially denatured, the sunflower protein concentrate could compete directly with soybean protein concentrate in the manufacture of ground meat products.

It is feasible to produce an undenatured sunflower protein concentrate by diffusion at 60°C or less. This protein product would have major applications where the functional property of high dispersibility is desired.

The diffusion process utilized large quantities of water, and the dilute solution of sugars, chlorogenic acid, soluble minerals, etc., would represent a serious problem in waste disposal and pollution control. The soluble constituents would have a high biological oxygen demand in a sewage treatment system. The process water could be concentrated by evaporation to about 50% dry basis for utilization in livestock feeds or as a substrate in fermentations. Recycling of the process water should reduce the requirement for fresh water, and molecular seiving, ultrafiltration and reverse osmosis are alternative methods for concentration of diffused solids. The total volume of waste water is determined by the solvent-to-kernel ratio and a ratio of 30:1 is presently being investigated in a counter-current diffusion system. Further studies on the utilization of the diffused solids in food, feed and industrial applications are required.

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Ms received 9/16/72; revised 11/18/72; accepted 11/20/72.

The research project was supported by grants from the Hantelman Agricultural Research Fund and the National Research Council of Canada. The authors are indebted to Mr. H. Braitenbach and Mrs. D. Knapp for technical assistance in conducting this investigation.

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METHIONINE SUPPLEMENTATION OF SOY MILK TO CORRECT CYSTINE LOSS RESULTING FROM AN ALKALINE SOAKING PROCEDURE

INTRODUCTION

THE STUDY of the effects of alkaline pH on protein quality have for the most part been limited to corn protein. Bressani et al. (1958) have reported the effects of lime (CaO) and heat during the production of tortillas on the nutritive value of zein. Of the essential amino acids they found decreases in leucine, cystine, histidine and lysine. DeGroot and Slump (1969) studied the effects of severe alkaline treatment on isolated soy protein and found significant changes in the amino acid levels and the net protein utilization as well as the formation of the amino acid derivative lysinoalanine. More severe treatment caused a decrease in digestibility.

In an earlier paper Badenhop and Hackler (1970) reported some of the effects of soaking soybeans in a sodium hydroxide solution as a pretreatment step in the preparation of soy milk. The results of that study indicated that an alkaline treatment with 0.05N NaOH produced a soy milk with a pH of 7.4 which had a significantly better flavor. A further increase in pH resulted in a poorer flavor, however. Other effects of alkaline pretreatment included an increase in available niacin, reduced trypsin inhibitor activity, reduced cystine content and decreased nutritive value as indicated by protein efficiency ratio measurements. The present investigation was undertaken to better define the effects of an alkaline soaking treatment on the nutritional value of the protein.

EXPERIMENTAL

FOUR SOY milk samples were prepared using the procedure outlined in the previous report (Badenhop and Hackler, 1970). Soaking solutions of 0.05N, 0.075N and 0.100N NaOH were prepared and the soybeans soaked for 2 hr at 50°C. A sample was also prepared using distilled water as a soaking medium. The fluid milk samples were divided into two lots one of which was filled into #10 cans and heat processed for 10 min at 121°C. All samples were then freeze-dried for chemical analyses and incorporation into dry test diets.

The dried samples were analyzed for protein (N × 6.25) by a slight modification of the micro-Kjeldahl procedure as outlined in AOAC (1940). Samples were prepared for amino acid analysis by acid hydrolysis of the protein using 6N HCl at 110°C for 22 hr. The hydrolysates were filtered, dried and taken up in pH 2.2 sodium citrate buffer. Amino acid composition of the samples was determined by ion-exchange column chromatography using a Technicon Amino Acid Autoanalyzer equipped with four columns containing type C-2 resin. Cystine was analyzed by conversion to cysteic acid using the method of Moore (1963) followed by chromatography. Tryptophan was determined using the method of Horn and Jones (1945).

Protein efficiency ratios (PER) were determined in growth studies using male weanling rats of the Holtzman strain. The soy milk supplements were used as the sole protein source in the test diets at a level of 10% crude protein. After the dietary ingredients were thoroughly mixed they were divided into two lots, one of which was fed as is while the other was supplemented with 0.35% of L-methionine. The diets were fed to groups of rats ad libitum for 28 days. The PER values obtained on the experimental diets were adjusted based on a control diet containing 10% casein. Other details of the experimental methods used in the rat growth studies have been reported previously (Hackler et al., 1963).

Trypsin inhibitor measurements were obtained using the procedure of Learmonth (1952) as modified by Van Buren et al. (1964). This method compares the retardation of gelatin liquefaction by the enzyme trypsin in the presence of various dilutions of the sample. Results are expressed as the percent of the activity present in the non heat-treated soybeans.

RESULTS & DISCUSSION

A PREVIOUS REPORT of the effects of alkali-soak treatment by Badenhop and Hackler (1970) indicated that the only amino acid which was significantly affected was cystine. The present study was concerned with establishing more clearly whether the availability of any of the essential amino acids is affected by the alkaline treatment.

Amino acid analyses

The results of essential amino acid analyses (Table 1) confirm that the amino acid most affected by alkaline conditions is cystine. Cystine was the only amino acid observed to decrease with increasing pH in both the raw and heat-processed soy milk samples. (The terms raw or unprocessed milk is used interchangeably to refer to soy milk not processed at 121°C.) The raw soy milk prepared at pH 8.97 contained 15% less cystine than the pH 6.50 soy milk. In the heat-processed samples the decrease was 25% at pH 8.97. A decrease was also noted in tryptophan (14%).

The amino acid, threonine, appears to increase slightly in soy milk prepared from beans soaked in NaOH (Table 1). Peptide bonds containing the amino groups of threonine have been found to be quite labile (Hill, 1965). For this reason one would expect to obtain complete release of threonine during the 22 hr hydrolysis procedure employed in the

Table 1—Essential amino acid composition of soy milk as influenced by an alkaline soaking procedure and heat processing for 10 min at 121°C

	10 min at 121°C				No heat treatment			
pH	6.50	7.21	7.81	8.97	6.50	7.21	7.81	8.97
Amino acid	(g/16g of nitrogen)							
Histidine	2.37	2.40	2.48	2.37	2.36	2.37	2.47	2.40
Lysine	6.69	6.75	6.65	6.70	6.79	6.90	6.84	6.78
Phenylalanine	5.08	5.02	4.98	4.92	5.01	5.05	5.00	5.02
Tyrosine	3.79	3.73	3.86	3.71	3.86	3.85	4.06	3.92
Tryptophan	1.95	1.92	1.89	1.70	1.79	1.81	1.91	1.84
Methionine	1.25	1.25	1.23	1.20	1.26	1.29	1.21	1.25
Cystine	1.50	1.51	1.39	1.13	1.70	1.63	1.64	1.45
Threonine	3.89	3.83	4.06	4.08	3.95	4.01	4.35	4.44
Leucine	7.85	7.93	7.91	7.90	7.88	7.91	7.92	7.99
Isoleucine	4.65	4.69	4.75	4.75	4.71	4.77	4.77	4.77
Valine	4.73	4.63	4.74	4.85	4.80	4.82	4.98	4.93

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preparation of the samples for analysis. However, threonine destruction during acid hydrolysis to the extent of 5–10% has also been reported (Rees, 1946). The soy milk data appear to suggest that the presence of a small quantity of NaOH has a protective effect on threonine. However, when gelatin and an amino acid standard were treated with alkali to confirm or deny the possible protective effect of alkali on threonine, no protective effect was observed (Table 2). Thus the observed increase in Table 1 must be an anomaly.

Results of the trypsin inhibitor measurements (Table 3) show that for the processed samples a heat process of 10 min at 121°C in #10 cans is sufficient to destroy at least 96% of the original trypsin inhibitor activity. In the raw milk samples the effect of pH on trypsin inhibitor is seen in that the activity dropped with each increase in pH. The activity present in the pH 8.97 sample was approximately one-half of that in the pH 6.50 sample.

Animal feeding study

Table 4 shows the relationship of the adjusted PER values from the rat feeding experiment to the pH of the fluid soy milk, as well as other growth data. The samples which were not supplemented with methionine confirm the results obtained in the earlier study (Badenhop and Hackler, 1970) in that the PER of the heat-processed, unsupplemented samples decrease with increasing pH. This decrease in nutritive value is in agreement with the results of amino acid measurements which have already been discussed. These two methods of nutritional evaluation indicate quite clearly that the additive effects of high pH and temperature quickly reduce the nutritive value of soy protein due mainly to cystine destruction. The raw soy milk samples were observed to improve in nutritive value with increasing pH which can be directly attributed to the increased lability of trypsin inhibitor at elevated pH (Table 3).

The effect of methionine supplementation is quite apparent from an examination of Table 4. The adjusted PER values obtained from the heat-processed samples were quite consistent except for the pH 8.97 sample. Increases in adjusted PER values of 28, 32, and 27% (10 min at 121°C vs. 10 min at 121°C + 0.35% methionine) were obtained for the pH 6.50, 7.21 and 7.81 samples, respectively. For the pH 8.97 sample, however, the increase in adjusted PER was 92%. This dramatic increase at pH 8.97 indicates that high temperature and pH may cause both a destruction and decrease in availability of sulfur amino acids. The raw milk samples were significantly improved by methionine supplementation at each pH. The percentage increase was unaffected by pH. The observed increases in adjusted PER due to addition of methionine to the non heat-treated samples were 54, 53, 49 and 52% for the pH 6.50, 7.21, 7.81 and 8.97 soy milks, respectively. The fact that the relative improvement was independent of pH is particularly interesting in view of the observation that the trypsin inhibitor activity is inversely related to pH (Table 3). This would indicate that the level of residual trypsin inhibitor activity has no affect on the relative improvement due to methionine supplementation.

The effect of trypsin inhibitor on cystine and methionine metabolism has been studied by Barnes and Kwong (1965). It appears from their work that an increase in the conversion of methionine to cystine is brought about by trypsin inhibitor. Therefore the apparent effect of methionine supplementation on the PER values observed for the raw sam-

Table 2—Effect of various pretreatments on threonine determined by acid hydrolysis

Gelatin	Threonine g/16g N
No pretreatment	1.66
pH ≈ 7	1.68
pH ≈ 9	1.64
Amino acid standard	**μM**
No pretreatment	0.72[a]
pH ≈ 9	0.72

[a]The sample hydrolyzed (amino acid standard) contained 0.8 μM of threonine; therefore, 90% of the threonine was recovered.

Table 3—Trypsin inhibitor activity of soy milk samples

pH	Trypsin inhibitor activity (%)[b]
6.50[a]	2.5
7.21[a]	4.4
7.81[a]	2.3
8.97[a]	2.2
6.50	50.0
7.21	42.2
7.81	36.7
8.97	26.7

[a]These samples were heat processed for 10 min at 121°C.

[b]Activity is expressed as the percent of that present in untreated raw beans.

Table 4—The effect of an alkaline soaking procedure and supplemental methionine on protein utilization by weanling rats[a]

pH	CP (%)	ADG (g)	ADF (g)	PER	Adjusted PER[b]
			No heat treatment		
6.50	10.00	1.51	9.35	1.62	1.44(.04)[a]
7.21	9.56	1.57	9.23	1.77	1.58(.08)[ab]
7.81	10.25	2.18	11.00	1.92	1.72(.09)[b]
8.97	10.38	2.10	10.26	1.96	1.75(.06)[b]
			No heat treatment + 0.35% L-methionine		
6.50	9.88	2.58	10.40	2.48	2.22(.07)[a]
7.21	9.81	3.13	11.79	2.69	2.41(.09)[ab]
7.81	9.81	3.55	12.59	2.86	2.56(.07)[b]
8.97	10.00	3.99	13.42	2.97	2.66(.08)[b]
			Processed 10 min at 121°C		
6.50	10.13	2.96	11.65	2.50	2.23(.06)[a]
7.21	10.13	2.64	11.30	2.31	2.06(.04)[a]
7.81	10.00	2.78	11.97	2.31	2.07(.03)[a]
8.97	10.13	1.45	8.53	1.65	1.47(.07)[b]
			Processed 10 min at 121°C + 0.35% L-methionine		
6.50	10.13	4.66	14.32	3.20	2.86(.04)[a]
7.21	10.25	4.13	13.20	3.04	2.72(.05)[a]
7.81	10.00	3.73	12.62	2.93	2.62(.06)[a]
8.97	9.81	4.17	13.45	3.16	2.82(.04)[a]

[a]Values within parentheses are the standard errors. CP = crude protein; ADG = average daily gain; ADF = average daily feed; and PER = protein efficiency ratio. Casein control diet: CP = 9.88; ADG = 3.53; ADF = 12.74; PER = 2.80. All adjusted PER values were corrected to the casein control diet = 2.50.

[b]Adjusted PER values within each grouping of four containing the same superscript are not statistically different at the 1% probability level as determined by Duncan's Multiple Range Test (Duncan, 1955).

ples of this study is probably the result of an interaction between the cystine content, total sulfur amino acid requirement and the trypsin inhibitor activity of the samples. Increasing the pH resulted in both a lower cystine content and lower trypsin inhibitor activity. Under these conditions an increase in PER would occur as a result of the lower trypsin inhibitor activity but since cystine is also limiting, the extent of the increase in PER will be diminished. While methionine supplementation should correct a cystine deficiency, it should be noted that the level of supplementation (0.35%) combined with the methionine and cystine already present results in a level of 0.65% or less for methionine plus cystine. This is more than the required level as found by Rama Rao et al. (1959) but only equal to or slightly less than the egg value used by Mitchell (1954) to calculate the essential amino acid index. If the metabolic conversion of methionine to cystine is affected by trypsin inhibitor activity as Barnes and Kwong (1965) have suggested, then in the lower pH samples, where the trypsin inhibitor activity is higher, more cystine would become available. The increased cystine level would tend to overcome part of the depressive effect of trypsin inhibitor and result in a compromise PER value. At higher pH the depressive effect of trypsin inhibitor is less, but the amount of available cystine is also less due both to cystine destruction at high pH and the decrease in conversion of methionine in the absence of trypsin inhibitor. Again a compromise PER would result.

In conclusion, it would appear that methionine supplementation is an effective means of improving the nutritive value of alkali-treated, heat-processed soy milk. Methionine supplementation also improved the nutritive value of raw soy milk. In no case, however, did supplementation of the raw milk make it equal in nutritive value to the supplemented, heat-processed soy milk. This indicates that methionine supplementation alone is not sufficient and that in order to obtain the most nutritious product both heat processing to destroy trypsin inhibitor and supplementation to correct cystine deficiency are required.

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Ms received 10/29/72; revised 12/15/72; accepted 12/19/72.

Journal Paper No. 1986, New York State Agricultural Experiment Station, Dept. of Food Science & Technology, Cornell University, Geneva, NY 14456. This research was supported in part by the Agency for International Development, Contract No. AID/csd-1815.

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GROWTH AND ENTEROTOXIN PRODUCTION BY VARIOUS STRAINS OF Staphylococcus aureus IN SELECTED FOODS

INTRODUCTION

NUMEROUS FOODS have been implicated as sources of enterotoxin produced by *Staphylococcus aureus* in outbreaks of food poisoning as indicated by Angelotti (1969) and Jay (1970). Four immunologically distinct staphylococcal enterotoxins (A–D) have been identified, with enterotoxin A being involved in about 75% of the cases of staphylococcal food poisoning according to Casman et al. (1967). Read and Bradshaw (1966) demonstrated that enterotoxin is heat stable and therefore is not inactivated by normal cooking.

The presence or absence of other microorganisms in a food may have a major effect on enterotoxin production. Zehren and Zehren (1968) reported that enterotoxin in cheese is usually associated with lack of good growth of the normal lactic culture. Peterson et al. (1962) found competitive organisms suppressed the growth of *S. aureus* at temperatures which permitted chicken pot pies to defrost.

In the presence of competitive microorganisms McCoy and Faber (1966) noted that good growth of *S. aureus* sometimes occurred without detectable amounts of enterotoxin being formed. Genigeorgis et al. (1971a, b) found that all meat samples which contained enterotoxin also contained high populations of *S. aureus*, but enterotoxin was not detectable in some samples which contained high populations.

The purpose of this investigation was to determine the potential for growth and enterotoxin production by *S. aureus* when inoculated into several foods.

MATERIALS & METHODS

Cultures

S. aureus strains 265, 243, 493 and 315 which produce enterotoxins A, B, C and D, respectively, were obtained from the late Dr. E.P. Casman of the Food & Drug Administration Microbiology Laboratory in Washington, D.C. Each strain produces only one enterotoxin. The lyophilized cultures were grown in BHI broth at 37°C. Sufficient inoculum of cultures in the stationary growth phase (incubated 12–24 hr) was mixed into the food with a Waring Blendor to yield an *S. aureus* population of about 10^5 cells/g.

Media

Mannitol Salt Agar (MSA) and Plate Count Agar (PCA) were used for enumerating organisms. Brain Heart Infusion (BHI) broth was used for growing the cultures used for inoculation.

Food

Commercial foods purchased in local markets or delicatessens were inoculated with *S. aureus* and examined at intervals for cell numbers and presence of enterotoxin. Canned vanilla pudding was used as a substrate for an intensive examination of growth and toxin production by the four strains of *S. aureus* at 19, 26, 37 and 45°C for as long as 120 hr. The frozen foods were defrosted at 5°C. All foods were placed in sterile, covered glass beakers, tempered to the incubation temperature before inoculation, and incubated without agitation in a constant temperature chamber. At selected times a portion of the food was removed aseptically to determine population and pH. Samples for enterotoxin analyses were collected at the conclusion of the incubation periods which varied from 48–119 hr; thus the time required to produce a measurable amount of enterotoxin was not determined, except in vanilla pudding which was subjected to a more detailed investigation. The portion used for determining enterotoxin was stored at −30°C until extraction could be performed.

Determination of pH

A Beckman pH meter (Model 1019) with a Corning single electrode was used to measure the pH.

Enumeration of bacteria

The samples were diluted as necessary in buffered distilled water with the first dilution made by weight and subsequent dilutions made by volume. To enumerate the staphylococci a 0.1 ml volume of the diluted sample was spread evenly on a pre-poured MSA plate with a sterile, bent glass rod. The plates were incubated about 36 hr at 37°C, and typical yellow pigmented, coagulase positive *S. aureus* colonies were counted.

The total bacterial population of the food before and after inoculation was determined by use of the pour plate technique using PCA. The plates were incubated at 37°C for 36 hr and all colonies were counted. This temperature was selected because *S. aureus* cells were an important component of the total population.

Enterotoxin extraction and determination

The enterotoxins were extracted from the foods and examined by the microslide gel-diffusion methods of Casman and Bennett (1965) and Casman (1967). Extracts with large amounts of starchy material could not be assayed accurately without precipitation of the starchy material by freezing.

RESULTS & DISCUSSION

BEFORE INOCULATION most of the foods used in this work contained less than 100 organisms/g and some contained less than 10/g. The *S. aureus* populations and total counts were determined every few hours throughout the incubation period but in the interest of brevity only the initial and terminal counts are included. Samples discarded because they contained no measurable enterotoxin after incubation for less than 119 hr were either spoiled, as indicated by high total counts in the pot pies, or had insufficient *S. aureus* populations to permit toxin production (Table 1). After inoculation and during incubation for 48–71 hr the *S. aureus* population decreased abruptly in all foods which had an initial pH within the range of 3.24–4.81 (Table 1); therefore, the samples were not examined for enterotoxin. Only slight changes in pH occurred and the total population at the end of incubation was insufficient to cause physical or organoleptic changes. Several frozen pot pies with an initial pH of 5.56–6.10 had terminal *S. aureus* counts ranging from 10^7 to 4.2×10^8/g after incubation for 96 hr. The terminal total counts varied from 2.6×10^8 to 2.0×10^9/g. The pH of all of the pot pies decreased during incubation. Since enterotoxin was not detected in any of the pot pies, it is apparent that good growth of enterotoxigenic *S. aureus* may occur without production of enterotoxin.

Several inoculated foods in the pH range of 5.51–6.60 with initial *S. aureus* counts of 10^2/g and initial total counts not exceeding 3×10^2/g supported substantial growth of *S. aureus* (Table 2). The terminal *S. aureus* counts after incubation for 68–119 hr varied from 1.7×10^7 to 3.9×10^9/g and the terminal total counts varied from 7.9×10^7 to 4.3×10^9/g. The decrease in pH ranged

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from 0.07–0.63 unit and is attributed to the lipolytic activity and the carbohydrate fermenting ability of *S. aureus*.

The data in Table 3 show the time in hours required to produce detectable amounts of enterotoxins A, B, C and D in canned vanilla pudding inoculated with the corresponding strains of *S. aureus* and incubated at 19, 26, 37 and 45°C. The populations associated with enterotoxin production tended to be lower at 45°C than at the other temperatures and strain 265 tended to produce measurable enterotoxin (Type A) at lower populations than the other strains. The initial total bacterial content of the pudding was less than 10 cells/g and was about 10^5 cells/g after inoculation. There was little variation between counts on PCA and MSA. The initial pH of the pudding was 6.37. When enterotoxin was first detected the populations of *S. aureus* in the samples varied from $0.7–41 \times 10^7$ cells/g and the pH varied from 5.56 with strain 493 incubated at 45°C, to 6.45 with strain 243 incubated at 19°C. Little correlation between enterotoxin production, population and pH was observed from 19–45°C. The incubation times needed for each of the four strains to produce detectable amounts of enterotoxin in vanilla pudding were similar at 19°C and at 37°C. At 45°C strains 265 and 315 produced measurable toxin in less time (24–31 hr) than strains 243 and 493 which required 48–72 hr.

The microslide gel-diffusion assay used in this investigation detects enterotoxin concentrations of about 1 μg/g of food according to Casman and Bennett (1965). Raj and Bergdoll (1969) suggest that about 20 μg of enterotoxin B, and considerably less enterotoxin A, may cause food poisoning symptoms in man although individual susceptibility is variable. Obviously an enterotoxin concentration of 1 μg/g in food is a potential health hazard.

Since enterotoxin was not detected in the pot pies in which good growth of *S. aureus* occurred (Table 1), it is apparent that growth of enterotoxigenic *S. aureus* may occur without production of enterotoxin. There was no apparent direct relationship between the kind of food and enterotoxin production, but some relationship existed between pH and entero-

Table 1—Commercial foods in which no enterotoxin was produced after inoculation with 10^5 cells per gram of S. aureus strains 265, 243, 315 and 493 and incubated at 21°C

Type of food	Initial pH (range)	Counts before inoculation (range)		Incubation time (hr)	Terminal pH (range)	Terminal counts (range)	
		S. aureus/g	Total/g			S. aureus/g	Total/g
Donut and jelly pastry cream fillings, salad dressing and creamed herring	3.24–3.90	< 10–50	$< 10–7.5 \times 10^2$	48–70	3.23–3.84	$<10–3.6 \times 10^3$	$10^2–3.8 \times 10^3$
Bavarian cream pastry filling and ham salad	4.75–4.81	< 10	$<10–6.2 \times 10^3$	48–71	4.75–4.80	$10–1.6 \times 10^5$	$10^2–5 \times 10^5$
Frozen beef, chicken, tuna and turkey pot pies and canned beef stew	5.56–6.10	$< 10^2 – >3 \times 10^4$	$< 10^2 –> 3 \times 10^5$	96	3.84–5.73	$10^7–4.2 \times 10^8$	$2.6 \times 10^8 – 2.0 \times 10^9$

Table 2—Commercial foods in which enterotoxin was produced after inoculation with 10^5 cells per gram of various strains of S. aureus and incubated at 21°C

Type of food	Initial pH	Counts before inoculation		S. aureus strain added	Incubation time (hr)	Terminal pH	Terminal counts	
		S. aureus/g	Total/g				S. aureus/g	Total/g
Banana cream pie (frozen)	6.13	$<10^2$	1.1×10^2	265	68	5.96	2.0×10^8	2.4×10^8
Chocolate cream pie (frozen)	5.91	$<10^2$	$<10^2$	265	68	5.46	4.3×10^7	1.1×10^8
Coconut cream pie (frozen)	6.60	$<10^2$	$<10^2$	265	68	5.97	5.0×10^7	8.0×10^7
Imitation whipped cream (pressurized)	6.46	<10	<10	493	69	6.28	1.7×10^7	8.3×10^7
Potato (frozen)	5.70	$<10^2$	$<10^2$	243	96	*	5.7×10^8	2.8×10^9
Sweet Potato (canned)	5.51	$<10^2$	$<10^2$	243	96	5.21	3.3×10^7	8.0×10^7
Rice pudding with raisins (canned)	6.30	$<10^2$	$<10^2$	493	119	6.23	7.8×10^7	7.9×10^7
Vegetable with liver (canned)	5.74	$<10^2$	$<10^2$	243	96	5.20	4.0×10^8	9.7×10^8
Sausage (frozen)	6.20	$<10^2$	3.0×10^2	243	96	*	3.9×10^9	4.3×10^9

*Data not available

Table 3—Population, pH and time required for formation of measurable enterotoxin in vanilla pudding inoculated (10^5 cells/g) with four strains of S. aureus and incubated without agitation

Incubation temp (°C)	S. aureus 265 (A toxin) Time[a] (hr)	pH	Count/g × 10^6	S. aureus 243 (B toxin) Time[a] (hr)	pH	Count/g × 10^6	S. aureus 493 (C toxin) Time[a] (hr)	pH	Count/g × 10^6	S. aureus 315 (D toxin) Time[a] (hr)	pH	Count/g × 10^6
19	50–84	6.26	31	50–84	6.45	240	50–84	6.32	410	50–84	6.17	270
26	25–48	6.21	48	25–48	6.27	190	25–48	5.74	390	20–25	6.12	420
37	15–22	5.92	16	15–22	6.07	400	15–22	6.06	41	15–22	5.99	350
45	24–31	5.90	30	48–72	6.23	7	48–72	5.56	9.6	24–31	6.00	90

[a]Incubation time in hours between the last negative and the first positive test for presence of enterotoxin

toxin production. Enterotoxin was produced in inoculated foods in the pH range of 5.51 (sweet potato) to pH 6.60 (coconut cream pie). No measurable enterotoxin developed in any food with a pH below 5.00.

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Ms received 9/14/72; revised 11/13/72; accepted 11/20/72.

Michigan Agricultural Experiment Station Journal Article No. 6070.

This investigation was partially supported by Public Health Service Grant No. FD 00163 of the Food and Drug Division.

Gratitude is expressed to the Microbiology Laboratory of the Federal Food and Drug Administration in Washington, D.C. for furnishing the purified enterotoxins and the balanced antitoxins used as standards in this investigation.

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EFFECT OF CARBOXYMETHYLCELLULOSE ON THE PROTEOLYSIS OF ALPHA$_S$-CASEIN BY IMMOBILIZED PEPSIN

INTRODUCTION

POLYANIONIC COMPOUNDS in general are known to interact readily with proteins (Behlheim-Terans, 1958; Boyd and Neuman, 1951; Cornwell and Kruger, 1961). Such interactions might interfere with the activity of biologically active proteins. It was found that chondroiten-4-sulfate and heparin inhibited peptic hydrolysis of proteins (Levey and Sheindfeld, 1954), and it was suggested that these inhibitors acted by binding directly to the enzyme.

A number of natural and artificial anionic polysaccharides are used extensively as food additives and several systems have been reported where such polyanions form complexes with the native proteins of the food (Hansen, 1966; Hansen, 1968; Lin and Hansen, 1970). The formation of such complexes might affect the digestibility of the proteins. Anderson and Watt (1959, 1959a) have found that the sulfated polysaccharides of the carrageenan group which are widely used as food stabilizers, inhibit pepsin activity due to inhibitor-substrate protein interactions resulting in depletion of available substrate. Vaughan et al. (1962) also examined the effect of carrageenans on pepsin activity and reported that higher carrageenan concentration and lower protein concentration than those usually found in foods were required for detectable inhibition.

Carboxymethylcellulose (CMC) is another widely used anionic polysaccharide and it has been shown to form complexes with both whey proteins (Hidalgo and Hansen, 1969, 1969a) and caseins (Cluskey et al., 1969; Valaris, 1972) of milk. It was of interest, therefore, to study the effect of the presence of CMC on peptic degradation of α_S-casein.

During this study, the enzyme was used in an insoluble form in anticipation that the information obtained will have some value in the future application of immobilized enzyme systems to food.

EXPERIMENTAL

Enzyme binding

Pepsin, 3X crystalline (Nutritional Biochemical Corporation) was bound to alkylamino derivative of porous glass, generously provided by H. H. Weetall, Corning Glass Work, Corning, N.Y. The glass derivative had a pore diameter of 550Å and a mesh size 40–80. The enzyme was coupled to the glass at pH 4.0 through amide bonds between the functional amino groups of the glass and free carboxyl groups of the enzyme, according to (Line et al., 1971). The efficiency of binding, expressed as percent of enzyme bound, was estimated from the difference in soluble enzyme activity in the supernatant liquid before and after the binding reaction.

In order to account for any inactivation that might have occurred during storage, under refrigeration, the activity of each preparation was tested from time to time during the period of its utilization. Samples of bound pepsin held for 40 days showed no significant loss of activity.

The assay used to determine pepsin activity during the binding process and the activity of the insolubilized derivative as a function of time during storage was the one described by Line et al. (1971). The substrate used in this assay was 2% hemoglobin from Nutritional Biochemical Corp., in 0.06N HCl. The reaction was terminated by the addition of 5% trichloroacetic acid (TCA). Next, the solution was filtered through a Whatman No. 1 filter paper and the absorbance of the filtrate was read at 280 nm against a substrate blank treated in the same way but containing no enzyme.

Determination of enzymatic proteolysis

α_S-casein provided by Dr. P.M.T. Hansen, Dept. of Food Science & Nutrition, The Ohio State University, was used as the protein substrate.

Solutions of the protein to be examined were made up to the desired substrate concentration, and the pH was adjusted to the assay pH (1.6) with HCl.

When proteolysis was carried out in the presence of carboxymethylcellulose (CMC), calculated amounts of protein and CMC solutions, at the desired pH, were mixed just prior to the enzymatic reaction. The CMC used was sodium carboxymethylcellulose (CMC) 7HP, with an average degree of substitution of 70%, which was purchased from Hercules Powder Co., Wilmington, Del.

Proteolysis was conducted at 37°C either (a) continuously, using an enzyme column, or (b) by a batch method, using the immobilized enzyme in suspension.

For the enzyme column-continuous method, 1g of immobilized enzyme derivative was packed in a jacketed Sephadex column, type 9/15. The void volume of the enzyme column was determined with blue dextran (Pharmacia Fine Chemicals AB, Uppsala, Sweden). The substrate solution was passed through the column with the reaction time being controlled by regulating the flow rate according to the equation:

$$\text{reaction time (min)} = \frac{\text{void volume (ml)}}{\text{flow rate (ml/min)}}$$

Aliquots of the eluate were collected, treated with equal volumes of 10% TCA, filtered through a Whatman No. 42 filter paper and the absorbance of the TCA soluble fraction was measured at 280 nm against a blank treated in the same way but not passed through the enzyme column. The results were expressed as Δ A 280/mg moist immobilized enzyme, minute.

For the batch method, essentially the same procedure was followed except that, a known weight of enzyme derivative was suspended in the substrate solution and the mixture was allowed to react in a water bath at 37°C under continuous stirring with a magnetic stirrer. The action of the enzyme was stopped by the addition of 10% TCA.

Binding of CMC to immobilized enzyme derivative

CMC solutions of known radioactive concentration, with and without protein substrate, were prepared and the pH adjusted to 1.6. The radioactive CMC was a mixture of carboxy ^{14}C-labeled 7HP carboxymethylcellulose obtained from Hercules Powder Co., diluted with nonradioactive material of the same type to give approximately 5,400 cpm/mg in 10 ml scintillation solvent.

The carboxy ^{14}C-labeled CMC substrate solutions were recycled through the corresponding enzyme column for 1 hr, during which aliquots were withdrawn from the eluate every 5 min. The changes in count rate in the eluate samples were measured by a scintillation method using as the scintillation medium Scintisol Complete (Isolab, Inc., Akron, Ohio). The sample of the original substrate solution which was not passed through the enzyme column, was used as a reference. A decrease in radioactivity in the eluate would reflect the extent of its binding by the immobilized enzyme derivative.

The same experiment was conducted with a column packed with akylamine glass which had been treated with boiling concentrated acetic anhydride for 10 min to inactivate free amine groups on the glass. All samples were measured over a 10 min period in a Tri Carb, Packard No. 3002 liquid scintillation spectrometer.

RESULTS

THE THREE IMMOBILIZED enzyme preparations used in these studies with

Table 1—Characteristics of immobilized enzyme preparations

Immobilized enzyme preparation	Binding efficiency %	Enzyme content[a] %	Activity of preparation Substrate 2% Hemoglobin Δ A 280/mg[b]/min
Pepsin-1	72.3	4.4	2.00×10^{-3}
Pepsin-2	88.6	4.2	0.95×10^{-3}
Pepsin-3	77.2	3.7	2.95×10^{-3}

[a]Dry basis
[b]mg Moist immobilized enzyme-glass derivatives

Table 2—Effect of CMC retained in pepsin column on the activity of the enzyme

α_S-casein conc (1%)	Original reaction velocity (Δ A/min)	Inhibited reaction velocity (Δ A/min)	Inactivation (%)
0.1	0.313	0.157	49.7
0.8	0.800	0.406	49.2

their binding efficiency, amount bound and enzymatic activity, are listed in Table 1. The amount of enzyme bound on the carrier varied from preparation to preparation and ranged from 3.7–4.4%. However, the variations in activity of the different preparations were significantly greater than what would be expected from the variations in the amount bound. Activities ranged from 0.95×10^{-3} Δ A/mg, min for Pepsin-2 to more than three times as much for Pepsin-3, i.e., 2.95×10^{-3} Δ A/mg, min.

Since the same crystalline enzyme and glass derivative were used in all binding experiments, this observation might indicate that some of the enzyme was inactivated during the binding process, the degree of inactivation depending on factors which were not identified.

Proteolysis of α_S-casein using the column procedure

In order to determine the effect of CMC on the activity of pepsin, the relationship between enzymatic reaction velocity and protein substrate concentration was studied as a function of CMC concentration in the substrate solution.

Preliminary experiments showed that α_S-casein-CMC complexes were not soluble above pH 1.6. From this point on, therefore, all experiments were conducted at pH 1.6, which was close to the pH optimum of the immobilized pepsin.

The substrate solutions contained from 0.1–0.8% α_S-casein and from 0.00–0.03% CMC in the sequence of 0.01, 0.03 and 0.02%. The same enzyme column was used for all experiments. Experiments with pure α_S-casein were conducted first, followed in order by substrates with 0.03% CMC and 0.02% CMC.

Lineweaver-Burk plots (1/v versus 1/s), for CMC concentrations of 0.00%, 0.03% and 0.02% are presented in Figure 1, Curves A, B and C, respectively. The plots for 0.00% and 0.03% CMC are linear in nature and have the same apparent Michaelis constant (app K_m) values and different maximum reaction velocity (V_{max}), as would be expected for noncompetitive inhibition. The plot for the 0.02% CMC is nonlinear and not subject to kinetic interpretation, suggesting a possible accumulative binding and the formation of either an enzyme-inhibitor (EI) or enzyme-substrate-inhibitor (ESI) complex on the column.

Trials were repeated with 0.1% and 0.8% solutions of α_S-casein following the trials with 0.02% CMC. The results (Table 2) were compared with the values obtained with the original column before the introduction of CMC-containing substrates. The comparison revealed approximately 50% decrease in protein hydrolysis at both substrate levels, indicating a loss of enzyme activity independent of substrate concentration.

Proteolysis of α_S-casein using the batch procedure

Since there were indications of accumulation of CMC in the enzyme column, the column system could not be used for further studies of the inhibitory effect. It was decided to continue the investigation with the batch method.

First, the activity of the immobilized enzyme was determined as a function of protein substrate concentration in the range 0.01–1.00% α_S-casein. There was a definite indication of inhibition above 0.02% substrate concentration which was not observed previously with the column. In all further work the substrate concentration was kept below the 0.2% level.

The effect of CMC was evaluated at 0.02% and 0.03% levels of addition. The

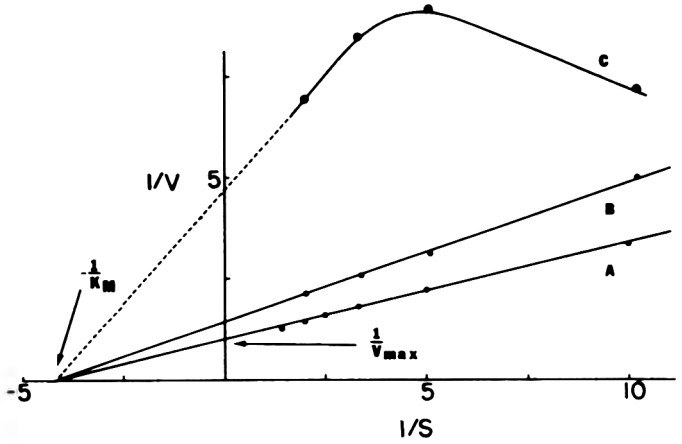

Fig. 1—Lineweaver-Burke plots of effect of CMC on the activity of pepsin against α_S-casein, (Column procedure). A = 0 CMC and 0.01% CMC; B = 0.03% CMC; C = 0.02% CMC.

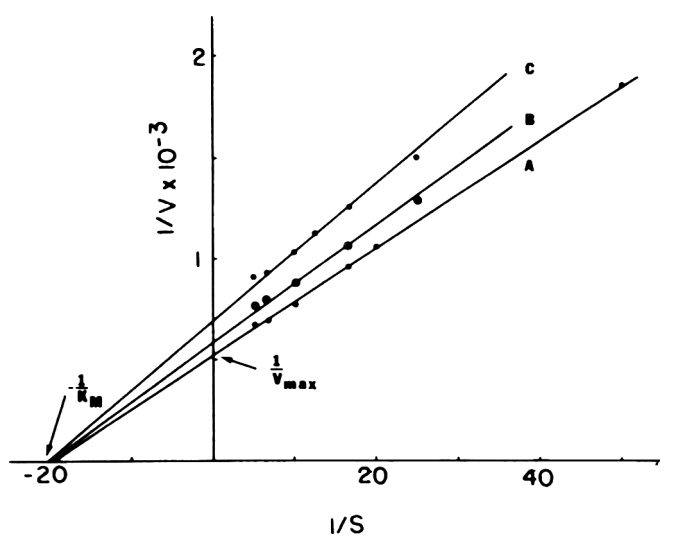

Fig. 2—Lineweaver-Burke plots of the effect of CMC on the activity of pepsin against α_S-casein (Bactin reaction). A = 0 CMC; B = 0.02% CMC; C = 0.03% CMC.

Table 3–Kinetic parameters for inhibition of pepsin by CMC

Inhibitor conc (mM)[a]	V_{max} (Δ A/mg, min)	K_I[b] (mM)	app K_m (mM)[c]
0.0×10^{-3}	1.92×10^{-3}	–	1.84×10^{-2}
1.0×10^{-3}	1.73×10^{-3}	8.65×10^{-3}	1.81×10^{-2}
1.5×10^{-3}	1.45×10^{-3}	4.94×10^{-3}	1.80×10^{-2}

[a]Molecular weight of CMC was taken as 200,000.
[b]Calculated from the equation:

$$\frac{1}{V'_{max}} = \frac{1}{V_{max}}\left(1 + \frac{I}{K_I}\right)$$

where V_{max} = inhibited reaction velocity and V'_{max} = uninhibited reaction velocity.
[c]Molecular weight of α_s-casein was taken as 28,600.

Table 4–Effect of treatment on the activity of pepsin-CMC column

Type of treatment	^{14}C-CMC removed as % bound	Column activity (Δ A280/min)
Original activity	–	1.2
After binding CMC	–	0.56
0.1N HCl	75	0.564
Buffer pH 5.0	1.7	0.61
1M NaCl	2.6	1.04

same procedure was followed as before, only this time, the substrate solution contained the indicated amount of CMC and the protein concentration ranged from 0.02–0.2%.

The double reciprocal plots, 1/v versus 1/s, for CMC concentrations of 0.00%, 0.02% and 0.03% are presented in Figure 2, Curves A, B and C, respectively. The V_{max} of the reaction decreased as the CMC concentration increased, whereas the app K_m of the reaction remained essentially unaffected by the presence of the inhibitor. The above observations were in good agreement with the results obtained with the pepsin column and also suggested a noncompetitive type of inhibition of immobilized pepsin by CMC.

The V_{max}, app K_m and K_I values for each CMC concentration are presented in Table 3. The K_I values for the two inhibitor concentrations differed by 3.7×10^{-3} mM.

Binding of CMC

In order to confirm the assumption made earlier that CMC binds directly on the immobilized pepsin derivative and to determine whether CMC binds on the enzyme itself or on carrier glass beads, radioactive CMC solutions were recycled through an immobilized pepsin column (Pepsin-3) and through a column packed with alkylamined glass carrying no enzyme on it. The glass used in the latter column was pretreated with acetic anhydride in order to achieve acylation of the functional amino groups.

About 20 ml of a 0.03% C^{14} CMC solution at pH 1.6 were recycled through each column for 60 min, with a flow rate of about 5 ml/min. Aliquots were withdrawn from the eluates every 5 min and were dissolved in the scintillation solvent for measurement of radioactivity.

Furthermore, in order to study the effect of the presence of substrate protein on the interaction of CMC with the enzyme derivative, the same experiment was repeated with a solution containing 0.03% radioactive CMC and 0.23% α_s-casein. For this experiment, a new pepsin column was prepared using approximately the same amount of the identical immobilized pepsin preparation, i.e., 1g of Pepsin-3.

The results are presented in Figure 3 as plots of count rate (cpm) versus reaction time. Results depicted by Curve A were obtained with the carrier column (glass alone); those for Curve B with the pepsin column in the absence of protein; and those for Curve C in the presence of 0.23% α_s-casein.

The count rate and, hence, the concentration of CMC in the solution passed through the glass column did not change over the 60 min period of recycling. In the absence of α_s-casein, the CMC solution recycled through the pepsin column exhibited a marked decrease in count rate. The CMC concentration started decreasing almost immediately as indicated by the reduction in count rate from 260 to 198 cpm after 5 min. The count continued to decrease until after 35 min when it reached a constant reading of 120 cpm. The overall reduction was approximately 50%. The presence of α_s-casein did not affect the binding of the CMC.

These observations provided direct evidence that about half of the CMC in the solution was retained in the enzyme column, interacting with the enzyme rather than with the carrier.

Removal of CMC from the pepsin column

The pepsin column containing the radioactive CMC was treated with a number of reagents in an attempt to dissociate the enzyme-inhibitor complex and restore activity. Washing with 0.1N HCl, acetate buffer pH 5.0 and 1M NaCl, in the indicated order, was employed. The release of CMC from the column was followed by measuring the increase in radioactivity in the eluate after each treatment. The effect of the various treatments on removal of CMC and on enzyme activity are recorded in Table 4. About 75% of ^{14}C-CMC was removed with the HCl treatment, whereas an increase in pH (buffer pH 5) and high ionic strength (1M NaCl) did not have any significant effect on further removal of ^{14}C-CMC. Neither low (pH 1.0 with 0.1N HCl) nor high (pH 5 with acetate buffer) pH levels were effective in reactivating the enzyme. High ionic strength (1M NaCl), on the other hand, resulted in almost two-fold increase in reaction velocity after the HCl treatment. Treatment with KCl before the acid treatment had no effect on the enzymatic activity. It is evident that treatment with HCl removed some of the inhibitor from the column but did not restore enzymatic activity, whereas treatment with NaCl reactivated the enzyme without removing any more CMC.

DISCUSSION

IT WAS DEMONSTRATED that CMC, at concentration levels at 0.02% and 0.03%, inhibited proteolysis of α_s-casein by immobilized pepsin.

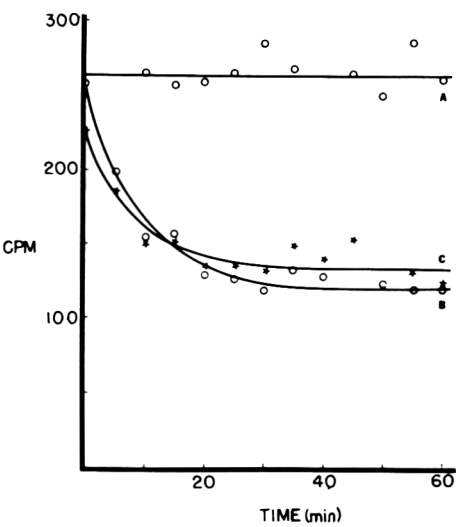

Fig. 3–Binding of ^{14}C-CMC to glass and glass bound pepsin. A = glass alone; B = glass bound pepsin in absence of substrate; C = glass bound pepsin in the presence of 0.23% α_s-casein.

The kinetic data obtained showed that CMC did not affect the app K_m of the pepsin reaction. V_{max}, on the other hand, decreased with increasing CMC concentration suggesting a noncompetitive type of inhibition. Experiments with radioactive CMC provided confirmatory evidence of noncompetitive inhibition since it was demonstrated that the CMC binding capacity of a pepsin column was essentially independent of substrate protein.

Noncompetitive inhibition would mean that the inhibitor formed inactive complexes with either the free enzyme, (EI), or the enzyme substrate complex (ESI). In that case, the K_I of the inhibition should represent the dissociation constant of the complexes according to the reactions:

$$EI \rightleftharpoons E + I$$

$$ESI \rightleftharpoons ES + I$$

$$K_I = \frac{[E] \times [I]}{[EI]} = \frac{[ES] \times [I]}{[ESI]}$$

However, there was evidence that the overall inhibitory effect of CMC on pepsin is more complicated than the pure noncompetitive type would dictate. The enzyme column, when saturated with inhibitor, gave a nonlinear Lineweaver-Burke plot, indicating that at high inhibitor concentration, the kinetic characteristics of the inhibition are changed. Moreover, some of the radioactive CMC was removed from the CMC treated column without simultaneous restoration of enzymatic activity, indicating a degree of irreversibility in respect to the inhibition. The independence of this inhibitory effect from the substrate concentration was also evidenced by the fact that following CMC saturation on a pepsin column, an eight-fold increase in protein substrate concentration did not affect the degree of inactivation.

These observations, together with the fact that the K_I values obtained from our experimental data, differed from one inhibitor concentration to another, may suggest that the inhibition is noncompetitive for low CMC concentration but changes to irreversible when the enzyme becomes saturated with inhibitor.

Anderson and co-workers (1959; 1959a; 1961; 1967; 1967a; 1968), in their studies on the antipeptic activity of carrageenans, have concluded that these polyions interact with the substrate protein through electrostatic attractions and, thus, inhibit pepsin activity by protecting the substrate molecules. This does not appear to be the case with CMC since it was demonstrated that CMC binds directly with the enzyme molecules.

There is a marked difference between the polyanionic character of CMC and carrageenans, CMC, as a polycarboxylic acid, is fully protonated at pH values around 1.5 and 2.0 where the present experiments were conducted whereas, carrageenan, possessing the strongly acidic sulfate groups, still bears a negative charge at these pH values. Thus, in the system, pepsin-CMC-α_S-casein at pH 1.6, pepsin is negatively charged (I.P. below 1.0), CMC is neutral, and α_S-casein is positively charged. At this pH, electrostatic attractions, therefore, between CMC and either the enzyme or the substrate protein should be excluded. The protonated —COOH groups on CMC, however, are very suitable for H-bonding. Consequently, the observed interaction between pepsin and CMC might be explained on the basis of H-bonding and other short-range forces rather than ionic bonding. In the case of carrageenans, the negative charge on both the enzyme and the inhibitor molecules would prevent any approach to the two species, so that no interactions would take place. However, electrostatic attractions between the opposite charges on the inhibitor and the substrate molecules could result in an inhibitor-substrate complex formation.

Although the mechanism of inhibition of pepsin by CMC could not be determined from the data obtained in this investigation, certain observations provide a basis for some speculation about the mechanism. It was observed that treatment of a pepsin column, saturated with CMC, with either 0.1N HCl or acetate buffer pH 5, did not increase the enzymatic activity, although some of the inhibitor was removed from the column. This could be an indication that some changes in the enzyme molecule, induced by the interaction with CMC, and related to the inactivation of the enzyme are not reversed merely by the removal of inhibitor. One may assume that th 1M NaCl, resulted in reactivation of the enzyme with no further release of inhibitor. One may assume that binding with CMC produces certain conformational changes in the enzyme molecule which are reversed only under conditions of high ionic strength, as might be provided by NaCl. That an enzyme molecule might be free to change conformation according to the environmental conditions even when it is covalently attached to an insoluble carrier has been demonstrated by Cho and Swaisgood (1972). They were able to induce reactivation of an unfolded enzyme subunit bound on porous glass by adding substrate and exposing the enzyme derivative to conditions favoring refolding. They successfully achieved reformation of the original quaternary structure by addition of free enzyme.

The results of this investigation suggest a possible adverse effect of CMC on the nutritional value of food proteins. However, this extrapolation of the data is not valid without suitable feeding trials.

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Ms received 10/14/72; revised 12/15/72; accepted 12/19/72.

Journal Series Article No. 97-72 of the Ohio Agricultural Research & Development Center. Supported in part by NSF Grant GI-32141.

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EFFECT OF CARBOXYMETHYLCELLULOSE ON THE PROTEOLYSIS OF ALPHA$_S$-CASEIN BY IMMOBILIZED TRYPSIN

INTRODUCTION

VALARIS AND HARPER (1973) provided the background information that serves as the basis for the investigation of the effect of carboxymethylcellulose (CMC) on the action of proteolytic enzymes. The earlier paper showed that CMC exerted an inhibitory effect on the proteolysis of α_S-casein by immobilized pepsin and that this effect was due to direct binding of the inhibitor on the enzyme. The inhibition was independent of substrate concentration.

Several polyanions, such as heparin, pancreatic RNA, polyglutamic acid and polycysteic acid have been shown to inhibit tryptic hydrolysis of partially denatured proteins (Dellert and Stahmann, 1955; Horwitt, 1940; Kornguth and Stahmann, 1960). Kornguth and Stahmann (1960) studied the inhibitory action of some of these polyions as a function of concentration, molecular weight, ionic strength and type of substrate. They found that polyglutamic acid inhibited tryptic activity on urea denatured proteins but not on native proteins. They concluded that the inhibitory effect was due to ionic interactions between the enzyme and the inhibitor.

In the present study, the effect of the presence of CMC on proteolysis of α_S-casein by immobilized trypsin was investigated. The enzyme was used in an insolubilized form to obtain some useful information about the function of immobilized enzymes and their applicability to food systems.

EXPERIMENTAL

TRYPSIN 2X crystalline, salt free, obtained from Nutritional Biochemical Corp., Cleveland, Ohio was bound through azo linkages (Campbell et al., 1951) to an arylamino glass derivative of 550 Å pore diameter and 40–60 mesh size, which was kindly provided by H.H. Weetall. The reaction was carried out at pH 8.0.

Benzoyl arginine-ethyl-ester (BAEE) from Nutritional Biochemical Corp. was used for assaying trypsin activity (Bergmeyer, 1965) in order to follow the binding process. The enzymatic esterolysis was conducted in tris buffer pH 8.0 and the absorbance of the solution was read at 254 nm at selected time intervals. The binding efficiency was 67.8% with the glass containing 4.10% trypsin on a dry weight basis. A known weight of immobilized enzyme was suspended in the substrate solution for a given period of time after which the reaction mixture was filtered and the absorbance of the filtrate was read. Insolubilized enzyme derivatives were stored as moist cakes at 5°C.

For proteolysis of α_S-casein by trypsin, the same method was used for the pepsin (Valaris and Harper, 1973) except that the reaction was conducted in tris buffer at pH 8.0.

To determine the combined action of pepsin and trypsin, the pepsin hydrolyzate of α_S-casein was used as substrate for trypsin. Initially, the substrate was treated with pepsin for 5 min., as described previously. The reaction was stopped by raising the pH with alkali and the mixture was filtered to remove the insoluble pepsin derivative. The pepsin hydrolyzate was then adjusted to pH 8.0 and allowed to react with the immobilized trypsin for predetermined periods of time after which tri-chloroactic acid (TCA) was added to terminate proteolysis. The absorbance of the TCA soluble fraction was measured at 280 nm.

RESULTS

Effect of CMC on the degradation of α_S-casein by immobilized trypsin

Trypsin bound on arylamine glass derivative was suspended in the substrate solution. Preliminary experiments had shown that 95–100 mg of moist immobilized enzyme were required to interact with 3 ml substrate solution to yield reliable absorbance readings. The reaction was conducted at pH 8.0 and 37°C for 10 min. The velocity of proteolysis was expressed in $\Delta A/mg, min$. The protein substrate concentration was between 0.04% and 0.4% while CMC concentration ranged from 0.00–0.05%.

Lineweaver-Burk plots, 1/v versus 1/s, derived by linear regression analysis for each CMC concentration, are presented in Figure 1. Curve A represents trypsin activity in the absence of CMC, as well as in the presence of 0.02% CMC. Curves B and C were obtained with 0.03% and 0.05% CMC, respectively. As shown in the graph, 0.02% CMC did not affect trypsin activity. Concentrations of 0.03% and 0.05% CMC, however, decreased the reaction velocity. It is also evident that the reduction in the reaction velocity decreased as the substrate concentration increased. All three curves intercept the 1/v axis at approximately the same point, indicating that the V_{max} of the reaction was unaffected by CMC.

The V_{max}, app K_m and K_I values derived from Figure 1 for each inhibitor concentration are presented in Table 1. The app K_m of the enzymatic reaction,

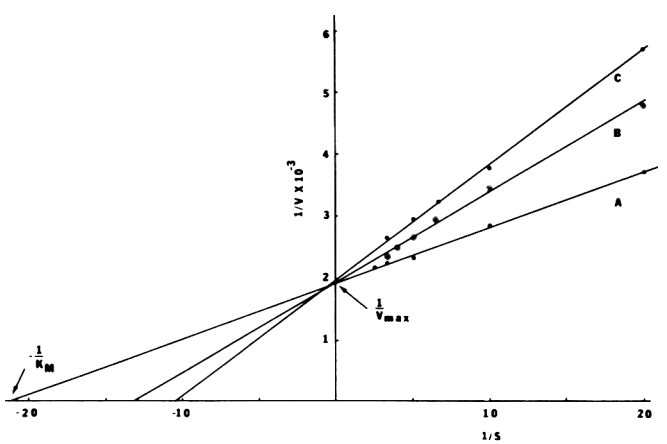

Fig. 1—Lineweaver-Burke plot of the effect of CMC on the action of trypsin against α_S-casein. A = 0 CMC and 0.02% CMC; B = 0.03% CMC; C = 0.05% CMC.

Table 1—Kinetic parameters for inhibition of trypsin by CMC

Inhibitor conc (mM)[a]	app K_m (mM)[b]	K_I[c] (mM)	V_{max} (ΔA/mg min)
0.0×10^{-3}	1.66×10^{-2}	–	0.525×10^{-3}
1.5×10^{-3}	2.67×10^{-2}	2.5×10^{-3}	0.513×10^{-3}
2.5×10^{-3}	3.32×10^{-2}	2.5×10^{-3}	0.500×10^{-3}

[a]Molecular weight of CMC was 200,000.
[b]Molecular weight of α_s-casein was 28,600.
[c]Calculated from the equation: $1/K'_M = 1/K_M[1 + (I/K_I)]$ where: K'_M = Michaelis constant for inhibited reaction, and K_M = Michaelis constant of uninhibited reaction.

on the other hand, was changed by the presence of CMC and increased with increasing inhibitor concentration. These observations suggested a competitive type of inhibition of trypsin by CMC. The fact that the K_I values obtained for two different inhibitor concentrations are identical is in agreement with the theoretical interpretation of the competitive inhibition.

Effect of CMC on the action of immobilized trypsin on α_s-casein partially hydrolyzed by pepsin

In order to study the effect of CMC on the overall proteolytic activity of immobilized pepsin and trypsin used in sequence, 0.3% α_s-caseine solutions with and without 0.05% CMC were reacted with pepsin for 5 min., Valaris and Harper (1973). The products of peptic hydrolysis were then used as substrate for trypsin. Substrate solution without CMC served as the control. The extent of peptic hydrolysis was measured in 3 ml aliquots withdrawn from each solution. As expected, proteolysis by pepsin proceeded faster in the absence than in the presence of CMC, giving 7.63×10^{-3} ΔA/mg, min for the control and 5.09×10^{-3} ΔA/mg, min^{-1} for the solution containing 0.05% CMC.

Next, aliquots of 3 ml from each peptic hydrolysate were treated with approximately 90 mg immobilized trypsin for predetermined periods of time. Aliquots of the corresponding peptic hydrolysates, not reacted with trypsin, were used as blanks. The results are presented in Figure 2 as plots of Δ absorbance/mg versus time. The time curves obtained in the absence and in the presence of 0.05% CMC coincided, indicating that CMC did not inhibit trypsin action on α_s-casein partially hydrolyzed by pepsin, although it had been shown above (in this paper) to inhibit tryptic proteolysis of intact α_s-casein.

Binding of CMC to immobilized trypsin

The direct binding of CMC to the trypsin derivative, as well as the effect of the presence of substrate protein on this binding, was investigated using a radioactive CMC solution at pH 8.0 (Valaris and Harper, 1973). Two columns packed with the same amount of immobilized trypsin derivative were used. The recycling time was 60 min and aliquots were withdrawn every 5 min. The results are presented in Figure 3 as plots of count rate versus time.

Curve A was obtained with 0.03% ^{14}C–CMC solution and Curve B with a solution of 0.03% ^{14}C–CMC and 0.28% α_s-casein. The 99% probable error was ± 14 cpm for the highest and ± 12 cpm for the lowest reading. With CMC only, the count rate decreased from about 300 cpm to 230 cpm after 15 min with an overall decrease of about 25–30%, indicating a definite binding of CMC to the trypsin. However, in the presence of α_s-casein (Curve B) no overall decrease in the count rate of the recycled CMC solution could be detected throughout the 60 min period. Apparently, at this concentration level, α_s-casein prevented complex formation between trypsin and CMC probably through interaction of CMC with α_s-casein.

As noted previously for pepsin, no ^{14}C–CMC binding was noted for acetic anhydride treated arylamine glass.

DISCUSSION

KINETIC DATA suggested competitive inhibition by CMC on the action of trypsin against α_s-casein. That CMC competed with α_s-casein for complexing with trypsin was confirmed by the fact that the presence of the protein substrate prevented the binding of CMC on a trypsin column.

All experiments with trypsin were conducted at pH 8.0 where CMC and α_s-casein are negatively charged while trypsin bears a net positive charge (I.P. 10.8). Electrostatic attraction, therefore, between the enzyme and the inhibitor is very possible in this case. Trypsin has been shown to contain a lysine residue which is very close to the active site of the enzyme whose ϵ-NH_2 group could be involved in ionic interaction with the polyanion. Thus, a mechanism similar to the one proposed by Kornguth and Stahmann (1960) for the inhibitory action of polyglutamic acid on trypsin might explain the action of CMC as well. One molecule of the polyanion could interact with the lysine residue adjacent to the active site and with another basic residue on the other side of the active site. It could thus form a block preventing access of the substrate to the active site. When α_s-casein is present in relatively high concentration levels, it might displace CMC from its complex with the enzyme, acting as another polyanion and thus reversing the inhibitory effect. This possibility is

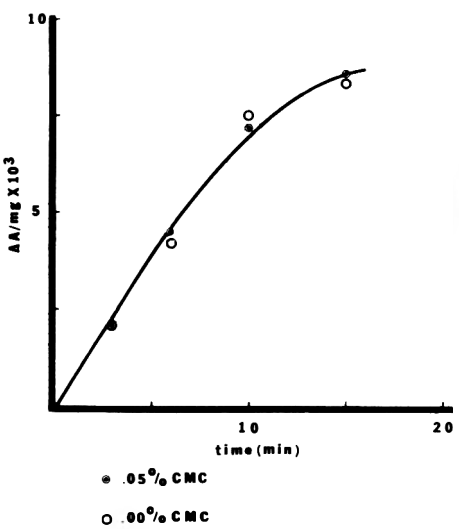

Fig. 2—Effect of CMC on the action of immobilized trypsin against α_s-casein pretreated with immobilized pepsin.

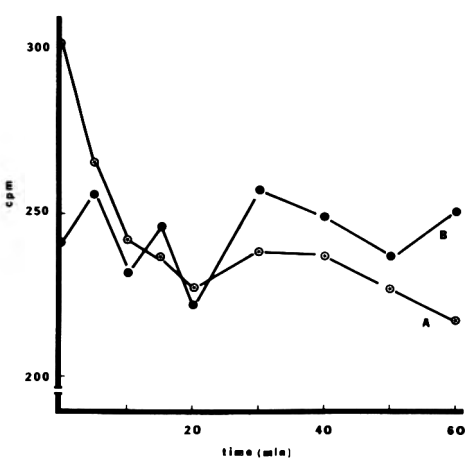

Fig. 3—Binding of ^{14}C–CMC to immobilized trypsin in the presence and absence of α_s-casein. A = no substrate; B = 0.28% substrate.

supported by the K_m and K_I relationship.

Like for pepsin (Valaris and Harper, 1972), CMC was found to inhibit trypsin activity on intact α_s-casein, but CMC failed to inhibt tryptic degration of α_s-casein partially hydrolyzed by pepsin. The assumption may be made that partial peptic hydrolysis of α_s-casein splits the protein molecule in peptides which expose a considerably greater number of bonds susceptible to tryptic attack than those available on the native protein. Generally, α_s-casein is characterized by a high degree of hydrophobicity (Hill and Wake, 1969). This protein, in its native state, has a random coil configuration. It is possible that this structure is stabilized through hydrophobic bonds in a way that arginine and lysine residues in the vicinity of the hydrophobic region become unavailable to the specific attack of trypsin. Pepsin preferentially splits peptide bonds of hydrophobic amino acids (Fruton, 1970) and this could result in destruction of the hydrophobic bonds exposing the hidden arginine and lysine residues.

The results of this investigation suggest that CMC would have no significant effect on the tryptic digestion of casein and that any nutritional significance of CMC would relate only to its effect of pepsin. The work also suggests that analysis of trypsin inhibition without regard to the prior effect of pepsin may lead to erroneous interpretation of the nutritional significance of trypsin inhibition.

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Ms received 10/14/72; revised 12/15/72; accepted 12/19/72.

Journal Series Article No. 98-72 of the Ohio Agricultural Research and Development Center. Supported in part by NSF Grant GI-32141.

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SOME EFFECTS OF BETA AMYLOLYTIC DEGRADATION OF PASTES OF WAXY MAIZE STARCH

INTRODUCTION

THE PROPERTIES of amylopectin starch (unmodified and modified) have great importance in a wide range of food systems as single ingredients. The regular starches of corn and wheat contain approximately 75% amylopectin and its properties thus influence the behavior of these starches as single food ingredients as well as the behavior of intact cereal flours. A notable example is the importance of amylopectin in the phenomena of bread staling (Schoch and French, 1947).

Changes of extensibility and cohesive properties of waxy maize starch pastes were observed by use of a devised Instron procedure (Henry and Katz, 1969) as these changes accompanied degradation of the amylopectin molecule by beta amylase. Other viscosity changes were also observed as digestion progressed; the amylopectin-iodine complex was measured spectrophotometrically. All changes were related to an average shortening of chain length, based on calculation from terminal end-group analysis measurement of liberated maltose.

EXPERIMENTAL

Gelatinization and incubation procedure

Waxy maize starch was gelatinized in the Brabender Amylograph (30 g/450 ml H_2O) and cooled to 35°C. An aliquot was removed representing zero time, 100 mg beta amylase (barley, lyophilized) (Nutritional Biochemicals Corp.) was added to the remaining slurry which was then incubated at 35°C in a constant temperature water bath. Sample aliquots were taken at 10 min intervals for up to 60 min and immersed in a boiling water bath to halt enzyme activity. The beta amylase was compared with alpha amylase (Calbiochem, 5,000 SKB units/g) under comparable incubation conditions on an equal weight basis. Complete dextrinization of starch (Kintner) was observed from alpha amylase in 19 min; none was observed from beta amylase after 20 hr.

Instron procedure

A procedure similar to that described by Henry and Katz (1969) was used to measure the cohesive and extensible properties of the starch pastes in this experiment. A roughened plexiglass disc was pulled away from the surface of the sample gel at a rate of 10 cm/min with the resultant force being recorded on the chart operating at the same speed. The dimensions of this contact disc are 0.64 cm by 2.54 cm (diam). The rupture of the gel column adhering to the contact disc was automatically marked on the recorder chart. A value for the area under the curve was obtained with an Instron Integrator.

The height of the Instron curve indicates the maximum force involved in extending the gel adhering to the contact disc (cohesiveness), while the integrated area of the curve indicates the amount of work required. The distance shown on the recorder between the beginning of extension and the rupture of the gel column denotes the extensibility of the gel.

Table 1—Average calculated unbranched chain length at progressive stages of beta amylolytic degradation of waxy maize starch[a]

Incubation time min	Maltose mg/g	Avg calc unbranched chain length
0	0	21
10	83	19.3
20	153	18
30	187	17.2
40	237	16.1
50	283	15.2
60	353	13.8

[a]Based on an average unbranched chain length of 21 calculated from data of J.C. Rankin and A. Jeanes, J. Am. Chem. Soc., 76: 4435 (1954).

Maltose measurement

Maltose measurement was made in accordance with the procedure for diastatic activity described by AACC (1969). 5-ml aliquots were used for the determinations.

Amylopectin-Iodine complex measurement

1 ml of starch solution was added to 5 ml 0.1N NaOH in a 100 ml volumetric flask. After 5 min, the sample was neutralized with 0.1N HCl using methyl orange as the indicator. 1 ml of KI/I_2 solution (2% KI, 0.2% I_2) was then added to develop the blue color. Distilled water was added to the 100 ml mark. A reference sample was similarly prepared by omitting the starch. The optical density was then measured at 550 nm on the Beckman D.U. Spectrophotometer. This wavelength corresponds to the peak absorbance of the amylopectin-iodine complex.

Alkaline viscosity

The procedure used was adapted from that of Meyers and Smith (1964). Quantities of digest were removed at specified intervals and made to 0.5% carbohydrate in 1.0M sodium hydroxide. 10-ml aliquots of these solutions were transferred to a vented Cannon viscometer (No. 1, C285) which was held immersed in a bath at 25°C. The time required for the solution meniscus to flow from the upper timing mark to the lower timing mark was recorded in seconds. An average of three readings was taken.

Brookfield viscosity

As amylolysis progressed, aliquots of the

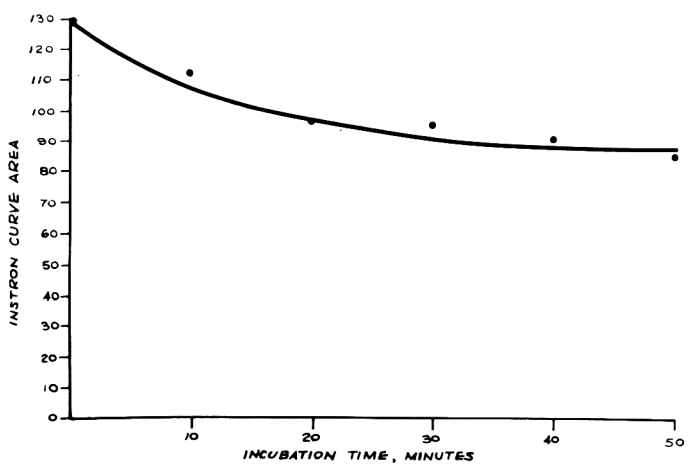

Fig. 1—Change of cohesive strength of amylopectin gel by beta amylase action.

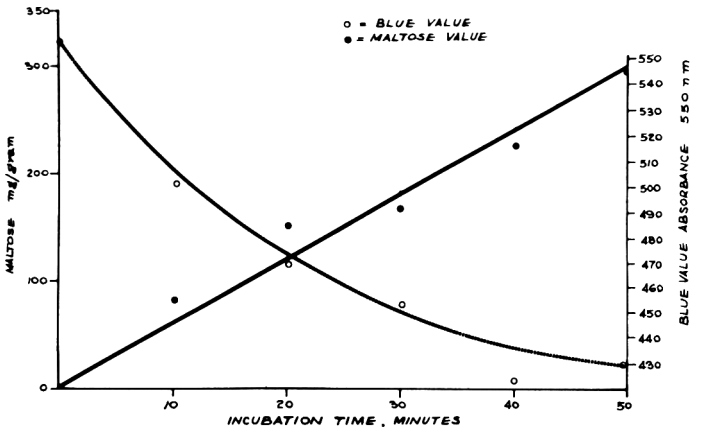

Fig. 2—Change in maltose value and amylopectin blue value by beta amylase action.

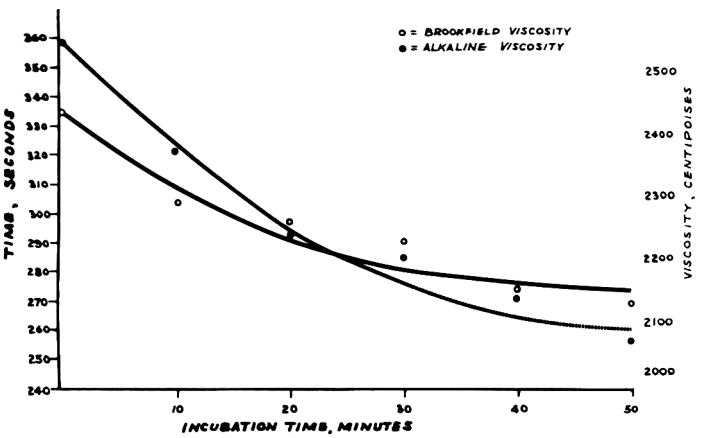

Fig. 3—Change in viscosity of amylopectin by beta amylase action.

starch solution were taken at 10-min intervals. The samples were immersed in a bath of boiling water to halt enzymatic activity. The samples were then cooled to 25°C and Brookfield viscosity measurements taken using the "T" spindle.

RESULTS & DISCUSSION

A LOWERING of the cohesive strength of amylopectin gel was observed as a consequence of beta amylase digestion, as shown in Figure 1.

Values of the ratio of terminal to nonterminal glucose units present in amylopectin molecules are reported in literature. This denotes average unbranched chain length of the molecule, and measurement of maltose permits calculation of average chain length shortening. These values are reported in Table 1.

The lowered absorbance of the blue amylopectin-iodine complex which followed the chain-shortening by beta amylase is shown in Figure 2. Decreasing viscosity in alkaline solution and in neutral solution as beta amylase digestion progresses is shown in Figure 3.

The Instron procedure described by Henry and Katz (1969) offers a good means of measuring changes in cohesive characteristics of the amylopectin paste as it is degraded by the action of beta amylase. These values are expressed in the curve resulting from gel extension. The curve peak height represents maximum force applied during gel extension; width of curve represents extensibility of gel; area of curve represents work applied during gel extension. In our experiments, curve area and extensibility diminished as beta amylase degradation proceeded, indicating loss of cohesive strength due to shortening of the branches.

A study made by Swanson (1948) on relation of polysaccharide structure to the color of the iodine complex showed that the intensity of the color was dependent on the length of the unbranched chains. Using beta amylase on an amylose substrate, the intensity of absorbance at the peak (650 nm) progressively diminished as amylolysis proceeded. A series of synthetic polysaccharides gave diminishing color with decreasing chain length, causing a distinct shift in absorption peak at chain lengths less than 20. Relating iodine color to lengths of segments in branched polysaccharides, Swanson (1948) observed that glycogen, of short chain length, gave faint color in comparison with starch amylopectin. Baldwin et al. (1944) obtained spectral data of various branched polysaccharides, whose variations of intensity could be attributed to the length of unbranched segments.

In light of the foregoing, it is not surprising that absorbance of the iodine complex diminished as amylolytic degradation proceeded. As Swanson (1948) predicted, it should be possible to use the measurement as a means of obtaining information of relative lengths of unbranched segments of various amylopectins.

Hunt et al. (1964) compared properties of corn amylopectin starches which had been progressively degraded by acid hydrolysis. Random cleavage of the molecule occurred and apparent molecular weight decreased over a range from over 8,000,000 to less than 200,000. Viscosity, as measured by an alkali fluidity technique, progressively diminished as hydrolysis proceeded. Iodine affinity, on the other hand, did not appreciably change until hydrolysis had proceeded to an advanced state.

Viscosity data obtained from our experiments suggest that part of the viscosity which was evident prior to beta amylase degradation, might be attributed to limited association of unbranched chain ends of neighboring molecules. It can be envisoned that certain minimal length is required before this can take place. Shortening this chain length will lessen the contribution to viscosity from this type of association. Thus it is not surprising to observe that viscosity decreased as chain shortening progressed, both in neutral and alkaline media.

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Ms received 11/10/72; revised 1/8/73; accepted 1/15/73.

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FLUORESCENT PRODUCTS IN A GLUCOSE-GLYCINE BROWNING REACTION

INTRODUCTION

THE DEVELOPMENT of fluorescent products in nonenzymatic browning reactions has been described (Burton et al., 1962a; Hannan and Lea, 1952; Hodge, 1953). Using glucose-amino acid model systems, Friedman and Kline (1950) and Overby and Frost (1952) showed that the fluorogens are precursors of the brown pigments but are not identical with them. In view of this nonidentity and the fact that inhibition of browning does not affect the formation of fluorescent products (Burton et al., 1962b, 1962c) and resultant deterioration of nutritional quality, it may be that measurement of fluorescence would be more useful than browning to assess nutritional quality. No definite attempts have been made to quantitate the fluorescent products and information on the nature of the fluorescent products and the chemical character of the fluorescent chromophore is scanty.

Aliphatic dialdehydes, such as malonaldehyde, react with primary amines to give Schiff-base derivatives that are fluorescent (Chio and Tappel, 1969). Such fluorescent compounds are widespread in biological systems, and are formed during lipid peroxidation of subcellular organelles in vitro (Chio et al., 1969; Dillard and Tappel, 1971). Chio and Tappel (1969) also showed that the fluorescent products measured are a family of compounds having the structure –N=C–C=C–N–. Burton et al. (1962a; 1963) showed that fluorescent products in a glucose-glycine browning system may be nitrogen containing carbonyl compounds. It was of interest, therefore, to ascertain whether these products contain fluorescent chromophores similar to those described by Chio and Tappel (1969).

This paper describes the measurement and partial identification of fluorescent products in a glucose-glycine browning system. The application of fluorescence measurements to assess browning in model systems and foods is also discussed.

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EXPERIMENTAL

ANALYTICALLY pure glucose and glycine, obtained from M/S Mallinckrodt Chemical Works, were used. The reaction system was based on that described by Burton et al. (1963). Aqueous solutions of glucose and glycine were mixed to give final concentrations of 2 and 1 molar, respectively, and the mixture was heated at 100°C (pH 5.0–5.2). Aliquots were withdrawn at regular time intervals. Absorbance was measured at 410 nm, and fluorescence was measured with an Aminco-Bowman spectrophotofluorometer (American Instrument Co., Inc.) calibrated with quinine sulfate. The slit arrangement for recording fluorescence spectra was slits 3, 4 and 6 set at 3, 1 and 3 mm, respectively. Under these instrument parameters, 1 μg of quinine sulfate/ml of 0.1N H_2SO_4 had a fluorescence intensity of 65 at the 0.3 meter multiplier setting.

Analytical tests

The effects of pH and of metal chelation on the fluorescent products were determined according to procedures developed by Malshet and Tappel (1972b). The effect of pH was studied by adjusting the diluted reaction mixture to pH 11.8, and then back to pH 4.9 with 1N NaOH and 1N HCl, respectively. To study the influence of a metal chelating agent, Europium (III) 2,2,6,6-tetramethyl heptane-1-3-dione ($Eu(DPM)_3$), obtained from Varian, was dissolved in chloroform:methanol (2:1). A small amount of the reaction mixture was lyophilized and the residue was dissolved in methanol. Decreased fluorescence caused by addition of an equal volume of 10^{-4}M $Eu(DPM)_3$ was compared with a control without $Eu(DPM)_3$.

Gel chromatography

An aliquot of appropriately diluted reaction mixture was applied to a Sephadex G-10 column (1.5 × 115 cm; V_o, 58–60 ml; flow rate 0.025 ml/min). Deionized water was used as eluant and 2-ml fractions were collected.

Fluorescence analysis of brown foods

The presence of similar fluorescent products was assessed in a few foods that undergo carbonyl-amine browning, including three breakfast cereals, toasted white bread, crust of bread and coffee. 10% aqueous extracts of these foods were analyzed for fluorescence.

RESULTS

Fluorescence development and its relationship with browning

The development of fluorescent products with increasing reaction time is shown in Figure 1. Fluorescence at 0 time was very low, and may have developed during the slight heating necessary to pre-

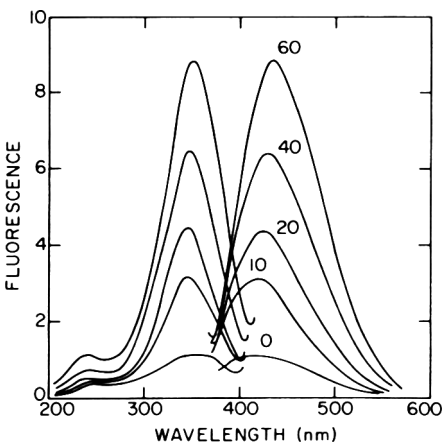


Fig. 1—Development of fluorescence with time in a glucose-glycine (2:1) browning reaction mixture, pH 5.0, heated at 100° C for 0, 10, 20, 40 and 60 min. The samples were diluted as follows (expressed as times dilution): 0 min: 8x; 10 min: 15x; 20 min: 40x; 40 min: 150x; 60 min: 250x.

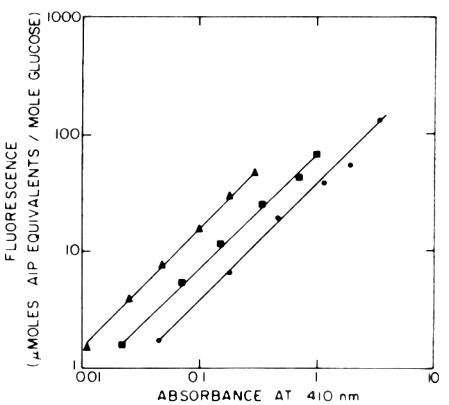


Fig. 2—Relationship between fluorescence and absorbance at 410 nm in the glucose-glycine system. Molar ratios of glucose:glycine are 2:1 (●); 1:1 (■); and 0.5:1 (▲). Fluorescence is expressed as μmole equivalents of glycine-1-amino-3-iminopropene (AIP) formed per mole of glucose.

pare the solutions. There was a 5-fold increase in the fluorescence within the first 10 min and, thereafterwards, it increased steadily, but the rate decreased after 20 min. After 120 and 180 min of reaction, the fluorescence increased only 3-fold and 2.5-fold, respectively, over that at 60 min.

Figure 2 shows that the logarithmic relationship between absorbance at 410 nm and fluorescence was maintained with molar ratios of glucose to glycine at 2:1, 1:1 and 0.5:1. The fluorescence yield is expressed as μmoles of glycine-1-amino-3-iminopropene (Chio and Tappel, 1969) per mole of glucose, and was 130 after 60 min of reaction with a 2:1 molar ratio of glucose:glycine. Decreasing the molarity of glucose solutions significantly decreased the fluorescence yield. Although the amount of fluorescent products was small, the inherent sensitivity of fluorescence measurements allowed measurement during the initial time periods when the absorbance at 410 nm was very low.

Identification of the fluorescent products

The products had excitation and emission maxima of 350 nm and 430 nm, respectively (Fig. 1). Malshet and Tappel (1973) have shown that the structure required for fluorescence is an electron donating group in conjugation with an imine. These compounds show decreased fluorescence at alkaline pH and when chelated by metals. These criteria can be used to identify this type of fluorescent chromophore. With the glucose-glycine products, a shift from pH 5.2 to pH 11.8 decreased the relative fluorescence by 35%. The fluorescence was restored upon readjustment to pH 4.9. Europium (III) 2,2,6,6-tetramethyl heptane-3-5-dione at 10^{-4} M in chloroform:methanol, caused a 9.8% decrease in the relative fluorescence.

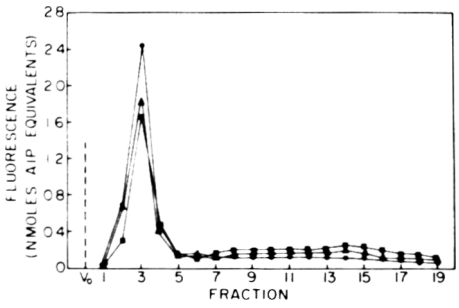


Fig. 3–Fractionation on Sephadex G-10 of fluorescent products from a glucose-glycine (2:1) reaction that was heated at 100° C for 60 min (●); 120 min (▲) and 180 min (■). In each case, one-half ml of the sample equivalent to 5 nmoles of glycine-1-amino-3-iminopropene was applied to the column. Column characteristics are described in the text.

Gel chromatography of the fluorescent products

Fractionation of the fluorescent products on a Sephadex G-10 column is shown in Figure 3. The major fluorescence was eluted in fractions 2–4 after the void volume in each fractionation, although later fractions also had low levels of fluorescence. Unreacted glycine eluted between fractions 17–22. With increasing reaction time, there was a decrease in the amount of fluorescent products in fractions 2–4 and a concomitant increase in the fluorescence of the smaller molecular-weight fractions. No free amine was present in fractions 2–4, showing that all the amino groups had reacted. The fluorescence spectra of chromatographed products (peak fractions) had maximum excitation at 390 nm and maximum emission at 470 nm, but were otherwise similar to those of the original, unchromatographed products. The effect of pH on fluorescence was the same as on the original product.

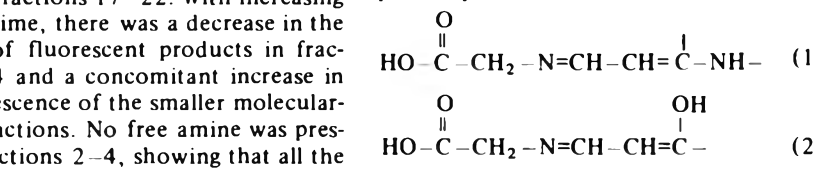
Fluorescence in brown foods

Application was made of these results to fluorescence measurements in brown foods. Most of the foods analyzed, primarily baked or roasted products, contained significant amounts of fluorescent products similar in character to those observed for the glucose-glycine products. Typical fluorescence spectra of aqueous extracts of toasted white bread and coffee are shown in Figure 4. The fluorescence decreased at alkaline pH. Fluorescence yield was not very high, and when expressed as nmoles of glycine-1-amino-3-iminopropene per g of food, it was 300 for toasted white bread and 700 for breakfast cereal.

DISCUSSION

THE PRINCIPAL AIMS of this study were to ascertain whether fluorescence could be used as a quantitative measure of the browning reaction and to partially identify the associated fluorescent chromophores. The observed linear relationship between fluorescence and browning (Fig. 2) is in agreement with previous reports (Pearce, 1950; Burton et al., 1963). The quantitative expression of the fluorescence yield, as μmoles of glycine-1-amino-3-iminopropene, coupled with the sensitivity of the fluorescence measurements, bring out the usefulness of these measurements of browning, particularly at earlier time intervals.

Fluorescence spectra of the products (Fig. 1) suggested that the chromophoric group is a Schiff-base in conjugation with an electron donating group (Chio and Tappel, 1969; Malshet and Tappel, 1972, 1973. On this basis, the effects of pH and of metal chelation can be explained. A shift in pH towards alkalinity dissociates the Schiff-base with resultant loss of the imine structure and a decrease in fluorescence. Reversal to acid pH restores the imine structure and the fluorescence. Chelation with $Eu(DPM)_3$ exerts a pull on the unpaired electrons of the electron donating group, which decreases the electron density around this group and leads to decreased fluorescence. Based on this information, structures for the chromophoric system could be:



(1)

(2)

Here, the groups –NH– and –OH serve as electron donating groups in conjugation with the Schiff-base. These fluorescent structures are formed by interaction of carbonyl and amine compounds (Malshet and Tappel, 1973). Interaction of glycine with unsaturated carbonyl compounds formed by the degradation of glucose would give rise to such structures in the glucose-glycine reaction system. This is further supported by the observations of Burton et al. (1963), who have shown that a decrease in conjugated unsaturated carbonyl content of a glucose-glycine system was concomitant with a decrease in the quantity of yellow, fluorescent substances. In view of the possible breakdown products of glucose, structure [2] is more likely to be present. The fluorescent products formed from an ethanolamine-sugar system are also suggested to be hydroxylic in nature (Burton et al., 1962a). Gel chromatography of the products (Fig. 3) showed that the fluorescent groups were located mainly in polymers.

The similarity of fluorescent products from the glucose-glycine system and those from the brown foods (Fig. 4) points to the feasibility of using fluorescence measurements to assess browning in foods. The fluorescent products are easily extractable, and the sensitivity of

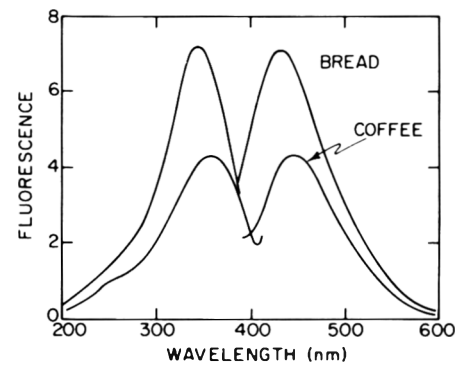


Fig. 4–Fluorescence spectra of extracts of toasted white bread (diluted 90x) and coffee (diluted 100x).

the method makes possible the measurement of small amounts of the fluorescent products.

The presence of fluorescent substances in model browning systems and in foodstuffs has been reported (Hannan and Lea, 1952; Burton et al., 1962a, 1963), and speculations as to whether the level of fluorescence could be used as a guide to the browning potentialities of foods have also been made (Friedman and Kline, 1950; Simonson and Tarassuk, 1952). The method described in this study uses spectral characteristics and chemical tests to essentially identify the character of the fluorescent chromophores in a glucose-glycine browning system. It also allows quantitation of this type of fluorescence in model systems and/or foods.

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Ms received 10/30/72; revised 12/11/72; accepted 12/12/72.

This research was conducted under a fellowship awarded by the International Atomic Energy Agency of the United Nations and administered by the National Academy of Sciences—National Research Council, Washington, D.C., and under U.S.P.H.S. grant AM 09933 from the National Institute of Arthritis and Metabolic Diseases.

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RHEOLOGICAL PROPERTIES OF SYRUPS CONTAINING GUMS

INTRODUCTION

FLUID FOODS may be categorized into two groups according to their rheological properties: Newtonian and non-Newtonian. Newtonian-type foods are homogenous mixtures such as oils and syrups, which exhibit no change in viscosity (resistance to flow) as the rate of shear (mechanical agitation) is increased. Non-Newtonian-type foods, however, are heterogeneous mixtures of substances, such as tomato catsup and various sauces, and do exhibit a change in resistance to flow (apparent viscosity) as the rate of shear is increased. Most types of fluid foods are of the non-Newtonian type.

In addition to thinning due to mechanical action, thinning is caused by elevation of the temperature. Thinning may be desirable for some products in certain instances; on the other hand, it may not be desirable. An example of a food product where it is desirable to maintain a fairly uniform viscosity over a wide temperature range is a fruit pie filling. In this case the filling must exhibit desirable flow properties when served either hot or refrigerated. Table syrup is an example of a Newtonian-type food which becomes thinner with increasing temperature.

The incorporation of hydrocolloids (gums) into fluid food systems may alter the rheological properties and flow rates. For instance, the flow properties of a Newtonian-type liquid may become non-Newtonian by the addition of a gum which exhibits non-Newtonian characteristics. This influence is of utmost importance when a gum or combination of gums is added to thicken the system (Farkas and Glicksman, 1967; Glicksman, 1969).

Many food-grade gums are available to the industry (Ganz, 1969; Glicksman, 1969). Each gum has unique properties and is selected for usage in a particular system on this basis. Some solutions of gums are Newtonian, e.g., low viscosity-type sodium carboxymethylcellulose (CMC) (Glicksman, 1963); some are dilatant, e.g., furcellaran (Farkas and Glicksman, 1967); and some are pseudoplastic, e.g., xanthan gum (Rocks, 1971). Solutions of dilatant-type gums become thicker and solutions of pseudoplastic-type gums become thinner as the rate of shear is increased. According to Rocks (1971), xanthan gum is the most pseudoplastic of the gums now available.

Most aqueous solutions of gums show a decrease in viscosity as the temperature is increased (Ganz, 1969; Glicksman, 1963). For example, the viscosity of certain solutions of CMC is reduced from 80–90% (calculated) when the temperature is raised from 10° to 70°C (Anon., undated). Contrariwise, the viscosity of xanthan gum solutions is reported to be more resistant to changes with increasing temperature. A solution of xanthan gum having a viscosity of 1000 centipoises (cps) is not reduced more than 100 cps between 10° and 70°C (Rocks, 1971).

The incorporation of gums into a food system may alter its rheological properties. According to Szczesniak and Farkas (1962), the mouthfeel imparted to a liquid or semiliquid product by the presence of a gum is especially important when gums are used at concentrations sufficient to affect the consistency of the food, as in the case when they are used as bodying and thickening agents. In such applications some fluid foods containing certain gums are slimy in the mouth and are difficult to swallow whereas the effect of other gums is hardly noticeable. The tongue provides the mechanical mixing action which would tend to cause non-Newtonian-type foods to become thinner but would cause no change on Newtonian-type food. These authors concluded that the degree of sliminess decreased with increasing deviations from the Newtonian character and that solutions of gums (or foods) exhibiting a high degree of shear-thinning are nonslimy in the mouth.

The experiment reported here was conducted to determine the effect of CMC and xanthan gums at three concentrations on the rheological properties and apparent viscosity of three 50% solids syrups as influenced by a temperature range between 26.7° and 71.1°C.

EXPERIMENTAL

THREE KINDS of syrups, sucrose, corn and a sucrose-corn syrup blend, were prepared to 50% solids. The sucrose syrup was prepared with table-grade sugar. "Regular" corn syrup of 42 dextrose equivalent (D.E.) and 82.2% solids (Hubinger Co., Keokuk, Ia.) and a sucrose-corn syrup blend (approximately 10% sucrose and 90% 55 D.E. corn syrup) of 75.6% solids were diluted to 50%.

Two gums were selected on the basis of results of a preliminary study in which four gums were studied; these were sodium CMC P-75-L, P-75-M and P-75-H (Du Pont, Inc., Wilmington, Del.), xanthan gum (Kelco Co., Clark, N.J.) and methylcellulose and methylcellulose 65 HG (Dow Chemical Co., Midland, Mich.). The gums selected for this study were CMC P-75-M and xanthan. Also, based on the preliminary studies, three concentrations of each gum were selected: CMC at 0.5, 1 and 1.25% and xanthan at 0.1, 0.2 and 0.3% (based on total weight of the syrup) (Dincer, 1972).

Before the water was added to the sugar (sucrose) or syrup, the appropriate amount of gum was dissolved in the designated amount of water. The aqueous solutions of gum and sugar or syrup were mixed with a magnetic stirrer for 2 hr.

The Brookfield Synchro-Lectric Viscometer, model LVF (Brookfield Engineering, Stoughton, Mass.) was used. All speeds, 6, 12, 30 and 60 rpm, and spindles Nos. 1 through 4 were utilized. The viscometer measures relative viscosities which are comparable to each other.

Table 1–Brookfield apparent viscosity in centipoise at 6 rpm for syrups containing sodium carboxymethylcellulose gum[a]

Syrup	Gum, %			Syrup mean
	0.5	1.0	1.25	
Sucrose	86f	427e	1064b	526j
Corn	80f	497d	1121a	566i
Sucrose-corn syrup blend	76f	457e	927c	487k
Gum mean	81z	460y	1037x	

[a]Each value of centipoise is the mean of the following number of observations: syrup, 45; gum, 45; syrup × gum, 15. Means within each factor not followed by the same letter are significantly different ($P < 0.05$).

The term "viscosity" is reserved for Newtonian fluids; "apparent viscosity", for non-Newtonian fluids. Thus, when the Brookfield viscometer is used, the term "comparative apparent viscosity" is understood to express the resistance to flow for fluids exhibiting non-Newtonian properties.

Measurements were taken at 26.7°, 37.8°, 48.9° 60° and 71.1°C. Temperature was maintained by placing the mixture in a water bath with an accuracy of ±0.02°C.

For measurement of apparent viscosity, 450 ml of the syrup were placed into a 600 ml beaker and allowed to equilibrate at the specified temperature before a reading was taken.

The data were analyzed by the analysis of variance as a factorial design (syrup × concentration of gum × temperature × replication: 3 × 3 × 5 × 3). A sample within each replication was tested at the five temperatures. Each value per replicate was the mean of three observations. Measurements of apparent viscosity are presented for 6 and 60 rpm; however, where the apparent viscosity-rate of shear relationships are presented, data for 12 and 30 rpm are included. Data for each rate of shear were analyzed separately.

RESULTS & DISCUSSION

ALL FACTORS at each rate of shear for both gums had a significant effect of apparent viscosity of the syrups at the level of probability of 0.01 or greater. The replication factor for each shearing rate was significant to at least the 0.05 level; this was caused probably by small deviations in the maintenance of the designated temperature and an inadvertant disproportionality in the admixture of gum and syrup. One observation of seeming importance is that of a decreasing residual error of the analysis of variance as the rate of shear was increased. This indicates that greater precision was obtainable at the higher rates of shear.

When CMC was added to the syrups, the apparent viscosity at 6 rpm was increased significantly as the level of gum was raised, but the magnitudes of increase were different among the syrups (Table 1). At 0.5% CMC no difference existed in apparent viscosity among the syrups. When the CMC content was raised to 1%, corn syrup showed a higher apparent viscosity than sucrose and sucrose-corn syrup blend among which no difference was found. At 1.25% CMC, corn syrup was the most viscous while the blend of syrups was the least viscous. Means for syrups indicate that corn syrup developed the highest overall apparent viscosity, while the blend of syrups developed the lowest.

Syrups containing CMC when tested at 60 rpm showed a progressive increase in apparent viscosity as the level of gum was raised (Table 2). At 0.5% CMC, sucrose was more viscous than the other syrups. This finding is a deviation from that observed at 6 rpm where there was no difference among the syrups. At 1% CMC, corn syrup was the most viscous just as it was at 6 rpm; sucrose syrup and the blend of syrups were not different. Results at 1.25% CMC are similar between 60 and 6 rpm in that the apparent viscosity is highest for corn syrup and lowest for the sucrose-corn syrup blend. Mean values for syrups at all levels of CMC show that the blend of syrups was less viscous than the other syrups among which no difference existed. The overall apparent viscosity values of the syrups at 60 rpm were slightly less than those at 6 rpm except for syrups containing 0.5% CMC.

Xanthan gum increased the apparent viscosity of syrups progressively as the level of gum was raised (Table 3). No differences occurred among the syrups at 0.1% xanthan. At 0.2% xanthan, sucrose was the most viscous and the blend of syrups was the least viscous; corn syrup was not different from either. At 0.3% xanthan, corn syrup was the most viscous and sucrose syrup was the least viscous. Overall means show that corn syrup was more viscous than the other syrups which were not different from each other.

Syrups containing xanthan gum and tested at 60 rpm showed an increase in apparent viscosity with each increase in the level of gum (Table 4). No differences in apparent viscosity existed among syrups containing 0.1% or 0.2% xanthan, but at 0.3% xanthan sucrose syrup was less viscous than the other syrups among which there was no difference. Mean values show that corn syrup was the most viscous and that sucrose syrup was the least viscous.

Some comparisons can be made between the influence of the two gums on the apparent viscosity of syrup. Xanthan

Table 2—Brookfield apparent viscosity in centipoise at 60 rpm for syrups containing sodium carboxymethylcellulose gum[a]

Syrup	Gum, % 0.5	1.0	1.25	Syrup mean
Sucrose	158f	424e	869b	484i
Corn	97g	461d	927a	495i
Sucrose-corn syrup blend	83g	424e	768c	425j
Gum mean	113z	436y	855x	

[a]Each value of centipoise is the mean of the following number of observations: syrup, 45; gum, 45; syrup × gum, 15. Means within each factor not followed by the same letter are significantly different ($P < 0.05$).

Table 3—Brookfield apparent viscosity in centipoise at 6 rpm for syrups containing xanthan gum[a]

Syrup	Gum, % 0.1	0.2	0.3	Syrup mean
Sucrose	295f	1003d	2008c	1102j
Corn	316f	966de	2216a	1166i
Sucrose-corn syrup blend	267f	937e	2133b	1112j
Gum mean	293z	969y	2119x	

[a]Each value of centipoise is the mean of the following number of observations: syrup, 45; gum, 45; syrup × gum, 15. Means within each factor not followed by the same letter are significantly different ($P < 0.05$).

Table 4—Brookfield apparent viscosity in centipoise at 60 rpm for syrups containing xanthan gum[a]

Syrup	Gum, % 0.1	0.2	0.3	Syrup mean
Sucrose	110d	257c	432b	266k
Corn	123d	286c	524a	311i
Sucrose-corn syrup blend	110d	265c	495a	290j
Gum mean	114z	269y	484x	

[a]Each value of centipoise is the mean of the following number of observations: syrup, 45; gum, 45; syrup × gum, 15. Means within each factor not followed by the same letter are significantly different ($P < 0.05$).

had a greater influence on increased thickening than CMC. Even though syrups are usually Newtonian in character, in some instances the addition of gums converted them to non-Newtonian. There was a difference, however, in the relative influence; xanthan gum at all concentrations caused the syrups to exhibit shear thinning (thinning as the rate of shear was increased) but CMC caused shear thinning of fairly large magnitude only at the highest level. CMC at 0.5% produced syrups that were more viscous at 60 rpm than at 6 rpm, thus, CMC at the lowest concentration caused the syrups to exhibit a dilatant-type viscosity.

Addition of CMC and xanthan gums caused corn syrup to exhibit the highest apparent viscosity. A qualification of this fact is that when CMC was added and tested at 60 rpm, sucrose syrup was not different from corn syrup. Conversely, the sucrose-corn syrup blend had the lowest apparent viscosity or was among the syrups having the lowest viscosity, except when xanthan-containing syrups were tested at 60 rpm.

As an average of both gums and the three levels of each, the syrups became thinner as the temperature was raised (Fig. 1 and 2). The apparent viscosity curves for corn syrup and the sucrose-corn syrup blend were essentially parallel to each other over the temperature range. The values for sucrose syrup were slightly lower as a whole than the values for corn syrup, but higher than the values for the sucrose-corn syrup blend. The rate of thinning due to heating was lowest for sucrose syrup. A close approach to linearity was evident when the log of apparent viscosity was plotted against temperature, especially between corn syrup and the blend of syrups.

The apparent viscosity values of syrups at the different temperatures as influenced by type and concentration of gum are presented in Figures 3 (6 rpm) and 4 (60 rpm). The syrups with xanthan gum had a lower rate of thinning than syrups with CMC. Thus, xanthan gum may be used to maintain a fairly uniform apparent viscosity of syrups when there is a moderate change in temperature. The respective percentage decreases in apparent viscosity for syrups with xanthan gum at 6 and 60 rpm when heated from 26.7° to 71.1°C were: 64 and 54 for 0.1% gum; 55 and 45 for 0.2% gum and 48 and 42 for 0.3% gum. The respective percentage loss of apparent viscosity for CMC-containing syrups at 6 and 60 rpm were: 75 and 83 for 0.5% gum; 90 and 85 for 1% gum and 91 and 86 for 1.25% gum. Thus, the behavior of syrups containing xanthan and CMC gums was analagous to that reported for aqueous solutions of the gums (Anon., undated; Rocks, 1971).

The rheology of syrups at rates of shear between 6 and 60 rpm was altered when gums were added (Fig. 5). There was a slight increase in apparent viscosity (dilatant-type) at 0.5% CMC and a decrease (pseudoplastic-type) at 1.25% CMC. Syrups with 1% CMC retained the Newtonian-type flow. Syrups with xanthan gum exhibited a pseudoplastic-type flow and the slopes of the curves for the different concentrations of gum were similar to each other. Thus, the principle on which Szczesniak and Farkas (1962) reported should apply in this study: since syrups with xanthan gum exhibited shear-thinning (reduction in apparent viscosity as the rate of shear is increased), they should be easier to swallow than syrups with CMC and should impart less sliminess in the mouth.

The appearance of the syrups containing the gums was similar to that encountered with gum-water mixtures. CMC forms solutions that are clear and essentially colorless while xanthan gum usually causes some opalescence (Anon., un-

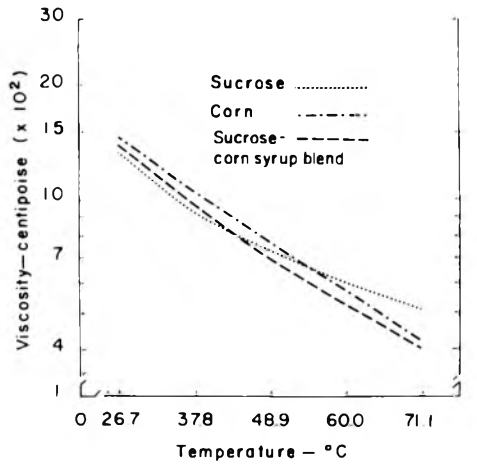

Fig. 1–Effect of temperature on Brookfield apparent viscosity of syrups containing sodium carboxymethylcellulose gum (0.5, 1, 1.25%) and xanthan gum (0.1, 0.2, 0.3%) at 6 rpm of shear. Means of 18 observations.

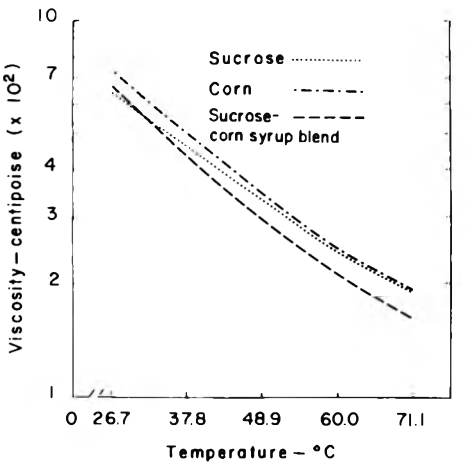

Fig. 2–Effect of temperature on Brookfield apparent viscosity of syrups containing sodium carboxymethylcellulose gum (0.5, 1, 1.25%) and xanthan gum (0.1, 0.2, 0.3%) at 60 rpm of shear. Means of 18 observations.

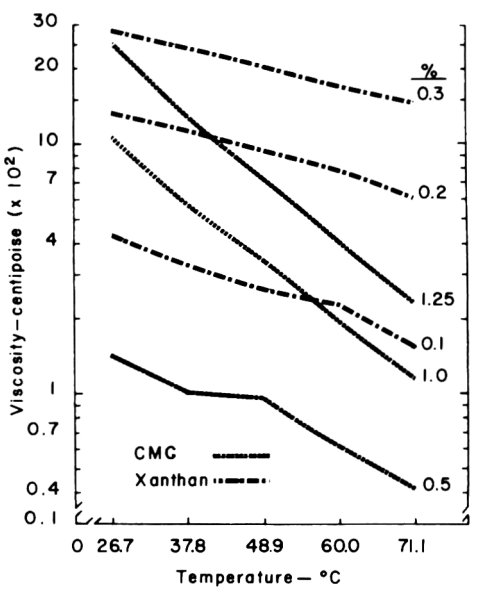

Fig. 3–Effect of gums and concentrations on Brookfield apparent viscosity of syrup (sucrose, corn, sucrose-corn syrup blend) as influenced by temperature at 6 rpm of shear. Means of 9 observations.

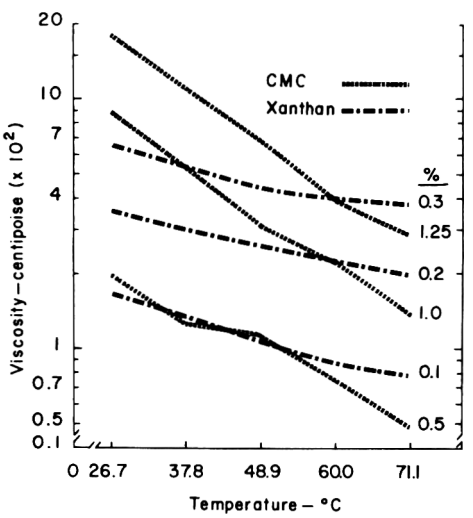

Fig. 4–Effect of gums and concentrations on Brookfield apparent viscosity of syrup (sucrose, corn, sucrose-corn syrup blend) as influenced by temperature at 60 rpm of shear. Means of 9 observations.

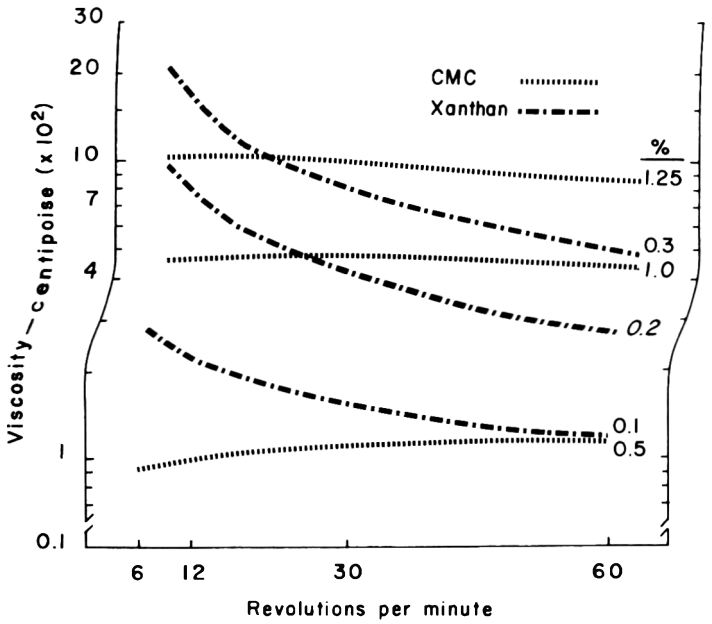

Fig. 5–Brookfield apparent viscosity of syrup (sucrose, corn, sucrose-corn syrup blend) at different rates of shear (rpm) as influenced by gums and concentrations. Means of 45 observations.

dated; Glicksman, 1969). The appearance of CMC-containing syrups was not noticeably different from the pure syrups, but xanthan-containing syrups were opalescent.

Syrups having 50% solids were utilized because of the inability to dissolve gums in full-strength syrups. According to Carlson and Ziegenfuss (1965), hydration of guar gum in a 65% sucrose solution was insignificant.

In conclusion, dilute syrups are thickened by the addition of gums. The choice of gum must be based, however, on the resultant properties that are imparted to the syrup by the gum. CMC imparted a thickening influence and a conversion of flow properties from Newtonian to dilatant at 0.5% CMC, while xanthan gum produced thickening that was greater than that produced by CMC, a conversion of flow properties from Newtonian to pseudoplastic, opalescence and the rate of shear-thinning was reduced when compared to the effect of CMC with increased temperatures.

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Ms received 9/1/72; revised 12/20/72; accepted 12/18/72.

JFS APPLIED SCIENCE and ENGINEERING

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VITAMIN RETENTION IN BEAN PRODUCTS: COOKED, CANNED AND INSTANT BEAN POWDERS

INTRODUCTION

INSTANT BEAN POWDER was developed at this Laboratory several years ago (Morris, 1961). More recently, a modification of the process was described by Kon et al. (1970). Due to recent increased commercial interest in these products, it appears that they may find their way into a number of commercial convenience food products (White and Kon, 1972). In view of the recent emphasis on nutrient content of processed foods, it becomes important to evaluate new food processes in terms of their effects on nutrient retention.

Schroeder (1972) reported the losses of vitamins and trace minerals resulting from processing and preservation of a variety of foods and suggested that vitamins such as pyridoxine and panthothenic acid and minerals such as chromium, zinc and manganese may have to be supplemented in diets of people eating processed foods. Numerous researchers (Farrer, 1955; Rajalakshmi et al., 1964; Hellendoorn et al., 1971; Rice and Benk, 1953; Hackler et al., 1964; and Guerrant et al., 1946) have verified that heating decreases the vitamin content of legumes by a variable amount ranging from 25–77%. Other researchers (Clifcorn, 1948; Cameron, 1955) have suggested methods of improving nutrient retention in canned and processed foods.

This paper reports on the retention of thiamine, pyridoxine, niacin and folacin in instant drum-dried pinto bean powder prepared by two different processes and compares the results with cooked and canned beans of the same variety. Protein efficiency ratios (PER) were also determined to assess protein quality of the processed beans.

MATERIALS & METHODS

Regular instant pinto bean powder

5 kg of raw pinto beans were soaked in water overnight. Representative samples of the soaked beans were sealed in glass jars and quickly frozen at −10°F. These served as controls for the drum-dried powder. The remainder of the beans were cooked in the soaking water for 1 hr at 210°F with constant stirring. The bean slurry was then passed through a Reeves disintegrator (screen size 1-16) to break up all particles. The cooked bean slurry was drum dried (double drum, 12 in. diam × 18 in. long at 260°F for 30 sec) yielding flakes which were further dried in a low humidity room (3% moisture) for 2 days. Samples of the dried powder were frozen for future vitamin analysis.

Acid treated instant pinto bean powder

5 kg of raw pinto benas were ground in a Wiley mill through a 2 mm screen. Samples of the ground beans were sealed tightly in glass jars and frozen immediately at −10°F. These samples served as the raw bean controls. The ground beans were added to water which was acidified with concentrated HCl to produce a slurry of about pH 3.5. The slurry was passed through a Reeves disintegrator (screen size 1-16) to break up all particles, cooked for 15 min at the acid pH, neutralized with NaOH to pH 6.0–7.0 and cooked for another 45 min. The cooked slurry was drum dried for 30 sec at 260°F followed by finish drying in a low humidity room (3% moisture) for 2 days. Samples of this powder were frozen for future vitamin analysis.

Canned beans

An 11.4 kg bag of pinto beans was thoroughly mixed and divided into 2 lots. Representative samples from both lots were ground in a Wiley mill through a 2 mm screen, and approximately 100g of the ground material was packaged and quickly frozen. This material served as the raw bean control for the canned products. One lot of beans was cooked in boiling water in a stainless steel pot until fully cooked (approximately 2 hr). The cooked beans were drained and weighed (215g) into 300 × 407 cans which were then filled to 0.5 in. from the top with fresh water. The cans were machine sealed, divided into three batches and treated as follows: (1) Frozen quickly at −10°F without further treatment; (2) Retorted in a rotary retort for 45 min at 250°F; (3) Retorted in a rotary retort for 90 min at 250°F.

Samples of the measured cook water were also quickly frozen for future vitamin analysis.

The other lot of beans was soaked overnight in water, drained, filled into 300 × 407 cans with fresh water as described above and exhausted in steam at 212°F for 15 min before sealing and retorting for 45 min at 250°F. All canned samples were frozen at −10°F, and the next day representative samples from each treatment were freeze-dried and ground in a Wiley mill through a 2 mm screen. The ground samples were then frozen for subsequent nutrient analysis. The moisture content of all samples was determined so that vitamin content could be expressed on a moisture free basis.

Protein efficiency ratio (PER)

Protein efficiency ratios were determined as described by Derse (1965) on specially formulated diets containing 10% protein supplied entirely by the different bean samples. The bean samples were prepared in the same manner as those used for the vitamin analysis.

Thiamine-thiochrome determination

In addition to extraction and acid hydrolysis of the bean powders, pepsin, a proteolytic enzyme, was used to free the thiamin-protein complexes, followed by takadiastase for the fission of vitamin B1 phosphoric acid esters as described by Strobecker and Henning (1966). Purification of extracts for thiochrome determinations was done according to the procedure described in "The Vitamins" (1971).

Pyridoxine-microbiological assay

The total pyridoxine content of the bean samples was done according to the AOAC method (1970). No attempt was made to separate the three different forms of pyridoxine. The microorganism used was *S. carlsbergensis* ATCC #9080. Difco #095113-12 was used as the pyridoxine inoculum medium. Free pyridoxine was determined according to the method of Toepfer et al. (1963). Distilled water was used to extract the bean samples instead of HCl used for the determination of total B_6 content.

Niacin-microbiological assay

Niacin content was determined microbiologically with *L. planterium* ATCC #8014 according to the method described by AVC (1951). The bacterial growth was measured turbidemetrically after 21 hr growth at 37°C. Difco 0 322-15-4 bacto-niacin assay medium was used. Free niacin was determined according

Table 1—Reproducibility of vitamin assay procedures

Sample[a]	Total vitamin content (mg/100g)			
	Thiamine	Pyridoxine	Niacin	Folacin
1	0.63	0.49	1.51	0.057
2	0.67	0.54	1.53	0.055
3	0.69	0.60	1.51	0.054
4	0.68	0.58	1.53	0.059
5	0.67	0.60	1.54	0.055
Mean	0.67	0.56	1.53	0.055
Standard deviation	±0.02	0.05	0.02	0.003
Coefficient of variation	3%	9%	1.3%	5.5

[a]Each of the five samples taken from the same lot of ground and thoroughly mixed beans

to the method of Snell and Wright (1941). Water instead of H_2SO_4 was used as the extractant.

Folacin-microbiological assay

Folacin content was determined microbiologically with *L. casei* ATCC #7469 by a procedure similar to that of Butterfield and Calloway (1972). The bean samples were extracted in 0.1M phosphate buffer (pH 6.1) containing 0.5% ascorbic acid (pH readjusted to 6.1 with sodium hydroxide). Total folacin content was determined with hog-kidney conjugase (Eigen and Shokman, 1963).

RESULTS & DISCUSSION

AN ATTEMPT was made to determine the degree of error introduced in the vitamin values by the specific procedures used for analysis. Table 1 shows the results for five separate subsamples from a uniformly ground sample of raw beans. The data show that the techniques used gave good repeatability on the same sample with coefficients of variation ranging from a low of 1.3% to a high of 9%. This indicates that fairly small differences among samples may be related to the processes to which they were subjected and to natural variation in the bean samples themselves.

The vitamin and PER values for the various bean products are shown in Table 2. It will be noted that the raw bean samples used in each of the three different basic processes (e.g., regular drum dried, acid processed drum dried and canned) showed a considerable variation in vitamin content. The variation in the three different lots of raw bean samples for the four vitamins was 13% for pyridoxine, 18% for niacin, 59% for thiamine and 100% for folacin. Since we were primarily concerned with the effects of different processing methods on vitamin retention rather than raw product variation, it was necessary to calculate percent retention based on the value for raw beans used in each process. In view of the present interest in nutritional labelling, however, it may be worth noting that label claims for these four vitamins would be difficult to make on the basis of raw product variation alone.

The main losses for niacin, pyridoxine and folacin occurred in the discarded cook water after 2 hr of cooking at 210°F. Retorting for 45 90 min at 250°F caused very little additional loss, suggesting that these time-temperature conditions did not cause significant breakdown or otherwise reduce the potency of these three vitamins. On the other hand, thiamine losses steadily increased with increasing retort time at 250°F clearly indicating the breakdown of this compound with prolonged heating. This was not surprising since the destruction of thiamine by heat processes has been reported previously (Cameron 1955). It is also clear that much large amounts of the vitamins except for folacin were retained in the soaked canned samples retorted for 45 min at 250°F than in the cooked canned samples retorted at the same time and temperature. Again this is mostly due to the large difference in amount of vitamins extracted by the cooking and soaking water (Table 2). A measure of the effect of retort time at 250°F was obtained by calculating percent retention for the retorted samples based on the vitamin values obtained after 2 hr of cooking. On this basis there was only a 2% loss in pyridoxine, 7% loss in niacin, 16% loss in folacin and a 57% loss in thiamine after 90 min of retorting at 250°F. Except for thiamine, these losses were minor compared to the initial losses in the cook water indicating that pyridoxine, niacin and folacin were not particularly sensitive to heat degradation as represented by 90 min of retorting at 250°F.

The process for making instant bean

Table 2—Effect of processing procedures on vitamin content and PER of pinto beans

Identification	Vitamin content—mg/100g dry wt								
	Thiamine	Pyridoxine		Niacin		Folacin		PER	
	Total	Total	Free	Total	Free	Total	Free	Actual	Corrected[b]
Instant bean powders									
Raw beans, soaked 24 hr	0.69	0.70	0.30	1.48	1.38	0.14	0.05		
Regular drum-dried powder	0.56	0.57	0.22	1.19	1.08	0.11	0.03	1.28 ± 0.10	0.92
Raw ground beans	0.54	0.69	0.18	1.49	1.36	0.19	0.07		
Acid processed, drum-dried powder	0.45	0.58	0.13	1.47	1.42	0.07	0.02	1.33 ± 0.30	0.91
Canned beans									
Ground raw beans	0.86	0.61		1.74		0.28			
Cook 2 hr, no retort	0.54	0.40		0.97		0.19		1.80 ± 0.22	1.26
Cook 2 hr, retort 45 min/250°F	0.37	0.39		1.02		0.17		1.51 ± 0.38	1.06
Cook 2 hr, retort 90 min/250°F	0.23	0.39		0.90		0.16			
Soak, blanch, retort 45 min/250°F	0.59	0.56		1.46		0.18		1.20 ± 0.09	0.84
Soaking water	0.046[a]	0.009[a]		0.42[a]		0.026[a]			
Cooking water	1.33[a]	0.31[a]		3.83[a]		0.416[a]			

[a]μg/ml

[b]Corrected to 2.50 casein

powders by either the regular method or the acid process did not appear to materially affect the ratio of free to total vitamin content. The regular method caused a slight decrease in percentage of vitamin in the free form for pyridoxine and niacin and somewhat more for folacin. The acid process caused similar decreases for pyridoxine and folacin. Apparently the acid extraction during part of the cooking process freed some of the bound niacin thus increasing the availability of this particular vitamin. However, a major part of the niacin content of the raw bean already exists in the free form and the effect of processing was minor. Neither the acid nor regular process for preparing drum-dried powder had much effect in increasing or decreasing free pyridoxine or folacin.

The most dramatic difference between the two drum-dried products was in folacin retention and to a lesser extent in niacin retention. The regular drum drying procedure caused a 19–21% loss in all four vitamins whereas losses for the acid process varied from a low of 1% for niacin to a high of 63% for folacin.

Comparing the powders with the canned products, it is apparent that powders prepared by the regular or acid procedure retained a substantially higher percentage of thiamine than any of the canned beans. In addition to large extraction losses during cooking prior to canning, retorting at 250°F severely degraded thiamine in the canned samples. Since there were no extraction losses prior to drying and the cooked bean slurry was in contact with the drums at 260°F for only 30 sec, it is reasonable to expect greater retention of the heat labile thiamine in the product with the shortest exposure to high temperature. Since pyridoxine was relatively stable during retorting at 250°F we would not expect any great differences in retention of this vitamin in powder or canned samples. The data (Table 2) indicate that retention of this vitamin was indeed similar for the powders and soak-blanch canned samples. The lower retention in the 2 hr cooked canned samples is almost entirely due to losses in the cook water and not to retorting as indicated by the same retention after 45 and 90 min of retorting at 250°F. Results for niacin were similar to those for pyridoxine—e.g., retention in the powders and soak-blanch canned samples were similar because niacin was not particularly sensitive to retorting at 250°F. The low niacin values in the cooked canned samples again are almost entirely due to the extraction losses during cooking. While folacin was much more stable to retorting than thiamine, more of this vitamin was lost as a result of retorting at 250°F than either niacin or pyridoxine. Therefore we would expect the reduced time-temperature exposure of the regular drum drying to result in somewhat better retention for this vitamin. The regular drum-dried powder and the soak-blanch canned samples retorted for 45 min at 250°F retained 79% and 64% of the original amount of folacin in the raw beans respectively. The biggest loss of this vitamin occurred in the acid processed drum-dried powder. Apparently partially cooking the ground beans in acid prior to drum drying was primarily responsible for the large loss of this vitamin. Therefore, retention of these four vitamins in the regular drum-dried powder is generally equal to or better than in the soak-blanch canned products and consistently better than in the cooked canned products. The same general statement applies for the acid processed powder except for folacin retention which is lower in the powder than in all canned products. The protein efficiency ratios shown in Table 2 indicate that there were no important differences between canned retorted beans and either of the powders, indicating that protein quality of the different products was similar. Considering both protein quality and vitamin retention, the regular drum-dried instant pinto bean powder was similar or superior to the canned product. Thus the convenience of a readily reconstituted dry powder is available without sacrifice in nutritional quality.

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Ms received 10/1/72; revised 11/18/72; accepted 11/20/72.

The authors gratefully acknowledge the valuable advice and assistance of Dr. John Garibaldi on microbiological assays and thank Dr. A.N. Booth for the protein efficiency ratios.

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

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SPLIT PEELED BEANS: PREPARATION AND SOME PROPERTIES

INTRODUCTION

BEANS have been an important food for many centuries. Together with other legume seeds they are an excellent source of protein and other nutrients. However, they require prolonged soaking, followed by a long cooking time. Over the years different methods of making beans quick-cooking and easier to use have been developed. Nielsen (1963) and Rockland and Metzler (1967a, b) reported the development of processes for preparing various types of quick-cooking dry beans. Steinkraus et al. (1964) reported the development of precooked dry bean products. All those treatments required soaking the beans, with subsequent drying or freezing. Complex processes raise the cost of bean products, eliminating them as a cheap source of protein.

This paper reports the development of an inexpensive mechanical way for making quick-cooking beans.

EXPERIMENTAL

Dry beans

California Small White (CSW) beans grown in the Salinas valley and pinto beans from Idaho were obtained from Trinidad Bean and Elevator Co., San Francisco. Sanilac beans were obtained from Michigan Bean Co., Saginaw, Mich.

Equipment

The peeling was done with a Ce-Co-Co Barley and Wheat polishing (debranning) machine made by Chuo Boeki Goshi Kaisha, Central Commercial Co. Ibaraki, Osaka, Japan. The removal of fines, powder and remaining peel was done by a Carter Dockage Tester made by Hart-Carter Co., Minneapolis, Minn.

Cooking time

This was measured by using the experimental bean cooker as described by Burr et al. (1968). It consists of a frame which holds 100 saddles, each holding one bean; different saddles were used for different size beans. A vertical plunger rests on each bean. Each saddle is perforated vertically and when a bean becomes sufficiently tender, the plunger penetrates the bean and drops a short distance through the hole in the saddle. The cooking time for a sample is taken as the time required for 50% or 95% of the beans to be penetrated.

Rat feeding

Protein efficiency ratio (PER) and digestibility were determined as described by Derse (1965) on specially formulated diets containing 10% protein supplied entirely by the respective bean samples. The bean samples used for feeding tests were prepared by cooking without prior soaking, and then freeze drying them.

Table 1—Efficiency of peeling

Fraction	CSW %	Sanilac %
Total yield	88	85
Of this:		
Unpeeled	8	1
Peeled whole	73	12
Peeled split	16	85
Peeled broken	3	2

Table 2—Effect of peeling on cooking time

	Time in min for 50% cooked	Time in min for 95% cooked
Soaked overnight		
CSW	27	40
CSW–peeled	20	30
Sanilacs	28	55
Sanilacs peeled	18	27
Pintos	26	31
Pintos–peeled	13	19
Unsoaked		
CSW	69	86
CSW–peeled	40	50
Sanilacs	75	99
Sanilacs–peeled	35	53
Pintos	82	25
Pintos–peeled	25	52

Vitamin analysis

Thiamine was determined by the thiochrome method as described by Gyorgy and Pearson (1967) and Strohecker and Hennings (1966). Niacin was determined turbidimetrically by the use of *Lactobacillus plantarum* ATCC #8014 as described by Association of Vitamin Chemists (1957). Pyridoxine was determined using *Saccharomyces carlsbergensis* ATCC #9080 by the method as described in AOAC (1970). Folic acid was determined using *Lactobacillus casei* ATCC #7479 as described by Jukes (1965).

RESULTS & DISCUSSION

THE CE-CO-CO Barley and Wheat polishing machine used to peel the beans is basically a tapered carborundum rotor turning inside a slotted screen. This mill did an excellent job of peeling CSW and Sanilac beans, but when pinto beans or red beans were run through results were not as good. This is primarily due to the fact that pinto beans and red beans are flat while CSW and Sanilac beans are rounder and smaller. We believe that changing the taper of the carborundum rotor will make it possible to use the bigger beans. As it was, too high a proportion of the larger beans was milled out and lost as powder. With CSW and Sanilac beans, peeled bean yield was 88% and 85%, respectively (Table 1). Those results are averages of three 4-kg peeling runs. Identical results were also obtained on a 28 lb lot prepared for commercial trial. The peeled beans were classified into fractions as indicated in Table 1. The results reported in this table are for single passage through the peeling machine. As can be seen from

Table 3—Effect of peeling on weight gain, protein efficiency ratio and digestibility by rats

Dietary source of protein[a]	Wt gain (±) std dev[b]	PER[c] Actual ± SD	PER[c] Corrected	% digestibility[d] Whole diet	% digestibility[d] Nitrogen
Casein	107 ± 22	3.47 ± 0.19	2.50	95	93
CSW beans	44 ± 7	2.00 ± 0.23	1.44	90	69
CSW beans–peeled	40 ± 6	1.88 ± 0.15	1.35	91	74
Sanilac beans	32 ± 4	1.57 ± 0.10	1.13	91	74
Sanilac beans–peeled	32 ± 9	1.53 ± 0.29	1.10	92	77

[a] All diets contained 10% protein–28 day assay
[b] Five male weanling rats per group, S-D strain, 21 days old with mean initial wt 56g
[c] PER = gain in wt/g protein eaten
[d] Digestibility = $\frac{\text{feed intake} - \text{fecal wt}}{\text{feed intake}}$; feces collected during second week

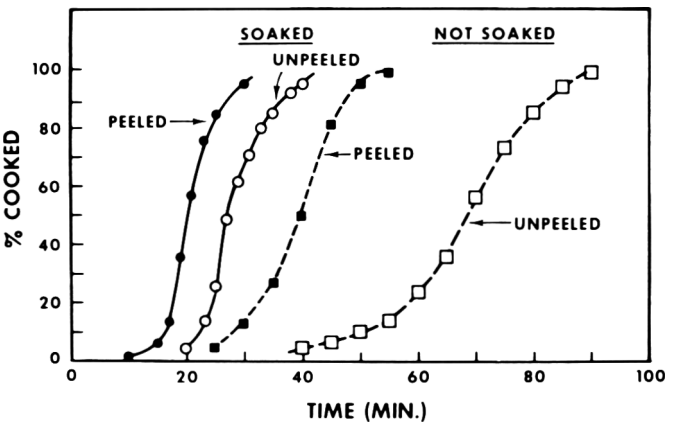

Fig. 1—Cooking rates for CSW.

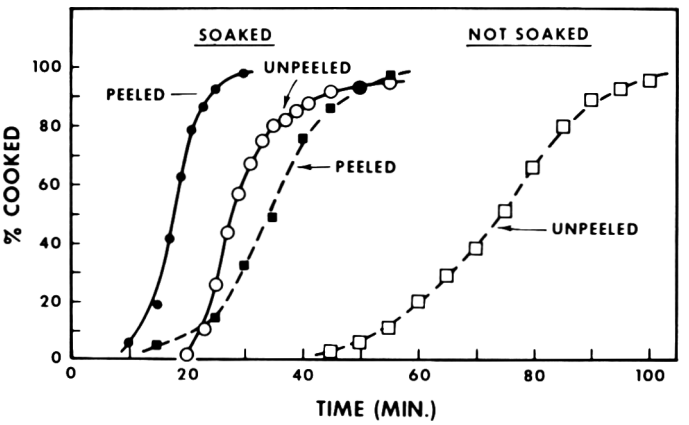

Fig. 2—Cooking rates for Sanilacs.

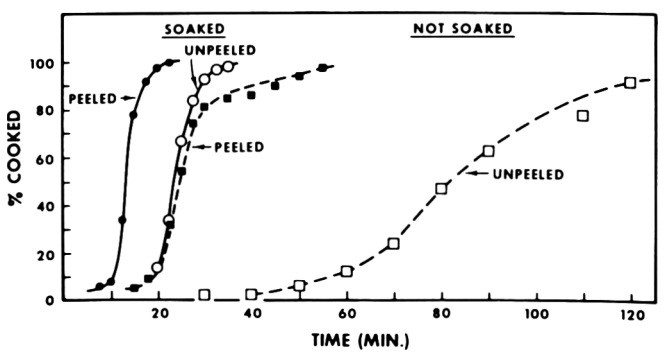

Fig. 3—Cooking rates for Pintos.

Table 4—Effect of peeling on residual vitamin content (μg/g dry wt)[a]

	Thiamin	Niacin	Pyridoxine	Folic acid
CSW	4.48	10.43	4.64	1.84
CSW peeled	5.45	13.38	5.79	3.32
Sanilacs	4.11	20.18	5.47	3.58
Sanilacs peeled	5.50	19.73	4.67	4.60

[a]Determined after cooking

the table, Sanilac beans are much more prone to splitting than are CSW beans. However, as seen in Figures 4 and 5 both look very attractive after peeling.

As mentioned before, beans normally require a long soaking before cooking. Even after over-night soaking, beans require almost an hour to cook. With the use of the experimental cooker described above, it was possible to determine cooking time objectively rather than subjectively. All the cooking results reported here are averages of three cooking runs. In all cooking experiments whole beans were used for the determination of cooking time except when peeled pinto beans were run. In the latter case split beans were used because no whole peeled pinto beans were obtained after peeling.

As can be seen from Table 2 and Figures 1, 2 and 3, it is apparent that cooking of both soaked and unsoaked beans is speeded up by the peeling process. When soaked beans are compared, peeling reduces the cooking time by 26% and 36% for CSW and Sanilacs, respectively. A bigger advantage of peeling is obtained when unsoaked beans are compared. Here the reduction in cooking time is about 42% and 53% for CSW and Sanilacs, respectively. The peeling of those beans results in a product that does not have to be presoaked and still cooks in reasonable time, about 45 min.

There was a strong feeling among bean processors that beans without skins would disintegrate during cooking to a puree-like product. As can be seen from Figures 4 and 5 both CSW and Sanilac beans kept their integrity after cooking. At this point they were almost all split but they were not mashed at all. The splitting is probably a major factor in contributing to the quicker cooking characteristics of those beans as the water has to penetrate only half the thickness as compared to unpeeled beans.

We were concerned about the loss of nutritional quality, due to the fact that during the peeling process some of the outer layers of the seeds were lost together with some of the germ, similar to that observed when rice is polished and wheat is bleached. For this reason we ran experiments to determine the protein efficiency ratio (PER) together with digestibility and growth rate by rats. From the results in Table 3 it is obvious that peeling did not affect these parameters significantly. As expected, the PER and weight gain for all beans were significantly lower than for casein, but both can be raised to the casein level by the addition of methionine to the bean diets, as shown by Kon et al. (1971) and others. Similar results can be obtained by supplementing the bean diets with a high methionine protein source, such as sesame seeds.

Because beans are an important source

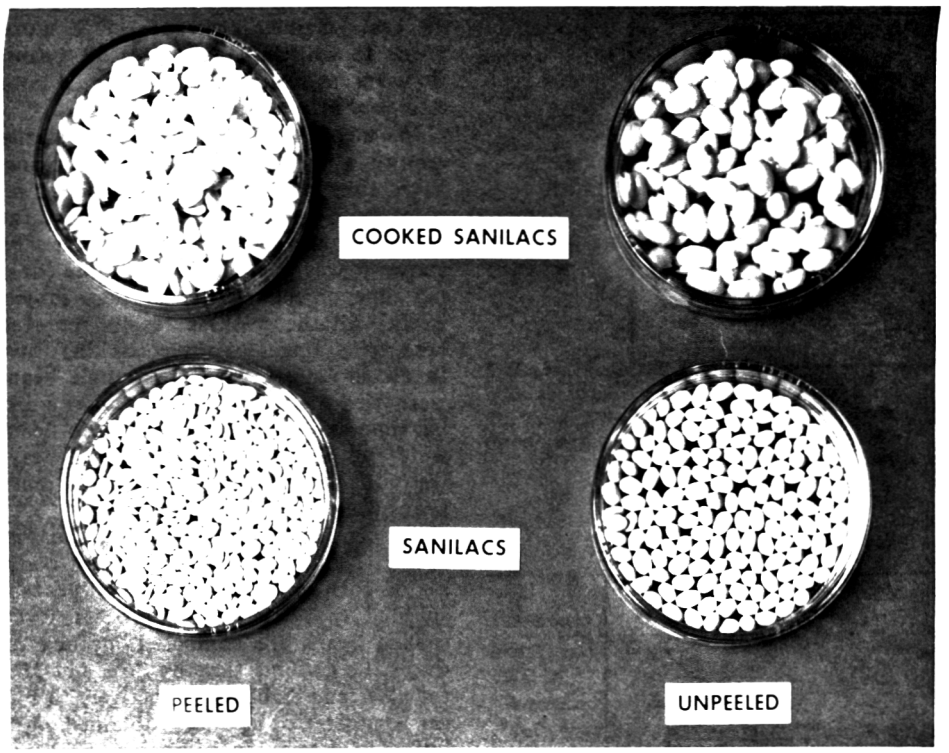

Fig. 4—Sanilac beans—peeled and unpeeled.

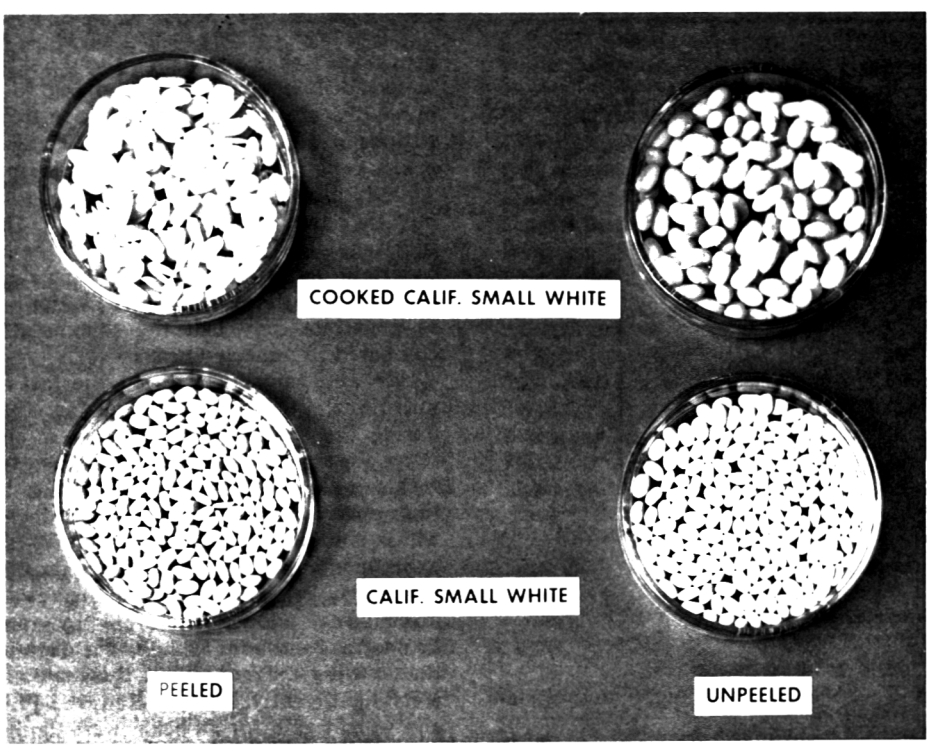

Fig. 5—California Small White beans—peeled and unpeeled.

of some of the water-soluble vitamins in addition to being a good source of protein we determined the vitamin content of the peeled beans and compared with the unpeeled beans. As shown in Table 4 there does not seem to be any loss in the vitamin content, due to peeling, of the four vitamins tested. Samples for vitamin analysis were taken from the same cooked beans that were incorporated into the diet for the PER experiment, so that the amount of residual vitamins reported in Table 4 is the amount of vitamins present in the ready-to-eat product.

Some lots of beans tend to be high in "hard shells," that is beans that do not hydrate well when soaked. This results in nonuniform cooked product because the unhydrated beans require much longer cooking time. The peeling treatment eliminates this problem, and all the beans hydrate and cook equally well.

Although we did not conduct a formal storage testing program of peeled beans, the beans used in the experiments reported here were stored for over a year at room temperature without any ill affects.

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

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BLOATER FORMATION IN BRINED CUCUMBERS FERMENTED BY Lactobacillus plantarum

INTRODUCTION

BLOATER DAMAGE in brine-fermented cucumbers causes serious economic losses to the pickle industry. Thus, attempts to determine the causes of bloating have been made by numerous investigators over the last several decades.

Structural characteristics of cucumbers have been suggested as an important factor relative to bloating susceptibility (Jones et al., 1941; Sneed and Bowers, 1970). It is commonly know that bloater damage is a more serious problem in larger sizes of cucumbers. Thus, structural changes in the fruit as it increases in size, especially increased skin thickness and enlargement and changes in texture of the seed cavity, have been thought to be associated with the occurrence of bloater damage. Piercing the fruit prior to brining is known to prevent bloaters (Etchells and Moore, 1971). Varietal differences in cucumbers (Jones et al., 1954) and growing conditions of the fruit (Wormley, 1939) also have been suggested as factors influencing bloater damage in the brined stock.

Brining conditions influence bloater damage in natural fermentations of cucumbers. Higher brine strengths during the active stage of the fermentation (Jones et al., 1941), the addition of lactic acid or sugar to the fermenting brine (Jones et al., 1940) and fermentations at higher temperatures (Samish et al., 1957), were shown to cause more bloater damage.

Numerous reports have shown a marked relationship between the occurrence of a gaseous fermentation, caused by yeasts and gas-forming bacteria, and the incidence of bloater damage (Jones et al., 1941; Etchells et al., 1952, 1945, 1953, 1968; Etchells and Bell, 1950).

A mechanism for bloater development was proposed by Etchells et al. (1968). They suggested that the fermentation gas, which is produced solely in the cover brine, diffuses into the cucumber in the dissolved state, is released from solution inside the fruit and accumulates in pockets at locations of structural weakness. This theory is consistent with the earlier finding that gas trapped inside bloated cucumbers was essentially of the same composition as that which evolved from the brine (Veldhuis and Etchells, 1939; Etchells and Jones, 1941).

It was shown recently that both the cucumbers and the bacteria in the fermenting brine contribute to the amount of CO_2 that accumulates in the brine (Fleming et al., 1973). CO_2 diffused from the cucumbers into the brine prior to the onset of bacterial fermentation. The concentration of CO_2 increased further with the onset of bacterial growth in the brine.

No work relating the concentration of dissolved CO_2 in the brine to bloating of cucumbers has been published. Quantitative data on CO_2 production has been based on the CO_2 that evolved from the fermentation (e.g., Veldhuis and Etchells, 1939; Etchells et al., 1945).

In the present work, relationships between brine concentrations of dissolved CO_2 and bloater damage of the fermented cucumbers were studied. Effects of physical treatments of the cucumbers prior to brining, cucumber size and incubation temperature on CO_2 concentrations in the brine, and bloater development, were of particular interest.

MATERIALS & METHODS

Cucumbers

Fresh cucumbers were obtained from nearby commercial pickling plants or from experimental plots of the Dept. of Horticultural Science at North Carolina State University. They were carefully selected for uniformity of size and shape and freedom from mold growth and mechanical damage.

Brining and description of the fermentation vessel

Cucumbers were hand-washed, weighed and packed in 1-gal jars. A semi-rigid plastic netting was placed over the cucumbers to prevent them from rising into the neck of the jar. A cover brine containing 14.5% NaCl, w/v, 0.8% sodium acetate (trihydrate), and sufficient glacial acetic acid to adjust the brine to pH 4.5 was added to the jars. After equilibration with the cucumbers, the brine was pH 4.7 (±0.2) and contained 6.7% NaCl and 0.4% sodium acetate. The final pack-out ratio averaged about 50:50, grams cucumber:ml brine. The number of cucumbers of a given size packed per jar was held as nearly constant as possible. For size no. 3, 13 cucumbers were packed and, for size nos. 2 and 1, about 35 and 70, respectively.

The jars were fitted with a "Twist-off" cap (White Cap Co., Chicago, Ill.) and assembly as shown in Figure 1. Several items were placed in the cap by means of rubber serum bottle stoppers. These stoppers were described previously (Etchells et al., 1964). One stopper was inserted for sampling. A graduated 250 ml, cylindrical dispensing burette, 37 mm diameter, (Fisher Scientific Co.), hereafter referred to as the expansion reservoir, was inserted through another stopper which had been bored. The orifice of the reservoir, which protruded through

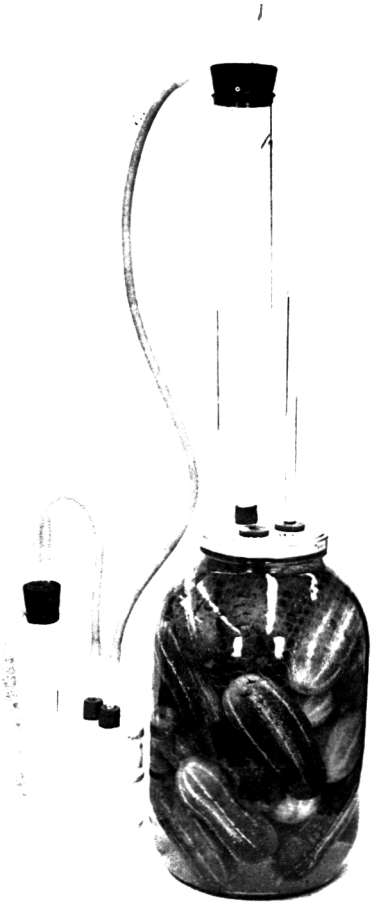

Fig. 1—Fermentation vessel with expansion reservoir and NaOH trap assembly. See text for details.

the stopper, had an internal diameter of 4 mm. This small opening allowed brine to rise in the reservoir during the fermentation, which minimized pressure build-up inside the jar. The small orifice greatly reduced surface exposure of the brine. An 8 mm diameter glass rod was fitted through another bored stopper and served as a plunger as well as support for the expansion reservoir.

When nitrogen purging of the jar contents was desired, a gas-dispersion tube was positioned through a fourth stopper in the jar cap so that the fritted portion rested near the bottom of the jar. Nitrogen was introduced by connection to the portion of the dispersion tube that protruded through the jar cap.

To trap evolved CO_2, the reservoir was connected to a trap containing standardized NaOH. The NaOH trap was protected from atmospheric CO_2 by means of a second NaOH trap and a tube of Ascarite. For most studies no attempt was made to trap evolved CO_2, and the reservoirs were loosely covered with a lid, which allowed any evolved gas to escape to the atmosphere.

The jar caps were heated to about 80°C to soften the sealing liner, and the caps with assemblies attached were then screwed onto the jars. It was convenient to first position the jar caps and then the expansion reservoirs. After this, sufficient cover brine was added to reach a level of 50 ml in the reservoir.

Inoculation

The brines and cucumbers were held at 24°C for 1 or 2 days to allow salt to diffuse in and fermentable nutrients to diffuse out of the cucumbers. Then, 5 ml of a culture of *L. plantarum* WSO which had been grown for 16 hr at 30°C in cucumber juice broth (Fleming and Etchells, 1967) was introduced by syringe through the sampling stopper.

Sampling and measurement of "expansion volume"

Brine samples were taken from the center depth of the jar by inserting a long needle through a sample port in the cap and along the inside of the jar. Duplicate 10 ml samples for CO_2 analysis and one 10 ml sample for turbidity and chemical analyses were taken with disposable 12-ml syringes. Trapped gas surrounding the cucumbers was freed by gentle movement of the plunger against the plastic netting placed on the surface of the cucumbers in packing. The cucumbers moved freely and any gas pockets external to the cucumbers rose to the top of the jar and out through the expansion reservoir. Small amounts of gas that remained under the cap were expelled by pressing on the jar cap several times. Then, the brine level in the graduate portion of the expansion reservoir was read. After correcting for the removal of brine due to sampling, net increases in volume were calculated and expressed as the "expansion volume," in milliliters. This rise in brine level that occurred during the bloating stage was attributed to gaseous expansion inside the cucumbers as proposed by Etchells et al., 1968. The volume of bloated cucumbers occupied as trapped gas, in percent, was estimated by dividing the volume which the cucumbers occupied at the time of brining by the expansion volume and multiplication by 100.

Evaluation for bloater damage

Brined cucumbers were cut longitudinally and examined for balloon-, honeycomb-, and lens-type bloaters as described and illustrated earlier (Jones et al., 1941; Etchells et al., 1968).

Subjective evaluations of bloater damage were on the basis of two criteria: (1) The percentage of cucumbers showing each of the three types of bloater defects was determined. When a cucumber had more than one type of defect, the balloon-type bloater was given priority in category placement. Lens and honeycomb categories gained priority over each other depending on the severity of each. (2) The degree or severity of damage was rated as none, slight, moderate or advanced.

Analyses

Analytical methods for determining percent titratable acidity (calculated as lactic acid), pH and percent NaCl in brines were described earlier (Etchells et al., 1964). Total reducing sugar was determined by the method of Sumner and Somers (1944), using glucose as the colorimetric standard.

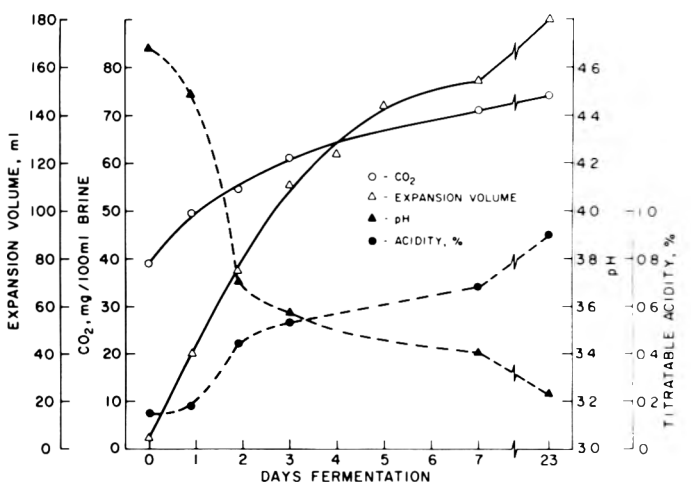

Fig. 2—Chemical changes and expansion volume increases during the fermentation of Explorer variety, size no. 3 cucumbers by L. plantarum WSO. Incubation was at 26.7° C.

Determination of CO_2 in brines

Dissolved CO_2 in brines was determined initially by the volumetric method of the AOAC (1965), contained in sections 11.053 through 11.055. Later, an application of the microdiffusion principle (Conway, 1957) was developed, which greatly increased the number of samples that could be analyzed. A 22-ml vial containing 5 ml of standardized NaOH (0.5N) was placed in an 8-0z jar. 10 ml of acid phosphate solution, as described in the AOAC method above, was pipetted into the bottom of the jar. The jar was closed with a "Twist-off" cap. The cap was fitted with a rubber serum stopper, through which 10 ml of brine was introduced by a syringe. The sample was injected so as to enter into the acid solution, care being taken to avoid spillage into the vial of NaOH. After holding the jars at 37°C for 24 hr, the vial was removed, 5 ml of 0.5M $BaCl_2$ added, and the solution titrated to the phenolphthalein endpoint with standarized 0.5N HCl. The concentration of CO_2 was calculated from the equivalents of base neutralized as described in the AOAC method.

RESULTS

CHEMICAL and volume changes that occurred during a typical fermentation of cucumbers by *L. plantarum* WSO are illustrated in Figure 2. Initial values indicate the conditions after a 2-day equilibration at 24°C. The brine was clear at this time, giving no visual evidence of microbial activity. Chemical and expansion volume changes were dramatic during the first 3 days after inoculation. "Expansion volume" increases were almost linear during this period. After this, the volume increased more gradually over the next several days. The CO_2 concentration increased from the value at equilibration, 39 mg/100 ml brine, to 74 mg/100 ml after 23 days' incubation, for a net increase of 35 mg/100 ml during the fermentation period. Measurement of evolved CO_2 during the 2–3 wk duration of the fermentations indicated that over 90% of the CO_2 produced in the brine was retained.

CO_2 accumulation and bloater development

A series of 1-gal jars of brined cucumbers, with expansion reservoirs attached, was prepared to study the time after brining of bloater development. The incubation temperature was 26.7°C. At specified times, duplicate jars were removed, expansion volumes determined, the brines analyzed and the cucumbers cut for examination of bloater damage (Table 1).

No bloating was evident in the cucumbers prior to inoculating the brine. After 2 days' fermentation, bloater damage was present in all of the cucumbers, 28% being balloon and 72% being of the honeycomb-type. The overall degree of damage for both types of bloaters was rated as only slight. The trapped gas volume inside the cucumbers amounted to 5.6% of the initial volume of the cucum-

Table 1–CO_2 accumulation and bloater development at intervals during the fermentation of cucumbers[a]

Fermentation time (Days)	Brine analyses				Expansion volume (ml)	Trapped gas volume inside cucumbers (%)	Bloaters found[b]	
	pH	Acid (%)	Sugar (%)	CO_2 (mg/100 ml)			Balloon (%)	Honeycomb (%)
0	4.78	0.20	0.56	31	25	1.7	0	0
2	3.79	0.62	0.29	61	117	5.6	28 (S)	72 (S)
4	3.54	0.86	0.19	74	156	7.5	32 (S-M)	68 (S-M)
6	3.61	0.78	0.09	71	180	8.6	22 (S-M)	78 (S-M)
13	3.38	0.99	0.06	79	245	11.6	28 (A)	72 (M)
21	3.46	0.96	0.04	78	216	10.0	21 (M-A)	72 (M-A)

[a]Size no. 3 Model variety cucumbers were fermented at 26.7°C. Duplicate gallon jars were analyzed at the times indicated.

[b]Values in parentheses indicate the severity of bloating: S, slight; M, moderate; A, advanced. When two letters are shown, the first indicates the degree of bloating in the majority of the cucumbers.

bers. As the fermentation proceeded to its latter stages, the trapped gas volume amounted to over 10%, and the subjective rating of bloater damage was moderate to advanced. No lens-type bloaters were found in this experiment. CO_2 increased in the brines from 31 mg/100 prior to inoculation to 79 mg/100 ml after 13 days' fermentation.

In another experiment, size nos. 1, 2 and 3 cucumbers were brined in separate jars and fermented at 26.7°C. No evidence of bloating was found for size no. 1 cucumbers, whereas, size nos. 2 and 3 were severly bloated after 3 wk of fermentation (Table 2). The CO_2 concentration in the brine of size no. 1 cucumbers was slightly lower at the time of inoculation, but the brines of all three sizes contained 78–80 mg CO_2/100 ml brine after fermentation for 3 wk. Fermentations of the three sizes of cucumbers proceeded at similar rates. The final percent titratable acidity was slightly lower in the brine of size no. 1 cucumbers (0.77%, pH 3.5) than in sizes 2 and 3 (0.88%, pH 3.3). The relationships between CO_2 concentration in the brine and expansion volume, determined at intervals during the fermentation, for the three sizes of cucumbers are illustrated in Figure 3. Although the concentrations of CO_2 in brines during the fermentation were similar for all sizes, the expansion volumes were greatest for the size no. 3 cucumbers at given concentrations of CO_2. Size no. 2 cucumbers had greater expansion volumes than did the no. 1 size.

Size no. 3 cucumbers were fermented at three different temperatures. Bloating occurred in 92% of the cucumbers fermented at 32.2°C, and balloon and honeycomb bloating had reached advanced stages after 3 wk (Table 2). The percentage of cucumbers affected was about the same at 26.7°C, but the severity of those bloated was not as great. At 21.1°C, no balloon bloating was evident and only 11% of the cucumbers had honeycomb defects. A plot of the relationship between CO_2 concentration in the brine and expansion volume for the fermentations at the three temperatures is given in Figure 4. Greater expansion volumes resulted at higher incubation temperatures for any given concentration of CO_2 in the brine. Although the fermentation rate was higher at the higher incubation temperatures, there were no appreciable differences in pH or titratable acidity after 3 wk.

Effects of physical treatments on bloater formation

Size no. 3 cucumbers were treated in various ways prior to brining. One lot was pierced on two sides to a depth of 1 in. with a bed of 20 gauge needles, 9 needles per square inch. A 1-mm thick layer of peel was removed from the entire surface of a second lot. A third lot was brined without any prebrining treatment. Fritted gas dispersion tubes were positioned in these jars so that gas would diffuse out into the brine near the bottom of the jars. After equilibration and inoculation of the brines, nitrogen gas was introduced continuously during the fermentation at a rate of about 5 ml per minute. The fourth lot of cucumbers served as a control and received the normal brining treatments.

Piercing of cucumbers eliminated bloaters. The CO_2 concentration was similar to that of the control at equilibration (36 mg/100 ml brine) and at the end of the fermentation (74 mg/100 ml brine). Only slight honeycomb bloating was evident in the peeled cucumbers. The CO_2 concentration was about 15 mg/100 ml less at equilibration and at the end of the fermentation when compared to the control.

Nitrogen-purged cucumbers also were free of bloaters. Upon introducing nitrogen, the CO_2 concentration decreased below the equilibration level and was less than 10 mg/100 ml brine after 1 day. It never increased above this concentration. The expansion volume did not increase greatly in any of the treatments, which was consistent with the absence of bloaters. None of the three treatments appreciably influenced acid development.

Table 2–Effects of cucumber size and incubation temperature on bloater damage of fermented cucumbers[a]

Cucumber size[c]	Incubation temperature °C	CO_2 in the brine (mg/100 ml)		Bloaters found[b]	
		At inoculation	Maximum reached	Balloon (%)	Honeycomb (%)
1	26.7	28	80	0	0
2	26.7	34	80	31 (M-A)	38 (S)
3	26.7	39	78	39 (M-A)	54 (M-A)
3	21.1	38	77	0	11 (S-M)
3	32.2	40	68	31 (A)	61 (M-A)

[a]Explorer variety cucumbers were used in this experiment. Examination for bloater damage was made after incubation for 3 wk.

[b]Values in parentheses indicate the severity of bloating: S, slight; M, moderate; A, advanced. When two letters are shown, the first indicates the degree of bloating in the majority of the cucumbers.

[c]Sizes of cucumbers: No. 1, 3/4–1-1/16; No. 2, 1-1/16–1-1/2; No. 3, 1-1/2–2 in. diam.

In another experiment, one-gallon jars of brined cucumbers were purged for 2-hr periods at specified times during the fermentation at a nitrogen flow rate of 80 ml/min. Typical effects of purging intermittently during the fermentation are shown in Figure 5. The expansion volume and CO_2 concentration were greatly lowered after purging on the third day of fermentation. The concentration of CO_2 in the brine was slightly higher the next day as was the expansion volume, but both again were reduced by a second purging. Bloater damage was reduced, but not eliminated, when CO_2 was removed by periodic purging during the first 4 days of fermentation.

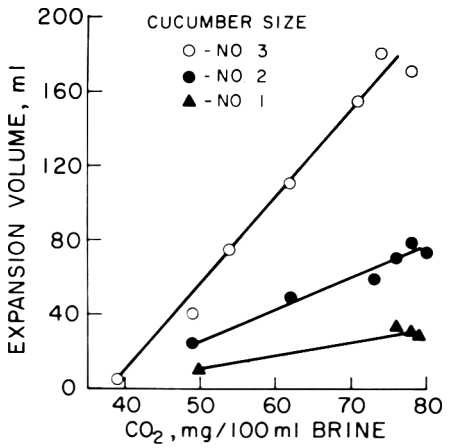

Fig. 3–Relationship between CO_2 concentration and expansion volume during the fermentation of three sizes of Explorer variety cucumbers. Incubation was at 26.7° C.

DISCUSSION

BRINE FERMENTATION of cucumbers with *L. plantarum*, a homofermentative lactic acid bacterium, may result in bloated stock as the foregoing results demonstrated. This finding was surprising as *L. plantarum* is considered to be a non-gas-former, in that it produces relatively little CO_2 in comparison with heterofermentative lactic acid bacteria. The amount of CO_2 which arose from the cucumbers plus that produced by *L. plantarum*, while undoubtedly low in comparison to "gaseous" fermentations caused by yeasts and other microbes that produce large amounts of CO_2, was largely retained in the brine and was sufficient to cause bloating. High retention of CO_2 in the brine probably was due to the small exposure of the brine surface to the atmosphere as imposed by the fermentation assembly. Also, maximum concentrations of CO_2 reached were 70–80 mg/100 ml brine, which is less than that required for saturation at 7% NaCl and 26.7°C. Under these conditions, CO_2 solubility is about 108 mg/100 ml, based on data tabulated by Quinn and Jones (1936).

Factors influencing the solubility of CO_2 may be related to bloater development in cucumbers. The solubility of CO_2 is reduced at higher temperatures and NaCl concentrations (Quinn and Jones, 1936). Brine pH also is a factor affecting solubility as it regulates the proportion of dissolved CO_2 of the "total CO_2 content" which includes CO_2, H_2CO_3, HCO_3^-, and $CO_3^=$.

Interestingly, visible bloater damage in size 3 cucumbers began early in the fermentation, after only 2 days, when the concentration of CO_2 was about 60 mg/100 ml brine (Table 1). Although this concentration is considerably less than saturation, the possibility that CO_2 solubility inside the cucumber was different from that in the surrounding brine cannot be disregarded.

The rise and overflow of brine from commercial brining tanks during an active fermentation is common, but the phenomenon has not been fully understood. Briners have attributed such occurrences to a too rapid fermentation. It is clear now, however, that such a rise in the brine level is due to gaseous expansion inside the cucumbers which results in bloater formation (Etchells et al., 1968). During the bloating stage, liquid is expressed from the tissue as is obvious from the desiccated appearance inside the cucumbers upon cutting. Also, the cucumbers become distended due to the internal gas pressure. Upon removal of CO_2 from the brine by nitrogen purging, it was found that the cucumbers lost their distended appearance and actually collapsed when balloon bloating had occurred. The cucumbers were moist inside, but a permanent cavity was present due to the tissue having been pressed toward the skin, and the liquid expressed, when it was under pressure. The rise in brine level, herein termed expansion volume, was used in the present work as a quantitative measure of bloater development. Such measurements proved valuable in that they provided a nondestructuve means of monitoring bloater formation during the entire fermentation.

A definite relationship between the expansion volume and the concentration

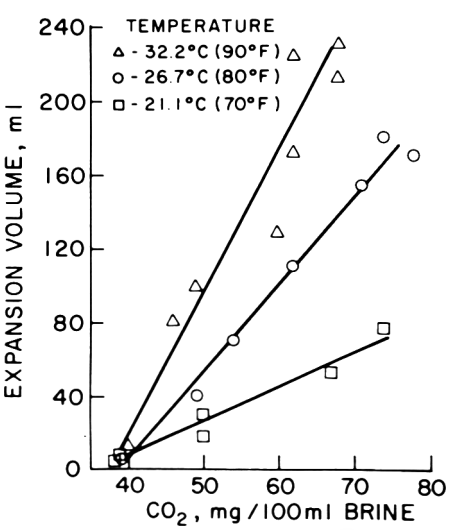

Fig. 4–Relationship between CO_2 concentration and expansion volume during the fermentation of size no. 3 Explorer variety cucumbers at three temperatures.

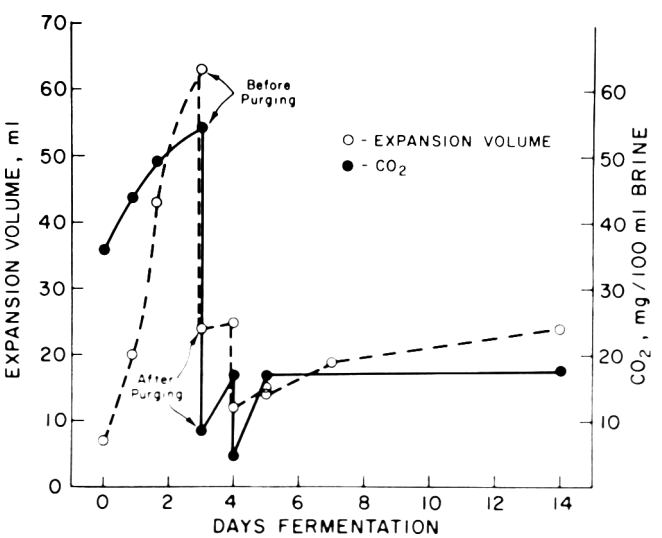

Fig. 5–Effect of nitrogen purging on reducing the CO_2 concentration and expansion volume of brined, Explorer variety cucumbers incubated at 26.7° C. The brine was purged for 2 hr on the third, fourth and fifth days of fermentation.

of CO_2 in the brine was obtained. Removal of CO_2 from the brine by sweeping with nitrogen during the fermentation of cucumbers prevented bloater development. This fact alone provided the most conclusive evidence that the concentration of CO_2 in the brine is related to bloater formation in cucumbers.

It would be desirable to establish the minimum or "critical" concentration of dissolved CO_2 in the fermenting brine which will cause bloating. However, it is clear that this "critical" concentration is variable and depends on the environmental conditions of the fermentation in addition to the variable characteristics of cucumbers.

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Ms received 7/13/72; revised 12/4/72; accepted 12/12/72.

Paper no. 3821, Journal Series, North Carolina State University Agricultural Experiment Station.

The authors express their appreciation to the Mount Olive Pickle Co., Mount Olive, N.C. and Perfect Packed Products Co., Inc., Henderson, N.C., for supplying cucumbers and other materials used in this work. We also thank Drs. C.H. Miller and R.L. Lower for supplying some of the cucumbers.

This investigation was supported in part by a research grant from Pickle Packers International (St. Charles, Illinois).

Reference to a company or product name does not imply endorsement by the U.S. Dept. of Agriculture.

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CARBON DIOXIDE PRODUCTION IN THE FERMENTATION OF BRINED CUCUMBERS

INTRODUCTION

BLOATER DAMAGE (hollow stock) in brined cucumbers has been attributed to the production of gases, particularly CO_2, in the fermentation brine (Etchells et al., 1968). Microorganisms that have been associated with gaseous fermentations of commercially brined cucumbers include yeasts (Etchells and Bell, 1950; Etchells et al., 1952; 1953), coliform bacteria of the genus *Aerobacter* (Etchells et al., 1945) and heterofermentative lactic acid bacteria (Etchells et al., 1968).

Previous work in this laboratory with pure and mixed cultures of lactic acid bacteria demonstrated that microbial control in cucumber fermentations can be achieved (Etchells et al., 1964). Recent studies have shown, however, that bloater development can occur, even when cucumbers are fermented by pure cultures of *Lactobacillus plantarum* (Fleming et al., 1973). *L. plantarum*, a homofermentative lactic acid bacterium, is referred to as a non-gas producer, although it is known to produce small amounts of CO_2 (Pederson, 1929). It has not, heretofore, been considered a contributor to bloater damage. It appeared that if CO_2 was responsible for bloater damage, it originated from either activity of *L. plantarum* or the cucumber tissue, or both.

The present work was undertaken to study the contributions of selected homofermentative lactic acid bacteria and the cucumber to the amount of CO_2 produced in brine fermentations.

MATERIALS & METHODS

SOURCES of the cucumbers used in this study were given previously (Fleming et al., 1973), as were the cultures of lactic acid bacteria and preparation of inocula (Etchells et al., 1964; Fleming and Etchells, 1967).

Brining of cucumbers

For fermentation by the natural microflora, cucumbers were packed in 1-gal jars. Brines containing various concentrations of NaCl were poured into the jars together with 3.5 ml of glacial acetic acid. The acid was added to retard growth of coliform bacteria (Etchells et al., 1964) and to favor fermentation by naturally-occurring lactic acid bacteria. The jars were closed with a cap fitted with a 250 ml dispensing burette which allowed the brine level to rise as the fermentation progressed. This system prevented a build-up of pressure in the jar and restricted loss of CO_2 from the brine (Fleming et al., 1973). Incubation was at room temperature (23°C ± 1°).

For fermentation by inoculation with pure cultures of bacteria, cucumbers were hand-washed and packed in 1-qt jars. The concentrations of NaCl, sodium acetate and acetic acid in the cover brine were adjusted so that, after equilibration with the cucumbers, the brine was pH 4.7 (±0.2) and contained 6.7% NaCl and 0.5% sodium acetate (Fleming et al., 1973). The final pack-out ratio was 1.3:1, grams cucumbers:ml brine. The jars were capped with "Twist-off" closures (White Cap Co., Chicago, Ill.), which gave a hermetic seal. The headspace volume was 40 ml. A 14-mm diameter rubber serum stopper was placed in each cap for the purpose of sampling (Etchells et al., 1964). Appropriate jars were pasteurized immediately after capping by submerging in a hot water bath as described by Etchells and Jones (1944). After allowing a 24 hr equilibration period at room temperature (23°C ±1°), the jars were inoculated through the sampling stopper with 2 ml of a broth culture of the desired species of bacteria and then incubated at 26.7°C for 3 wk.

Sampling

Headspace gas samples and brine samples when under pressure were taken with a gas-tight needle (22 gauge) and syringe assembly (Hamilton Co.). Otherwise, 10-ml brine samples were taken with 12-ml disposable plastic syringes. Headspace pressures were taken with a pressure gauge equipped with a needle for insertion through the rubber stopper in the jar caps (Hamilton Co.). The jars were equilibrated to 24°C prior to sampling for headspace pressure and gas.

Analyses

Analytical methods for determining titratable acidity, pH, percent NaCl, percent reducing sugar and dissolved CO_2 were described or referred to previously (Fleming et al., 1973).

Sugars in aqueous extracts of fresh cucumbers were identified by thin layer chromatography on cellulose powder MN 300 (Brinkmann Instruments). The plates were developed in a solvent system of formic acid:butanone:t-butyl alcohol:water, 15:30:40:15. Reducing sugars were detected by spraying the plates with aniline phthalate (Stahl, 1969; reagent no. 10). Nonreducing sugars were detected by spraying with silver nitrate-sodium hydroxide (Stahl, 1969; reagent no. 234).

Carbon dioxide in quart jars of cucumbers was determined at the completion of fermentation. An estimate of the total amount in the jars was made by assuming that the concentration of CO_2 dissolved in the brine surrounding the cucumbers was the same as that dissolved inside the tissue. Headspace CO_2 also was included in the total, and was determined by the same method used for dissolved CO_2. To test the validity of the above assumption, the total CO_2 in the jars also was determined by sweeping the entire CO_2 from the jar contents with nitrogen and trapping in standardized NaOH. The values obtained by the latter procedure and

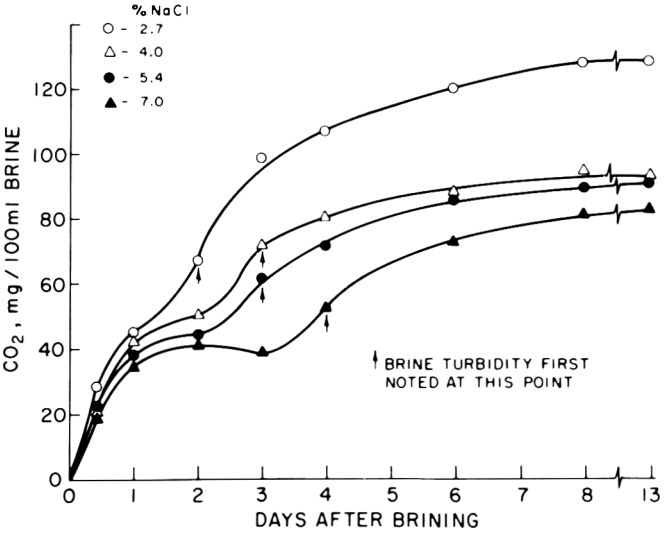

Fig. 1—CO_2 accumulation in cucumber brines undergoing fermentation by natural microflora at four concentrations of NaCl. See footnote "a" of Table 1 for details.

Table 1–Brine analyses of cucumbers undergoing fermentation by natural microflora at four concentrations of sodium chloride[a]

NaCl concentrations in the cover brine			Days after brining								
			2			13			36		
Initial (%)	After 2 days (%)	After equilibration (%)	pH	Acid (%)	Sugar (%)	pH	Acid (%)	Sugar (%)	pH	Acid (%)	Sugar (%)
5.5	2.9	2.7	4.39	0.20	0.37	3.31	0.99	0.00	3.26	1.00	0.00
8.4	4.3	4.0	4.20	0.18	0.38	3.15	1.02	0.00	3.12	1.02	0.00
11.4	5.8	5.4	4.40	0.16[b]	0.38	3.19	0.90	0.07	3.13	1.00	0.00
14.5	7.2	7.0	4.36	0.16[b]	0.42	3.21	0.78	0.18	3.17	0.85	0.12

[a]SMR variety cucumbers, 1-1/16 to 1-1/2 in. diameter, were used. The pack-out ratio was 1g cucumber:0.9 ml brine. Values are averages of duplicate 1-gal jars; CO_2 concentrations are given in Figure 1. Standard deviations for replicate jars were: pH, 0.09; % acid, 0.01; % sugar, 0.05; % salt, 0.1.

[b]This amount of acid was due to the acetic acid added, 3.5 ml/gal, at the time of brining.

those obtained from calculations based on dissolved CO_2 in the cover brine as described above agreed within 6%.

RESULTS

Dissolved CO_2 in brines fermented by natural microflora

CO_2 concentration increased in the 7% NaCl w/v, brine (equilibrated concentration) up to 2 days, and did not increase further until the fourth day, when a slight visual turbidity due to microbial growth was noted (Fig. 1). The concentration of CO_2 after two days was about 40 mg/100 ml brine and was considered to represent the CO_2 that diffused from the cucumbers. Numerous studies since have consistently confirmed that about 30–40 mg of CO_2/100 ml brine is present after equilibration when fresh cucumbers are brined to equilibrate at 7% NaCl. This concentration of CO_2 has been found when the cucumbers were carefully washed and the brine chlorinated, as well as when the cucumbers were brined, unwashed, as in the present experiment.

When visual turbidity began in the 7% brine, there was an abrupt increase in dissolved CO_2 which subsequently reached a maximal concentration of 82 mg/100 ml (Fig. 1). Lower concentrations of NaCl resulted in higher concentrations of CO_2, but the relative proportions arising from the cucumber and from the bacteria were not as apparent since the plateau in CO_2 concentration prior to microbial growth was less prominent at lower brine strengths. No yeasts were detected by microscopic examination of the brines during the first 2 wk after brining.

Brine assays for samples taken at 2, 13 and 36 days after brining are given (Table 1). The data indicate that acid-forming bacteria were active during the fermentations as about 1% acid was present in the 2.7 and 4% brines after 13 days. The acid was lower in the 5.4 and 7% brines, and residual sugar was present, whereas, no sugar remained in the 2.7 and 4% brines. Sugar remained in the 7% brine after 36 days. The combination of salt and low pH (3.1–3.2) prevented further fermentation by the lactic acid bacteria.

CO_2 in pure culture fermented cucumbers

Relative contributions of the cucumber tissue and the fermenting bacteria in the brine to the total amount of CO_2 produced in pure culture fermentations were studied. Four quart-jar lots of cucumbers (three jars per lot) were treated as indicated in Table 2 and examined after the completion of fermentation.

Headspace pressures of the jars, taken at 24°C just prior to sampling, were 5.5 psi for pasteurized, fermented cucumbers and 9 psi for unheated, fermented cucumbers. The pasteurized, uninoculated controls had a slight vacuum.

Jars of pasteurized, unfermented cucumbers contained 30 mg CO_2/100g cucumbers while the pasteurized, fermented product contained 114 mg CO_2/100g (Table 2). The difference, 84 mg CO_2/100g cucumbers, represented the CO_2 produced by *L. plantarum* WSO. Unheated, fermented cucumbers contained 175 mg CO_2/100g. Subtracting the level of CO_2 produced by the culture in the pasteurized, fermented cucumbers from this amount gave 91 mg CO_2/100g cucumbers, which represented the CO_2 contributed by the cucumber tissue.

CO_2 contributed by the cucumbers included that which was present in the fruit at the time of brining plus that formed by respiratory and/or fermentative activity of the tissue subsequent to brining. The initial level of CO_2 present in pastuerized cucumbers (Table 2) may not represent that which was present in the fresh cucumbers. The pasteurizing process may have caused a loss of CO_2 or the heating may have resulted in the formation of CO_2.

Table 2–CO_2 produced by cucumbers and Lactobacillus plantarum WSO during brine fermentation[a]

Treatment of cucumbers	CO_2 in headspace gas (mg/100 ml)	CO_2 in brine (mg/100 ml)	CO_2, mg/100g cucumber		
			Total	Produced by L. plantarum	Produced by cucumbers
Pasteurized, not inoculated	22.0 (1.3)	15.7 (0.7)	30.1 (1.2)		
Pasteurized, inoculated	81.8 (1.8)	57.9 (1.5)	114.3 (3.4)	84	
Not heated, but chlorinated and inoculated[b]	125.4 (0.0)	90.2 (1.3)	175.4 (2.0)		91
Not heated, not chlorinated, but inoculated[b]	128.7 (1.9)	91.3 (1.9)	178.4 (2.9)		94
Contribution of sources of CO_2 to total, in percent[c]				48%	52%

[a]Model variety cucumbers, 1-1/16 to 1-1/2 inches diameter, were used. The pack-out ratio was 1.3g cucumber:1 ml brine. Data are based on averages of triplicate jars sampled after three weeks of fermentation. Standard errors of the means are given in parentheses.

[b]The cover brine contained 100 ppm available chlorine, added as calcium hypochlorite, before pouring over the cucumbers.

[c]See text for explanation of computations.

Table 3–Production of CO_2 by three cultures of lactic acid bacteria during the pure culture fermentation of pasteurized, brined cucumbers[a]

Bacteria	Brine analyses				CO_2 produced by bacteria per g sugar fermented[b] (mg)
	pH	Acid (%)	Residual sugar (%)	CO_2 (mg/100 ml)	
Uninoculated control	4.67 (0.01)	0.28 (0.00)	1.46 (0.01)	15.7 (0.7)	
L. plantarum WSO	3.39 (0.02)	1.23 (0.03)	0.12 (0.03)	57.9 (1.5)	34.6 (1.0)
L. plantarum 442	3.58 (0.03)	0.98 (0.03)	0.48 (0.05)	56.2 (0.8)	45.9 (2.1)
P. cerevisiae 39	3.91 (0.01)	0.70 (0.01)	0.75 (0.02)	63.8 (2.4)	75.2 (5.0)

[a]Standard errors of means are given in parentheses. See footnote "a" of Table 2 for other details.
[b]Values are based on total CO_2 in the jars which included dissolved CO_2 and that present in the 40 ml headspace. Amount of sugar fermented was calculated as the difference between residual sugar in inoculated and uninoculated controls.

Since the unheated cucumbers undoubtedly contained a small number of contaminating microorganisms, their contribution to the CO_2 produced in unheated cucumbers cannot be wholly disregarded, but CO_2 from this source was thought to be very small. Very little difference was noted in CO_2 concentration between the unheated cucumbers that received chlorination and those that did not (Table 2). Microscopic examination revealed a homogenous population of short rods characteristic of *L. plantarum* WSO. No yeasts were detected.

Overall, the fermentation of unheated cucumbers resulted in 48% of the CO_2 being derived from metabolic activity of *L. plantarum*WSO and 52% from the cucumber tissue.

Three cultures of homofermentative lactic acid bacteria were compared regarding amounts of CO_2 produced in pure culture fermentations of pasteurized cucumbers (Table 3). *L. plantarum* WSO and *L plantarum* 442 produced similar amounts of CO_2, while *P. cerevisiae* 39 produced slightly more. Expressed on the basis of mg of CO_2 produced per gram of sugar fermented, however, *P. cerevisiae* 39 produced over twice as much and *L. plantarum* 442 about 30% more CO_2 when compared to *L. plantarum* WSO. Nearly all of the sugar was fermented by *L. plantarum* WSO during the 3 wk of fermentation while 0.48 and 0.75% sugar remained in brines fermented by *L. plantarum* 442 and *P. cerevisiae* 39.

Since sugar content in the cucumbers was determined as reducing sugar, it was essential to establish the identity of the sugars present. Sucrose is fermentable by the lactic acid bacteria, but would not have been detected by the reducing sugar assay.

Thin layer chromatography of extracts from fresh cucumbers indicated that glucose and fructose constituted the major portion of sugars present. Only traces of other unidentified sugars were found. Sucrose was not detected. These findings are consistent with unpublished data collected by C. L. McCombs for numerous varieties of pickling cucumbers (personal communication).

DISCUSSION

THE CUCUMBER TISSUE and homofermentative lactic acid bacteria have not been seriously considered as sources of CO_2 in the brine of fermenting cucumbers. Present results show that the concentration of CO_2 resulting from these combined sources is as high as that reached when bloater damage has been shown to occur, 60–80 mg/100 ml brine (Fleming et al., 1973). While this level of CO_2 is undoubtedly low compared to fermentations characterized by yeasts, coliforms and heterofermentative lactic acid bacteria, a further reduction in the amount present would appear desirable.

There was variation among the homofermentative lactic acid bacteria regarding the amount of CO_2 produced. Strains of *L. plantarum* produced lower levels of CO_2 than did *P. cerevisiae*. Production of CO_2 by homofermentative lactic acid bacteria has not been studied extensively, but amounts reported herein appear to be consistent with those reported for *L. plantarum* (Pederson, 1929). Apparently small amounts of CO_2 are inevitable, although CO_2 is not an end product of the major metabolic pathway in *L. plantarum* (Wood, 1961).

Amounts of CO_2 in cucumbers prior to brining probably vary, depending on the physiological state of the fruit and the storage conditions prior to brining. Temperature and duration of storage have an important influence on the respiratory rate of cucumbers (Eaks and Morris, 1956). The respiratory activity of cucumbers submerged in brine has not been reported. Such activity probably would be limited by the amount of oxygen present and the acid and NaCl which diffuse into the tissue from the surrounding brine. Therefore, prebrining as well as brining treatments undoubtedly influence the amount of CO_2 originating from cucumbers after they are brined.

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Ms received 9/10/72; revised 12/4/72; accepted 12/12/72.

Paper no. 3766, Journal Series, North Carolina State University Agricultural Experiment Station.

The authors express their appreciation to the Mount Olive Pickle Co., Mount Olive, N.C. and Perfect Packed Products Co., Inc., Henderson, N.C., for supplying cucumbers and other materials used in this work. We also thank Drs. C.H. Miller and R.L. Lower for supplying some of the cucumbers.

This investigation was supported by a research grant from Pickle Packers International, Inc. (St. Charles, Ill.).

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FIELD TESTS OF SALT RECOVERY SYSTEM FOR SPENT PICKLE BRINE

INTRODUCTION

EACH YEAR the food processing industry in this country uses an estimated 200 million pounds of sodium chloride to brine products such as olives, pickles, cauliflower, beets, cabbage, etc. (The estimate is based on production figures in USDA Agriculture Statistics, 1971, industry figures for the cured stock to fresh-pack ratios, the product-to-brine ratios and average salt concentration for the spent storage brines.) At $13 per ton f.o.b. this represents a recurring expenditure of over $1.25 million a year.

Because of the nature of the brining operation, almost all of the salt used during the year is discarded when the spent processing brine is discharged into local streams, ponds and sanitary sewer systems. Until recently this practice has gone unchallenged. But times have changed. A greater concern for the environment has brought about a search for viable alternatives that will permit disposal of the spent brine without causing water pollution.

An estimated 90% of the waste brine comes from the pickling of cucumbers. The spent brine is generated during a salting operation in which the freshly harvested material is stored in salt brine and allowed to ferment before further processing.

In the pickling industry, salinity is measured in terms of percent saturation at 60°F., or salometer degrees. Typically, the initial or starting brine has a strength of 40° salometer (10-1/2% NaCl). After 4–6 wk, the concentration is gradually increased to a final value of from 60° to 70° (16–18-1/2% NaCl) at the rate of from 1 to 6 degrees per week (Cruess, 1958; Etchells et al., 1950; Veldhuis and Etchells, 1941). This "strong brine" is drained from the storage tanks and discarded after the salt stock is removed for further processing. The corrosive nature of sodium chloride and the fact that the waste stream is a mixture of a nonbiodegradable salt and organic solids makes the disposal problem a particularly difficult one.

There are obvious advantages to recycling the salt rather than discarding it. Reusing the salt would certainly obviate the disposal problem.

The salt in the spent brine can be

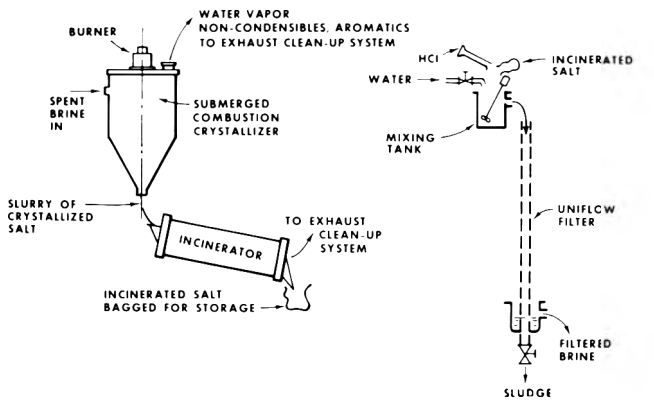

Fig. 1—Salt reclamation process.

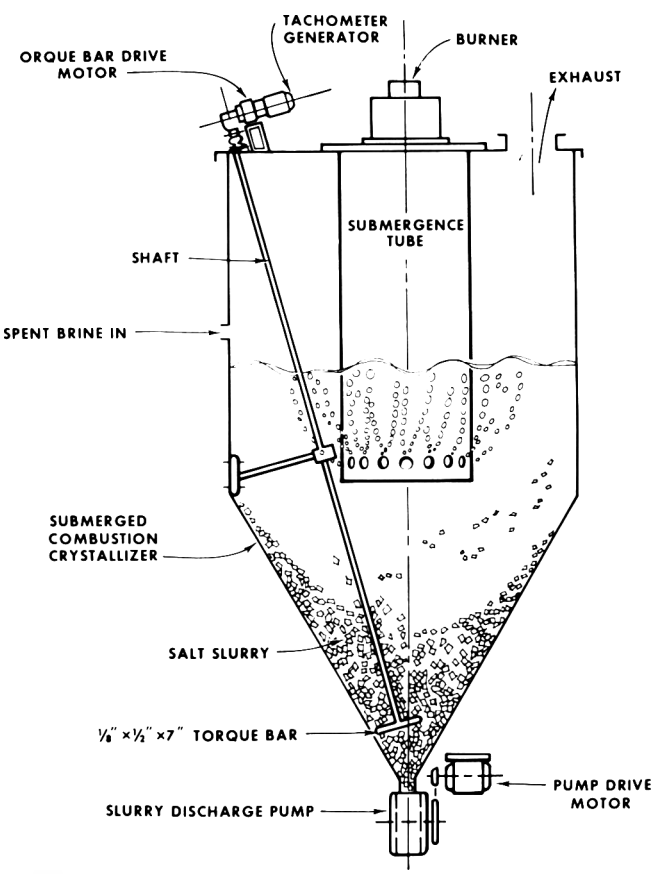

Fig. 2—Cross-sectional diagram of submerged combustion crystallizer.

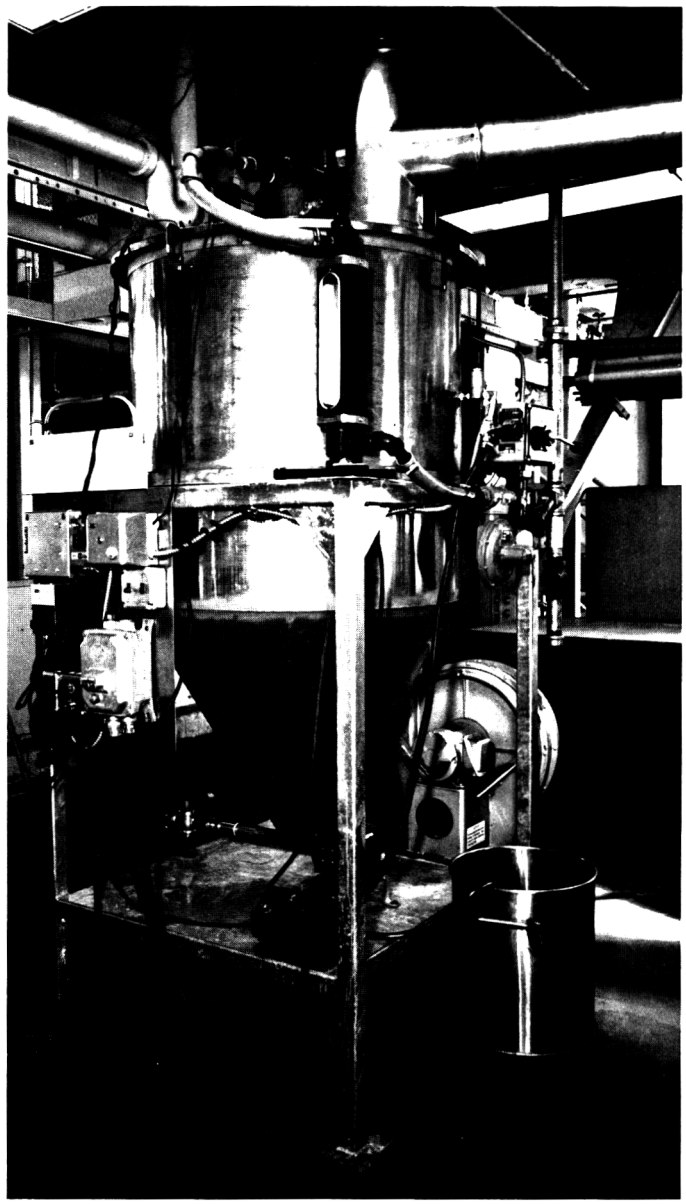

Fig. 3—Pilot scale submerged combustion crystallizer.

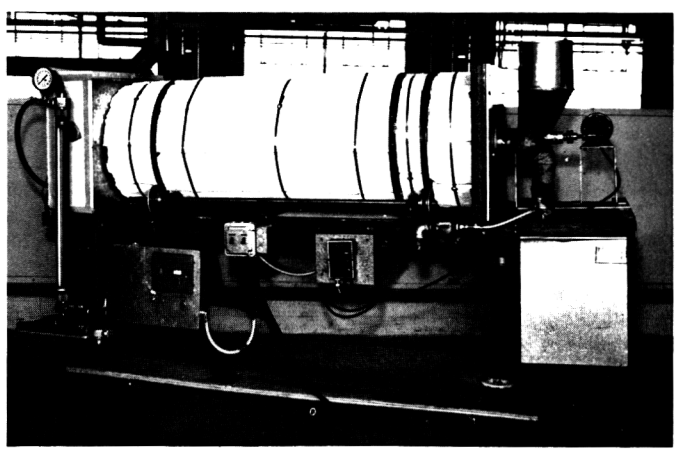

Fig. 4—Pilot scale rotary incinerator.

made re-usable simply by removing the water to re-crystallize the NaCl and then ridding the crystals of organic contaminants. This technique was first used to reclaim salt from spent olive brine (Lowe and Durkee, 1971). The salt recovery system is shown diagrammatically in Figure 1. Briefly, the process involves the use of a submerged combustion crystallizer to produce a slurry containing about 60% NaCl. The slurry also contains organic contaminants which are then destroyed by incinerating the slurry at 1200°F for 5 min. During the past year, use of the method was successfully extended to "strong" pickle processing brine.

EXPERIMENTAL

Equipment

The experimental work was conducted at the Isleton, Calif. salting station of the H.J. Heinz Co. using the pilot scale submerged combustion crystallizer and rotary incinerator described by Lowe and Durkee (1971). The crystallizer is shown in Figures 2 and 3; the incinerator in Figure 4.

During the tests, salt brine was pumped into the crystallizer body filling it to a depth of 15 in. above the discharge holes in the submergence tube bringing combustion gases from the burner to the liquid being evaporated (see Fig. 2). Salt is crystallized as water evaporates from the brine, and settles down to the bottom of the crystallizer where it is removed as a slurry. The crystallizer operates continuously by controlling the flow of brine into and the discharge of slurry from the unit.

Crystallizer exhaust

The exhaust stream from the crystallizer contains water vapor, combustion gases, excess air, entrained liquid droplets and aromatic volatiles from heating the spent brine. To avoid atmospheric contamination by the droplets and aromatic volatiles, a 4-stage exhaust clean-up system was employed (see Fig. 5). The first stage, a liquid-vapor cyclone, was designed to remove 95–98% of the entrained droplets and return the liquid, essentially saturated brine to the crystallizer body. The cyclone also breaks up small amounts of foam that may occasionally get beyond the submerged combustion vessel.

The second stage, a liquid-vapor and gas contactor, consists of a 6-in. thick wire-mesh packed column wetted by water sprayed from a full cone nozzle. The contactor or scrubber was designed to remove offensive odors and residual entrained brine droplets. The effluent from the scrubber was sent to waste.

The third stage, a wire mesh entrainment separator, was selected to prevent all but a small amount of liquid from being discharged into the atmosphere.

The fourth stage is an activated carbon filter used to adsorb residual odors that may by-pass the water scrubber.

The effectiveness of the exhaust clean-up system was determined by sampling and condensing in alcohol-dry ice traps, the vapor from various parts of the exhaust stream as indicated in Figure 5, and by collecting the effluent from the water scrubber. The oxygen requirements for the waste streams were determined by COD

and 5-day BOD measurements and by measuring the organic carbon concentration in a Beckman Model 915 carbon analyzer and calculating the oxygen demand on the basis of 100% conversion to CO_2.

Slurry control

Automatic hands-off operation of the crystallizer requires control of both brine and slurry levels within the vessel. The brine level determines the rate at which new brine is brought into the crystallizer, the slurry level the rate at which salt slurry is drawn from the bottom of the vessel.

The mechanism used to sense and control slurry level in the pilot crystallizer consisted of a small (1/20 hp) shunt wound D.C. motor rotating a single-bladed torque bar, a tachometer generator directly coupled to and mounted integral with the shunt motor, a slurry discharge pump, a standard variable speed controller for the torque bar motor, and a special variable speed controller for the slurry discharge pump motor electrically coupled to the tach. generator. The control scheme is shown diagrammatically in Figure 2.

The interface between the slurry and the brine above it is not a well defined one. Salt crystals are held in suspension by the brine, with a much heavier concentration of crystals at the bottom than at the top of the vessel. As more salt collects in the area of the rotating torque bar, the bar slows down, the decrease in speed depending on the speed-torque characteristics of the shunt-wound motor. This in turn slows down the directly coupled tachometer generator so that a reduced voltage signal is sent to the slurry pump discharge motor controller. The change in input signal to the controller speeds up the discharge pump.

The opposite effect, a decrease in salt concentration results in an increase in the input signal and a corresponding decrease in the rotational speed of the slurry discharge pump. The rotating torque bar thus continuously monitors the concentration of salt in the slurry at the bottom of the crystallizer and triggers adjustments of the slurry discharge rate to maintain a preset concentration of crystals in the salt slurry.

The rotating torque bar serves one other function. It keeps the salt crystals in suspension so that the slurry can flow down into the discharge pump. If stirring is inadequate solid salt accumulates and compacts along the sloping walls of the crystallizer, in time clogging the inlet to the discharge pump.

Forming

Pickle brine has a tendency to foam when heated by submerged combustion. The degree of foaming varies, depending in part, on the kind and amount of organic contaminants in the waste stream. If foaming is severe, raw brine will be carried over into the exhaust vapor-gas stream. Foaming also prevents proper operation of certain types of liquid level controllers. This is the case, for example, with electric eye sensors.

Foaming can be controlled by the addition of a defoamer to the waste brine stream. The defoamer used in the experimental work was Dow Corning FG-10 food grade emulsion at a dosage level of 100 ppm.

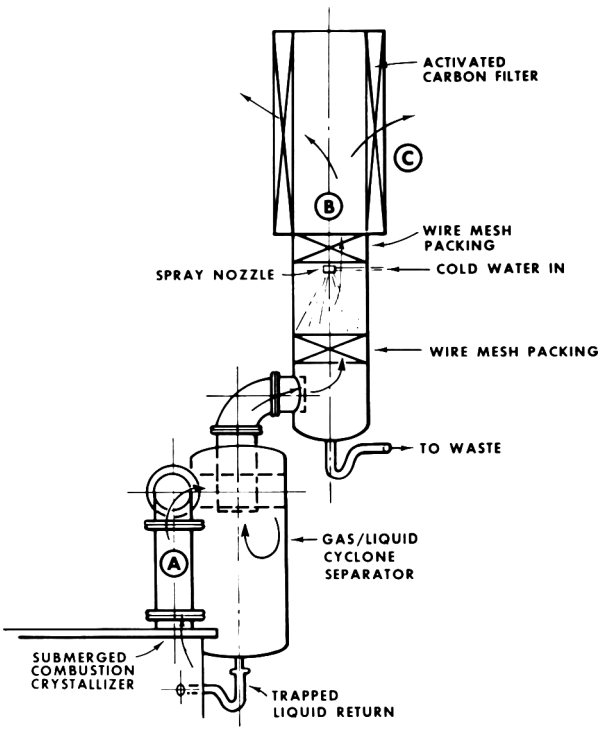

Fig. 5—Crystallizer exhaust clean-up system.

General

The spent brine used in the experiments was taken from a 9000 gal wood tank in which size 4 cucumbers had been stored during the previous 10 months.

Water used to make up storage brine was supplied to the plant by a water company from local wells. Solar salt was used to make up the original storage brine.

The tests were conducted over a 20-day period in April-May, 1972. Operation was continuous during the tests and all test data were taken under steady state conditions. Grab samples of the slurry, crude salt and scrubber effluent were taken at periodic intervals and pooled for analyses. Representative samples were taken of the plant water, spent storage brine and solar salt. Condensible waste streams were sampled continuously during the data taking period. Samples for BOD, COD and other organic analyses were packed in dry ice for shipment back to the laboratory and transferred to −10° F storage pending analyses.

Elemental analyses for the data in Table 1 were by atomic absorption spectrophotometry, total nitrogen by the Kjedahl method, chloride by titration, sulfate by gravimetric method and total solids by drying in a forced draft oven and finishing in an air oven at 100°C.

Table 1—Composition of salts, slurry and solutions

	Solids %	SpGr @ 60°F	pH	Na, % dwb	Cl, % dwb	NaCl, % ($Na_{dwb} \times 2.54$)	Ca, % dwb	K, % dwb	Mg, % dwb	SO_4, % dwb	Protein, % ($N_{dwb} \times 6.38$)	Lactic acid ppm	Combustible organic, % dwb
Plant water	.05	1.0	8.2	27.2	34.8		9.2	.36	3.3	3.6			
Spent brine	18.0	1.126	3.6	35.9	55.6	91.2	.12	.74	.08	.27	2.1	540	5.3
Slurry	60.0		3.6	35.8	56.9	91.1	.08	.64	.07	.20			
Solar salt				38.1	59.0	97.1	.05	.06	.04	.23			
Crude salt (reclaimed)				37.5	57.2	94.7	.06	.46	.04	.22			

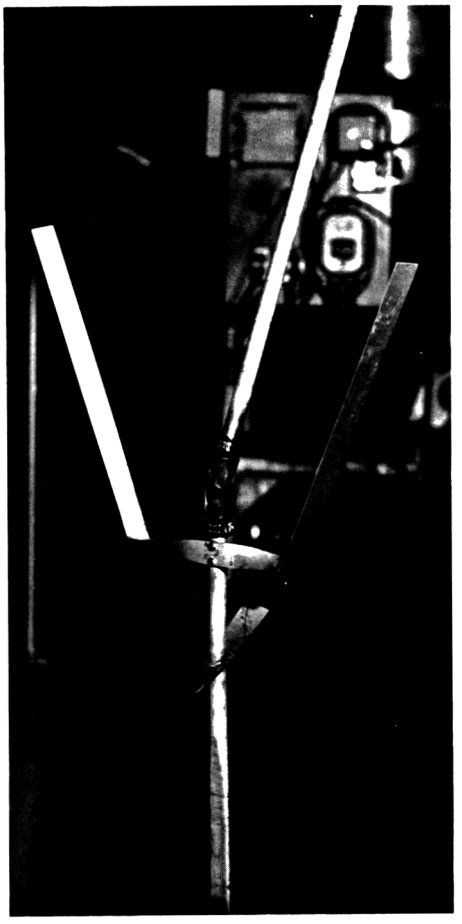

Fig. 6—Improved torque bar.

RESULTS & DISCUSSION

Brine and plant water

The spent brine used in the tests contained 18% solids. Most of it was sodium chloride as might be expected, with measurable amounts of K, Ca, Mg and SO_4 and 5.3% combustible organic matter on the dry weight basis. BOD for the waste stream was 8550 ppm; the COD 13,000–16,000 ppm (see Tables 1 and 2).

The plant water contained 136 ppm Na, 46 ppm Ca, 1.8 ppm K, 16.5 ppm Mg, and 18 ppm SO_4 (see Table 1 for equivalent percentages, dry weight basis).

Exhaust clean-up

The efficiency of odor and brine droplet removal by the exhaust clean-up system depends to a large extent on good contact between the exhaust stream and the scrubber water. The spray nozzle in the scrubber was selected to operate with a pressure of at least 20 psi. Water pressure at the plant varied from about 5 psi to over 30 psi depending apparently on water usage in other parts of the plant. There were times when there was no flow at all when service was interrupted without notice by the water company. During the test, data were recorded only when conditions appeared to be stable, but it was not possible to continuously monitor the water pressure at the scrubber.

Measurements made at about 20 psi indicate a scrubber water usage rate of approximately 12 gph.

The data in Table 2 indicate almost all of the organic contaminants removed by the exhaust clean-up system came out in the water scrubber and very little if any in the carbon filter. BOD was 550 ppm for the condensibles exhausted to atmosphere. However, condensibles only represent an estimated 45% of the total exhaust stream, so that the BOD for the vapor-gas stream discharged to the atmosphere was approximately 250 ppm.

The chromatographic data are inconclusive. Formic acid, formaldehyde and acetaldehyde are breakdown products from thermal degradation of lactic acid. Formic acid does not appear to be one of the contaminants in the exhaust stream. There is a possibility that formaldehyde is one of the contaminants and a stronger possibility that acetaldehyde is in the vapor-gas stream in detectable quantities.

Salt carry-over into the atmosphere appears to be high. Sodium chloride level in the condensible portion of the exhaust stream was 1500 ppm (0.15%). On the basis of 45% condensibles, this is equivalent to 675 ppm NaCl in the total stream.

Slurry level control

The torque bar used during the tests was 7 in. long, 1/2 in. wide and 1/8 in. thick rotating at 80–100 rpm to sweep a cylindrical path 7 in. in diameter by 1/2 in. high. Slurry control with the torque bar was sporadic primarily because of inadequate stirring. Salt build-up was substantial at times, both above and below the stirred area.

An improved torque bar is shown in Figure 6. Designed to keep the vessel walls clear of salt deposits and with blades pitched to insure a positive flow of slurry into the pump suction, the new torque bar was tested in a subsequent series of experiments and found to be a great improvement over the original. Slurry discharge control was positive and stable. Slurry consistency was adjustable over a wide range of salt levels simply by changing the set point on the torque bar motor controller. Nominal speed for the torque bar was 20–21 rpm.

Foaming

The use of Dow Corning FG-10 defoamer minimized but did not eliminate foaming. As a consequence, the submerged combustion crystallizer was operated at less than rated capacity to assure a carry-over-free vapor discharge. The use of a defoamer alone might have been enough to prevent foam carry-over had the freeboard or vapor space above the liquid level in the crystallizer been a foot higher than the 20–21 in. called for in the original design.

The 3-ft diam by 5 ft 7 in. tall pilot submerged combustion unit was designed to liberate 500,000 BTU/hr using natural gas (1,000 BTU/ft^3). Assuming a thermal efficiency of 86.7% (Lowe and Durkee, 1971) rated evaporative capacity of the crystallizer is theoretically about 50 gal of water an hour. Because of the foaming problem, the rate was reduced to 40 gal

Table 2—Properties of process streams

	Solids %	pH	NaCl, % (Na_{dwb} × 2.54)	O_2 demand, ppm (C org. × 2.67)	COD ppm	BOD ppm	Formic acid[a]	Formaldehyde[a]	Acetaldehyde[a]
Spent brine	18.0			13000	16300	8550			
Reconditioned brine	9.56			230					
Crystallizer condensibles									
At sample point:									
A) (see Fig. 5)		4.54		2680	2770	1190	N,N	P,P	P,P
B) (see Fig. 5)		3.75		670	750	560	P,N	F,P	P,F
C) (see Fig. 5)		5.00	.15	650	830	550	N,N	P,F	P,P
Crystallizer scrubber effluent	.32	3.90	.22	1210	1100	760	F	VF	N

[a]Qualitative chromatographic analyses: N = Negative; P = Positive; F = Faint; VF = Very faint; F,P = 1st sample faint, 2nd sample positive.

an hour. At this rate, the experimentally determined thermal efficiency was 84.0%.

Leaching

The data in Table 1 show a marked difference in potassium concentration for the solar salt used to make up the original brine solution and the crude or reclaimed salt. Fresh cucumber contains 160 mg potassium, 6 mg sodium, and 25 mg calcium per 100g edible portion (Watt and Merrill, 1963). After 12 months of storage, salt stock (size 4) contained 150 mg potassium per 100g edible portion. A sample of the spent brine from which the salt stock was drawn contained 150 mg potassium per 100g brine. The figures indicate a portion of the potassium in the cucumber leached out into the storage brine to raise the potassium level in the recycled salt. Repeated recycling should in time increase the amount of potassium in the reclaimed salt until the potassium level in the spent brine reaches equilibrium with the potassium in the fresh cucumber. This is not expected to lead to any difficulties.

The experimental data show substantially no change in the amount of Ca, Mg and SO_4 in the sodium chloride salt after one recycle.

Reconditioned brine

Reclaimed salt was dissolved in plant water to produce a solution containing 10% solids (9.56% NaCl). The "reconditioned brine" showed a pH of 10.0 and therefore needs to be neutralized with HCl before it is used (Lowe and Durkee, 1971). Filtration is also necessary to remove suspended carbon that was produced during incineration of the slurry. (See Fig. 1). This can be done with a simple cloth filter such as the Uniflow filter described by Popper, 1970.

Incineration

The sole purpose for incinerating the slurry is to destroy the organic contaminants in the spent brine. This can be done in any number of different ways so long as the slurry is heated at 1200°F for at least 5 min. A rotary type incinerator was used in the experimental work simply because of its ease of fabrication.

Most of the reclaimed or product salt left the incinerator properly heat treated. Occasionally however, slurry would roll into a ball and pass through the incinerator without adequate treatment. Such pieces were sorted out by hand and removed from the final product.

A simple wetted column or scrubber was used to reduce offensive odors in the incinerator exhaust. The scrubber was obviously inadequate for the purpose but no further studies were made of the exhaust problem because of manpower limitations.

Storage tests

The reclaimed salt produced during the field tests was originally scheduled for use in brining pickles during the 1972–73 season. In June of 1972, a major disaster struck the town of Isleton where the salt was stored. A levee surrounding the town broke, inundating the area. The reclaimed salt was lost in the flood, delaying storage studies until a new supply of recycled material could be produced. Storage studies are now under way however, and results will be reported at a later date.

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Ms received 11/18/72; revised 1/2/73; accepted 1/8/73.

The authors thank Dick Harrison and Linda Luis of the H.J. Heinz Co., Tracy, Calif. laboratory for the BOD and COD determinations, and from the Western Regional Research Lab., Joyce Hudson for the carbon analyses, Millie Gee for the chromatographic data, Eufemio Ducay for the elemental analyses by atomic absorption and Mona Gauger for the Cl, SO_4, nitrogen and solids determinations.

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EFFECTS OF DIFFERENT SUBMERGENCE TIMES IN HOT CALCIUM CHLORIDE ON PEELING EFFICIENCY OF TOMATOES

INTRODUCTION

A METHOD of peeling tomatoes by submerging them in a hot solution of calcium chloride ($CaCl_2$) was developed by Childs et al. (1948). They reported that the underskin or mesocarp of the tomato was not removed, therefore the finished product had a better appearance and color, due partially to the fact that the veins of the tomatoes were not exposed. They further report that higher drained weights and original shape were obtained and the firm whole tomatoes could be sliced in a manner similar to whole fresh tomatoes. The authors did not mention the amount of calcium absorbed by the tomatoes.

Heddins and Burns (1965) compared Childs' $CaCl_2$ peeling method with sodium hydroxide (NaOH) and hot water peeling and reported that fruit scalded in water were softer than those scalded in NaOH solution and were considerably softer than those scalded in $CaCl_2$ solution. The fruit scalded in $CaCl_2$ possessed an attractive red color but the white vascular bundles were exposed on fruit scalded in NaOH and the white color of these bundles reduced the redness of the fruit.

Stephens et al. (1967) used a rotary drum peeling machine to submerge tomatoes in a hot $CaCl_2$ solution and reported that the variety used continued to absorb calcium if allowed to remain in the peeling solution after the skin ruptured. They also found that tomatoes peeled in calcium chloride and covered with juice made from the peel trimmings and pieces of tomatoes (tailover juice), which had been peeled in calcium chloride, exceeded the permissible amount of 0.026% of the weight of the finished canned tomatoes, which is allowed by the Food and Drug Administration.

Saldana et al. (1971) compared the peeling efficiency of $CaCl_2$ liquid nitrogen and hot water on Chico, M-110 and M-121 tomato cultivars and reported that although the tomatoes which were peeled in $CaCl_2$ were firmer than tomatoes peeled with liquid nitrogen or hot water, it was difficult to control the amount of calcium absorbed by the tomatoes from the hot peeling solution.

The purpose of this investigation was to determine the ideal submergence interval by submerging several tomato cultivars in a boiling calcium chloride solution for varying lengths of time in order to obtain maximum peel removal with minimum calcium absorption.

EXPERIMENTAL

CHICO, Chico Grande, Chico III, and La Bonita tomato cultivars were harvested from plantings at the Texas A&M University Agricultural Research & Extension Center, Weslaco, Texas. Tomatoes were selected at the time of harvest to be firm, red-ripe, uniform size, free of cracks, skin blemishes and insect damage. Approximately 200 lb of tomatoes were harvested twice from each planting. The fruit was washed and all cracked or damaged tomatoes made into juice to be used to cover the canned tomatoes. Seven 12-lb samples of tomatoes were weighed for each treatment.

The first sample of tomatoes was dipped 1 min in boiling water, hand peeled and 300g packed into each of 12 No. 303 plain tin cans. A 30gr NaCl tablet was added to each can. The second sample was prepared like the first except a 30gr tablet containing 80% NaCl and 20% $CaCl_2$ was added to each can. Samples number 3, 4, 5, 6 and 7 were divided into 6-lb batches and the duplicate batches submerged for 20, 25, 30, 35 and 40 secs, respectively, in a boiling 42% $CaCl_2$ solution (b.p. 121.1°C) according to the procedure of Childs et al. (1948). The temperature of the peeling solution remained constant when 6-lb batches were submerged. After removal from the $CaCl_2$ solution the tomatoes were washed with a stiff spray of tap water and the peeled fruit counted and recorded as tomatoes that ruptured. The skin of the tomato was considered to be ruptured when it had split and could be removed from the fruit with a stiff spray of tap water. The skin on those tomatoes that did not rupture was split with a knife and removed by hand. The peeled tomatoes of each peeling treatment were packed 300g to each of 12 No. 303 plain tin cans to which 1.56g (24gr) NaCl had been added. All cans were filled with hot tomato juice, exhausted to a center-can temperature of 71°C, closed, processed 25 min in boiling water, cooled in tap water, then stored at 20°C to allow the tomatoes and juice to equilibrate.

Drained weight of the tomatoes was determined according to the procedure in the United States Standards for Grades of Canned Tomatoes (USDA, 1964). Firmness was determined on 200g of drained tomatoes with an Allo-Kramer Shear Press operated with a 1,000-lb proving ring, a 50% setting of the Varian 10-in. strip recorder and a 1 min time setting for the full stroke of the instrument. The area under the time-force curve on the recorder was measured with a self-compensating planimeter and the area was used as a measure of firmness.

The drained tomatoes from the firmness determinations were blended without the cover juice for 2 min in a Waring Blendor, deaerated and used in the determinations of pH, titratable acidity, Brix and color. Percent calcium was determined on both the blended tomatoes and the cover juice.

Titratable acidity was determined by titrating 10g of the blended tomato sample diluted with 100 ml of distilled water to a pH of 8.2 using 0.1567N sodium hydroxide and a Corning Model 10 pH meter. The pH was measured with the same instrument. A Bausch and Lomb refractometer was used to determine the °Brix.

Color notations were made with a Gardner Color Difference Meter which had been standardized with a tomato red color plate: Rd 5.36, a 30.4, b 14.6. The a/b ratio recommended by Robinson et al. (1952) was used for color comparisons.

Calcium content of the blended tomatoes and juice samples was determined with a Perkin Elmer Model 303 Atomic Absorption Spectrophotometer using the procedure described by the manufacturer of the instrument (1971). Preparation consisted of weighing 10g of blended sample, drying at 90°C, ashing overnight at 500°C, then taking the ash up in 5% HCl containing 1% lanthanum.

Data were subjected to an analysis of variance as described by Steel and Torrie (1960).

RESULTS & DISCUSSION

FOUR TOMATO cultivars, Chico, Chico Grande, Chico III and La Bonita were submerged for different intervals of time in a boiling 42% solution of $CaCl_2$ to determine peeling efficiency. Tables 1 through 4 show that the percentage of the tomatoes which ruptured and peeled at a specific submergence time was not the same for all cultivars. These results are in agreement with Heddins and Burns (1965). They noted a cultivar dependence on the amount of peel removed from Homestead and Chico tomatoes by submerging in boiling calcium chloride.

Data are not included in the tables, but it was noted that the percentage of tomatoes from the first harvest, that ruptured in the hot $CaCl_2$ solution was greater than the percentage of tomatoes that ruptured from the second harvest. This condition was true for the five submergence time intervals for each of the four cultivars. The peel of these varieties grown under the conditions in this area became more difficult to remove with hot $CaCl_2$ as the picking season progressed.

The change in the skin texture of the tomatoes due to maturity could have resulted in the peel being difficult to remove. It has been noted by Juven et al. (1969) that the conditions required for the satisfactory peeling of Roma variety of tomato with NaOH differed very conspicuously depending upon the season of maturation of the fruits and their relative maturity.

The drained weight of the tomatoes shown in the tables increased as the submergence time in the $CaCl_2$ solution increased. Except for Chico, the drained weight of the $CaCl_2$-peeled fruit for all peeling treatments was statistically greater than the water-peeled fruit. It is difficult to explain why the drained weight of Chico variety which had been submerged in a hot $CaCl_2$ solution was not greater than the drained weight of water-peeled fruit. Godfrey (1940) and Kertesz et al. (1940) dipped peeled tomatoes into a $CaCl_2$ solution and noted that the drained weight increased but tomatoes subjected to the dipping treatment lacked uniformity. Godfrey (1940) stated that some tomatoes from the same can were soft and others were firm. Kertesz et al. (1940) found that the amount of calcium taken up by the tomatoes increased as the dipping temperatures were raised. Although these investigators were dipping peeled tomatoes into an approximate 2% warm $CaCl_2$ solution rather than the 42%, 121.1°C temperature solution used in this investigation, the same conditions could have existed to cause the drained weight of Chico variety to be inconsistent.

The total amount of calcium taken up by a No. 303 can of tomatoes increased as the submergence times increased, but the amounts taken up were not uniform among submergence times. The increase in the amount of calcium taken up by Chico Grande and Chico III was partially dependent upon the percentage of tomatoes that ruptured in the solution. However, the rupture percentage and calcium absorbed by Chico and La Bonita variety was inconsistent. The influence of the percentage of tomatoes that ruptured in the solution on the amount of calcium taken up by the tomatoes could be due to skin structure and pectic substance differences among the cultivars. Voisey et al. (1970) reported that the cracking resistance of the skin of three tomato cultivars studied does not appear to be governed by skin thickness. Cracking resistance is governed by skin strength and its ability to stretch and these factors may be governed by the penetration of the cutinized outer layer into the cells of the skin. Loconti and Kertesz (1941) concluded that tomatoes treated with $CaCl_2$ formed calcium-pectate with the pectic substances in the tomatoes. Luh et al. (1960) reported that two pear-shaped cultivars were higher in total pectin and protopectin contents than were three round-shaped cultivars and Stephens et al. (1970) found that Chico and Chico Grande pear shaped tomatoes were higher

Table 1—The percentage ruptured fruit, calcium content and firmness of treated Chico variety tomatoes[a]

Treatment	Tomatoes that rupture %	Net wt in can g	Drained tomatoes Wt g	Drained tomatoes Ca^{++} %	Drained tomatoes Wt Ca^{++} g	Cover juice Wt g	Cover juice Ca^{++} %	Cover juice Wt Ca^{++} g	Total amt Ca^{++} in can g	Permissible amt Ca^{++} per can[b] g	Shear press values sq in.
Water peel (Control)		433[b]	310[ab]	.010[a]	.031	121[b]	.010[a]	.012	.043		.44[a]
Water peel $CaCl_2$ added		432[b]	301[a]	.037[d]	.111	128[b]	.038[d]	.049	.160	.155	.79[c]
$CaCl_2$ peel											
Dip 20 sec	40[a]	431[b]	307[ab]	.014[a]	.043	119[b]	.016[b]	.019	.062	.155	.61[b]
Dip 25 sec	53[ab]	425[a]	300[a]	.018[b]	.054	123[b]	.020[b]	.025	.079	.153	.67[b]
Dip 30 sec	66[bc]	422[a]	309[ab]	.017[b]	.053	121[b]	.019[b]	.023	.076	.152	.68[b]
Dip 35 sec	77[cd]	421[a]	315[b]	.024[c]	.075	106[a]	.025[c]	.027	.102	.152	.91[c]
Dip 40 sec	92[d]	420[a]	315[b]	.035[d]	.110	103[a]	.037[d]	.038	.148	.152	1.12[d]

[a]Values followed by the same letter are not significantly different at the 5% level by Duncan's multiple range test.
[b]The permissible amount of calcium for each can was determined by multiplying the net weight by 0.026% and adding the natural calcium in the tomatoes to the product.

Table 2—The percentage ruptured fruit, calcium content and firmness of treated Chico Grande tomatoes[a]

Treatment	Tomatoes that rupture %	Net wt in can g	Drained tomatoes Wt g	Drained tomatoes Ca^{++} %	Drained tomatoes Wt Ca^{++} g	Cover juice Wt g	Cover juice Ca^{++} %	Cover juice Wt Ca^{++} g	Total amt Ca^{++} in can g	Permissible amt Ca^{++} per can[b] g	Shear press values sq in.
Water peel (Control)		435[a]	277[a]	.009[a]	.025	158[c]	.008[a]	.013	.038		.52[a]
Water peel $CaCl_2$ added		437[a]	301[bcd]	.036[c]	.108	135[ab]	.035[e]	.047	.155	.151	.77[c]
$CaCl_2$ peel											
Dip 20 sec	32[a]	433[a]	293[b]	.015[b]	.044	139[b]	.014[b]	.019	.063	.150	.61[b]
Dip 25 sec	59[b]	436[a]	296[bc]	.017[bc]	.050	138[b]	.017[bc]	.023	.073	.151	.63[b]
Dip 30 sec	68[bc]	440[a]	308[cd]	.022[cd]	.068	132[ab]	.023[d]	.030	.098	.152	.80[cd]
Dip 35 sec	83[cd]	439[a]	312[d]	.023[d]	.071	126[a]	.022[cd]	.028	.099	.152	.83[d]
Dip 40 sec	93[d]	437[a]	313[d]	.040[e]	.125	124[a]	.040[e]	.050	.175	.151	1.10[e]

[a]Values followed by the same letter are not significantly different at the 5% level by Duncan's multiple range test.
[b]The permissible amount of calcium for each can was determined by multiplying the net weight by 0.026% and adding the natural calcium in the tomatoes to the product.

in the acid soluble pectic fraction than La Bonita or Homestead-24 varieties which are round shaped. The skin of La Bonita appeared to be different from Chico, Chico Grande and Chico III and the fruit possibly contained different types and amounts of pectic substances.

Adding calcium to the can in the form of a 30gr salt tablet increased the total amount of calcium in each can of tomatoes slightly more than would be permissible by the definitions and standards of the Food and Drug Administration (1956). The standard states that, "calcium salts (may be added) in a quantity reasonably necessary to firm the tomatoes, but in no case such that the amount of the calcium contained in such salts is more than 0.026% of the weight of the finished canned tomatoes." A slight excess of calcium in these tomato samples does not alter the results of the experiment but is mentioned because the tomatoes treated with a 30gr salt tablet is included in the tables to show that tomatoes peeled with a $CaCl_2$ solution utilize the calcium more efficiently than tomatoes peeled with hot water and canned with a tablet containing calcium added to the can. Peeling tomatoes in hot water probably destroyed the ability of the pectic substance in the outside portion of the tomatoes to pick up calcium. Although the temperature of the $CaCl_2$ peeling solution was much higher than the hot water, the calcium was immediately available to the exposed cells beneath the ruptured skin of the tomatoes before the heat had time to destroy the pectic substances and reduce their ability to take up calcium. A similar condition was noted by Brown et al. (1970) in canned tomatoes peeled with liquid nitrogen compared with tomatoes peeled with hot water with calcium added to the cans. They believed that the heat of the peeling water destroyed some of the ability of the outside layers of tissues to be firmed by calcium. Chico, Table 1, was the only one of the cultivars that did not take up more calcium from the peeling solution than is permissible by the definitions and standards of the Food and Drug Administration. This phenomenon is not in agreement with the results of Stephens et al. (1967) indicating that most of the tomatoes of Chico ruptured within 40 sec after being submerged in hot $CaCl_2$ but if the tomatoes remained in the hot peeling solution for an additional 20 sec they continued to take up calcium.

The shear press values of the tomatoes increased as the submergence times in the peeling solution increased; however, the increases were not uniform between the submergence time intervals.

There were slight differences in pH, titratable acidity, °Brix and color among the varieties but there were no consistent differences which could be attributed to the submergence times in the hot $CaCl_2$. The pH of all treatments of La Bonita, 4.8 4.9, was slightly higher and Chico III, pH 4.3 4.5, was lower than comparable treatments of the other cultivars.

Table 3—The percentage ruptured fruit, calcium content and firmness of Chico III tomatoes[a]

Treatment	Tomatoes that rupture %	Net wt in can g	Drained tomatoes Wt g	Drained tomatoes Ca^{++} %	Drained tomatoes Wt Ca^{++} g	Cover juice Wt g	Cover juice Ca^{++} %	Cover juice Wt Ca^{++} g	Total amt Ca^{++} in can g	Permissible amt Ca^{++} per can[b] g	Shear press values sq in.
Water peel (Control)		438^{ab}	284^{a}	.007^{a}	.020	153^{c}	.006^{a}	.009	.029		.40^{a}
Water peel $CaCl_2$ added		435^{ab}	293^{ab}	.034^{cd}	.100	141^{b}	.035^{cd}	.049	.149	.142	.70^{b}
$CaCl_2$ peel											
Dip 20 sec	31^{a}	443^{b}	296^{b}	.012^{ab}	.036	146^{bc}	.012^{ab}	.018	.054	.141	.43^{a}
Dip 25 sec	54^{b}	437^{ab}	309^{c}	.016^{ab}	.049	128^{a}	.017^{b}	.022	.071	.143	.51^{a}
Dip 30 sec	68^{c}	435^{ab}	310^{c}	.026^{c}	.081	124^{a}	.028^{c}	.035	.116	.142	.75^{b}
Dip 35 sec	92^{d}	433^{a}	314^{c}	.038^{d}	.119	119^{a}	.039^{dc}	.046	.165	.142	1.04^{c}
Dip 40 sec	90^{d}	433^{a}	310^{c}	.047^{e}	.146	123^{a}	.048^{e}	.059	.205	.142	1.03^{c}

[a]Values followed by the same letter are not significantly different at the 5% level by Duncan's multiple range test.

[b]The permissible amount of calcium for each can was determined by multiplying the net weight by 0.026% and adding the natural calcium in the tomatoes to the product.

Table 4—The percentage ruptured fruit, calcium content and firmness of treated La Bonita tomatoes[a]

Treatment	Tomatoes that rupture %	Net wt in can g	Drained tomatoes Wt g	Drained tomatoes Ca^{++} %	Drained tomatoes Wt Ca^{++} g	Cover juice Wt g	Cover juice Ca^{++} %	Cover juice Wt Ca^{++} g	Total amt Ca^{++} in can g	Permissible amt Ca^{++} per can[b] g	Shear press values sq in.
Water peel (Control)		454^{a}	290^{a}	.008^{a}	.023	163^{d}	.007^{a}	.011	.034		.36^{a}
Water peel $CaCl_2$ added		458^{ab}	310^{bc}	.035^{d}	.109	143^{b}	.035^{d}	.050	.159	.153	.55^{bc}
$CaCl_2$ peel											
Dip 20 sec	53^{a}	462^{c}	307^{b}	.013^{ab}	.040	155^{cd}	.013^{ab}	.020	.060	.154	.45^{ab}
Dip 25 sec	69^{a}	460^{c}	313^{bcd}	.017^{b}	.055	146^{bc}	.016^{b}	.023	.078	.154	.59^{c}
Dip 30 sec	92^{b}	455^{ab}	319^{cde}	.026^{c}	.083	135^{ab}	.024^{c}	.032	.115	.152	.77^{d}
Dip 35 sec	90^{b}	456^{ab}	325^{e}	.044^{e}	.143	130^{a}	.042^{e}	.055	.198	.153	1.09^{e}
Dip 40 sec	98^{b}	459^{bc}	324de	.039de	.126	133^{a}	.039^{dc}	.051	.177	.153	1.05^{e}

[a]Values followed by the same letter are not significantly different at the 5% level by Duncan's multiple range test.

[b]The permissible amount of calcium for each can was determined by multiplying the net weight by 0.026% and adding the natural calcium in the tomatoes to the product.

but these differences were not satistically significant. These data are not in agreement with Saldana et al. (1971). They reported a slightly lower pH in most samples of salad pack tomatoes treated with calcium chloride added to the cans of tomatoes or if the tomatoes were peeled in the hot $CaCl_2$. They attributed the decreases to possible increases in methylesterase activity.

The °Brix was a little higher in most of the samples treated with $CaCl_2$ than it was in the water-peeled control sample, but the differences were not statistically significant.

The small inconsistent differences in color as measured on the blended tomato samples by the Gardner Color Difference Meter was probably due to differences in internal color of the tomatoes. The visual color of the whole tomatoes peeled in $CaCl_2$ appeared to be redder than tomatoes peeled in hot water but the instrument did not detect a difference, because the color notations were made on the blended samples rather than the whole fruit. Brown et al. (1970) noted that tomatoes peeled in liquid nitrogen had a redder visual color than tomatoes peeled in boiling water but the Gardner Color Difference Meter did not detect the difference possibly because the color notations were made on the blended tomatoes.

Information reported by Childs et al. (1948), Heddins and Burns (1965) and Stephens et al. (1967) indicates that peeling tomatoes in a hot $CaCl_2$ solution has some advantages over the conventional hot water peeling method. However, according to the results of this investigation, there are also some disadvantages which the canning plant operator must consider if he intends to use $CaCl_2$ as a peeling media for tomatoes. The use of the process will require constant supervision by a trained operator if a high percentage of peeled tomatoes is obtained from different varieties harvested on different dates throughout the season. More important, it was impossible in this study to control the amount of calcium taken up by the tomatoes from the hot peeling solution by controlling the submergence time.

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Ms received 8/5/72; revised 11/16/72; accepted 11/20/72.

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AQUEOUS PROCESSING OF FRESH COCONUTS FOR RECOVERY OF OIL AND COCONUT SKIM MILK

INTRODUCTION

COCONUT PROTEIN represents a substantial source of potentially available food protein in a number of tropical countries. Currently, worldwide growth of coconuts results in about 200,000 metric tons of coconut protein annually (USDA, 1971). This protein is currently not usable as food because of unsanitary processing of copra. Consequently efforts have been undertaken to process fresh coconuts to recover food-grade protein. However, the processes developed have been marked by inefficient recovery of oil and protein (Rajasekharan and Sreenivasan, 1967).

The purpose of this work is to describe an economical method for the pilot plant processing of coconuts. The processing is designed to produce coconut skim milk, a nutritious product currently produced in the kitchen or on a larger scale by rather rudimentary technology (Salon and Maniquis, 1969). The pilot plant processing as presented is a development of preliminary work involving bench-scale processing (Hagenmaier et al., 1972).

EXPERIMENTAL

Pilot plant operation

A simplified flow diagram for the process is shown in Figure 1. Pilot plant operation was performed on a scale of approximately 100 lb of coconut meats per day. The coconuts were purchased as husked nuts from a local grocer, shelled manually and the coconut water saved for use in processing. Washed meats were ground in a hammer mill (The Fitzpatrick Co.) with 0.25 in. diam holes in the screen, followed by grinding in a disc attrition mill (Bauer Bros. Co.) with the discs set at minimum clearance.

The ground coconut was briefly mixed with heated coconut water (80°C), or with a corresponding amount of heated tap water, when the coconut water was not used. The mixture was pressed at 50 psi in a pulp press, against a screen with holes 0.01 in. diam. Extraction with hot water, followed by the pressing, was repeated twice more. The two materials resulting from the pressing operation are the liquid filtrate (called coconut milk), and the solids (called residue). The residue came from the press at 70 ± 10% moisture, and was dried in a rotary dryer, in a flow of hot air (inlet air is ca. 100°C). The dried residue is a final product.

The coconut milk was filtered through a vibrating screen (120 mesh) to remove the last traces of residue. The pH of the filtered milk was raised to 7.0 (from 6.3) with sodium hydroxide. The milk was pastuerized (ca. 1 hr at 60°C), then centrifuged in a three-phase, desludging type separator (Westfalia). This model develops a centrifugal force of 4,700 × G at the radius of the hole in the disc stack, has a bowl capacity of 3.2 liters and was operated with a feed of 8 liters per minute. The milk was centrifuged until the solids capacity of the bowl was reached, the feed to the separator stopped, and the bowl flushed with aqueous phase for 4 min before desludging. Complete desludging was performed once for every 20 kg of coconut meats used.

Three phases emerge from the separator: (1) The lightest phase, usually called coconut cream, which is ca. 65% oil on a wet basis; (2) The aqueous phase, usually called coconut skim milk, which is a solution of the soluble components of the coconut, with some suspended particles. With counter-current washing of the residue, the aqueous phase would contain ca. 9.5% solids as it comes from the separator; and (3) A solids phase, consisting mostly of insoluble protein, which is dried to give a final product.

The coconut cream is an oil-in-water emulsion with average oil globule size of ca. 10 μm in diameter (Hagenmaier et al., 1972). This emulsion was diluted with oil to reduce the moisture content, then agitated with a high velocity pump to invert the emulsion. Best results were achieved when the temperature was raised to ca. 80°C before the agitation. After inversion of the emulsion, oil and water were separated by first allowing a gravity separation (which took several minutes) into two phases: an aqueous phase and an oil phase. The aqueous phase,

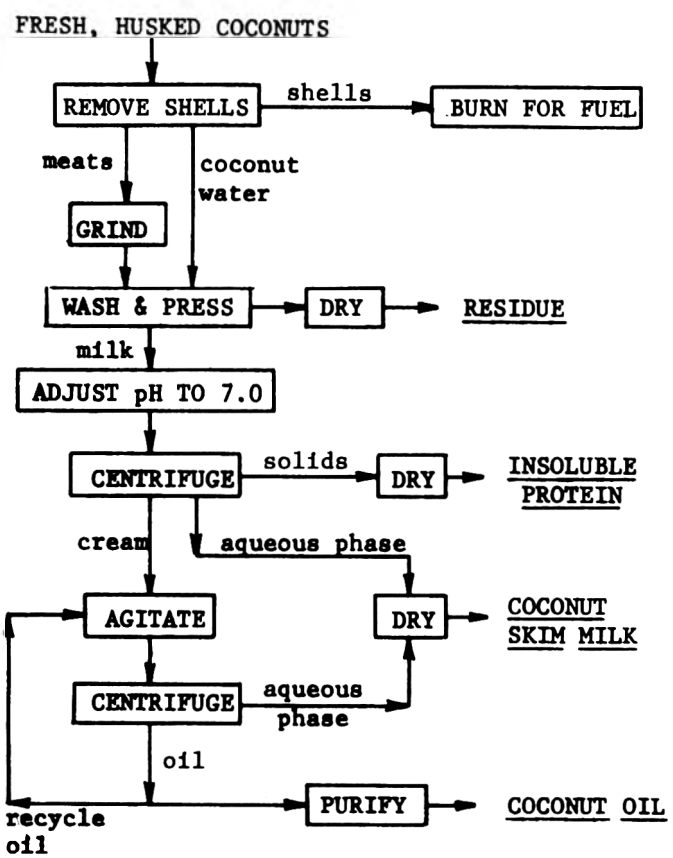

Fig. 1—Simplified flow diagram for the aqueous processing of coconuts. The shells were discarded in our operations, but would be burned for fuel in commerical processing.

after centrifugation, has approximately the same composition as the aqueous layer from the three-phase separation of the milk. The oil phase was purified by centrifugation, heating to drive off residual moisture, and decanting the oil from settled solids.

The insoluble protein recovered from centrifugation of coconut milk was dried by freeze drying. The coconut skim milk was dried by spray drying, with an air outlet temperature of ca. 85°C, and air inlet temperature of ca. 170°C.

Analytical methods

Standard methods of analysis were used for nitrogen and crude fiber. Crude fat was based on ether extraction of dried samples, except for the soluble coconut fraction, which required extraction with a chloroform-methanol mixture (Bligh and Dyer, 1959). Ash analysis followed heating for 2 hr at 600°C, except for the soluble coconut fraction which was ashed overnight at 550°C. Soluble carbohydrate was determined by measurement of reducing sugars in a clarified aqueous extract. The measurement followed inversion for 1 hr at 60°C in 0.7M HCl.

RESULTS & DISCUSSION

Products

The gross analyses of the products are shown in Table 1. All values are the averages of measurements on at least two independently prepared samples. The reliability expressed represents the estimated 95% confidence level for the sum of both analytical errors and variations in analyses due to small changes in processing parameters.

The analysis of coconut oil is not included in Table 1. Analysis of the final oil product showed it to contain 0.4 ± .1% free fatty acids, with an iodine number of 7.5. The oil was centrifuged to clarity in the laboratory and some precipitate was obtained, with the amount of nonoil solids being 0.4 ± .2%. Coconut oil from aqueous processing is therefore superior to oil obtained by crushing copra, which varies widely in analysis. A typical value for free fatty acid content of crude coconut oil from copra would be ca. 5%.

The residue is a fibrous material, which contains small particles of dark testa. Preliminary work shows that the testa can be separated to give a very light-colored product. The protein products are light brown or tan. All products can be obtained almost white if the coconuts are pared.

The products from processing of coconut meats are shown in Table 2. Any product losses that may result from spray drying are not included, because spray-drying losses are expected to be almost negligible in a commercial operation. All results are the averages of at least two independent experiments, performed after many preliminary experiments that were done to determine optimum procedures and operating parameters. The results reflect the product losses resulting from multiple transfer of material between containers and processing equipment.

It is presumed that such losses would be negligible in a continuous process. Therefore a material balance was assumed to calculate the products from the continuous, large-scale aqueous processing of fresh coconuts, with coconut water being used to extract coconut milk from the fiber (by counter-current extraction). Fresh husked nuts are assumed to be 44% meats, 31% water and 25% shells (wet weights). The results of this calculation are shown in Table 3.

These results indicate that 69% of the total protein is in the skim milk fraction, and 99% of the crude fiber is in the residue fraction. This separation of protein from crude fiber is considered one of the principal advantages that aqueous processing has, as compared to dry processing of coconuts.

Similar calculations show that the coconut skim milk also contains 83% of the salts (as estimated from ash analyses) and 97% of the soluble carbohydrates of the coconut. The skim milk fraction therefore contains the bulk of salts, sugars and protein of the coconut.

The preparations of products was performed repeatedly, with slight changes in

Table 1–Typical compositions of dried products

	Residue	Insoluble protein	Skim milk[a]
% Crude protein (N × 6.25)	3.4 ± 0.6	52 ± 10	25 ± 2
% Crude fat	24 ± 3	19 ± 9	5.1 ± 1.5
% Crude fiber	25 ± 4	2.0 ± 0.5	0.03 ± 0.03
% Ash	0.6 ± 0.1	9 ± 2	9 ± 1
% Moisture	5 ± 3	4 ± 2	0.5 ± 0.3
% Carbohydrate, total[b]	60 ± 3	unknown	unknown
% Carbohydrate, soluble	0.3 ± 0.05	7 ± 3	45 ± 3

[a]Composition with coconut water used in processing. See text for composition without use of coconut water. Dehydrated skim milk was stored in desiccator to maintain low moisture content.
[b]By analysis, after digestion in 72% sulfuric acid

Table 2–Experimental recovery of different components in the processing of coconut meats[a]

Component / Product	Fraction of component in product (%)		
	Total mass	Oil	Protein
Residue	17[b]	5.8	7.0
Insoluble protein	2.5	0.7	20
Skim milk	16	1.2	65
Oil	59	85	0
Total % recovered	94	93	92

[a]For pilot plant processing of coconut meats only; coconut water was not used in the processing.
[b]The standard deviation for each result is approximately 2% of the value in the table.

Table 3–Products from processing, as calculated from experimental recovery, product compositions and material balance

Product	Mass of product	Protein in product	Oil in product
Residue	43 kg[a]	1.5 kg	10 kg
Insoluble protein	5.7	3.6	0.7
Skim milk	44	11.8	0.6
Oil	142	0.0	142
Totals	234	16.9	153

[a]All results are on a dry weight basis. The starting material is 1000 kg of husked coconuts (wet weight basis).

the processing. The observations considered most significant are the following:

Effect of pH. Coconut milk was centrifuged at pH values from 6.4–8.1. The protein content of the skim milk fraction was observed to be independent of pH over this range. Furthermore, no significant change in composition of the insoluble protein was observed from pH 7.0–8.0.

Use of coconut water. It was observed that only the skim milk fraction was significantly changed in composition if coconut water was not used in the processing. When coconut water was discarded, instead of being used in processing, the skim milk fraction contained 30 ± 2% crude protein; 37 ± 3% soluble carbohydrates; and 9 ± 1% ash (see Table 1).

Temperature. Milk temperatures of 60 ± 5°C were used in the processing. Laboratory results indicate that significant heat coagulation of coconut protein does not occur below 75°C in the solvent system of the aqueous processing (pH 7.0, 8% suspension of the skim milk fraction).

The temperature used during spray drying (air outlet temperature of 85°C) did not reduce protein solubility. Based on four measurements each, it was observed that 89 ± 5% of the nitrogen was dissolved for a spray-dried sample, versus 85 ± 5% for a freeze-dried control and 88% for a single measurement on an undried control (at 25°C, pH 7.0, ca. 6% solids in suspension).

Estimation of product costs

In making the cost estimate oil and the skim milk fraction were considered to be the main products of the aqueous processing of coconuts, and were made to carry all costs. Cost of the skim milk fraction was calculated as total costs minus income from oil sales. Total costs were taken as raw material costs plus processing costs. The costs were calculated for a hypothetical processing plant, operating in the Philippines, which would process 125 metric tons per day of husked coconuts.

Purchase prices of fresh, husked coconuts in the Philippines from 1962 to 1971 averaged $34.20 per metric ton, according to information from the United Coconut Assocation of the Philippines (1971) and Emata (1972). This price includes $0.70 per metric ton estimated as transportation costs. Crude oil prices in the Philippines, also for the period 1962–1971, averaged $0.113 per lb.

Processing costs for the hypothetical processing plant were estimated to total $15.80 per ton of husked coconuts processed. This cost includes labor, equipment depreciation, interest on borrowed capital, maintenance, utilities, administration and other miscellaneous expenses. The manual shelling of coconuts is the largest processing expense, accounting for 22% of total processing costs. The estimated total equipment cost is $650,000. The total installed plant cost is taken as 2.5 times the equipment cost or $1,600,000.

Based on these estimates, which conservatively assume 90% recovery of oil (results in Table 3 indicate 93% oil recovery) and 69% recovery of protein, it was estimated that the dried skim milk fraction would cost $0.16/lb to produce. It should be borne in mind that any income from other products would reduce the estimated cost of the soluble fraction. It might also be pointed out that assumption of zero income from products 1 and 2 has merely been a bookkeeping operation. It is anticipated that products 1 and 2 would, in fact, be marketable commodities.

An alternate process

An alternate process has also been considered, in which the skim milk fraction is sub-fractionated into two components: an acid-precipitated fraction, and an acid-soluble fraction. Our results have indicated that pH 4.3 is optimum for protein precipitation. This agrees well with data of Khaund (1971) on preparation of islates from hexane-defatted coconut meal.

The pilot plant separation of the two fractions was accomplished by adjustment of the aqueous phase to pH 4.3, followed by centrifugation at ca. 50°C. The freeze-dried, acid precipitated fraction, prepared without using coconut water in the processing, contained 65 ± 6% crude protein, 24 ± 4% oil, 2 ± 1% ash, 0.15 ± .05% crude fiber and 3.5 ± 1% soluble carbohydrates. The acid soluble fraction (also prepared without using coconut water) contains 13 ± 2% crude protein and 13 ± 3% ash. It is estimated that with use of coconut water the acid-precipitated fraction would not change significantly in composition, but the acid soluble fraction would change to a composition of 10% crude protein and 13% ash. From experimental data and material balance considerations, it was estimated that 46% of the total coconut protein would be recovered in an acid-precipitated protein fraction. If 1000 kg of husked coconuts were processed, 12 kg of the acid-precipitated fraction would be recovered, which would contain 7.8 kg of crude protein (see Table 3).

Production costs for this alternate process would be about the same as for the process resulting in the coconut skim milk. If oil and the acid-precipitated protein are taken as the only marketable products, then the cost of producing the acid-precipitated fraction would be $0.59 per pound.

In future reports, detailed chemical analysis of the products will be given, together with some biological evaluation, information on stability and physical properties, and discussion of possible food uses.

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Ms received 10/19/72; revised 12/4/72; accepted 12/12/72.

Presented at the 32nd annual Meeting of the Institute of Food Technologists in Minneapolis.

This research was supported by the Agency for International Development.

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DEMINERALIZATION OF UNTREATED COTTAGE CHEESE WHEY BY ELECTRODIALYSIS

INTRODUCTION

DEMINERALIZED whey solids can be used as a food ingredient for products such as geriatric and dietetic foods, candy, cereals, bread, sherbet, coffee whiteners and ice cream (Anonymous, 1969). Demineralized whey mixed with skim milk has a composition similar to human milk and can be used as a formula for infants (Al and Wiechers, 1952; Wilson, 1960).

Conventionally dried whey produced by evaporation, spray and drum drying has a high salt and lactic acid content which limits the amount utilized for human consumption. One process that has been used to reduce the high salt concentration is electrodialysis. Although there are commercial electrodialysis installations for cheese whey such as Foremost Dairies, Inc. (Craig et al., 1971) and Purity Products Co. (Anonymous, 1970), the process factors are not well defined. Most of the work has been centered on desalination of salt water (Friedlander and Rickles, 1966; Mason and Kirkham, 1959; Rapier et al., 1963). It is the purpose of this study to provide an insight into the efficiency of the process as influenced by flow conditions, temperature, current and pH of the whey.

MATERIALS & METHODS

DEMINERALIZATION of the raw cottage cheese whey was studied in a unit provided by Ionics, Inc. The rectifier control unit was wired for 220 volt, 3 phase, 60 cycle AC input power with an output rating of 150 volts at 30 amps. A variac was used to maintain the desired voltage across the stack assembly.

The membranes used were of two basic types—anion membranes (Ionics 111 EZL-219) and cation membranes (Ionics 61 CZL-183). The anode electrode was platinum coated tantalum (Tirreloy B) and the cathode electrode was a silvery finished platinum (Hastelloy C). The diluate and concentrate spacers between the membranes were cast polyethylene.

The life of a membrane was difficult to predict as it depended upon the various operating process parameters. In our experiments, it was necessary to ensure that any changes in membrane flux from experiment to experiment was a function of the process variables and not a result of fouled membranes.

The membrane flux determination was accomplished by demineralizing an approximate 4% NaCl solution by batch plug flow operation with external staging on 18 cell pairs at a fixed temperature of 25°C, voltage of 25v and flow rates of 12.50 ml/sec in the diluate and the concentrate streams. A volume of 6,000 ml NaCl solution was used for demineralization. Membrane flux determinations were conducted both before and after each experiment.

The polarization of the membranes was determined at various flow rates by assembling a stack with nine membranes, all of the anion type, with spacers aligned such that the solution to be demineralized was fed into every inter-membrane compartment while the electrode stream was fed to the two electrode compartments. All anion membranes were used because the anion membranes polarize sooner than the cation membranes (Mason and Kirkham, 1959). One platinized-platinum foil was inserted in the third spacer from the cathode and another in the third spacer from the anode. The platinum tabs were connected to a high impedance voltmeter. Batch plug flow operation was conducted at a constant flow rate and at a constant temperature after membrane equilibration. Membrane equilibration was accomplished by running the solution to be tested through the membrane stack for 30 min with a maximum reading of 0.2 amperes. This portion of the test solution was then discarded.

The voltage across the five cell pairs measured by the voltmeter connecting the two platinum tabs was determined for corresponding increases in amperage. A break in the plot of the ratio of voltage across tabs to corresponding current (V/I) versus the reciprocal of the current (1/I) indicated the polarization point. Limiting current density could then be calculated at the polarization point.

The undiluted raw cottage cheese whey was adjusted to the desired pH by the addition of concentrated NaOH or concentrated HCl. The pH adjusted whey was then allowed to equilibrate by storing at 40°F for 12 hr. The diluate (product) tank and the concentrate (waste) tank were charged with approximately 6,000 ml of the pH adjusted cottage cheese whey after membrane equilibration. Membrane equilibration was accomplished by running the pH adjusted cottage cheese whey through the membrane stack for 30 min with a maximum reading of 0.2 amperes. This portion of the whey was then discarded. The electrode stream was Na_2SO_4 solution with the same conductance as that of the whey. The pH of the electrode stream was then adjusted to 1.5 and 3.0 with concentrated H_2SO_4.

Demineralization of the pH adjusted cottage cheese whey was accomplished by batch operation with external staging, plug flow (no agitation), and a constant temperature of 25 ± 3°C. Flow rates in the diluate and the concentrate streams were increased slowly so that the pressure differential between any two lines did not exceed 1.0 psi. Batch operation was carried out at a constant voltage of 85v across 18 cell pairs and a constant flow rate of 20.80 ± 0.2 ml/sec (Re=1983) in both the diluate and the concentrate streams. Flow rate in the electrode stream was adjusted so that there was approximately the same line pressure as in the diluate and the concentrate stream. Demineralization proceeded for 45 min with sampling of the ef-

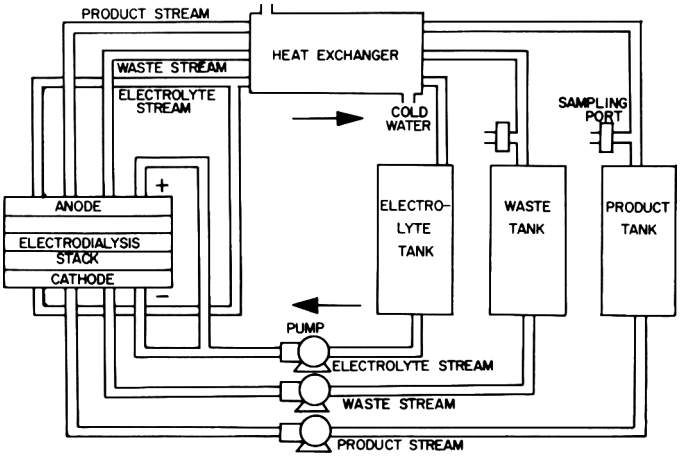

Fig. 1—Schematic diagram of the experimental equipment used in the demineralization of cottage cheese whey.

fluent diluate stream at even intervals during multiple passes through the stack assembly. Flow rates were checked and maintained at a constant value during the demineralization run. Figure 1 illustrates the schematic of the experimental setup used for the demineralization of cottage cheese whey.

The samples were analyzed for the following: (1) total solids by the Mojonnier Method (Mojonnier and Troy, 1965); (2) total noncombustible ash (AOAC, 1970); (3) total nitrogen by the micro Kjeldahl Method (AOAC, 1970); (4) nonprotein nitrogen (Bradstreet, 1965); (5) pH on a Corning pH meter; (6) calcium ions with a Beckman Calcium Electrode; and (7) sodium, potassium, calcium, magnesium, chlorine and phosphorus elements by Neutron Activation Analysis at the University of Wisconsin-Madison by the services of the Nuclear Engineering Supervisory Personnel.

RESULTS & DISCUSSION

PRELIMINARY EXPERIMENTS were done on NaCl solution to determine the parameters to be studied in the demineralization of cottage cheese whey. An approximate 1–4% NaCl solution was demineralized with temperature, fluid flow rate, operating streams and current as the process parameters. Cheese whey was used to study the effect of the type of recirculation.

The results were analyzed by observing both the rate and extent of demineralization. Generally, the rate was determined during the first 20 min and was a measure of fraction demineralized per unit of time. Whereas, the extent of demineralization was the fraction demineralized at the end of 45 min. Thus, in the discussion both the rate and extent of demineralization are analyzed.

It was observed that an increase in the temperature from 25°C to 30°C produced a change of 4% demineralization in the final demineralized product. The rate of demineralization for the two temperatures was not appreciably affected. A process temperature of 25°C was selected for the demineralization of whey, since it was easy to maintain a constant temperature throughout the experiment by a heat exchanger system. This was necessary to prevent any permanent changes in the components of the whey, especially the protein, and to get maximum use of the membranes. A heat exchanger was needed as temperature of the fluid increased during the demineralization because of energy being expended in the stack.

No experiments were conducted on the effect of velocity on NaCl demineralization, but experiments conducted on cottage cheese whey (pH 4.52) showed that an increase in fluid velocity of 30% (16.0 ml/sec to 20.80 ml/sec) produced a change of only 3% in demineralization at the end of 45 min. The rate of demineralization for the two velocities did not change significantly. Velocity had no ef-

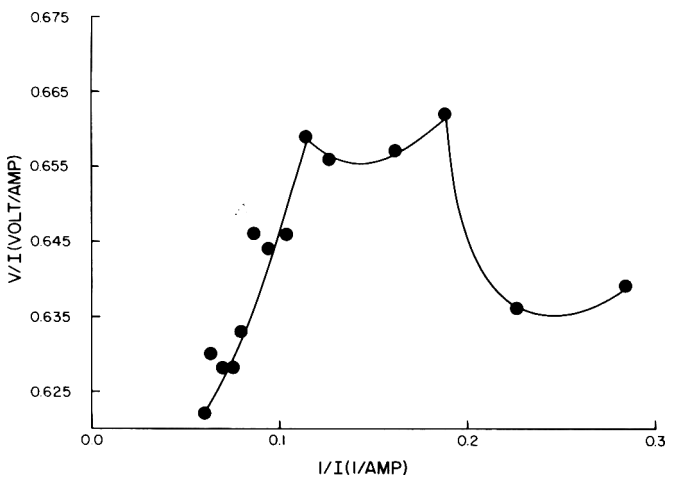

Fig. 2—Concentration polarization for 1% NaCl solution at a temperature of 25 ± 3° C and an average flow rate of 23.50 ml/sec.

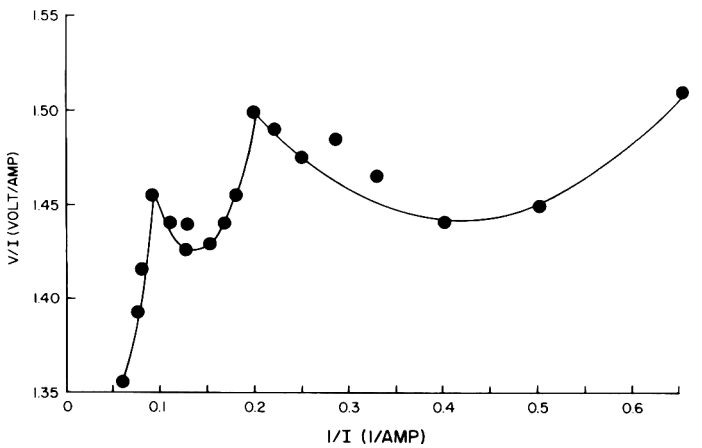

Fig. 3—Concentration polarization for cottage cheese whey (pH 4.62) at a temperature of 25 ± 3° C and an average flow rate of 24.20 ml/sec.

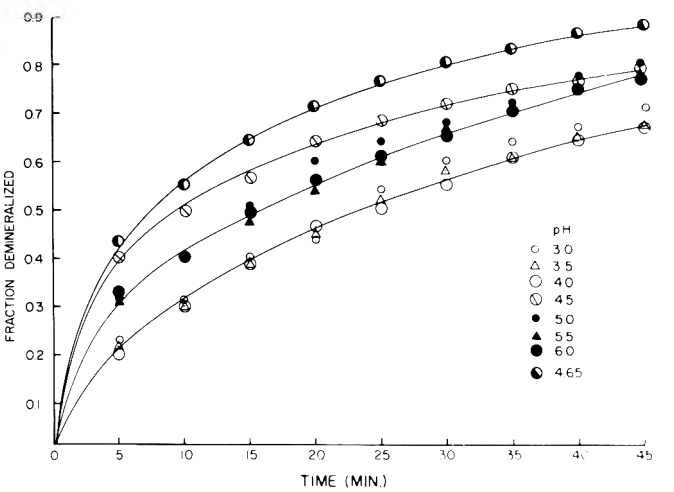

Fig. 4—Fraction demineralized of cottage cheese whey versus time for various initial pH.

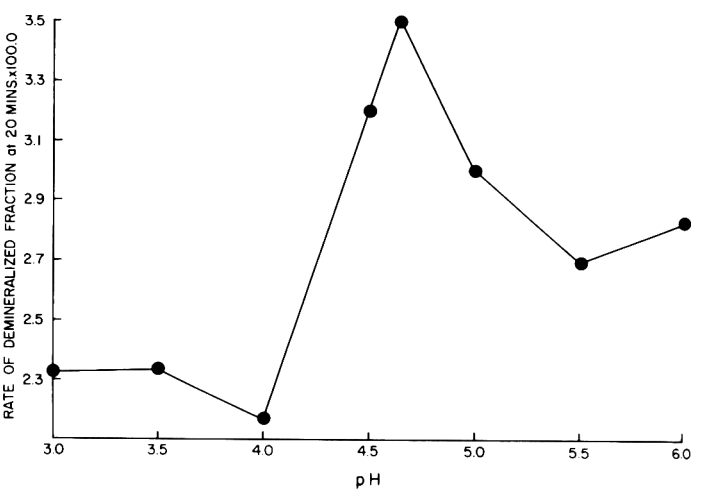

Fig. 5—Fraction demineralized at 20 min versus initial pH of cottage cheese whey.

Table 1–Percentage of element removed as function of time analyzed by neutron activation analysis and calcium ions by Beckman electrode[a]

pH	Time (min)	Na %	K %	Cl %	Ca %	Ca^{++} Beckman	Mg %
3.5	0	100.00 (144 ppm)	100.00 (262.5 ppm)	100.00 (1015.5 ppm)	100.00 (278 ppm)	100.00 (355 ppm)	100.00 (34.5 ppm)
	10	37.16	0.77	67.41	3.60	9.30	69.56
	20	55.21	–	81.98	3.42	27.89	42.08
	30	68.06	72.39	91.04	1.44	21.70	20.29
	45	79.17	70.00	97.10	25.90	24.79	26.09
4.5	0	(143.5 ppm)	(285 ppm)	(331.5 ppm)	(278.5 ppm)	(389 ppm)	(31 ppm)
	10	28.23	17.55	55.51	–	0.00	12.91
	20	49.13	35.09	80.22	6.39	8.75	45.17
	30	65.51	79.48	92.31	19.04	8.75	30.65
	45	80.49	92.11	94.88	40.76	8.75	4.84
5.5	0	(208.5 ppm)	(415 ppm)	(264.5 ppm)	(295 ppm)	(355 ppm)	(37.5 ppm)
	10	31.42	36.03	69.38	2.04	21.70	50.67
	20	49.89	51.09	83.94	30.85	21.70	21.34
	30	68.11	72.78	92.63	32.38	21.70	33.33
	45	81.54	86.99	96.79	60.00	27.89	33.33
6.0	0	(340 ppm)	(535.5 ppm)	(263 ppm)	(296 ppm)	(355 ppm)	(28 ppm)
	10	53.24	50.42	70.92	9.63	21.70	12.50
	20	67.65	–	84.79	28.05	21.70	1.79
	30	77.65	71.34	91.64	35.98	27.89	17.86
	45	88.08	73.58	95.06	50.85	37.47	35.72

[a]Error: Na: 5%; K: 10%; Cl: 6%; Ca: 2%; and Mg: 40% for 1.0 standard deviation.

fect on fraction demineralized and rate of demineralization because both the velocities used resulted in Reynold's numbers that indicated laminar flow ($Re < 2100$). To promote turbulence, spacers designed by Ionics have baffles in the flow stream to minimize concentration polarization at the membrane surface. As a result, the highest permissible flow rate of 20.80 ± 0.5 ml/sec (Re=1983) was chosen for the demineralization of cheese whey. The limitations of the stack assembly and equipment prevented higher flow rates than the one chosen.

Concentration polarization is mainly responsible for fouling of stack membranes, and extra energy for transport of H^+ and OH^- ions (water splitting) must be supplied, resulting in high current densities. It was therefore necessary to determine the limiting current at which concentration polarization for the stack membranes would occur.

Concentration polarization curves (V/I versus 1/I) drawn for the 1% NaCl system could not conclusively identify the occurrence of the concentration polarization point due to two inflection points in the polarization curve. The first inflection point is termed false polarization (Fig. 2) (Rapier et al., 1963; Cowan and Brown, 1959). Rapier et al. (1963) postulated that false polarization is due to the long tortuous flow path encountered in an externally staged system where the rate of demineralization is far greater at the entrance than at the exit of the electrodialysis stack. Concentration polarization experiments made with cottage cheese whey (pH 4.60–4.64) exhibited similar false polarization (Fig. 3). The apparent limiting current determined for cottage cheese whey (pH 4.62) in Figure 3 was 10.42 amps for 5 cell pairs. Each cell pair has an effective area of 220 cm^2 and the apparent limiting current density was calculated to be 9.48 ma/cm^2. Maximum current density determined by V/I versus 1/I curves imposed high stack voltages, greater than 150v, and the maximum current density could not be used due to voltage limitations in the stack equipment. The maximum permissible voltage was calculated by conductivity measurements on the cottage cheese whey using the relation recommended by Ionics (Anonymous, 1967).

$$V_{max\ 64°F} = (1.4 + 0.02R)n$$

$$V_{max} = V_{max\ 64°F} / [1.0 + (0.01)(t - 64°F)]$$

where, R = Resistance of the cottage cheese whey (Ohm-cm); n = Number of cell pairs; and t = Temperature of cottage cheese whey (F).

Studies were made by having distilled water in the concentrate (waste) stream and the product to be demineralized in the diluate (product) stream. It was observed that the initial current in the stack was nearly zero due to absence of current conducting ions in the waste stream and then eventually increased to a maximum as the ions were transferred to the waste stream. Therefore, in order to have an initial maximum current density, identical solutions were used in the diluate and the concentrate streams.

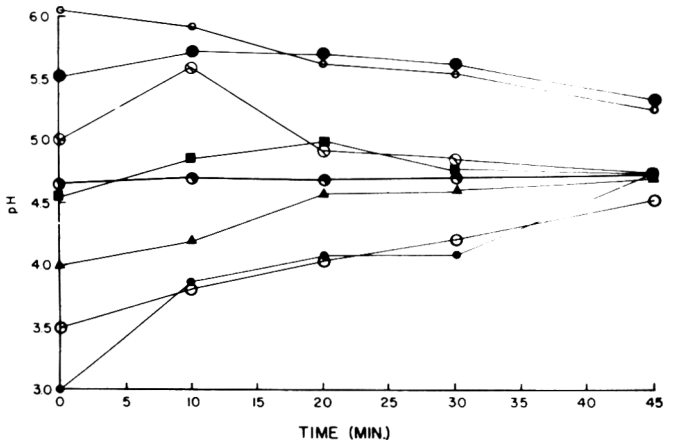

Fig. 6–pH Changes during the demineralization of cottage cheese whey.

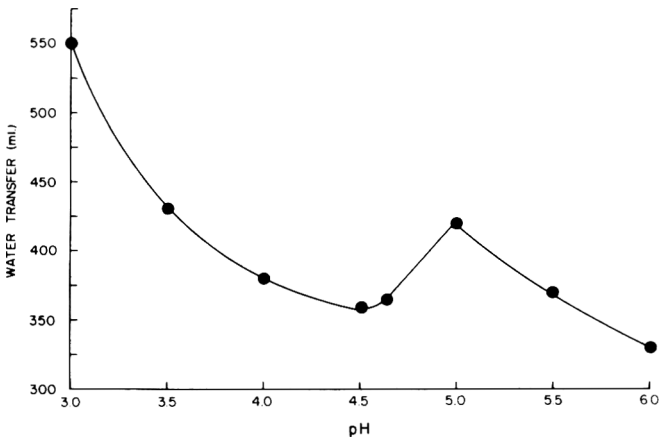

Fig. 7–Water transferred to the concentrate stream after 45 min as a function of initial pH of cottage cheese whey.

Two modes of operations were possible: (1) batch system with plug flow in the product tank (no agitation); and (2) batch system with agitation. The experiments showed that the initial demineralization rates were much higher for the batch system with plug flow even though both modes of operation attained the same total demineralization in 45 min. This phenomena was to be expected since the concentration gradient was much higher in a batch system with plug flow after each successive pass through the stack assembly than with the batch system with agitation. Violent agitation was undesirable since it caused foaming of whey. Consequently, a batch system with plug flow was selected as the mode of operation.

The experimental design for the demineralization of cottage cheese whey after the preliminary experiments was thus reduced to a study of rate and extent of demineralization as a function of whey pH at a constant flow rate, temperature and voltage.

The data on fraction demineralization of cottage cheese whey as a function of pH were checked for the order of reaction to determine the kinetics of the demineralization process. Unaccomplished fraction demineralization $[1.0 - (C_o - C_t)/C_o]$ as a function of time (t) was plotted on a semilogarithmic plot for various initial pH values of cottage cheese whey. C_o was the initial ash content in whey and C_t the ash content in whey at time t. A curvilinear relationship was obtained indicating that the demineralization process did not follow first order kinetics.

The ash in cheese whey is composed of various mineral elements and the demineralization of cottage cheese whey results in the removal of these elements. One hundred percent removal of mineral elements is not possible but some are removed faster than others. Sodium, potassium, calcium, magnesium, chlorine, and phosphorus were analyzed in the demineralization of cheese whey at various initial pH values for various times as presented in Table 1. No definite conclusions could be made on phosphorus due to the interference by the presence of aluminum. Neutron Activation Analysis (N.A.A.) suggested that the major transport in the demineralization of cottage cheese whey was sodium, potassium, and chlorine.

Figures in Table 1 suggest that the most rapid transport rate and the greatest amount of ion transported was for chloride followed by sodium and potassium. This would be expected since there are fewer small anions available for movement than cations. Thus, the chloride ion would be removed faster than sodium or potassium. The relative rate of transport of the three elements did vary as a function of pH with its effect being most marked with potassium. Perhaps this is due to changes in hydration of the ion as a function of pH. Calcium was removed to a small extent and magnesium was not at all affected. The slight removal of Ca^{++} ions was further supported by the Ca^{++} ion analysis by the Beckman Calcium Electrode. Calcium was removed to a greater extent at pH 5.5 and pH 6.0 than at pH 3.5 and pH 4.5. This behavior was believed to be attributed to the breakdown of the whey system by precipitation, visibly observed on the anion membranes in the concentrate stream at pH values above 4.5.

The dependence of the demineralization of cottage cheese whey on the initial pH of the whey is illustrated in Figure 4. Below pH 4.65, the fraction demineralization obtained at various intervals of time was well below that of pH 4.65. The smaller fraction demineralization can be explained by the presence of a positive charge on the proteins. Proteins retain a positive charge below their isoelectric point and may act as blocking barriers to the smaller positive ions. N.A.A. showed a major transport of sodium, potassium, and chlorine (Table 1). The reduction in transport of potassium does not fully explain the effect of lowered pH on the rate of demineralization. However, it may be that these cations have an effect on other species present which were not monitored. This is under investigation.

Above pH 4.65, the observed rate of demineralization was higher than for pH below 4.65. The rate differences may be due to the relative effects of transfer rates in cations and anions. A pH below 4.65 may reduce cation transfer rates more than a pH above 4.65 affects anion transfer rates. A smaller fraction demineralization can be explained by the presence of a negative charge on the proteins above its isoelectric point. Above pH 4.65, chlorine removal (the only anion monitored in these experiments) was not noticeably reduced (Table 1). However, other elements, including phosphorus, could have been influenced. The highest fraction demineralization was observed at pH 4.65. Native whey proteins have been shown to have an isoelectric point at pH 5.1 (Webb and Johnson, 1965). Stuiber (1966) and Metwally (1964) found that the optimum yield of whey proteins by heat coagulation from industrially treated cottage cheese whey was at pH 4.5. This may suggest that the isoelectric point of the whey proteins was lowered due to partial denaturation. Whey used in the experiments was vat pasteurized as well as subjected to heat and pH changes during the cheese making. It was, therefore, believed that the isoelectric point of the partially denatured whey proteins may have been lowered to pH 4.65. Maximum demineralization would be obtained near the isoelectric point as no interference should be experienced in the transport of cations and anions due to a neutral charge on the protein molecule.

To more graphically illustrate the effect of pH on the rates of demineralization, the fraction demineralized at 20 min was plotted as a function of pH (Fig. 5). Clearly, the more acid cheese wheys (below pH 4.65) are demineralized at a lower rate than those cheese wheys above pH 4.65. Also, the maximum demineralization rate was at pH 4.65.

The pH changed continuously with time in the demineralization of cottage cheese whey due to differential rates of removal of ionic species as shown in Figure 6. The smallest change in pH values with time occurred at pH 4.65. Runs made with initial pH values from pH 3.0 to pH 4.0 and pH values from pH 5.0 to pH 6.0 tended to approach the final value of pH 4.65. It was believed that at pH 4.65, the rate of removal of acidic components equaled the rate of removal of alkaline components. At initial pH values from pH 3.0 to pH 4.0, the rate of removal of acidic components was believed to be faster than the removal of alkaline components and vice versa in the case of pH values from pH 5.0 to 6.0.

Water transfer or electro-osmosis is important since it is an indication of the amount of effective work done by the current in the removal of ash in an electrodialytic process. Figure 7 is a plot of water transfer as a function of pH. It was observed that the water transferred from the diluate stream into the concentrate stream was a minimum at pH 4.65 with no breakdown in the whey system. The minimum water transfer means that the maximum effective work done by the current was in the removal of ash in whey. High electro-osmosis is indicative of more work done by the current in the transfer of water across the membranes thereby negating the primary objective in an electrodialytic process. Above pH 4.65, water transfer was expected to rise indefinitely but in actual experiments water transfer dropped below the minimum. The unexpected behavior was believed to be due to the breakdown of the whey system by precipitation at the anion membranes in the concentrate stream. Precipitation at the anion membranes was visually observed as a white precipitate at the time of dismantling the membranes from the stack. Increased precipitation was observed from pH 5.0 to pH 6.0 suggesting that the whey system was more unstable as the initial pH increased above pH 4.65.

Membranes checked for constant membrane properties showed very small changes (less than 5%) from the original rate of demineralization for NaCl solution.

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Ms received 7/20/72; revised 12/30/72; accepted 1/8/73.

Research supported by the College of Agricultural & Life Sciences, University of Wisconsin, Madison.

The authors thank the Stauffer Chemical Co. for its support of the Stauffer Fellowship and Ionics, Inc. for making available the electrodialysis unit.

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DIFFERENCE TASTE THRESHOLDS FOR SODIUM CHLORIDE AMONG YOUNG ADULTS: AN INTERLABORATORY STUDY

INTRODUCTION

OFTEN, a large variability in sensory response to chemical stimuli is encountered among subjects within the same laboratory (Berg et al., 1955; Pangborn, 1959, 1970; Gregson and McCowen, 1963; Ekman and Åkesson, 1964). This is true for simple chemical compounds as well as for complex food systems. For example, absolute detection thresholds for the taste of sodium chloride ranged from 0.0058–0.468% within the same laboratory (Knowles and Johnson, 1941). Such variability can be of critical importance if threshold data or similar responses from different laboratories are to be intercompared.

The investigation reported herein was undertaken to determine the degree to which differential sensitivity to a simple taste sensation differed among groups of subjects and among laboratories in widely-separated locations. The idea of this study was conceived by the authors during an interdisciplinary symposium on principles and methods of sensory evaluation of foods held in Sweden in 1968 (Drake, 1969; Pangborn, 1969). Initially, a laboratory in Japan also participated, but the investigator was not able to complete the study due to unforeseen difficulties. The present study is confined to simple model solutions. However, three of the present authors intend to continue by performing interlaboratory comparisons of responses to the taste properties of selected food systems.

EXPERIMENTAL

Laboratories

The experiments were conducted in the laboratories of the authors. These are referred to below as I–IV for the laboratories in Sweden, USA, Poland and The Netherlands, respectively. All of these laboratories had extensive previous experience with psychophysical studies of taste responses and all but the Dutch laboratory also conduct sensory analyses of foods.

Material

Samples of NaCl from one and the same batch, manufactured by Zoutindustrie, The Netherlands, were sent to the four laboratories. A specification of the amounts of impurities in the salt is given in Table 1. The salt was dissolved in double-distilled water.

Test procedure

Taste difference thresholds were measured by a method of constant stimuli, described in detail by Galanter (1962).

Before the experiments were started, a written plan of the study was distributed which was approved by the participants. After completion of the experiments, three methodological differences (for one laboratory as to time order of experimental series, and for another as to randomizations and time interval between successive pairs) were discovered. These differences were considered negligible as compared to other possible sources of error. A description of the plan is given below.

Two experimental series, below called 1 and 2, were performed with the standard concentrations 0.30% and 0.80% NaCl, respectively. In the former case, nine equidistant concentrations ranging from 0.26–0.34% NaCl, were used, and, in the latter case, the same number of equidistant concentrations ranging from 0.72–0.88% NaCl were used.

In each session the subjects were presented with nine pairs of samples containing salt. The subject's task was to taste each pair and indicate which of the two samples was the saltier. Eight pairs consisted of one sample of the standard concentration and one sample of a higher or lower concentration. The ninth pair consisted of two samples of the standard concentration and was included as a check of order effects within pairs.

Each subject completed five sessions within each experimental series. The results from the first session were considered introductory and were not included in the final calculations which thus involved four replicates. At laboratories I–III, the pairs within sessions were presented in a counterbalanced, randomized order which varied among subjects, whereas at laboratory IV all subjects received the samples in the order "comparison sample–standard sample" in sessions 1 and 3, and in the opposite order in sessions 2 and 4. At laboratories I, II and IV, half of the subjects started with series 1 and the other half with series 2, and at laboratory III, all subjects started with series 1. In all cases one experimental series was completed by each individual subject before the next one was started.

Samples of 20–30 ml were served in 50 ml glass beakers coded with two-digit random numbers. All solutions were presented at room temperature (20–22°C). Double-distilled water was provided for oral rinsing between pairs or, if desired, between tastings. Swallowing of samples or of rinse water was not allowed. At laboratories I–III, the time interval between pairs was at least 30 sec, whereas at laboratory IV the corresponding successive time interval was more than 1 min.

As closely as possible from a practical point of view the final four sessions in each experimental series were held on four consecutive days and at the same time of day for each subject. The tests were ordinarily performed between 8 and 11 a.m. or between 1 and 3 p.m.

Table 1—Amounts of impurities in the NaCl used[a]

Impurity	Amount
Water	0.1–0.2%
Sulfate	0.1–0.2%
Potassium	0.1%
Calcium	20–40mg/kg
Copper	0.1–0.2mg/kg
Iron	1–2mg/kg
Magnesium	0.1–1mg/kg
$K_4Fe(CN)_6$	1–3mg/kg

[a]According to information from the Manufacturer

Subjects

At each laboratory, between 43 and 72 subjects participated in the experiments. The subjects were students or laboratory technicians ranging from 18–25 yr of age. Most subjects had no previous experience in taste testing experiments, whereas a few had some experience in sensory evaluation of foods. The numbers of male and female subjects who participated within each laboratory are indicated in the second column of Table 2.

Evaluations

The percentages of correct identification of

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the saltier sample within pairs were transferred to percentages of saltier than the standard (cf., Pangborn et al., 1967). Then, the untransformed percentages, and the percentages transformed to probit values, were plotted separately against salt concentration. Just noticeable differences (jnd) and points of subjective equality (pse) were calculated from straight lines fitted to the data according to the method of least squares. The jnd was determined as half the distance between 25% and 75% saltier, and the pse as the concentration corresponding to 50% saltier. The Weber ratio (Wr) was calculated as the ratio between the jnd value and the standard concentration value. The significance of differences between slopes of the fitted straight lines for the total percentages was tested by t-tests.

RESULTS & DISCUSSION

TABLE 2 GIVES the jnd, Wr and pse values obtained for percentages and for probit values for males and females as well as for the total number of subjects within each laboratory. Also, the totals for all laboratories are given.

The curves obtained for percentages calculated for all subjects within each laboratory as well as for all laboratories are shown in Figures 1 and 2 for the lower and higher standard concentration, respectively. The probit values obtained at the two standard concentrations for male and female subjects as well as for all subjects are plotted in Figures 3 and 4.

Checks of the procedure

In all cases, the pse values given in Table 2 show deviations smaller than 1% from the standard concentration. These deviations were considered small enough to conclude that there was no systematic error in the procedure.

To balance out possible order effects within pairs, all pairs were presented equally often in both orders. To obtain an independent check of order effects, one of the nine pairs presented in each session consisted of two equal samples of the standard concentration. This check showed that there were no significant order effects.

jnd values

Almost all response curves had the expected sigmoid shape. The evaluation of jnd values should therefore be done from fitted sigmoid curves rather than from fitted straight lines. Besides visual smoothing, such a fit could be made by determining the parameters in a mathematical formula by applying, e.g., the method of least squares. A suitable formula, which has previously been suggested in the psychophysical literature, is the cumulative normal distribution. This formula implies that, with the same accuracy, the fitting of a sigmoid curve to experimental data can be replaced by the much simpler fitting of a straight line to probits.

In this work, jnd values were calcu-

Table 2—Just noticeable differences (jnd), Weber ratios (Wr) and points of subjective equality (pse) obtained for salt at four laboratories

Laboratory[a]	Subjects No.	Sex	At standard concentration: 0.30% NaCl: No. of tests	Untransformed data: jnd[b]	Untransformed data: Wr	Untransformed data: pse[b]	Probits: jnd	Probits: Wr	Probits: pse	0.80% NaCl: No. of tests	Untransformed data: jnd	Untransformed data: Wr	Untransformed data: pse	Probits: jnd	Probits: Wr	Probits: pse
I	39	M	156	0.023	0.077	0.299	0.022	0.073	0.298	156	0.053	0.066	0.797	0.050	0.063	0.797
	33	F	132	0.022	0.073	0.300	0.020	0.067	0.300	132	0.052	0.065	0.799	0.048	0.060	0.800
	72	tot.	288	0.023	0.077	0.300	0.021	0.070	0.299	288	0.053	0.066	0.798	0.049	0.062	0.798
II	28	M	112	0.025	0.083	0.300	0.023	0.077	0.300	112	0.050	0.063	0.806	0.046	0.058	0.808
	30	F	120	0.022	0.073	0.303	0.019	0.063	0.303	120	0.044	0.055	0.806	0.039	0.049	0.808
	58	tot.	232	0.023	0.077	0.302	0.022	0.073	0.304	232	0.047	0.059	0.806	0.042	0.053	0.808
III	15	M	60	0.030	0.100	0.301	0.029	0.093	0.301	60	0.068	0.085	0.804	0.067	0.083	0.804
	28	F	112	0.026	0.087	0.298	0.025	0.083	0.299	108[c]	0.055	0.069	0.795	0.052	0.065	0.794
	43	tot.	172	0.027	0.090	0.299	0.026	0.087	0.299	168	0.059	0.074	0.798	0.058	0.073	0.797
IV	29	M	116	0.032	0.107	0.303	0.032	0.107	0.303	116	0.111	0.139	0.793	0.116	0.145	0.792
	29	F	116	0.032	0.107	0.300	0.030	0.100	0.301	116	0.081	0.101	0.794	0.083	0.104	0.795
	58	tot.	232	0.032	0.107	0.302	0.031	0.103	0.302	232	0.094	0.118	0.794	0.097	0.121	0.793
Total	111	M	444	0.026	0.087	0.300	0.025	0.083	0.301	444	0.063	0.079	0.800	0.062	0.078	0.800
	120	F	480	0.025	0.083	0.300	0.023	0.077	0.301	476	0.055	0.069	0.801	0.053	0.066	0.800
	231	tot.	924	0.026	0.087	0.300	0.024	0.080	0.300	920	0.058	0.073	0.800	0.057	0.072	0.801

[a] I—Sweden; II—USA; III—Poland; IV—Netherlands
[b] In % NaCl
[c] Based on 27 subjects

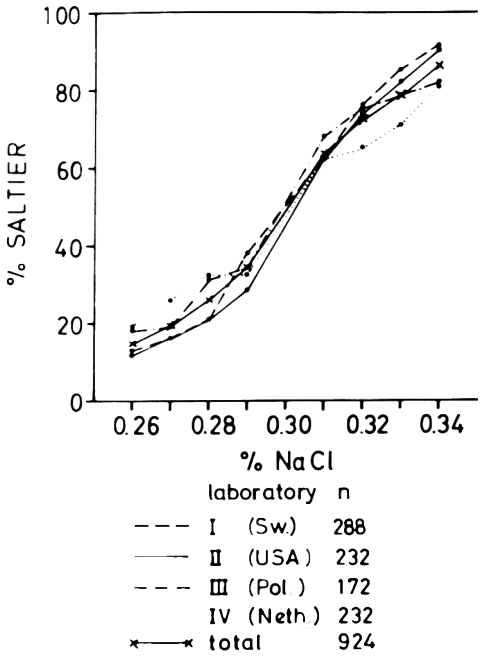

Fig. 1—Plot of "percent saltier" against salt concentration in experiment 1 (0.30% NaCl) for all subjects within each laboratory.

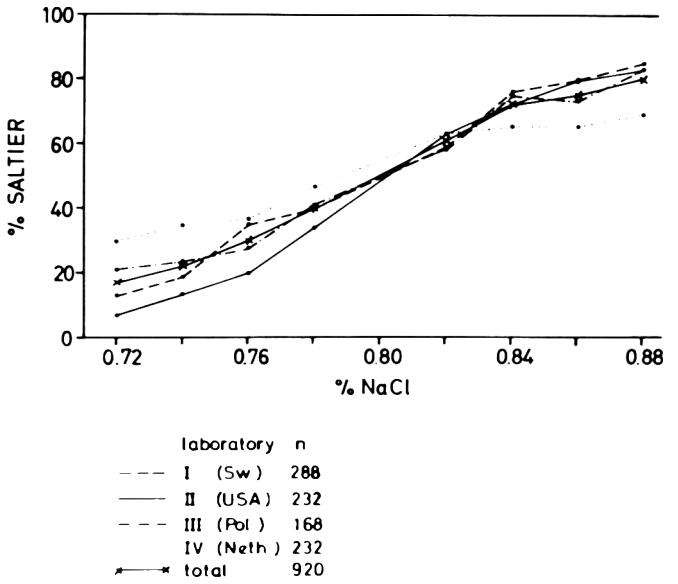

Fig. 2—Plot of "percent saltier" against salt concentration in experiment 2 (0.80% NaCl) for all subjects within each laboratory.

lated for untransformed data as well as for probits. As expected, the transformation gave a better fit but yielded only slightly lower jnd values. The latter fact can be explained by the choice of the concentration ranges which, for practical reasons, were selected to cover the most informative part of the response curve. To accommodate a comparison of jnd values based on untransformed data with those based on transformed data as well as with earlier published data, values for both types of data are given in Table 2.

For probit transformed data the jnd values obtained for the four laboratories at the standard concentration of 0.30% NaCl were: 0.021, 0.022, 0.026 and 0.031% NaCl. For these values, there were no significant differences ($p > 5\%$) among the first three laboratories, whereas the fourth value was significantly higher than the pooled value for the other three laboratories ($p < 1\%$). Corresponding jnd values at the standard concentration of 0.80% NaCl were: 0.049, 0.042, 0.058 and 0.097% NaCl. The value for laboratory III was here significantly higher than the ones for laboratories I and II ($p < 5\%$ and $p < 1\%$, respectively) which latter values did not differ significantly from each other ($p > 5\%$), whereas the value for laboratory IV was significantly higher than any of the other three values ($p < 1\%$–$p < 0.1\%$).

Weber ratios

For probit transformed data, the Weber ratios for the four laboratories varied between 0.070–0.103 at the standard concentration of 0.30% NaCl, and between 0.053–0.121 at the standard concentration of 0.80% NaCl. Within laboratories I–III, the Weber ratios were higher at the lower standard concentration, whereas the reverse held at laboratory IV. The ratios between the Weber ratios at the two standard concentrations were for the four laboratories 1.12, 1.38, 1.19 and 0.85 and for the totals, 1.11.

Pilgrim et al. (1955), and Schutz et al. (1957) determined the differential sensitivity for NaCl using a method of constant stimuli with single sample presentations requiring the subjects to categorize the taste intensity of the samples. At the concentration of 0.95% NaCl, Pilgrim et al. (1955) obtained a Weber ratio of 0.119, whereas Schutz et al. (1957) obtained the Weber ratios of 0.131 and 0.121 at the concentrations of 0.40 and 1.10% NaCl, respectively. The differences in the level of these Weber ratios and the ones reported in the present work are most probably due to differences in methodology. This implies that no certain conclusion can be drawn as to differences between these three groups of subjects.

The observed decrease of ca. 10% in Weber ratio between the two standard concentrations is in agreement with the decrease found by Schutz et al. (1957). This indicates that the Weber law holds only approximately in this concentration range.

Differences between males and females

At the lower standard concentration of 0.30% NaCl, three of the four laboratories obtained for females slightly lower jnd values based on probits, whereas the fourth laboratory obtained equal values for males and females. However, in neither case was the difference between the two sexes significant ($p > 5\%$). At the higher standard concentration of 0.80% NaCl, the values were consistently lower for females at all four laboratories. At this concentration the difference between males and females was significant at the 5% level at laboratories II and IV, and at the 10% level at laboratory III, whereas the corresponding difference was not significant at laboratory I. A sign test, applied to the rank order of the differences between males and females, showed that the probability was less than 5% that, by chance alone, one would have obtained the same direction of the differences between males and females in seven out of the eight cases. It was therefore concluded that the jnd values for females were slightly lower than for males. However, no explanation can be given for the fact that there were larger differences between the sexes at the higher standard concentration.

Very few scientific articles report sex differences in sensitivity for salty taste, and these articles deal only with absolute or recognition thresholds and not with difference thresholds. E.g., Tilgner et al. (1959) found that women had higher sen-

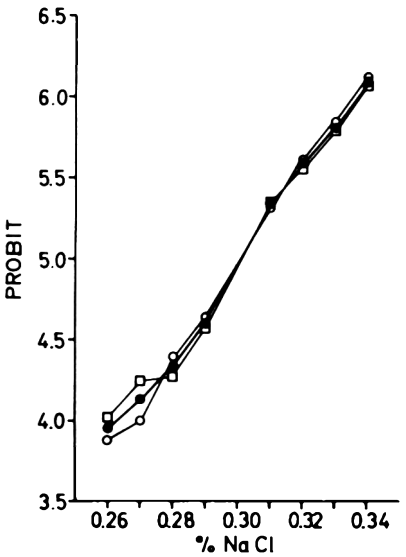

Fig. 3—Plot of probit values against salt concentration for responses of all male and all female subjects in experiment 1 (0.30% NaCl). Subjects: □ males, 444; ○ females, 480; ● total, 924.

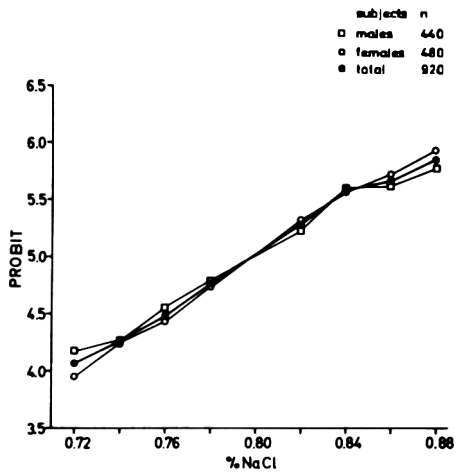

Fig. 4—Plot of probit vlaues against salt concentration for responses of all male and all female subjects in experiment 2 (0.80% NaCl).

sitivity than men for sweet and salty tastes but not for sour taste. Bourlière et al. (1958) found that the recognition threshold for salt was higher for men than for women over a wide age range. The results on sex differences reported in the present work support these earlier findings.

Differences between laboratories

The data for laboratories I–III were in more or less close agreement, whereas the data for laboratory IV were significantly different. Even with this difference, however, the degree of agreement is higher than could be expected when considering the large variations among published data on taste thresholds. This finding is reassuring for food scientists who want to compare threshold data obtained at various laboratories.

No unequivocal explanation for the consistently deviating values at laboratory IV can be given. The two observed methodological differences from the other laboratories (not randomized serving order within sessions, and longer time intervals between successive pairs) were considered too small to account for the observed differences. However, available knowledge, which unfortunately cannot be quantified, makes it plausible that the subjects at laboratory IV were less motivated than those in the other three laboratories. E.g., adherence to the agreed plan implied that the subjects at this laboratory, contrary to the ones at the other three laboratories, had to perform the experiments at times which involved an extra effort that might have influenced their motivation to participate.

CONCLUSION

THIS INTERNATIONAL study on differential taste thresholds showed a certain consistency between groups of subjects at widely separated laboratories. The occurring differences were assumingly caused by factors that were difficult to control in spite of careful experimental planning. Especially, the motivation of the subjects was difficult to estimate and keep under control.

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Ms received 10/5/72; revised 11/19/72; accepted 12/3/72.

Appreciation is extended to the technicians and subjects from each laboratory. We thank Dr. C.Å. Åkesson at the Swedish Institute for Food Preservation Research for helpful discussions. Dr. O. Wouters, Central Institute for Nutrition and Food Research, Zeist, The Netherlands, is thanked for providing all participants with salt.

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FLAVOR THRESHOLDS FOR FATTY ACIDS IN BUFFERED SOLUTIONS

INTRODUCTION

THRESHOLD LEVELS were determined for flavor of free fatty acids in water (Patton, 1964), in oil (Feron and Govignon, 1961), in butteroil (Siek et al., 1969) and in butter (McDaniel et al., 1969). For butanoic and hexanoic acids, the thresholds were lower in oil than in water. The reverse trend was apparent for octanoic and decanoic acids, as a markedly greater concentration was indicated for the threshold in oil than in water.

The influence of pH on thresholds for fatty acids was not mentioned in the above studies. However, several investigators recognized the importance of pH in foods of which fatty acids are important constituents. Tuckey and Stadhouders (1967) found that rancid flavor was detected at a lower level of fat hydrolysis in an acidified or in a cultured product than in milk at the normal pH. A synthetic butter culture flavor concentrate, developed by Lindsay et al. (1967), had an undesirable acetic acid-like flavor when the pH was below 4.5 but not when the pH was in the range of 4.6–4.8. Bills et al. (1969) stated that acidification of solutions of sodium salts of fatty acids resulted in an intensified aroma.

Fatty acids with 14 or more carbon atoms are assumed to influence flavor of foods less than the short-chain fatty acids which have distinctive aromas (Lea, 1963). The flavor of rancid milk has been attributed chiefly to the free fatty acids from butanoic to dodecanoic with no single fatty acid being credited with a major influence (Scanlan et al., 1965). Ethanoic acid, although the amount is variable, is the most abundant of the free fatty acids found in Cheddar cheese. In Roquefort cheese, octanoic and decanoic acids are present at high levels (Anderson and Day, 1965; Day, 1966). Decanoic and dodecanoic acids may be responsible for "soapy" flavors in foods containing coconut or palm oil from which these acids might be liberated by lipolysis (Lea, 1963).

Ethanoic, butanoic, hexanoic, octanoic and decanoic acids are representative of fatty acids which play a dominant role in the characteristic flavor of dairy products and other foods. This study was designed to determine the concentration at which the flavor of these fatty acids are detectable (recognition threshold) in citrate-phosphate buffer systems at pH levels of 3.2, 4.5 and 6.0.

EXPERIMENTAL

FOR ALL SOLUTIONS, glass distilled water was used. Water was distilled in 13-liter lots. The first two and the last three liters of water were discarded.

Citrate-phosphate buffer

Citrate-phosphate buffer was selected because this system did not introduce any ions which were foreign to food products. Stock solutions of 0.1M citric acid ($C_6H_8O_7 \cdot H_2O$) and 0.2M dibasic sodium phosphate ($Na_2HPO_4 \cdot 7H_2O$) were used in preparing the three buffers in 2000-ml lots. For pH levels of 3.2, 4.5 and 6.0, the milliliters of citric acid:disodium phosphate were, respectively, 168.8:31.2, 115.0:85.0, and 78.8:121.2. To minimize objectionable flavor of buffer at pH 3.2, half strength buffer was used. This was not possible with decanoic acid, because of difficulty in dispersing the acid in the half strength buffer.

Fatty acids

Reagent grade fatty acids were used. Addition of fatty acid to buffer changed the pH no more than 0.1 unit in every case. Butanoic, hexanoic and octanoic acids were vacuum distilled, and decanoic acid was purified by three ethanol-water recrystallizations. Fatty acids were made into stock solutions as follows: 3000 ppm ethanoic or butanoic acids in water; 600 ppm hexanoic or octanoic acids in buffer solutions; 35.5 ppm decanoic acid in pH 3.2 buffer; 70 ppm decanoic acid in pH 4.5 buffer; and 350 ppm decanoic acid in pH 6.0 buffer. Since the solubility of decanoic acid in water is very limited and it exists as a solid at room temperature, it was dispersed (approximately 30°C) by submerging the container of acid and buffer in an ultrasonic bath (Mettler ultrasonic cleaner, Model ME 1.5) for at least 30 min. From the stock solutions, appropriate dilutions (logarithmic increments in concentration) for sensory evaluations were made in citrate-phosphate buffer.

Sensory evaluation

Sensory evaluations were conducted in individual booths. Students and staff served as judges. No attempt was made to classify judges as to age, sex, or previous experience in sensory testing. A total of 50 or more observations (representing at least 25 individual judges) for each concentration of fatty acid at each pH were obtained. To facilitate serving samples, no more than seven judges were seated at one time.

A brief orientation was given at the beginning of all sessions. Two reference samples were served to familiarize the judges with the characteristics of the buffer system and the buffer plus fatty acid (highest concentration of fatty acid that was served in that session). Judges were not required to swallow samples, but they were instructed to be sure the sample contacted all parts of their mouths. Distilled water at room temperature was provided for rinsing the mouth between samples.

Samples (10 ml) were poured in 6-oz glasses and served (ambient temperature, ca 25°C) in pairs, each consisting of a buffer and a buffer plus fatty acid. Because of volatility, ethanoic, butanoic, hexanoic and octanoic acid:buffer samples were poured immediately before serving each tray. Glasses were not covered, but no more than 5 min elapsed between pouring and serving. Decanoic acid:buffer samples were dispensed with syringes into the glasses within 1 hr of serving. Three or four concentrations of one fatty acid at one pH level were presented randomly on a tray. Order of serving buffer only and buffer plus fatty acid within each pair was randomized. Each judge was required to circle the code number on the score sheet which corresponded to the sample which contained the fatty acid. No quality judgments were requested.

Preliminary work revealed that judges tended to rely on aroma for detection of fatty acid at low pH levels, and taste or flavor at high pH levels. Therefore, for butanoic and decanoic acids, taste and aroma, as well as, flavor thresholds were determined. For these fatty acids, three pairs of samples were served on each tray. The first pair was evaluated for taste only, and nose clips were used to prevent aroma from influencing this judgment. The second pair was judged for aroma only, three sniffs per sample. The third pair was evaluated (without nose clips) for flavor in the mouth. At each session, the order of serving three or four concentrations of fatty acid was randomized for test (aroma, taste, flavor) and for judge.

Calculations

A linear regression of positive responses was calculated for each fatty acid at each pH (Snedecor and Cochran, 1967). The threshold was designated as the concentration at which 50% of the judges was able to recognize ($P = 0.05$) the presence of the fatty acid (Amerine et al., 1965).

After thresholds were determined, amounts

of ethanoic, butanoic, hexanoic, octanoic and decanoic acids present in acid form were calculated by applying the Henderson-Hasselbalch equation.

RESULTS & DISCUSSION

Threshold determinations

It was not possible to establish recognition thresholds for ethanoic acid because the amount exceeded the buffer capacity of the system. At pH 3.2, 4.5 and 6.0, concentrations of 102 ppm, 60 ppm and 21 ppm were below threshold. Higher concentrations were not tested by sensory methods because they lowered the pH of the buffer system more than 0.1 unit. Observations of researchers in this laboratory suggested that the flavor of the citrate-phosphate buffer system was more like ethanoic than the other acids included in this study. Perhaps the flavor of the carrier was confused with that of ethanoic acid.

In general, the trends were similar for all other fatty acids. Increased concentrations were required for recognition of the fatty acids as the pH was raised. Within pH levels 3.2 and 4.5, threshold concentrations of fatty acids increased, then decreased, as carbon chain length of the acid increased. This trend did not hold for pH 6.0 (Fig. 1). Trends were not changed by transformation of data from ppm to molar concentration (Table 1).

Table 1–Flavor thresholds[a] for fatty acids in buffered solutions

	Butanoic		Hexanoic		Octanoic		Decanoic	
pH	ppm	Molar ($\times 10^{-5}$)	ppm	Molar ($\times 10^{-5}$)	ppm	Molar ($\times 10^{-5}$)	ppm	Molar ($\times 10^{-5}$)
3.2	0.4	0.5	6.7	5.8	2.2	1.5	1.4	0.8
4.5	1.9	2.2	8.6	7.4	8.7	6.0	2.2	1.3
6.0	6.1	6.9	27.1	23.0	11.3	7.8	14.8	8.6

[a]50% identification, P = 0.05, n = 50 (Amerine et al., 1965) for each concentration.

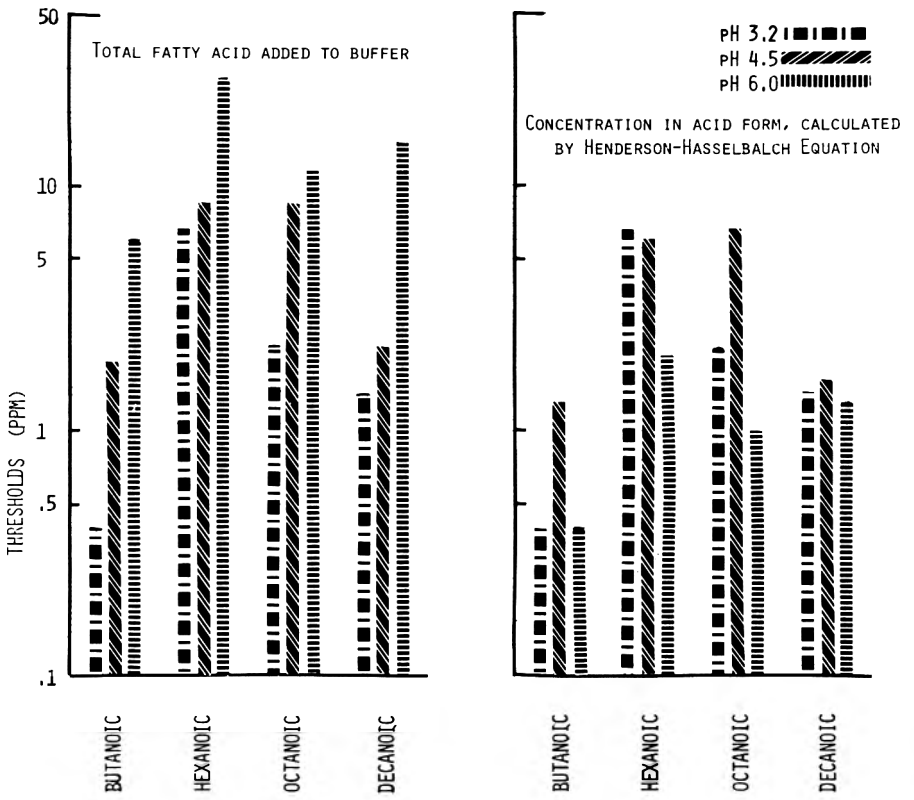

Fig. 1–Thresholds for fatty acids in buffered solutions (50% identification, P = 0.05, n = 50 for each concentration).

Calculated amounts of fatty acid in acid form

The pK_a values reported by West and Todd (1955) for ethanoic and butanoic acids were, respectively, 4.7 and 4.8. The pK_a values determined for 100 ppm (according to the methods described by West and Todd) for hexanoic, octanoic and decanoic acids were as follows: 4.9, 5.0 and 5.0.

The amounts of fatty acids calculated (by the Henderson-Hasselbalch equation) as existing in the acid form at the thresholds differed less among the pH levels than did amounts as measured when the solutions were prepared. With the exception of hexanoic acid, a greater amount of the acid form was required for recognition when the pH of the system was 4.5, which was closer to the pK_a for these fatty acids, than the other pH levels tested (Fig. 1). It is possible that the sodium salt of hexanoic acid contributed flavor which was detected and confused with the flavor of the acid. The amount of the sodium salt increased as pH increased.

The calculated thresholds for butanoic, hexanoic, octanoic and decanoic acids at pH 6.0 were much lower than thresholds reported by Patton (1964) for these fatty acids in water. There are a number of factors which might explain this difference including evaporation of fatty acids, number and selection of subjects, as well as, sensory techniques and experimental design. Dawson et al. (1963) pointed out that thresholds for the four primary tastes (sucrose, tartaric acid, sodium chloride and caffeine) were lowest when a paired test was used, next lowest by triangles, and highest by single stimulus methods. Patton (1964) used a single stimulus method and sprayed the subject's tongue with the fatty acid solution.

In our work, undue influence of individual responses to interactions among fatty acid flavors, pH and buffer components was prevented by obtaining a large number (50) of observations per concentration. Thus, trends would more likely represent the population in general than would tests involving a small number of subjects.

Aroma, taste and flavor thresholds

The extremes of the fatty acid series, butanoic and decanoic, were investigated in regard to the taste and aroma components of flavor. However, it was not possible to establish the threshold for taste of decanoic acid because the amount of fatty acid required exceeded the dispersion capacity of the system. Also, the amount of butanoic acid needed for recognition of the taste at pH 6.0 was greater than the buffer capacity. At pH 3.2 and 4.5, taste thresholds for butanoic acid were markedly greater than flavor or aroma thresholds. Little difference was found between flavor and aroma thresholds for either butanoic or decanoic acids. The flavor threshold was slightly higher than the aroma threshold for butanoic

Table 2—Taste and aroma thresholds[a] (ppm) for butanoic and decanoic acids in buffered solutions

	Butanoic acid		Decanoic acid	
pH	Taste	Aroma	Taste	Aroma
3.2	40.7	0.3	>35.5	1.7
4.5	86.6	0.9	>60.3	2.3
6.0	>102.3	4.8	>102.3	16.0

[a] 50% identification, P = 0.05, n = 50 (Amerine et al., 1965) for each concentration.

acid, and the reverse was true for decanoic acid (Tables 1 and 2). Thus, sensory evaluation of fatty acids need not include testing for aroma, taste and flavor. Either aroma or flavor would be adequate for most purposes. However, as volatility of fatty acids changes, the relationship between the flavor and aroma thresholds may be expected to change also.

Implications

Aroma appears to play a more important role than taste in the flavor of the C_4 to C_{10} fatty acids, since aroma and flavor thresholds were more similar than taste and flavor thresholds. Taste thresholds of fatty acids in model aqueous buffer systems are of interest from a theoretical standpoint, especially in comparisons with nonaqueous thresholds of fatty acids, and may have some practical application in interpreting the flavor impact of high levels of fatty acids measured in products such as cured cheeses (Bills et al., 1969) and butter (McDaniel et al., 1969).

The contribution of a given level of butanoic, hexanoic, octanoic and decanoic acids to flavor of foods may be expected to increase as pH of the product decreases. No doubt, subliminal levels of fatty acids also play a role in the total flavor. However, consideration of pH in relation to contribution of fatty acids to flavor is essential to product development.

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Ms received 10/12/72; revised 11/25/72; accepted 11/30/72.

Contribution from the Missouri Agricultural Experiment Station Journal Series No. 6526.

The authors acknowledge Dr. Gary Krause, Missouri Agricultural Experiment Station Statistician, for advice on the experimental design and assistance with analyses of data.

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PRESERVATION OF CHANNEL CATFISH WITH SOME SELECTED CHEMICALS

INTRODUCTION

EXTENSION of shelflife in such highly perishable food commodities as fresh chilled or iced channel catfish is imperative in order to offer the consumer a consistent, high-quality product. Properly cultured catfish, when freshly slaughtered and prepared, have a pleasantly mild flavor which is complimented by a firm, moist texture. As spoilage proceeds, aroma and flavor become flat, aromatic, or stale; characteristics described as musty, marshy, or kerosine-like may then develop (Boggess et al., 1971), depending to some degree upon the environment under which the fish were cultured. Lastly, putrid, sour and acrid aromas and flavors are detectible. The rate of development of undesirable organoleptic characteristics depends on many interacting factors. Autolysis in fish tissue plays a role early in the spoilage sequence, facilitating entrance and proliferation of bacteria. The initial number as well as the type of microflora present on the fish at the time of slaughter or introduced at some stage in processing will greatly influence the rate of spoilage and thus the shelflife of chilled or iced catfish.

The use of antibacterial agents to control the growth of bacteria on fish and fishery products has been described (Tomiyasu and Zenitani, 1957; Tarr, 1954). Antibiotics have been investigated in attempts to determine their value as preservatives for fish. Included in a long list of largely ineffective antibiotics are polymyxin B, circulin, bacitracin, subtilin, neomycin, gramicidin, metholyl gramicidin, streptomycin, chloromycetin, tyrothricin, penicillic acid, penicillin G, rimocidin, terramycin and aureomycin. Of these, aureomycin (chlortetracycline) (Lee et al., 1967; Boyd and Southcott, 1968) and terramycin (Lennon, 1972) are reported to exhibit significant bacteriostatic action on fish flesh. Sulfonamides, nitrofurans, organic acids, chlorine-liberating compounds and sodium nitrite have been employed with varying degrees of success in preserving fish and fishery products. Furia (1971) has shown that the storage life of fresh fish can be increased substantially by using alkali metal, ammonium and amine salts of ethylenediaminetetraacetic acid (EDTA) and ethylenetriaminepentaacetic acid in dips. Sodium salts of EDTA have also been reported to extend refrigerated storage of haddock fillets (Levin, 1967; Power et al., 1968).

Applications of these chemical agents and antibiotics can be readily accomplished by dipping or spraying. Use of germicidal or antiseptic ice has, in addition to bactericidal and bacteriostatic effectiveness, an advantage for its low temperature effect.

The present study was designed to evaluate the preservative action of 2,4,4′-trichloro-2′-hydroxydiphenyl ether, methyl 2-chloroacetoacetate and the pentasodium salt of diethylenetriaminepentaacetic acid on chilled channel catfish. Increase in refrigerated storage times which might be attributed to these chemical agents would be of potential benefit to the catfish industry.

EXPERIMENTAL

CHANNEL CATFISH *(Ictalurus punctatus)* were grown in tank culture under controlled conditions to about 700g live weight at Skidaway Institute of Oceanography, Savannah, Ga. The fish were transported in aerated tanks by truck to the Food Science Lab. in Experiment, Ga., where they were held without feeding for 24 hr. Slaughtering consisted of first stunning the fish with alternating current followed immediately by evisceration, decapitation, skinning and washing.

Dipping

Fish were treated after slaughter with dips according to the protocol listed in Table 1. The pentasodium salt of diethylenetriaminepentaacetic acid (Na_5DTPA) was prepared from diethylenetriaminepentaacetic acid. Solutions of 2,4,4′-trichloro-2′-hydroxydiphenyl ether (THDE; Irgasan DP 300, trade name of Ciba-Geigy Corp., Ardsley, N.Y. 10502) and methyl 2-chloroacetoacetic acid (MCA; U.S. patent 3,044,884, Shell Development Co., A Div. of Shell Oil Co., Modesto, CA 95352) were prepared by serial dilutions of the concentrated chemicals. After draining the catfish to remove excess dip solution, each fish was packaged individually at atmospheric conditions in polyethylene bags and stored at 0°C. Fish from each dip treatment were subjected to sensory evaluations, microbial enumeration, texture, pH, and moisture determinations after 0, 3, 6, 8, 10, 13, 16 and 22 days storage.

Microbial enumeration

To determine the potential bacteriostatic effects of various preservatives used in this

Table 1—Summary of treatments[a]

Treatment	Chemical(s) used
1	2% NaCl, control
2	5% ethanol in 2% NaCl, control
3	0.33% Na_5DTPA in 2% NaCl
4	0.67% Na_5DTPA in 2% NaCl
5	1.0% Na_5DTPA in 2% NaCl
6	10 ppm THDE in 5% ethanol plus 2% NaCl
7	20 ppm THDE in 5% ethanol plus 2% NaCl
8	30 ppm THDE in 5% ethanol plus 2% NaCl
9	33 ppm MCA in 2% NaCl
10	67 ppm MCA in 2% NaCl
11	100 ppm MCA in 2% NaCl
12[b]	20 ppm THDE in 5% ethanol plus 2% NaCl/0.33% Na_5DTPA in 2% NaCl
13[b]	10 ppm THDE in 5% ethanol plus 2% NaCl/0.67% Na_5DTPA in 2% NaCl
14[b]	67 ppm MCA in 2% NaCl/0.33% Na_5DTPA in 2% NaCl
15[b]	33 ppm MCA in 2% NaCl/0.67% Na_5DTPA in 2% NaCl

[a]Catfish were dipped in solutions containing pentasodium salt of diethylenetriaminepentaacetic acid (Na_5DTPA) or methyl 2-chloroacetic acid (MCA) or NaCl alone for 3 min and in solutions containing 2,4,4′-trichloro-2′-hydroxydiphenyl ether (THDE) in ethanol plus NaCl or ethanol plus NaCl alone for 30 sec.

[b]Slashes in treatments 12–15 indicate two dips.

study on the microflora of catfish, swabs of a 10 cm^2 area in the anterio-dorsal region of the fish were made after various storage times. Appropriate dilutions of each swab were made in sterile 0.1% peptone and the organisms were recovered in Standard Methods Agar (BBL) using the pour-plate technique. Incubation was at 21°C and counts were made after 4 days.

Sensory evaluation

Sensory ratings were made by a trained six-judge panel. Ratings were performed for appearance, aroma and color, using a nine-point hedonic scale. A rating of 9 indicated excellent; a 1 indicated extremely poor. The breakpoint in quality was considered as a 5 rating, in which case the panel members neither liked nor disliked the particular quality being judged.

Texture measurement

Two samples, each approximately 1.5 cm thick and weighing about 6g, were removed from fillets of fish. Caution was taken to assure that the samples were free from skin and bones. A Food Technology Corp. Recording Shear Press Model TP-1 was used to assess textural quality of the fish muscle. A 3000-lb transducer ring was used and the down stroke time of the shear blade was 30 sec. A range setting of 50 was employed throughout. Shear values were calculated as kg of force per g of fish tissue.

pH and moisture determination

Surface pH values were determined on all fish as they were removed from storage using a Corning Model 12 pH meter.

A 5-g portion of homogenized fish fillet was dried at 70°C in a vacuum oven for 24 hr and the moisture content was determined by difference.

Statistical analyses

Data were subjected to Duncan's multiple range test (Duncan, 1955) and significance was expressed at the 95% confidence level.

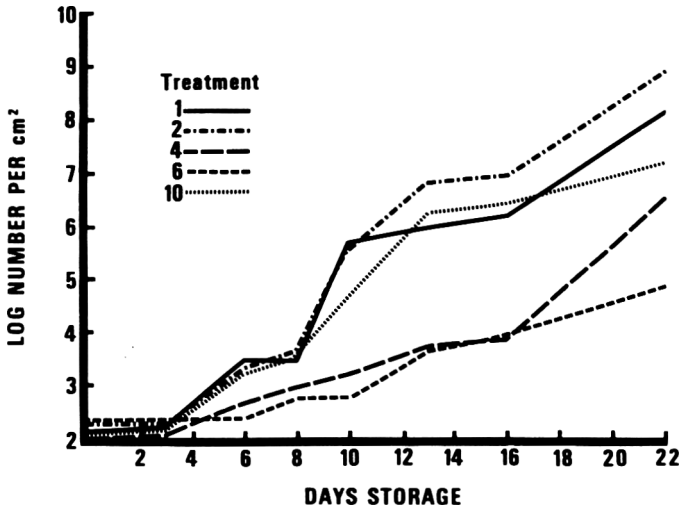

Fig. 1—Microbial counts on chilled channel catfish subjected to five dip treatments and stored for 0, 3, 6, 8, 10, 13, 16 and 22 days at 0° C.

Table 2—Appearance ratings[a] for chilled channel catfish subjected to 15 different treatments and held at 0° C for 0, 13, 16 and 22 days

	Treatment[b]		Days stored at 0°C			
			0	13	16	22
1	Control		8.67a	7.83ab	6.83abcd	3.33d
3	Na_5DTPA –	.33%	7.83a	7.50abc	6.33cd	4.67bc
4		.67%	8.17a	7.67abc	7.50abc	6.00ab
5		1.00%	7.83a	8.00ab	7.00abcd	6.00ab
9	MCA –	33 ppm	7.50a	6.17de	7.83ab	5.00bc
10		67 ppm	8.17a	5.83de	7.83ab	5.67b
11		100 ppm	7.33a	7.67abc	6.33cd	4.67bc
14	Na_5DTPA	(0.33%) + MCA (67 ppm)	7.50a	7.67abc	8.00a	5.17b
15		(0.67%) + (33 ppm)	7.33a	7.67abc	6.50bcd	5.50b
2	Control, 5% ethanol		8.17a	8.50a	3.83e	3.83cd
6	THDE –	10 ppm, 5% ethanol	8.50a	6.33cde	7.00abcd	5.83b
7		20 ppm, 5% ethanol	8.33a	7.67ab	7.50acd	1.83e
8		30 ppm, 5% ethanol	8.00a	5.33e	6.33cd	5.83b
12	Na_5DTPA	(0.33%) + THDE (20 ppm)	7.50a	6.83bcd	6.00d	7.17a
13		(0.67%) + (10 ppm)	7.83a	7.50abc	6.83abcd	6.00ab

[a]Values in the same vertical row bearing the same letter are not significantly different ($P < 0.50$).

[b]All treatments contain 2% NaCl

RESULTS & DISCUSSION

TABLE 1 has been compiled to summarize the dip treatments employed in this study. Frequent reference to this table by the reader will aid in his clearer understanding of the various comparisons. For simplicity, only data from 0, 13, 16 and 22 days storage are presented in Tables 2 through 5. Few differences were observed for any of the sensory ratings at 3, 6, 8 and 10 days storage. Likewise, only data from treatments 1, 2, 4, 6 and 10 are shown in Figure 1. These data are representative of the other treatments, as will be discussed below. Occasionally, sensory ratings for a particular organoleptic quality were increased slightly over ratings from the previous evaluation date. These exceptional data are probably due to slight variability in fish at the initiation of the study rather than to an actual improvement with storage time. Significant differences in color for treatments 7 and 15 are most likely due to variability in the raw product rather than to the treatments. In general, the greatest decline resulting in greater numbers of significant differences in aroma ratings were detected after 13 days storage (Table 4) while appearance (Table 2) and color (Table 3) ratings did not drop as sharply until after 16 days storage. Total sensory rating scores presented in Table 5 reflect an overall judgement which includes appearance, color and aroma.

Treatments 1 and 2

Few significant differences between control treatments 1 and 2 were found in appearance, color and aroma (Tables 2, 3 and 4, respectively). Total sensory scores indicated that the treatments were significantly different only on the 16-day storage evaluation, where treatment 2 scored lower than treatment 1 (Table 5). The 5% ethanol in 2% NaCl dip was therefore not considered to have a greater preservative effect than the 2% NaCl alone. Figure 1 shows that bacterial counts were, in fact, higher for the alcohol-treated fish after 10 days storage. Microbial counts from both treatments exceeded 10^8 per cm^2 of fish surface after 22 days storage. Treatment 2 was included in the study as a control for treatments 6, 7, 8, 12 and 13, all of which contained 5% ethanol. The alcohol was necessary to solubilize 2,4,4′-trichloro-2′-hydroxydiphenyl ether (THDE) in water at room temperature. Catfish panelled for aroma from treatment 2 scored no higher than 13th out of 15 treatments for any storage time. An

aromatic, sweet fragrance was often noted as objectionable early in storage while low scores after 13 days storage were probably due to decomposition products as a result of high microbial populations. Interestingly, the sweet fragrance was noted with less frequency on fish representing other alcohol-containing treatments. This phenomenon might be explained through reduction in volatile ethanol due to its reacting in some fashion with THDE.

Treatments 3, 4 and 5

Although salts of ethylenediaminetetraacetic acid (EDTA) have shown limited promise for use as bacteriostatic agents, they have been shown to extend the storage life of haddock fillets up to 11 days (Levin, 1967; Power et al., 1968). Their use in controlling adverse color development in canned fish and shellfish is a common practice. Diethylenetriaminepentaacetic acid (DTPA) is structurally similar to EDTA but it forms stronger metal chelates. An attempt at controlling off-flavor developments which are accelerated by relatively high levels of such trace metals as iron, zinc and copper in catfish might, therefore, be more efficiently accomplished by DTPA treatment. Furia (1971) has reported that Na_2DTPA, when applied as a dip, was more effective than Na_2EDTA, which in turn was more effective than controls, in extending storage life of haddock. The data presented in Tables 2, 3 and 4 indicate that Na_5DTPA, at dip concentrations of 0.33, 0.67 and 1.0% in 2% NaCl (treatments 3,4 and 5, respectively), will insure quality for longer storage times than the control. Few significant differences in appearance, color and aroma were found among the three Na_5DTPA concentrations, however, indicating that the lower (0.33%) concentration was sufficient to extend preservation. No statistically significant differences in total sensory ratings among treatments 3, 4 and 5 were observed (Table 5).

Surface counts revealed some differences in bacterial population early in storage as a result of Na_5DTPA dips. Figure 1 shows bacterial counts on fish from treatment 4 which are representative of data collected from treatments 3 and 5. It appears that Na_5DTPA does suppress total microbial growth, especially in early storage. However, the relative levels of specific genera of bacteria present on Na_5DTPA-treated versus control fish may change throughout storage; thus, different bacterial populations may account for significant differences in aroma ratings at 16 and 22 days storage. In other words, the less objectionable aromas detected in Na_5DTPA-dipped fish at 16 and 22 days may be a result of lower numbers of those organisms naturally producing foul-smelling aromatics and not entirely upon the metal chelating capacity of Na_5DTPA. Since no attempt was made to identify the microflora on any fish, we can merely speculate.

Treatments 6, 7 and 8

The evaluation of THDE as a preservative on catfish was made in light of its proven bacteriostatic action on such potentially toxic organisms as *Bacillus cereus, Staphylococcus aureus, Escherichia coli* and salmonellae (Savage, 1971). To date, THDE has been restricted to use in nonfood items which include toilet and laundry soaps, cosmetics, deodorants and fabrics. Tables 2 through 5 show that THDE at concentrations of 10, 20 and 30 ppm (treatments 6, 7 and 8, respectively) has only a limited preservative action. Significant differences from the control treatment in sensory ratings did not establish a definite pattern for a particular treatment as storage time increased. Significant differences observed late in storage are based on ratings below a level where fish would be considered accept-

Table 3—Color ratings[a] for chilled channel catfish subjected to 15 different treatments and held at 0° C for 0, 13, 16 and 22 days

	Treatment[b]		Days stored at 0°C			
			0	13	16	22
1	Control		7.83ab	8.50a	6.83abc	3.83c
3	Na_5DTPA –	.33%	7.17ab	7.33ab	6.50abc	5.50b
4		.67%	8.00ab	7.67ab	7.83a	5.50b
5		1.00%	6.83ab	7.50ab	7.50ab	5.00bc
9	MCA –	33 ppm	7.33ab	6.50bc	7.50ab	5.50b
10		67 ppm	8.00ab	6.17bc	7.83a	5.67b
11		100 ppm	7.67ab	7.17ab	5.67cd	5.17bc
14	Na_5DTPA	(0.33%) + MCA (67 ppm)	7.00ab	7.33ab	7.83a	5.50b
15		(0.67%) + (33 ppm)	6.67b	7.33ab	6.33bcd	6.00b
2	Control, 5% ethanol		7.83ab	8.50a	4.33e	5.67b
6	THDE –	10 ppm, 5% ethanol	8.17ab	6.17bc	7.00abc	5.17bc
7		20 ppm, 5% ethanol	8.33a	7.33ab	7.83a	2.33d
8		30 ppm, 5% ethanol	7.00ab	5.17c	5.83cd	5.67b
12	Na_5DTPA	(0.33%) + THDE (20 ppm)	7.17ab	6.33bc	5.00de	7.67a
13		(0.67%) + (10 ppm)	7.00ab	7.00b	6.00cd	7.33a

[a]Values in the same vertical row bearing the same letter are not significantly different ($P < 0.50$).
[b]All treatments contain 2% NaCl

Table 4—Aroma ratings[a] for chilled channel catfish subjected to 15 different treatments and held at 0° C for 0, 13, 16 and 22 days

	Treatments[b]		Days stored at 0°C			
			0	13	16	22
1	Control		8.17a	7.50a	5.00defg	1.17f
3	Na_5DTPA –	.33%	8.17a	7.50a	7.17a	2.33def
4		.67%	8.00a	7.17a	7.00ab	5.17ab
5		1.00%	8.17a	7.17a	7.00ab	2.67de
9	MCA –	33 ppm	7.50a	7.33a	4.50fg	1.83ef
10		67 ppm	7.67a	6.83a	5.00defg	5.17ab
11		100 ppm	7.17a	7.00a	4.83efg	3.00cde
14	Na_5DTPA	(0.33%) + MCA (67 ppm)	7.33a	7.67a	7.17a	2.83de
15		(0.67%) + (33 ppm)	7.83a	7.50a	6.67abc	3.00cde
2	Control, 5% ethanol		7.50a	6.67a	3.67g	1.67ef
6	THDE –	10 ppm, 5% ethanol	8.17a	7.00a	6.00abcde	2.67de
7		20 ppm, 5% ethanol	8.50a	7.00a	7.17a	1.00f
8		30 ppm, 5% ethanol	7.67a	6.33a	5.33cdef	3.50cd
12	Na_5DTPA	(0.33%) + THDE (20 ppm)	7.83a	7.33a	5.67bcdef	6.33a
13		(0.67%) + (10 ppm)	7.67a	7.16a	6.33abcd	4.33bc

[a]Values in the same vertical row bearing the same letter are not significantly different ($P < 0.50$).
[b]All treatments contain 2% NaCl

able for human consumption. Although there are differences when comparing treatments 6, 7 and 8 to each other, again no definite trend exists which establishes one concentration of THDE as superior for maintaining organoleptic quality of catfish.

The bacteriostatic effect of THDE is shown in Figure 1. Data from treatment 6 are presented and are representative of treatments 7 and 8. Total counts were substantially lower for THDE-treated fish throughout storage; a maximum count of less than 10^5 per cm^2 fish surface was obtained after 22 days storage. Minimum inhibitory concentrations of THDE are greater than 300 ppm for *Pseudomonas* spp. (Savage, 1971). Treatments 6, 7 and 8 were undoubtedly ineffective in suppressing the growth of odor-producing pseudomonads naturally present and capable of proliferation on fish at refrigerated temperatures. Thus, undesirable aromas which were noted in sensory evaluations and contributed to the general lack of preservation were probably largely due to *Pseudomonas* spp. It might be noted that we do not know the exact concentration of THDE on the fish surface immediately after dipping. Since fat is exposed at the fish surface and THDE is lipophylic, there is a partitioning of the THDE onto the surface during the dip. The extent of partitioning taking place as a result of our method of application is not known.

Treatments 9, 10 and 11

Methyl 2-chloroacetoacetate (MCA) was evaluated at 33, 67 and 100 ppm (treatments 9, 10 and 11, respectively) for its ability to retard catfish spoilage. Sensory ratings are listed in Tables 2 through 5. All treatments gave sensory ratings significantly higher than the control at 22 days storage for appearance (Table 2) and total scores (Table 5). Treatments 9 and 10 were rated significantly higher for color (Table 3) and treatments 10 and 11 scored significantly higher for aroma (Table 4) when compared to the control at 22 days storage. Many scores were near or above the point of "neither like nor dislike" on the hedonic scale. There were no significant differences among the three treatments at any time during storage when considering overall sensory evaluations as shown in Table 5.

Upon removal of catfish from individual storage pouches in preparation for quality evaluations, it was noted that the small amounts of liquid present in pouches containing fish treated with MCA remained clear and red for up to 13 days storage. All other treatments resulted in brown or gray, turbid liquid after 3 days storage. Apparently MCA has some effect on the oxidation of hemoglobin.

None of the treatments containing only MCA in 2% NaCl was demonstrated to be antibacterial. Data from treatment 10 are representative of treatments 9 and 11 and are presented in Figure 1.

Treatments 12 and 13

Furia (1971) demonstrated a marked increase in storage life of haddock fillets as a result of the combined effects of either 2-hydroxy-3′,4,4′-trichlorodiphenyl ether or 2-hydroxy-4,4′-dichlorodiphenyl ether with Na_5DTPA. Treatments 12 and 13 resulted in extended storage life of catfish as evidenced by high sensory ratings which were significantly different from both control treatments (1 and 2) at 22 days storage (Tables 2 through 5). Few differences were observed between treatments 12 and 13. Catfish from treatment 12 were rated more acceptable than those from treatments 3 and 7; however, few significant differences were noted between treatment 13 and treatments 4 and 6.

Microbial levels on fish from treatments 12 and 13 generally paralleled treatments 3 through 8 during most of the storage period. Representative data from treatments 4 and 6 are presented in Figure 1. It may be concluded, therefore, that combinations of Na_5DTPA and THDE employed in this experiment were no more effective than either chemical alone in controlling bacterial growth.

Treatments 14 and 15

Organoleptic scores from treatments 14 and 15 were significantly higher than control (treatment 1) scores after 22 days storage (Tables 2 through 4). With the exception of appearance and color at 16 days storage, there were no significant differences in ratings between treatments 14 and 15. In addition, the combinations of Na_5DTPA and MCA in treatments 14 and 15 did not result in higher total organoleptic ratings when compared to fish treated with dips containing individual chemicals (compare treatment 14 with 3 and 10; 15 with 4 and 9 in Table 5).

Microbial counts for fish representing treatments 14 and 15 were similar to those obtained from the Na_5DTPA-treated fish (treatments 3, 4 and 5). Therefore any antibacterial effect of treatments 14 and 15 is contributed by Na_5DTPA and not MCA.

Texture, pH, and moisture

Shear press values were reduced from about 7.0 kg of force per g of fish tissue when the project was initiated to about 3.5 at 22 days storage. There was no indication that specific treatments were advantageous in maintaining textural quality of channel catfish.

Evidently the decomposition products formed during catfish spoilage had enough buffering capacity to prohibit great changes in pH. The pH values during storage were observed to range between 6.2 and 7.0 with no increasing or decreasing trend noticeable as a result of individual treatments.

Moisture content of fish tissue fluctuated between 71 and 75% during the storage period. Again, no significant trends were observed among treatments.

Table 5—Total sensory ratings[a] for chilled channel catfish subjected to 15 different treatments and held at 0° C for 0, 13, 16 and 22 days

	Treatments[b]	Days stored at 0°C			
		0	13	16	22
1	Control	8.22a	7.94a	6.22bcd	2.78fg
3	Na_5DTPA — .33%	7.72a	7.50abc	6.67abcd	4.17cde
4	.67%	7.94a	7.50abc	7.44ab	5.56bc
5	1.00%	7.61a	7.56abc	7.17abc	4.56bcde
9	MCA — 33 ppm	7.44a	6.67abcd	6.61abcd	4.11de
10	67 ppm	7.94a	6.28cd	6.39abcd	5.50bcd
11	100 ppm	7.39a	7.28abc	5.61d	4.28cde
14	Na_5DTPA (0.33%) + MCA (67ppm)	7.28a	7.56abc	7.67a	4.50cde
15	(0.67%) + (33 ppm)	7.28a	7.50abc	6.50abcd	4.83bcde
2	Control, 5% ethanol	7.83a	7.89ab	3.94e	3.72ef
6	THDE — 10 ppm, 5% ethanol	8.28a	6.50bcd	6.67abcd	4.56bcde
7	20 ppm, 5% ethanol	8.39a	7.33abc	7.50ab	1.72g
8	30 ppm, 5% ethanol	7.55a	5.61d	5.83cd	4.83bcde
12	Na_5DTPA (0.33%) + THDE (20 ppm)	7.50a	6.83abcd	5.56d	7.06a
13	(0.67%) + (10 ppm)	7.50a	7.22abcd	6.39abcd	5.89ab

[a]Values in the same vertical row bearing the same letter are not significantly different ($P < 0.50$).

[b]All treatments contain 2% NaCl

CONCLUSIONS

NO SINGLE TEST, objective or otherwise, can predict the total quality of fish.

Variables in judging include the fish's growth environment, diet, method of slaughtering, handling, storing and preparing; individual people or entire cultures may judge a fish as excellent to poor, depending upon habit and background. Odor has recently been suggested as a reliable index to assess quality of refrigerated cod (Charm et al., 1972). However, if microbial counts are to be considered as an indication of wholesomeness and quality, then odor is not always a reliable index. Nonproteolytic strains of toxigenic *Clostridium botulinum* may produce toxin without a corresponding release of undesirable volatiles. Data presented in this study have revealed a lack of correlation between aroma or overall sensory ratings with total microbial counts. The THDE sensory data indicate few significant differences from control data but a substantial suppression in the rate of total bacterial growth. On the other hand, MCA resulted in sensory ratings significantly higher than the control but showed limited promise as a bacteriostat when used at the levels employed in this study. Increased storage life obtained from MCA dips may be due to inhibition of fish tissue autolysis ordinarily evident at high microbial populations.

The results of this study indicate that Na_5DTPA, THDE and MCA exhibit preservative action on chilled channel catfish. Since none of the chemicals are presently approved by the FDA as food additives, an extensive study involving each chemical individually and in combination with other potentially bacteriostatic compounds should be undertaken in order to arrive at the most effective and safe levels for their usage.

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Ms received 10/6/72; revised 11/17/72; accepted 11/20/72.

Appreciation is expressed to J. Andrews, Skidaway Institute of Technology, Savannah, for supplying the catfish and to D. W. Parvin for assistance in statistical design and analyses. We are grateful to Ciba-Geigy Corp. for gratis samples of diethylenetriaminepentaacetic acid and 2,4,4'-trichloro-2'-hydroxydiphenyl ether and to Shell Development Co. for methyl 2-chloroacetoacetic acid used in this study.

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MUSCLE QUALITY, COOKING METHOD AND AGING VS. PALATABILITY OF PORK LOIN CHOPS

INTRODUCTION

NUMEROUS STUDIES and observations indicate that there is a significant incidence of pale, soft, exudative (PSE) muscle among pork carcasses (Stringer, 1970). Studies to date on the palatability of PSE pork report conflicting results. Some researchers (Huffman et al., 1968; Deethardt and Tuma, 1971; Judge et al., 1960; and Merkel, 1971) have found PSE muscle to be more tender than normal or dark, firm, dry (DFD) muscle. Others have reported that DFD muscle is the most tender and PSE muscle least tender (Lewis et al., 1962; Kauffman et al., 1964; Searcy et al., 1969; and Sayre et al., 1964). Research is in general agreement that PSE is the least juicy of the muscle types while DFD is the most juicy.

No studies comparing household and deep fat methods of cooking pork were found in the literature. Because investigators often fry pork, a comparison is needed to determine if frying is a judicious method to use in palatability studies.

Likewise, little work has been published on the effect of aging pork. Gould et al. (1965) and Buchter and Zeuthen (1971) suggested that aging does increase pork tenderness. However, Harrison et al. (1970) concluded that aging pork loins was of little benefit to organoleptic properties.

Because work in each area is inconclusive, this study was designed to investigate the effect of type of musculature (PSE, normal, DFD), cooking method (oven broiling, deep fat frying), loin section (anterior, posterior) and aging (48 or 144 hr) on palatability of pork loin chops.

EXPERIMENTAL

TEN PORK LOINS each of PSE, normal and DFD musculature were selected 24 hr postmortem at a commercial packing plant. Sections were cut from two muscle locations. Section 1 extended from the 10–13th thoracic vertebrae; section 2 consisted of a 12.8 cm section forward from the 5th lumbar vertebra. One section from each loin was aged at 0–4°C for an additional 24 hr (48 hr total postmortem aging) before it was cut into chops. The other section was aged for a total of 144 hr postmortem. Four 3.2 cm chops cut from each section were wrapped after aging, frozen, and held at −30°C until 37 hr before testing when they were thawed at 4°C. All external fat was removed from each chop prior to testing.

Each loin was assigned a color score on the basis of the Wisconsin standards outlined by Forrest et al. (1963). Light reflectance of the chops was measured at 525 nm using a Bausch and Lomb Spectronic 20 equipped with a color reflectance attachment. Hart's turbidity method (transmission value) as modified by Dekker and Hulshof (1971) was used to assess differences in muscle protein solubility. Oven broiling and deep fat frying were the cooking methods used. Oven broiling at 177°C was a modification of the method described by Cover and Hostetler (1960). Oven temperature and internal meat temperature were recorded on a Honeywell Multipoint temperature recorder. Chops were cooked to 77°C and cooking time was recorded. For deep fat frying, a 15.2 cm glass thermometer was inserted into the center of the longissimus muscle. Chops were fried to 77°C in corn oil heated to 110°C in an institutional fryer. The lower temperature was chosen to reduce protein hardening of muscle surfaces. Cooking time was noted. Total cooking loss was calculated for all chops. For oven-broiled chops, total cooking loss was subdivided into drip loss and evaporation loss.

Firmness of raw and cooked chops at 22° ± 1°C was measured using a Universal Precision Penetrometer with timer equipped with a single bell attachment (15 mm diam). Depth of penetration in 5 sec was measured to the nearest 0.1 mm. Three readings were taken on each side of the chop. For each raw and cooked chop, the pH of a slurry prepared from 20g of finely chopped muscle and 40 ml of distilled water was read on an expanded scale Beckman pH meter. Total moisture of both raw and cooked longissimus muscle was determined by drying at 65°C under a vacuum equivalent to 70 mm Hg for 15 hr. Percent total moisture was determined by expressing weight lost during drying as a percentage of initial weight. Press fluid and bound water were also measured on both raw and cooked longissimus muscle. Percent press fluid was determined by expressing weight lost during pressing as a percentage of initial weight (Sanderson and Vail, 1963). Bound water was measured by drying the pressed samples as described above and expressing weight lost during drying as a percentage of unpressed sample weight. Twelve 1.3 cm cores were used for Warner-Bratzler shear determination of each chop. Shear force, read in pounds, was converted to kg.

A five-member sensory panel judged warm samples of the longissimus muscle (0.3 cm × 1.9 cm × 5.1 cm) for tenderness, juiciness and flavor using a 5 point hedonic scale (5 = most desirable). Four samples were judged at one time. Data were analyzed using analysis of variance and Newman-Keuls sequential range test was used to separate significant means (Steel and Torrie, 1960).

Table 1—Objective measurements of variation in muscle quality

Measurement	PSE	Normal	DFD	Std error[a]	F value
pH, raw	5.38[f]	5.56[g]	6.07[h]	.059	20.7**
pH, cooked	5.61[f]	5.84[g]	6.26[h]	.043	99.6**
Color score[b]	1.1[f]	3.1[g]	3.9[h]	.11	175.5**
% Reflectance 525 nm	33.4[f]	24.6[g]	19.5[h]	.67	109.9**
Transmission value	77.6[f]	36.3[g]	14.6[h]	5.73	31.2**
Firmness, raw[c]	90.6[f]	88.8[f]	81.9[g]	2.19	4.3*
Firmness, cooked[c]					
Oven[d]	17.5[f]	20.9[f,g]	23.4[g]	1.44	4.2*
Fry[e]	17.9	20.6	20.3	1.44	1.1

[a]Based on Error Mean Square from Analysis of Variance
[b]Based on Wisconsin color standards (Forrest et al., 1963)
[c]Penetration, 0.1 mm
[d]Oven broiled at 177°C
[e]Deep fat fried at 110°C
[f,g,h]Means in same line bearing a common superscript are not significantly different ($P < 0.05$)
*F value significant at 5% level
**F value significant at 1% level

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RESULTS & DISCUSSION

Muscle quality

Table 1 indicates that the three types of musculature, PSE, normal and DFD were distinct based upon criteria of raw and cooked muscle pH, color score, percentage reflectance, transmission value and firmness of the raw longissimus muscle. The large transmission value of PSE muscle indicates that the water soluble proteins were less extractable. The PSE chops had the lowest pH, lowest color score, greatest percentage reflectance and the least firmness in the raw state. After cooking, PSE muscle was the most firm, probably due to the loss of fluid surrounding but not bound to the muscle proteins. The coagulated fibers then pack closely together, increasing firmness.

In agreement with Sayre et al. (1964), it was observed that, in the oven, PSE pork cooked the slowest and DFD pork cooked the fastest. When cooking time was expressed as min/g to eliminate the effects of weight variations, the results also indicated that the PSE chops cooked the slowest (Table 2). However, PSE chops which were deep fat fried cooked the most rapidly. Differences between the three types of musculature were not significant when cooking time by deep fat frying was reported in min/g. Sayre et al. (1964) postulated that a cooling effect of large evaporation losses was responsible for the slower cooking rate of PSE pork when chops were oven broiled.

Numerous investigators (Judge et al., 1960; Lewis et al., 1962; Sayre et al., 1964; Kauffman et al., 1964; Searcy et al., 1969; Deethardt and Tuma, 1971; and Merkel, 1971), have reported that PSE pork has greater cooking losses than normal or DFD pork. Table 2 shows that differences in total cooking loss between PSE, normal and DFD muscle were significant only for oven-broiled chops. PSE chops had greater total losses than either normal or DFD chops; DFD chops had the least. Since all external fat was removed, evaporation accounts for most of the cooking losses.

Although total moisture is significantly different between PSE and DFD and between normal and DFD muscle, the differences are small (Table 3). After heating, total moisture is inversely related to cooking losses. Cooked PSE muscle had the least total moisture (highest cooking loss) and DFD muscle had the greatest total moisture (least cooking loss). Press fluid did not vary significantly between types of musculature in the raw chops. However, press fluid yields of both oven-broiled and deep fat fried chops varied significantly between PSE, normal and DFD muscle. PSE muscle had the lowest press fluid yields and DFD the highest (Table 3). In the raw state, normal and DFD pork had higher percent bound water than PSE muscle ($P < 0.05$) but DFD and normal muscle did not differ significantly. After cooking, regardless of the method used, percent bound water in all samples was similar at approximately 23% of sample weight.

A significant interaction ($P < 0.05$) for cooking method versus muscle type indicated that the differences in Warner-Bratzler shear values among PSE, normal and DFD muscles were greater when chops were oven broiled than when they were deep fat fried (Table 4 and Fig. 1). Warner-Bratzler shear values for oven-broiled PSE chops were significantly less ($P < 0.05$) than shear values for DFD chops. Frying, however, toughened all chops, leaving no significant differences between PSE, normal and DFD muscle. Nevertheless, PSE chops required the least shear force. Several researchers have indicated that high pH DFD muscles have the lowest shear values (Lewis et al., 1962; Kauffman et al., 1964; and Searcy et al., 1969). This experiment confirms the findings of Huffman et al. (1968), Deethardt and Tuma (1971) and Merkel (1971) that PSE pork has the lowest shear values.

In agreement with Warner-Bratzler shear determinations, the sensory panel scored oven-broiled PSE chops significantly higher in tenderness than the other muscle types (Table 4). However, fried DFD chops were rated more tender than either normal or DFD chops although the differences were not statistically significant ($P < 0.05$). PSE chops scored lowest for juiciness and DFD chops received the highest scores regardless of the cooking method used. There were no significant differences in flavor scores for PSE, normal and DFD chops prepared by either oven broiling or deep fat frying. Judge et al. (1960), Deethardt and Tuma (1971), and Merkel (1971) also found that PSE muscle was the most tender as judged by a sensory panel. However, other workers have found that porcine muscle of high pH was most tender (Kauffman et al., 1964; Lewis et al., 1962; and Sayre et al., 1964). Previous research is in agreement that DFD pork is significantly more juicy than normal or PSE pork and that PSE pork is the least juicy (Kauffman et al., 1964; Lewis et al., 1962, Sayre et al., 1964; and Merkel, 1971).

Cooking method

The most striking difference between cooking methods is in the time required for the meat to reach an internal temperature of 77°C. Oven-broiled chops required three to four times longer to cook than did fried chops, 48.9 vs. 13.5 min,

Table 2—Cooking data for PSE, normal and DFD muscle

Measurement		PSE	Normal	DFD	Std error[a]	F value
Total cooking time						
(min)	Oven[b]	56.3[d]	49.5[e]	40.9[f]	1.86	17.3**
	Fry[c]	12.6[d]	13.7[e]	14.4[e]	.35	5.1*
Cooking time						
(min/g)	Oven	0.31[d]	0.26[e]	0.22[f]	.010	25.4**
	Fry	0.07	0.07	0.08	.002	2.6
Total cooking loss (%)						
	Oven	29.8[d]	26.1[e]	18.7[f]	.98	33.5**
	Fry	23.0	22.2	20.2	.93	2.41
Drip loss (%), Oven		1.7[d]	1.8[d]	1.3[e]	.12	4.1*
Evaporation loss (%), Oven		28.1[d]	24.3[e]	17.4[f]	.99	29.9**

[a]Based on Error Mean Square from Analysis of Variance
[b]Oven broiled at 177°C
[c]Deep fat fried at 110°C
[d,e,f]Means in same line bearing a common superscript letter are not significantly different ($P < 0.05$)
*F value significant at 5% level
**F value significant at 1% level

Table 3—Moisture determinations of PSE, normal and DFD muscle

Measurement	PSE	Normal	DFD	Std error[a]	F value
Total moisture, raw (%)	71.0[d]	71.9[d]	73.5[e]	.40	9.8**
Total moisture, cooked (%)					
Oven[b]	59.8[d]	61.7[d]	65.0[e]	.72	13.5**
Fry[c]	59.8[d]	61.0[d,e]	62.6[e]	.63	5.0*
Press fluid, raw (%)	54.1	52.7	52.8	.73	1.0
Press fluid, cooked (%)					
Oven	42.0[d]	44.2[d]	48.6[e]	.92	13.5**
Fry	41.9	43.6	45.3	.75	4.9*
Bound water, raw (%)	23.5[d]	26.2[e]	27.4[e]	.67	8.7**
Bound water, cooked (%)					
Oven	23.8	23.8	22.4	.44	2.7
Fry	23.3	23.5	23.4	.37	.04

[a]Based on Error Mean Square from Analysis of Variance
[b]Oven broiled at 177°C
[c]Deep fat fried at 110°C
[d,e]Means in same line with common superscript letter are not significantly different ($P < 0.05$)
*F value significant at 5% level
**F value significant at 1% level

Table 4–Palatability of PSE, normal and DFD muscle

Measurement		PSE	Normal	DFD	Std error[a]	F value
Warner-Bratzler shear (kg)						
	Oven[b]	2.3[e]	2.7[e]	3.2[f]	.16	7.8**
	Fry[c]	3.2	3.3	3.3	.18	.2
Tenderness[d]	Oven	3.8[e]	3.1[f]	3.2[f]	.11	11.3**
	Fry	2.5	2.7	3.2	.19	3.2
Juiciness[d]	Oven	2.0[e]	2.7[f]	3.1[g]	.13	16.3**
	Fry	2.2[e]	2.6[f]	3.0[g]	.14	8.0**
Flavor[d]	Oven	2.9	3.2	3.2	.14	2.2
	Fry	2.8	3.1	3.1	.12	2.8

[a]Based on Error Mean Square from Analysis of Variance
[b]Oven broiled at 177°C
[c]Deep fat fried at 110°C
[d]5-point hedonic scale: 5 = most desirable, 1 = least desirable
[e,f,g]Means in same line bearing a common superscript letter are not significantly different ($P < 0.05$)
**F value significant at 1% level

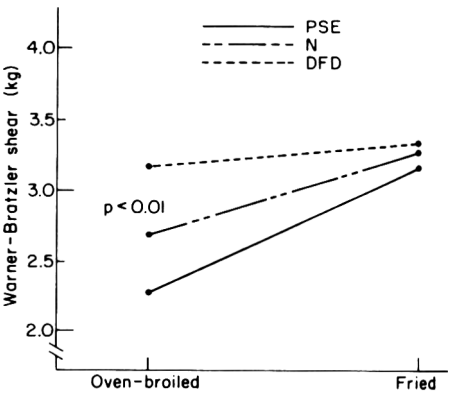

Fig. 1–Effect of muscle type and cooking method interaction on Warner-Bratzler shear values.

or 0.26 min/g vs. 0.07 min/g. While oven-broiled chops had greater total cooking losses, the difference between methods was not significant (24.9% vs. 21.8%). In view of the much longer cooking time for oven broiling, losses during frying per unit of time were large. Schock et al. (1970) likewise found no significant difference between broiling and frying for cooking loss of beef. In the present study, oven-broiled samples had a greater percentage of total moisture (62.2% vs. 61.2%, $P < 0.05$) and press fluid (45.0 vs. 43.6, $P < 0.05$), lower Warner-Bratzler shear values (2.7 kg vs. 3.3 kg, $P < 0.01$) and higher tenderness scores 3.4 vs. 2.8, $P < 0.05$) than did fried samples. There were no significant differences between cooking methods for muscle firmness, pH, bound water, juiciness or flavor.

The most interesting result of the comparison of cooking methods was in the interaction between cooking method and muscle type as it affected Warner-Bratzler shear values (Fig. 1). There were also significant interactions in the results for cooking time, cooking loss, total moisture, press fluid yield and tenderness scores. These data suggest that oven broiling is a more appropriate cooking method to use if differences among samples are to be detected.

Loin section and aging

Palatability did not differ between the anterior and posterior loin sections used in this study. Increasing the aging period from 2 to 6 days had no significant effect on any of the results in this experiment.

A slight increase in tenderness as a result of aging was noted for normal and DFD chops, but PSE chops showed decreased tenderness. However, the changes in shear values and tenderness scores were not large or consistent enough to be significant. Gould et al. (1965) reported that as aging period lengthened from 2 to 12 days, tenderness of loin chops increased. Likewise, Buchter and Zeuthen (1971) suggested that aging for 6 days increases the tenderness of pork cutlets of normal musculature but has little effect on PSE cutlets. The results of this experiment lead to the same conclusion as that reached by Harrison et al. (1970): holding pork loins for longer than 1 to 2 days postmortem is of little benefit to palatability.

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Ms received 10/24/72; revised 12/30/72; accepted 1/8/73.

Financial support provided by National Science Foundation Traineeship Program. Journal Paper No. 4912 of the Purdue University Agricultural Experiment Station. This paper is based on a study conducted by Mary E. Bennett for the M.S. degree at Purdue University.

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QUALITY CHANGES IN PRE-RIGOR BEEF MUSCLE AT −3°C

INTRODUCTION

LOCKER (1960), working with beef muscles, was one of the first investigators to establish a definite relationship between tenderness of a muscle and its state of contraction. He found an inverse relationship between tenderness and degree of contraction, which was especially significant in muscles of rather low contents of connective tissue. It was subsequently shown by Herring et al. (1964, 1965a, 1965b) that contraction of beef muscle, whether the result of thaw rigor (Newbold, 1966), cold shortening (Locker and Haygard, 1963), or normal rigor, is accompanied by decreases in sarcomere length and tenderness. Additional studies by Marsh and Leet (1966) showed that maximum toughness occurred at about 40% shortening, and that percent drip loss increased greatly as shortening exceeded this value.

If beef muscle is frozen pre-rigor and stored at a low subfreezing temperature, rapid thawing will result in a highly undesirable phenomenon called thaw rigor (Newbold, 1966). Thaw rigor is characterized by unusually rapid rates of biochemical changes (ATP depletion, glycogen breakdown, lactic acid accumulation and a decline in pH), extreme muscle contraction and excessive drip loss. It has been demonstrated in lamb (Marsh and Thompson, 1958) and beef (Marsh et al., 1968) that the adverse toughening and high drip loss accompanying thaw rigor can be prevented if the muscle is raised to just a few degrees below its freezing point for several hours prior to thawing. Under these conditions of time and temperature, normal glycolytic changes and rigor mortis will go to completion, while enough ice exists to physically prevent the muscle from shortening. Thus, a muscle postmortem must be given enough time prior to freezing (e.g., chilling) or at high subfreezing storage temperatures to complete the biochemical and physical changes accompanying normal rigor mortis, or upon thawing it will undergo thaw rigor and its associated undesirable effects.

In this study, beef muscle was excised soon after slaughter, frozen, and stored for various times at high subfreezing temperatures so as to allow continuation of glycolysis. Although previous studies have established that quality defects can be avoided by storing muscle frozen pre-rigor at high subfreezing temperatures, still unknown at these temperatures are the commercially important interrelationships among shortening, tenderness and drip, and the range of time-temperature conditions yielding satisfactory quality. This study provides information relevant to these points.

EXPERIMENTAL

STERNOMANDIBULARIS (neck) muscles were removed from one side of freshly slaughtered (45 min postmortem) cutter grade cattle at Oscar Mayer, Inc., Madison, Wis. Excised muscle strips (length 17–29 cm, width 2–3 cm and weight 91.4–168.3g) were measured for length to the nearest half centimeter, labeled, wrapped in aluminum foil, frozen in crushed dry ice (solidified carbon dioxide) and transported to the University of Wisconsin. These muscles were then wrapped in tightly-closed polyethylene bags and given one of six treatments: (1) stored in dry ice, or (2) immersed in an ethanol-water refrigerated bath at −3°C for 4, 6, 10, 14 or 24 hr, immersed in liquid nitrogen for at least 20 min, and stored in dry ice (5 days or less). A storage temperature of −3°C was chosen since Behnke et al. (1973a) established that the rate of glycolysis in beef muscle attained a subfreezing maximum at about this temperature.

The sternomandibularis muscle from the

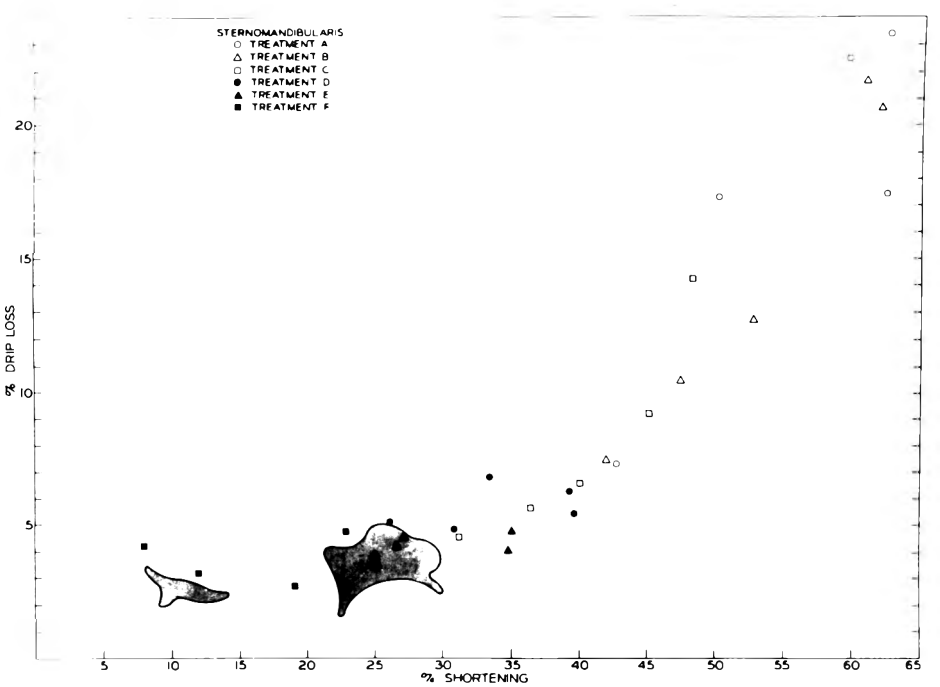

Fig. 1—Effect of percent shortening on percent drip loss of excised sternomandibularis muscles. Treatments: A = frozen pre-rigor in crushed dry ice (−78°C) and stored 1–5 days. B = frozen pre-rigor (as in A), immersed in a refrigerated bath at −3°C for 4.0 hr, then stored in dry ice. C = frozen pre-rigor (as in A), immersed (as in B) for 6.0 hr, then stored in dry ice. D = frozen pre-rigor (as in A), immersed (as in B) for 10.0 hr, then stored in dry ice. E = frozen pre-rigor (as in A), immersed (as in B) for 14.0 hr, then stored in dry ice. F = frozen pre-rigor (as in A), immersed (as in B) for 24.0 hr, then stored in dry ice. The irregular-shaped, shaded areas represent the range of 30 control values. Control muscles were chilled on the carcass for 24.0 hr in circulating air at 4–7°C, excised, wrapped and frozen post-rigor in crushed dry ice.

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other side of each carcass was left in place (on the carcass) and chilled for 24 hr in cool air at 4–7°C. Muscles treated in this manner served as controls. After a 24-hr chilling period these muscles were excised, measured for length to the nearest half centimeter, labeled, wrapped in aluminum foil, frozen in crushed dry ice, transported to the University of Wisconsin and stored in dry ice. 30 cows, sufficient for five replicates for each of the above six treatments, were used in this study.

While still frozen, all muscles were weighed to the nearest tenth of a gram. The muscles were then thawed on paper towels for 90 min at room temperature (25°C), measured for length and weighed as previously described. Values for percent shortening and percent drip loss of each thawed muscle were calculated from this data.

The muscles were then labeled, wrapped twice in aluminum foil and cooked for 60 min in steam at 100°C (0 psig). Neck muscles were then cut into standard-size pieces (6.5 cm length × 2.1 cm width × 1.0 cm thickness) using a stainless steel template. These standard-size muscles were weighed to the nearest hundredth gram (range 9.58–11.65g), labeled and wrapped in polyethylene bags to prevent dehydration until tenderness values were determined (no more than 3 hr delay). A Lee-Kramer shear press was used to assess tenderness of the samples as described in a previous paper (Behnke et al., 1973b).

RESULTS

A PLOT OF percent drip loss versus percent shortening of sternomandibularis muscles after various times at −3°C (Fig. 1) illustrates that: (1) shortening from about 6–35% had no detectable effect on percent drip loss; (2) increased shortening between about 35 and 63% was accompanied by large increases in percent drip loss; (3) muscles which were held at −78°C (treatment A) and those that were held at −3°C for 4 hr (treatment B) exhibited much greater shortening and larger drip losses than the controls; (4) muscles which were held at −3°C for 6–10 hr (treatments C and D) exhibited somewhat greater shortening and somewhat larger drip losses than the controls; and (5) muscles which were held at −3°C for 14 or 24 hr (treatments E and F) exhibited essentially the same shortening and drip losses as the controls.

A graph of the relationship between tenderness (lb shear/g) and percent shortening of sternomandibularis muscles after various times at −3°C (Fig. 2) shows that: (1) shortening from about 6–20% had no detectable effect on tenderness; (2) increased shortening between about 20 and 43% was accompanied by large decreases in tenderness; (3) increased shortening between about 43 and 63% resulted in increasing tenderness; (4) muscles which were held at −78°C (treatment A) and those that were held at −3°C for 4 or 6 hr (treatments B and C) exhibited severe shortening, and their tenderness values ranged from greater than (between 32 and 50% shortening) to about equal to (between 52 and 63% shortening) those of the controls; (5) muscles which were held at −3°C for 10 or 14 hr (treatments D and E) exhibited somewhat greater shortening and somewhat less tenderness than the controls; and (6) muscles which were held at −3°C for 24 hr (treatment F) exhibited essentially the same shortening and tenderness values as the controls.

The data in Figure 3 illustrate that shortening of sternomandibularis muscles decreased as holding time at −3°C was increased from 0 to 24 hr. Muscles held at −3°C for 24 hr (treatment F) shortened significantly ($p < 0.05$) less than muscles held at −78°C (treatment A) or those held at −3°C for 4 hr (treatment B), and were about the same length as control muscles (treatment X) which remained on the carcass for 24 hr at 4–7°C. This latter finding suggests that shortening of beef muscles can be equally well minimized by either freezing beef pre-rigor and holding it for 24 hr at −3°C or by holding beef for 24 hr at 4–7°C (unfrozen).

DISCUSSION

THE RESULTS of Figure 1 (percent drip loss versus percent shortening) and Figure 2 (tenderness versus percent shortening) are in good agreement with those obtained by Marsh and Leet (1966) with unfrozen beef muscle.

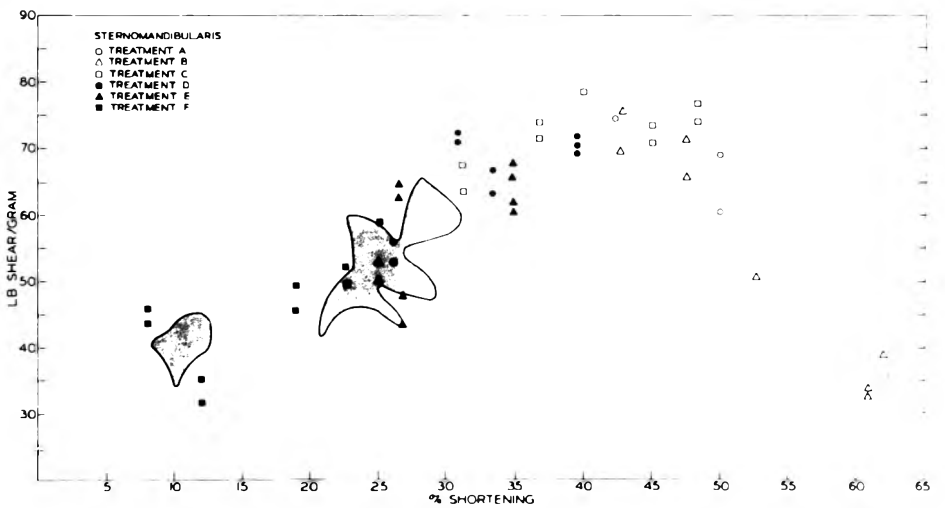

Fig. 2—Effect of percent shortening on tenderness of excised sternomandibularis muscles. Treatments: See legend of Figure 1.

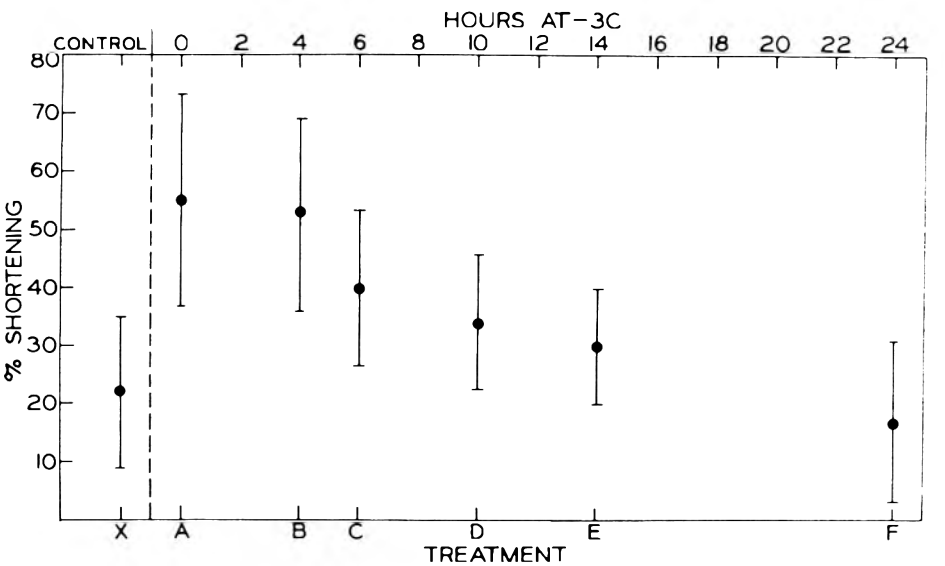

Fig. 3—Effect of time at −3°C on percent shortening (mean ± 95% confidence interval) of excised sternomandibularis muscles. Treatments: X = chilled on the carcass for 24.0 hr in circulating air at 4–7°C, excised, wrapped, then frozen post-rigor in crushed dry ice (control). A through F: See legend of Figure 1.

Although storage of pre-rigor beef muscle at −3°C results in rapid glycolysis (Behnke et al., 1973a), this does not, as shown here, necessarily cause toughness. This finding is in agreement with a statement by Lawrie (1968) indicating that prevention of shortening (e.g., by freezing the muscle) will prevent an increase in toughness, despite a rapid rate of ATP depletion.

An obvious question concerns the relationship that these results with excised muscle have to whole carcasses or parts of carcasses. Although Marsh and Leet (1966) showed that excised muscle held at a constant adverse temperature will not toughen if physically restrained from shortening, they also showed that localized toughening can occur if the restrained muscle is cooled nonuniformly. In the latter situation, the overall length of the muscle remained constant, but contraction and increased toughness occurred in the more rapidly cooled areas, and compensating elongation occurred in more slowly cooled areas. They pointed out that nonuniform cooling of carcasses would not be uncommon because insulating constituents (fat, bone) are distributed nonuniformly. They also mentioned two additional factors which would tend to make carcass muscles susceptible to shortening: (1) some muscles such as sternomandibularis are severed during dressing; and (2) other muscles such as longissimus dorsi are firmly attached to the skeleton at one end only. Studies with lamb (McCrae et al., 1971) and with poultry (Behnke et al., 1973b) support the contention of Marsh and Leet that pre-rigor animal carcasses exposed to unsuitable temperature treatments can develop undesirable toughness, presumably because of substantial contraction of certain muscles or portions of muscles.

Also of concern is whether important muscles of a beef carcass will behave like sternomandibularis. Although extensive information is not available on this point, there are indications that the important longissimus dorsi does behave very much like sternomandibularis (Locker and Haygard, 1963; McCrae et al., 1971; Marsh et al., 1968).

It would appear therefore that the results obtained in this study with excised sternomandibularis would have at least some relevance to muscles which have not been removed from the skeleton. If so, commercial application of these results appears possible. In the event that freezing becomes a common means of preserving retail cuts of beef, it would seem advantageous in terms of bacteriological quality and ease of cutting to utilize a −3°C holding treatment following freezing, in place of a normal chilling procedure prior to freezing. Beef carcasses could be frozen immediately following slaughter then cut up in a room maintained at perhaps 5–10°C. While in this room the beef temperature would rapidly rise to and remain near −3°C (because −3°C approximates the temperature of the freezing-thawing plateau), thereby allowing glycolysis to proceed rapidly without undesirable consequences. Following cutting and the minimum holding period (probably no more than 14 hr) the beef would be returned to normal frozen storage. It is likely that the time needed at −3°C would be much less than 14 hr since extensive glycolysis would occur during the long period required to freeze large beef carcasses. Since it is possible that problems with cold shortening would be encountered during freezing of large pre-rigor carcasses, commercial trials are needed to assess the feasibility of the procedure suggested above.

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Ms received 10/21/72; revised 12/26/72; accepted 12/28/72.

Presented at the 32nd Annual Meeting of the Institute of Food Technologists in Minneapolis.

Research supported by the College of Agricultural & Life Sciences, University of Wisconsin-Madison; by U.S. Public Health Service grant No. FD00115; and by U.S. Public Health Service fellowship No. ES 38605 (to James Behnke). The willing cooperation of Oscar Mayer and Co., Madison, Wis. is gratefully acknowledged.

JFS RESEARCH NOTES

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A Research Note

STORAGE BEHAVIOR OF ARTIFICIALLY WAXED GREEN SNAP BEANS

INTRODUCTION

SEVERAL TECHNIQUES have been used to extend the storage life of perishable fruits and vegetables. These include: (1) low temperature storage (Edmond et al., 1964); (2) modified atmosphere storage (Kidd, 1915); and (3) application of a protective coating on the surface of the tissue (Platenius, 1939; Ford and Alban, 1951; Hartman and Isenberg, 1956). The first two methods have been extensively studied and applied to nearly all fruits and vegetables; however, the third technique has only been applied to certain products, and, therefore, the applicability of this technique is rather limited. It was the objective of this study to determine the effect of two wax coatings on the shelflife of a rapidly respiring vegetable. Snap beans were selected since they exhibit a high respiration rate and consequently have a relatively short storage life. The heat of respiration is about 10,000 BTU per ton per 24 hr at 40°F and, consequently, the storage life is 8–10 days (ASHRAE, 1968). Such parameters as respiration rate, color, general appearance and microbial infection of the waxed beans are examined.

EXPERIMENTAL

TWO DIFFERENT wax emulsions were obtained from FMC Corp. (Florida Div., Lakeland, Fla.): Sta-Fresh 715 (an anionic polyethylene emulsion with 20% polyethylene solids) and Sta-Fresh 800 (an emulsified vegetable oil). Preliminary experiments with filter paper were conducted to determine the effect of these wax emulsions on carbon dioxide permeability. An air cell was used consisting of two chambers separated by Whatman No. 2 filter paper which had been waxed on one side with one of the test emulsions. The test solution was a 3/1 dilution (i.e., three parts water to one part concentrated emulsion) and the filter paper was dried 24 hr at room temperature prior to use. After the filter paper was positioned between the two chambers, one chamber was pressurized to 14.7 psig with an air-mixture of known percent carbon dioxide and oxygen. The temperature was maintained at 5°C. At regular time intervals small samples were withdrawn from the other chamber and analyzed for carbon dioxide and oxygen in a Quintron gas chromatograph, Model H-3.

For the green snap bean experiments, the product was harvested one day and transported to the laboratory for testing the next. The beans were thoroughly washed with cold tap water and rinsed for 1 min with a 200 ppm active chlorine solution. Approximately 500-g samples were dipped in the diluted (3/1) emulsion solution for 2 min, shaken for 3–5 min to drain off excess emulsion and finally dried for about 15 min in front of a fan. Weighed samples, approximately 100g, of treated beans were put in jars, sealed with rubber stoppers and placed in chambers at 4.4°C or 7°C. At 24-hr intervals, a 100-ml sample of gas from each jar was analyzed for CO_2 and O_2. The jar was inverted for 1 hr to allow renewal of the atmosphere in the jar.

Color of the beans was measured on the Hunter Color and Color Difference Meter. Beans cut into small pieces were spread onto a glass petri dish, covered with a black cylinder and the "L", "a" and "b" values recorded.

Microbial infection, general appearance and crispness of the beans were assessed subjectively. Weight losses of the beans were not measured since most of the water loss was due to moisture migration from the beans to the surface of the storage containers. This was not considered as transpiration losses and thus was not measured.

RESULTS & DISCUSSION

BOTH WAX EMULSIONS reduced the CO_2 permeability of filter paper. The anionic polyethylene (Sta-Fresh 715) reduced the first order response to CO_2 permeability by 4.75%; whereas, the emulsified vegetable oil (Sta-Fresh 800) reduced CO_2 permeability by approximately 43%.

The respiration data (based on CO_2 evolution) for untreated beans and beans treated with the emulsified vegetable oil and stored at 4.4°C are shown in Figure 1. The waxed and unwaxed control beans behaved similarly. The higher rate of respiration for the first day of storage reflects the high initial temperatures of the beans. For up to 10 days' storage, the evolution of CO_2 for the waxed beans was lower than for the unwaxed. Analysis of covariance indicated a significant difference at the 90% confidence level. The significant increase in apparent respiration after 10 days' storage was due to microbial growth (including mold) on the surface of the beans. These same trends were also evident in the oxygen consumption data. At 7°C, the vegetable oil coated beans exhibited the same respiration rate as the untreated beans.

An analysis of the data for the beans coated with the anionic polyethylene emulsion (Sta-Fresh 715) showed no sig-

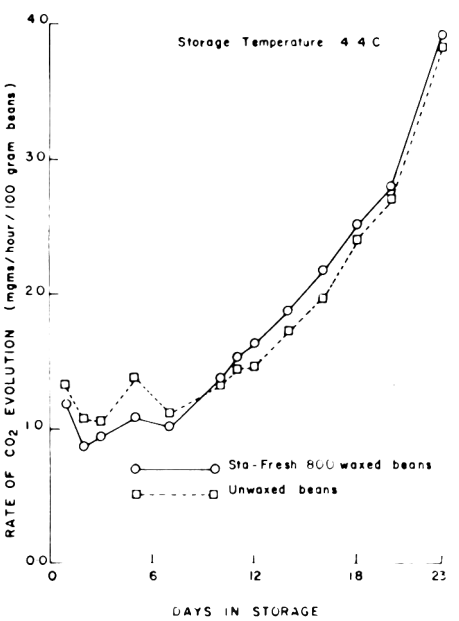

Fig. 1—Rates of CO_2 evolution of waxed beans (with Sta-Fresh 800) and control beans, stored at 4.4°C for 23 days.

nificant difference from untreated, control beans at both 4.4 and 7°C.

At 4.4°C, the vegetable oil coated beans were generally in better condition with less browning and were crisper than the control. The unwaxed beans showed signs of white fungus attack about 2 days before there was any noticeable fungus on the waxed beans. Color analysis indicated a darker color due to increased browning for the unwaxed beans.

Additional detail on this research is available (Singh, 1972).

CONCLUSIONS

RAPIDLY RESPIRING vegetables such as snap beans have a very short storage life. Wax emulsions provide a thin coating on the vegetable and may reduce respiration rates. A pilot test on an emulsified vegetable oil coating (Sta-Fresh 800) and an anionic polyethylene emulsion (Sta-Fresh 715) indicated that although both significantly reduced CO_2 permeability of filter paper, the vegetable oil should be more effective in reducing respiration rates of vegetables.

Tests with green snap beans waxed with the vegetable oil emulsion (Sta-Fresh 800) and stored at 4.4°C did show a lower respiration rate as compared to the unwaxed control beans. The waxed beans were crisper and had less browning for the first 14-day storage period compared to control.

The anionic polyethylene coating (Sta-Fresh 715) did not significantly reduce respiration rates of the snap beans at either 4.4 or 7°C. This suggests that the storage life of green snap beans may be extended slightly and higher quality maintained by using an emulsified vegetable oil coating and storing at approximately 5°C.

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Ms received 10/6/72; revised 12/18/72; accepted 12/21/72.

Research supported by the College of Agricultural & Life Sciences, University of Wisconsin-Madison.

The authors express their thanks for the financial grant by the Biotron Committee, University of Wisconsin-Madison.

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

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A Research Note

EFFECTS OF HEAT PROCESSING ON THE RETENTION OF VITAMIN B_6 IN LIMA BEANS

INTRODUCTION

RECENTLY, the nutritional effects of heat processing of foods have been an issue of concern. The vitamin and mineral contents of a variety of heat-processed foods have been presented in the literature (Clifcorn, 1948; Ives et at., 1946; Orr, 1969; and Teply et al., 1953). Among these, little data are available for the effects of heat processing on vitamin B_6 (Everson et al., 1964; Lushbough et al., 1959; Richardson et al., 1961; Schroeder, 1971).

Vitamin B_6 can be obtained from a variety of foods to fulfill the Recommended Dietary Allowance of 2 mg/day for adults. The three forms of the vitamin [pyridoxine (PN), pyridoxal (PL) and pyridoxamine (PM)] are widely distributed in nature with the activity residing mainly in the phosphorylated form. Normally, PN and PL are the major forms found in plant products whereas PL and PM are predominant in animal products (Schroeder, 1971). The present study was conducted to investigate the effects of commercial heat processing conditions on the retention of these various forms of vitamin B_6.

EXPERIMENTAL

TWO VARIETIES of dry large lima beans were heat processed (Raab, 1972). After soaking the beans were blanched in water or steam at 100°C for 10 min. Cans were filled with 250 g of blanched, rinsed beans and 230 ml brine containing 1.5% NaCl, 3.0% sugar, and 0.1% monosodium glutamate.

The cans containing the White Ventura Nematode-resistant variety were heat processed at 116°C for 34, 42, or 48 min corresponding to F_o lethal values of 6, 8 and 10 assuming an initial temperature of 60°C (Lewis, 1972). Total vitamin B_6 was determined after each heat treatment and compared to that of the dry beans.

The White Ventura (65) variety, grown in three California localities, was retorted at 119°C for 30 min (F_o of 8) after water blanching. Dry and canned samples were analyzed for total vitamin B_6 and for the three vitamin B_6 forms after chromatography. A Dowex AG 50W-X8 ion exchange column was used to chromatograph extracted samples. Several methods of chromatographic separation resulted in poor resolution (MacArthur and Lehmann, 1959; Hedin, 1963; Storvick et al., 1964; Toepfer and Lehmann, 1961; Toepfer and Polansky, 1970). The type and amount of eluant buffers and the rate of flow were varied in an attempt to improve resolution (Raab, 1972).

Ground, dry bean samples or canned beans plus brine homogenate were assayed for vitamin B_6. The experimental procedures for sample extraction and microbiological assay with *S. carlsbergensis* were those of Toepfer and Polansky (1970) with certain modifications (Raab, 1972). Standard curves were made for each of the three vitamin B_6 forms, both chromatographed and nonchromatographed. Values for total vitamin B_6 were interpolated from the PN standard curve and reported as units of PN per gram of dry weight. To assume equal activity of all three forms for man, using the PN standard curve, may introduce some error. The response of the yeast to PM was reported to be 60–70% of that of PL and PN.

RESULTS & DISCUSSION

THE EFFECT of varied heat processing treatments on total vitamin B_6 is shown in Table 1. Water- and steam-blanched beans were analyzed after the given heat treatments. Analysis of variance shows no significant difference among the four steam-blanched samples or among the four water-blanched samples. There is also no significant difference between water-blanched as compared with steam-blanched samples processed for any one of the four lengths of time. The difference between the vitamin B_6 contents of dry and canned samples on a dry weight basis is statistically significant at the 1% level, however. The data show that, within the range of heat treatments employed, heat processing after blanching did not result in decreased vitamin B_6 content. Therefore, loss of vitamin B_6 occurred during blanching. To compute retention, the vitamin B_6 content of the dry beans was considered as 100% retention. In the water-blanched beans, 76–81% of the vitamin was retained and 83–87% in the steam-blanched beans. Steam blanching may have somewhat improved retention, but the difference between steam and water blanching was not significant. Blanching losses may occur as a result of leaching of the water soluble vitamin. If so, a reduction in blanching time might be beneficial to increase vitamin B_6 retention.

The data in Table 2 report the effect

Table 1—Vitamin B_6 values for large lima beans (White Ventura Nematode-resistant variety) after varied heat processing treatments[a]

Heat treatment min at 116°C	Samples: Water blanched	Steam blanched
	μg pyridoxine/g[b]	
0	5.99 ± 0.17	6.53 ± 0.31
34	5.76 ± 0.22	6.54 ± 0.27
42	6.14 ± 0.07	6.25 ± 0.22
48	5.89 ± 0.27	6.25 ± 0.20
Dry bean	7.56 ± 0.17	

[a]Each value represents an average of three extractions and assays ± standard deviation.
[b]Dry weight basis corrected for solids added in brine.

Table 2—Vitamin B_6 values for dry and canned large lima beans (White Ventura 65 variety) grown in three localities[a]

Locality	Samples: Dry	Canned
	μg pyridoxine/g[b]	
Westley	7.14 ± 0.57	5.92 ± 0.29
King City	7.72 ± 0.07	6.16 ± 0.30
Santa Maria	7.85 ± 0.31	5.79 ± 0.27

[a]Each value represents an average of three to seven extractions and assays ± standard deviation.
[b]Dry weight basis corrected for solids added in the brine

of heat processing on total vitamin B_6 in water blanched White Ventura (65) variety beans. Dry and canned samples from each of the three localities were analyzed. No significant difference in total vitamin B_6 content was apparent in this study among lima beans grown in the three localities. The vitamin B_6 values for the dry beans are slightly greater than others in the literature for dry large lima beans (Polansky, 1969). However, the data compare favorably to those in Table 1 for dry beans and water-blanched heat-processed beans. Calculations show 70–80% retention of the vitamin in the canned beans plus brine homogenate for each locality.

The data obtained for the chromatographed samples had large standard deviations. Presumably, the inconsistency of the results from experiment to experiment was due in large part to inadequate resolution of the three vitamin B_6 forms. A general trend in samples from all three localities, however, was the similar distribution of the vitamin B_6 forms with PN and PL being the predominant forms. The similar distribution of the three forms in dry and canned samples indicated that the relative proportions were not changed by heat processing.

The significance of this research is the stability of vitamin B_6 under the given heat processing conditions after blanching. Study over a wider range of heat treatments would be of interest. Further investigation of the effects of heat processing on the individual forms of the vitamin should be undertaken.

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Ms received 9/19/72; revised 12/18/72; accepted 12/21/72.

This work was supported in part by Graduate Training Grant No. FD-0008, U.S. Public Health Service.

The donation of the lima beans by Carl L. Tucker, Dept. of Agronomy, University of California, is gratefully acknowledged.

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A Research Note
COMPOUNDS CONTRIBUTING TO HEAT-INDUCED BITTER OFF-FLAVOR IN AVOCADO

INTRODUCTION

THE OCCURRENCE of off-flavors in heated avocado (Cruess et al., 1951) prevents thermal processing, and greatly limits the utilization of the fruit in processed foods. Several authors (Harrold, 1931; Bilger et al., 1932; Bates, 1970) have attempted to identify the source of the off-flavor, but none has succeeded in isolating relevant pure compounds. The mechanisms of heat-induced off-flavor have generally been considered to be extremely complex and a result of synergistic effects between several compounds. Current increased interest in avocado processing has led us to attempt to define more clearly the components contributing to this off-flavor.

Kashman et al. (1969) have reported the isolation of an interesting series of compounds containing a 17 carbon chain, and one member of the series was found to be active in inhibiting certain gram positive bacteria (Neeman et al., 1970). Our studies indicate an additional role for these interesting materials.

MATERIALS & METHODS

RIPE AVOCADO of the Ettinger variety were carefully peeled, pitted and sliced. The thin slices were heated at 100°C for 15 min and stored at a temperature of −45°C for 16 hr. Freeze drying was carried out at −18°C to +20°C over 7 hr at 0.1 atmosphere pressure.

The dried avocado (40g portions) were extracted three times in a blender, each time with a fresh portion of absolute ethanol (300 ml). An average of 26g (65%) of extracted lipids were thus obtained from each 40-g portion of dried avocado, after vacuum filtration of the pulp.

The ethanolic filtrate was concentrated at 40–50°C at reduced pressure to one half its original volume, and stored at −28°C for 16 hr. The precipitated triglycerides were removed by centrifugation at 10,000 rpm for 30 min. This process was then repeated twice more on the ethanolic solution before evaporation to dryness. In this way, approximately 80% of the total lipids were removed by winterization. The ethanol-soluble fraction was characterized as bitter and the precipitated triglycerides as non-bitter.

The ethanol-soluble lipids were then fractionated by column chromatography with both silica gel (70–325 mesh, 50g adsorbent per g lipids) and fluorosil (100–200 mesh, 70g adsorbent per g lipids) employing solvent mixtures of increasing polarity as eluent (hexane, benzene, chloroform, methanol). The fractions obtained were then examined organoleptically and by TLC, and further separations were carried out on the bitter fractions.

Flavor evaluations were performed by a taste panel, trained to recognize bitterness in the form of solutions in safflower oil at 0.2g/100 cc. All samples were evaluated in the form of a triangle test with solutions of quinine serving as the standard in each test. Each sample was given a score of 4 (very bitter), 3 (bitter), 2 (slightly bitter) or 1 (not bitter), and an average score was then calculated for each sample.

RESULTS & DISCUSSION

CONDITIONS for investigating the chromatographic separation of the cold ethanol-soluble lipids from heated avocado

Table 1—Fractions obtained from silica gel column

Fraction	Amount (g)	Eluent	No. of tasters	Average taste score
I	1.15	benzene	12	1.58
II	0.07	benzene	–	–
III	2.57	benzene	10	1.00
IV	0.05	benzene	–	–
V	0.07	benzene-$CHCl_3$(1:1)	–	–
VI	0.39	$CHCl_3$	10	2.10
VII	1.21	$CHCl_3$	9	1.89
VIII	0.43	$CHCl_3$	14	2.36
IX	1.74	$CHCl_3$-MeOH(9:1)	12	2.33
X	0.35	$CHCl_3$-MeOH(9:1)	14	1.65
XI	0.76	$CHCl_3$-MeOH(9:1)	12	3.00
XII	0.85	$CHCl_3$-MeOH(4:1)	14	2.36
Not recovered	0.36	–	–	–
Total	10.00			

Table 2—Fractions obtained from fluorosil column

Fraction	Amount (g)	Eluent	No. of tasters	Average taste score
A	0.06	hexane	–	–
B	0.54	hexane	12	1.50
C	0.16	hexane	–	–
D	0.02	hexane	–	–
E	0.01	hexane-$CHCl_3$(9:1)	–	–
F	0.05	hexane-$CHCl_3$(3:1)	–	–
G	2.14	hexane-$CHCl_3$(2:1)	12	1.50
H	1.10	hexane-$CHCl_3$(2:1)	–	–
I	0.44	hexane-$CHCl_3$(2:1)	11	2.45
J	0.79	hexane-$CHCl_3$(1:1)	14	2.50
K	0.36	hexane-$CHCl_3$(1:2)	13	2.23
L	0.31	hexane-$CHCl_3$(1:2)	10	2.90
M	1.79	$CHCl_3$	10	3.10
N	1.10	$CHCl_3$-MeOH(19:1)	10	3.30
O	0.76	$CHCl_3$-MeOH(19:1)	10	2.80
Not recovered	0.37	–	–	–
Total	10.00			

were examined with neutral alumina, basic alumina, silica gel and fluorosil. The latter two absorbents were found to be most effective in leading to relevant separations (as evidenced by TLC) and high recovery of the lipids. The crude fractions obtained from these initial separations were evaluated by the taste panel, and results are shown in Tables 1 and 2.

The bitter fraction XI was further fractionated on a second silica gel column to give 0.199g of 1-acetoxy-2,4-dihydroxy-n-heptadeca-16-en (1), m.p. 56°C (methanol). The infrared spectrum of the compound in chloroform has bands at 3490–3430 cm^{-1}, 3290 cm^{-1} (hydroxyl groups), 1735 cm^{-1} (acetate) and 1640 cm^{-1} (double bond). The mass spectrum demonstrates unequivocally that the acetate group is in the 1-position, and the NMR spectrum as well as elemental analysis are also in agreement with this structure.

Fraction 0 was also further separated on a silica gel column to give 0.269g of 1,2,4-trihydroxy-n- heptadeca-16-en (2), m.p. 56.5–58.0°C. The infrared spectrum of (2) contains a broad band at 3520–3420 cm^{-1} (hydroxyl groups), but no band in the ester region. The NMR, mass spectrum, and elemental analysis are also in agreement with this structure.

Further chromatographic separation of fraction M showed that both compounds 1 and 2, as well as other unidentified components were present.

In addition to compounds (1) and (2), we have prepared 1-acetoxy-2-hydroxy-4-keto-n-heptadeca-16-en (3) by chromic oxide oxidation of (1) according to Kashman et al. (1969), and all three of the pure compounds were evaluated by the taste panel. Compounds (1) and (2) were found to be moderately bitter (average scores of 2.95 and 3.00 respectively), whereas compound (3) was nonbitter (average score of 1.50).

In an additional experiment, non-heated avocado flesh was extracted in the cold with absolute ethanol, and after precipitation and removal of the triglycerides, the solvent was evaporated at room temperature. A control sample of heated avocado flesh was likewise treated as described. TLC comparison of the two lipid mixtures demonstrated conclusively that the concentrations of (1) and (2) were greatly increased by the heat treatment. In fact, only a barely detectable trace of (1) was found in the lipids of the avocado that had not been treated. It seems likely, therefore, that these compounds make some contribution to the heat-induced bitter off-flavor of avocado.

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Ms received 8/29/72; revised 11/29/72; accepted 12/5/72.

We are indebted to the Israel Fruit Marketing Board for financial support of this work and to A. El-Chasid, A. Even-chen and G. Yitzchaki for technical assistance.

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A Research Note

ALPHA-AMYLASE IN SWEET POTATOES. A Comparison Between the Amyloclastic and Chromogenic Starch Methods of Analysis

INTRODUCTION

ALPHA-AMYLASE ACTIVITY is frequently determined by an amyloclastic method which is based on the rate of enzymatic hydrolysis of a soluble starch substrate. This method depends on the fact that as sample α-amylase fragments the substrate, there is a progressive decrease of the blue color formed when iodine is added. A typical amyloclastic procedure is that of the AOAC (1960) which with certain modifications (Briggs, 1961) is used extensively. However, the AOAC or similar procedures are not ideal due to technical deficiencies such as difficulty in reproducible preparation of the starch substrate and considerable operator attention for each assay.

Recently insoluble derivatives of starch have been developed which can serve as substrate for α-amylase assay (Rinderknecht et al., 1967). These chromogenic substrates, prepared by reacting starch with organic dyes, are hydrolysed by α-amylase causing a release of color into the solution. The amount of color released is proportional to enzyme activity in the sample. Although measurement of α-amylase activity with chromogenic starch substrates has received considerable attention in medical research (Hall et al., 1970), little has been published on the use of this type substrate in food or agricultural areas. Recently, however, an assay for α-amylase in malt was reported which employed chromogenic starches as substrate (Barwald et al., 1970).

During our investigation of α-amylase levels in sweet potato varieties, it became evident that a simpler more rapid procedure was needed. Consequently, we decided to develop a chromogenic starch method applicable to sweet potatoes and compare it with the AOAC procedure.

MATERIALS & METHODS

SWEET POTATOES were obtained from a root maintenance collection of the N.C. Agricultural Experiment Station at Clayton, N.C. The roots were harvested and cured at 90–95°F and 85% relative humidity for 7 days. After curing, they were stored at 60°F until used. The varieties used were Centennial, Porto Rico, Pelican Processor, Australian Canner and Jewel.

The roots were hand peeled, grated and juice equivalent to 23–25% of the weight was obtained by squeezing the grated roots in a Carver Press. The juice was centrifuged at 3000G for 10 min (5°C) and the supernatant collected. Toluene was added as a preservative. Amylase assays were run at 40°C and 60°C on the juice or dilutions thereof. The 60°C assay was run on several varieties at two different times while the 40°C assay was run on the same varieties at one time period only.

Alpha-amylase assays

Chromogenic starch method. Amylopectin Azure (Calbiochem) was the substrate used. A 2% slurry of substrate was prepared using 0.02M phosphate buffer (pH 6.0) containing 0.3% NaCl. The substrate was placed in a water bath at the reaction temperature for 15 min. Juice samples or dilutions (0.3 ml) were put in test tubes and placed in the water bath for 0.5 min. The substrate was swirled until a homogenous suspension was obtained and then 2.7 ml was rapidly pipetted into the sample tube.

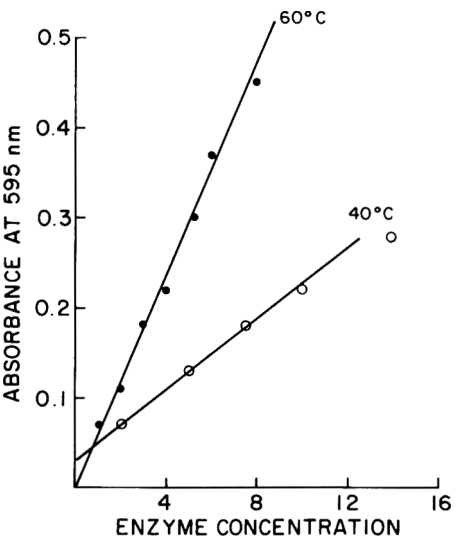

Fig. 1—Effect of enzyme concentration on the hydrolysis of amylopectin azure substrate (2% pH 6.0). Juice from Porto Rico variety sweet potatoes incubated 15 min at 40°C and 60°C. The enzyme concentration shown is a dilution factor times 100 of a stock juice sample.

The tube was shaken and a timer started. Other samples were treated the same way with the reaction being started at 1 min intervals. After exactly 15 min, the reaction of the first tube was stopped by adding 1.2 ml of 5% v/v trichloracetic acid followed by vigorous mixing. Following the same sequence and time interval, other samples were assayed. Blanks were prepared by adding trichloracetic acid solution to the juice sample prior to substrate addition. Several blanks were run with each batch of samples. After stopping the reaction, the tubes were centrifuged for 3 min in a clinical centrifuge and filtered through Whatman #2 paper. Absorbance was then measured at 595 nm. The calculations are as follows:

$$\text{APA Amylase Units per ml} = \frac{As_{595} - As_{595}\ (\text{blank})}{As_{595}\ \text{of 0.1M } CuSO_4} \times \text{Dilution factor.}$$

The 0.1M copper sulfate solution is used as an arbitrary standard so that results using different spectrophotometers can be compared (Hall et al., 1970).

AOAC method. The method used was similar to that as described in AOAC (1960) except that 60°C and 40°C were used as the assay temperatures and all solutions were buffered to pH 6.0. Enzyme activities are expressed as dextrinizing units (DU) per ml of juice.

RESULTS & DISCUSSION

ALTHOUGH OPTIMUM reaction conditions for assay of sweet potato α-amylase have been described for the AOAC procedure (Ikemiya and Deobald, 1966) further studies were necessary before the chromogenic starch method could be applied. Time-reaction course studies showed that enzyme activity is linearly related to reaction time up through 20 min. Further studies using sweet potato juice as the enzyme source focused on the relationship between enzyme levels and the amount of blue color released (Fig. 1). At an assay temperature of 60°C, linear kinetics are observed up to 0.45 absorbance units. At 40°C, however, deviation is noted. Extrapolation to "zero" enzyme concentration gives an absorbance of about 0.03 units. In addition the linear portion at 40°C extends only to 0.225. Consequently, at this temperature a correction factor must be applied by

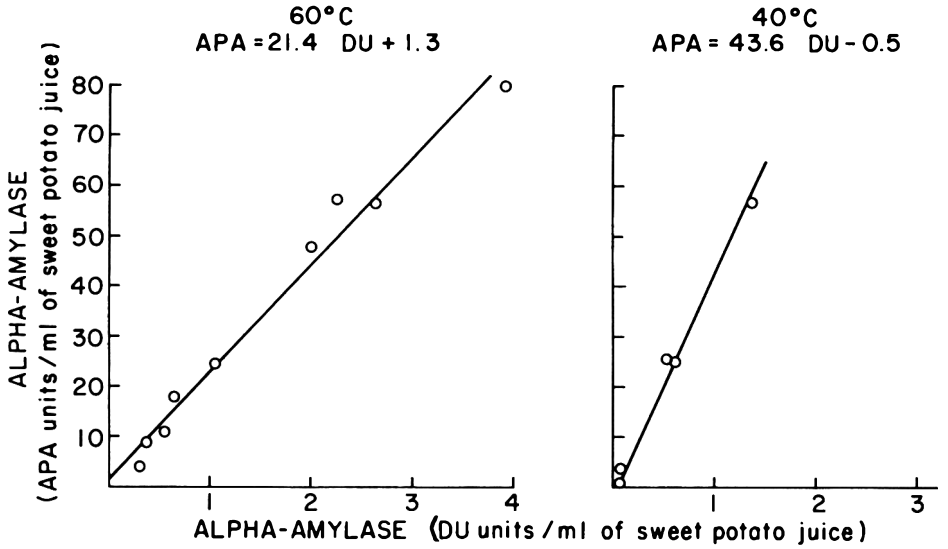

Fig. 2—Comparison of α-amylase activity in sweet potato varieties as determined by Blue Starch (APA Units) and AOAC (DU Units) methods. Assays run for 15 min at 40°C and 60°C. Regression equations are listed for each temperature.

subtracting the absorbance at "zero" enzyme concentration (about 0.03) from the actual absorbance of the sample. When this correction is applied, the method agrees well with the AOAC method which is linear at 40°C (Fig. 2). Samples having absorbancies greater than 0.450 at 60°C or 0.225 at 40°C must be diluted to bring them within these limits. The experiments described above indicate that the chromogenic starch method is valid for sweet potato α-amylase when performed within the prescribed limits.

Several sweet potato varieties were assayed for α-amylase by both the AOAC and chromogenic starch (blue starch) methods and the results compared in Figure 2. It should be noted that there is only slight deviation of the data points from the regression lines at both 40°C and 60°C.

Since sweet potatoes are a rich source of β-amylase, any method for assay of α-amylase must be free from interference by β-amylase. The AOAC method eliminates this problem by using β limit dextrin as the substrate. Our data show significant correlation for enzyme activities measured by the two methods (Fig. 2). This is convincing evidence that the blue starch method is not affected by β-amylase and that α-amylase activities from this newly developed method are comparable to those measured by the AOAC procedure. The chromogenic starch method shows adequate precision with a standard deviation of ± 4.7%.

The level of α-amylase is extremely important when sweet potatoes are processed into dehydrated flakes. Fresh-dug roots are starchy and give poor quality flakes unless considerable starch conversion occurs (Hoover, 1967). Since conversion times are governed by α-amylase activity, a knowledge of enzyme levels is of significant value in determining optimum processing conditions (Deobald et al., 1968). Presently the AOAC method is used for this determination. However, the simplicity, precision and sensitivity of the chromogenic starch method make it readily adaptable as a routine method for controlling conversion in flake production.

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Ms received 10/28/72; revised 1/2/73; accepted 1/8/73.

We thank Dr. Daniel T. Pope of the Dept. of Horticultural Science, North Carolina State University for making the sweet potato varieties available to us. In addition, we thank Dr. Pope and Dr. Leaton H. Kushman of the U.S. Dept. of Agriculture for constructive advice while this study was in progress.

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A Research Note
ASCORBIC ACID CONTENT OF ARTIFICIALLY RIPENED TOMATOES

INTRODUCTION

TOMATOES are a good source of ascorbic acid. In fact, the 6.5 million tons of tomatoes produced annually (1971) in this country could provide approximately one-third of the recommended dietary allowance for Americans. An average value for the ascorbic acid content of tomatoes is 23 mg per 100g of fresh fruit (USDA, 1963).

In a previous communication (Malewski and Markakis, 1971) we reported that four cultivars of tomatoes grown in the field reached their highest ascorbic content about 6 wk after anthesis (flower opening) and just before the entire fruit turned red. Except for processing or local use, however, tomatoes are harvested at an earlier stage of development, usually mature-green, and shipped to large markets where they are ripened. In this note we report on the ascorbic acid content of tomatoes ripened off the vine.

Two specific reports on the effect of storage on the ascorbic content of unripe tomatoes are those by Scott and Kramer (1949) and Craft and Heinze (1954). Both groups used the Rutgers variety in their studies but the first group reported loss of ascorbic acid during storage of green-mature tomatoes at 70°F and riper tomatoes at 35–50°F, while the second group found no pronounced change in the ascorbic acid content of mature-green tomatoes stored at 32°, 40°, 50°, 65° and 75°F for periods up to 14 days.

MATERIALS & METHODS

THIRTY 4-in. seedlings of the tomato cultivars No. 71-816 and 71-817, supplied by the Horticulture Dept. of this University were planted on June 25, 1971 on the University farm. Individual blossoms were tagged a month later and tomatoes were harvested when a whitish area appeared around the blossom end of the green fruit, about 5 wk after anthesis. The tomatoes were stored in stainless steel cabinets thermostatically controlled at 55°, 60°, 65° and 70°F and with relative humidity of 86–92%. At 0, 2, 4, 6, 8 and 10 days from the day of harvesting three tomatoes were randomly sampled and analyzed for ascorbic acid by the 2,6-dichloroindophenol method (AOAC, 1970). Three titrations on a composite liquid sample were applied. The replicate titrations differed by less than 5% from each other. An analysis was also made on tomatoes left on the vine until they turned almost fully red and tomatoes left 1 wk longer.

Table 1—Ascorbic acid content (mg/100g fruit) of two tomato cultivars harvested mature-green and ripened at four different temperatures

Cultivar	Temp. °F	Days in storage 0	2	4	6	8	10
71-816	55	23.2	22.7	21.3	20.2	17.1	16.8
	60	23.2	22.8	20.6	19.0	17.9	17.6
	65	23.2	22.9	21.1	18.2	–	–
	70	23.2	22.9	20.0	18.2	–	–
71-817	55	23.6	22.9	21.1	19.7	16.6	16.0
	60	23.6	22.5	20.7	19.1	17.0	16.6
	65	23.6	23.1	19.9	18.0	–	–
	70	23.6	22.5	19.2	17.9	–	–

RESULTS & DISCUSSION

THERE WAS a decline in the ascorbic acid content of the tomatoes with time of storage at all temperatures and for both cultivars (Table 1). Regarding the progress of ripening, on the sixth day of storage, approximately 90% of the fruit stored at 70°F was fully red, 80% of the fruit at 65°F, 50% of those at 55°F and 40% of those at 40°F. The color of the tomatoes stored at the two lower temperatures continued to turn red up to the tenth day, while the texture remained firm; some loss in firmness was observed in the tomatoes stored at the higher temperatures beyond the sixth day.

Tomatoes left on the vine and analyzed when they were almost fully red (40 days from anthesis) contained 24.8 and 25.2 mg/100g fruit for cultivars No. 71-816 and 71-817, respectively. One week later, the fruit on the vine were overripe and contained 19.2 and 18.8 mg/100g, respectively. It may be concluded that table-ripe tomatoes of the cultivars, and under the conditions of growth and ripening used in this study, contained one-fourth to one-third more ascorbic acid when they were ripened on the vine rather than artificially. This finding should not be surprising since other varietal characteristics (aroma, taste, etc.) of fruits develop to a different degree when ripening occurs on the plant rather than artificially.

During December, 1972, 2-lb samples of tomatoes ripened artificially at three different commercial ripening establishments (as deduced from the labels) were obtained from local food stores; they contained 14.4, 14.6 and 14.8 mg of ascorbic acid per 100g of fruit.

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Ms received 12/19/72; accepted 1/8/73.
Journal Article No. 6192 of the Michigan Agricultural Experiment Station.
The authors appreciate Prof. S. Honma's assistance.

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A Research Note

THE USE OF SQUID IN MEAT EMULSIONS

INTRODUCTION

THE FISH SAUSAGE industry has become a major food source in a number of countries particularly Japan. Ueno (1963) reported that Dr. Wataru Sinidu developed the first fish sausage in Japan in 1937. Eiichi (1963) gave the procedure for making the same fish sausage. Analyses of the resulting sausage showed moisture, 68.8%; fat, 5.9%; and protein, 15%. Not over 25% of squid is used in making these sausage or poor elasticity will result. He stated that pork skins may be added to strengthen the elasticity. These publications do not specifically define elasticity. In some cases it appears to refer to fat emulsifying capacity rather than to the water-holding capacity and at times it refers to the final texture of the finished product. Squid is readily distributed throughout the world oceans, yet it is one of the least expensive protein foods. Taniknrva et al. (1960) found that of all ingredients used in Japanese fish sausage, squid had the lowest bacterial counts.

The objectives of this study were to determine the potential of squid as an emulsifying agent and to determine the effect of varying amounts of squid in various formulations on the characteristics of the fish sausage produced.

MATERIALS & METHODS

THREE DIFFERENT LOTS of frozen squid were obtained and permitted to thaw sufficiently to enable separation of the individual squid. The head sections were removed and evisceration was then performed. Skinning of the squid was conducted with care being taken to eliminate the pigment pores located under the epidermis as they will turn red when heated. The flesh was ground five times through a 3 mm plate and thoroughly mixed. The ground squid was packaged in 1 lb packs and immediately frozen until ready for use.

The analyses for moisture, fat and protein were determined according to AOAC (1965). The salt-soluble protein content was determined by a modified procedure of Saffle and Galbreath (1964). A 125-g sample of squid was added to 500 ml of 3% saline and the pH adjusted to 6, with 0.1N HCl. The mixture was blended for 1 min in an Osterizer and permitted to stand 3 min. The sides of the jar were swabbed and the slurry was blended for one additional min and then centrifuged at 10,000 × G for 10 min at 2°C. The solute was filtered through glass wool and again centrifuged at the same speed, time and temperature. The solute was filtered through glass wool and the protein content determined. Results were expressed as percent of the total protein which was salt soluble. The procedure of Carpenter and Saffle (1964) was used to determine the emulsifying ability of the salt-soluble protein in squid. The protein contents of the salt-soluble extracts were adjusted at 10 mg per ml. The emulsifying ability was expressed as ml of oil emulsified by 100g of salt-soluble protein. The binding efficiency of the squid was determined by multiplying the percent of the total protein which was salt-soluble times the ml of oil emulsified per hundred mg of salt-soluble protein. The formulations which were used are presented in Table 1.

The processing procedure for formula 1 was to place all of the squid, ice and salt in a silent cutter and to chop for 3 min. The remainder of the ingredients was placed in the cutter and chopped until a temperature of 10°C was reached. The mixture was passed through a colloid mill and stuffed in 24 mm NoJax casings. The processing procedure for formula 2 was to add all the ingredients except one-half of the squid to the silent cutter and chop to a temperature of 10°C. The mixture was passed through a colloid mill, placed back in the silent cutter and the second half of the squid was added and chopped for 1-1/2 additional min and stuffed into 24 mm NoJax casings. The processing procedure for formula 3 was to place all the ingredients except the squid in the silent cutter and chop to a temperature of 10°C. The mixture was passed through a colloid mill, placed back in the silent cutter and the squid added. The mixture was chopped for 1-1/2 additional min and stuffed into 24 mm NoJax casings. The processing procedure for formula 4 was that the pork skins were soaked for 24 hr in 4°C water. The soaked skins were ground through a 1/2-in grinding plate and then through a 1/8-in. grinder plate. All the ingredients except the squid for formula 4 were placed in a silent cutter and chopped to 10°C. The squid was added and the entire emulsion was chopped for 1-1/2 additional min. The emulsion was placed in metal molds and cooked in 60°C water for 30 min. The temperature of the water was increased to 85°C until the internal temperature of the product was 80°C. Emulsions prepared according to the first four formulations were placed in a smokehouse at a temperature of 39°C for 30 min and then the temperature of the smokehouse was raised 10°C every 15 min until a temperature of 77°C was attained. The product remained in the smokehouse at this temperature until an internal temperature of 71°C was reached. The product was then showered with cold water for 5 min and placed in a 4°C cooler over night.

RESULTS & DISCUSSION

THE CHEMICAL analyses, protein-moisture ratio, pH, percent of the total protein which was salt-soluble and the amount of oil which could be emulsified by 100 mg of salt-soluble protein of the squid are gived in Table 2.

The most expensive ingredients in red meat emulsions are those having a high protein content and having a high percentage of the protein which is salt-soluble. Squid had an analysis of 17.7% protein which is comparable to lean cow meat which has a protein analysis of

Table 1—Formulations

Ingredients	Batch No. 1	2	3	4	5
Squid (lb)	2.3	2.3	1.15	1.15	1.0
Pork fat (lb)	1.0	1.0	1.0	1.0	0.5
Ice (lb)	1.1	0.8	1.1	0.8	0.8
NFDM[a] (lb)	0.3	0.3	0.3	0.3	0.3
Flour (lb)	0.19	0.19	0.19	0.19	0.19
NaCl (lb)	0.1	0.1	0.1	0.1	0.1
Seasoning (g)	6	6	6	6	6
Beef (lb)	–	–	1.15	1.15	–
Pork skins (lb)	–	–	–	0.5	–
Flounder (lb)	–	–	–	–	2.5

[a]NFDM = Nonfat dried milk

Table 2–Analyses of squid

	No.1	No.2	No.3
Percent moisture	79.83	79.78	79.71
Percent protein	17.44	17.71	17.63
Percent fat	.34	.34	.41
Protein-moisture ratio	4.78	4.51	4.52
pH	6.5	6.5	6.5
% Salt-soluble protein	69.27	72.68	67.52
ml Oil emulsified per 100 mg salt-soluble protein	32.4	32.1	32.5
Emulsifying efficiency	25.66	25.51	25.71

17–19%. However, the protein-to-moisture ratio for squid is 4.5 which is higher than the 3.5–3.6 average found in most red meats. This would indicate that the amount of ice or water added to the formulation should be less if squid is used than in the case of red meats. The percent of the total protein which was salt-soluble averaged 69.8% for the squid as compared to only 38.2% for cow meat as shown by Saffle and Galbreath (1964). It is apparent that there is almost twice as much salt-soluble protein available to act as an emulsifier for squid as compared to cow meat which is considered to be one of the better emulsifying types of red meats in emulsions. The ml of oil emulsified per 100 mg of salt-soluble protein for squid was 32.4. This value is comparable to cow meat which was reported to be 36.6 ml by Carpenter and Saffle (1964). The binding efficiency of squid is 25.66 as compared to 13.98 for cow meat. These data indicate that squid should have high fat emulsifying properties in actual meat emulsions.

The flesh of raw squid is tough and rubbery in texture, but an interesting phenomenon occurs when it is placed in a silent chopper in the presence of salt and ice. After chopping for a minute or two the squid is soupy in texture and actually can be poured from one container to another. The protein is apparently so soluble in the presence of salt that most of the squid will go into solution and leave very little material for structure or texture properties.

In the case of the product made with formula number one, the finished product had no evidence of fat separation; however, the sausage was extremely soft and mushy. Because of the extremely soft texture the second formulation was prepared by emulsifying all the ingredients except one-half of the squid. The second half of the squid was then added to the other emulsified ingredients and the mixture was given only an additional chopping time of 1-1/2 min. In addition, the amount of ice was decreased. This was done to decrease the time that all the ice and salt would be in contact with all the squid. It was hoped that the solubilization of the squid would be decreased. However, the finished product was extremely poor in texture and was more like a paste. Because of the poor texture the third formulation was attempted in which one-half the squid was replaced by lean beef. However, again the texture was extremely poor but was somewhat better than products from formula number two or three. In an attempt to improve texture, pork skins were added to formula four. The use of pork skins is fairly common in red meat emulsions particularly in plants covered by state inspection. In addition, it has been reported that pork skins in fish sausage sometimes add to the elasticity of the fish emulsion. However, the product made according to formulation number four still had poor texture properties and would not be accepted commercially. It was firmer in texture than products made from the formulations one, two or three. The last formulation was attempted to prepare a fish spread. The fish spread prepared according to the last formulation had desirable texture and appeared to have some merit.

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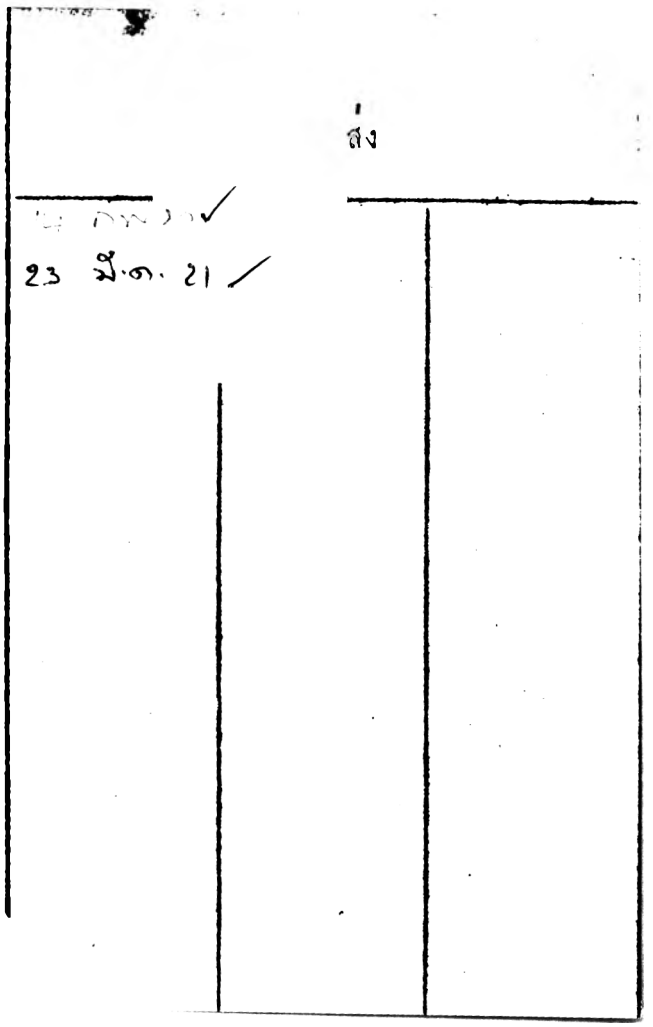
Ms. received 10/10/72; revised 12/15/72; accepted 12/19/72.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