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JOURNAL of FOOD SCIENCE

BASIC SCIENCE

1101 Blueing discoloration in canned crab meat (Cancer magister)—*J.K. Babbitt, D.K. Law and D.L. Crawford*

1104 Reaction of hydrogen peroxide with myoglobins—*K.S. Morey, S.P. Hansen and W.D. Brown*

1108 Oxidation-reduction potential and growth of Salmonella and Pseudomonas fluorescens—*J.L. Oblinger and A.A. Kraft*

1113 Factors associated with postmortem increase of extractable Ca in chicken breast muscle—*R. Nakamura*

1115 Substrate inhibition of chicken muscle lactate dehydrogenase as a function of temperature —*J.D. Ehmann and H.O. Hultin*

1119 Temperature dependence of the Michaelis constant of chicken breast muscle lactate dehydrogenase — *J. D. Ehmann and H.O. Hultin*

1122 Effect of copper binding on the autoxidation of oxymyoglobins—*M. Bembers, N.Y. Zachariah, L.D. Satterlee and R.M. Hill*

1124 Influence of epinephrine and calcium upon glycolysis, tenderness and shortening of sheep muscle—*A.M. Pearson, W.A. Carse, C.L. Davey, R.H. Locker, C.J. Hagyard and A.H. Kirton*

1128 The association of protein solubility with physical properties in a fermented sausage—*J.T. Klement, R.G. Cassens and O.R. Fennema*

1132 Direct enzymatic conversion of lactose to acid: Lactose dehydrogenase—*D.G. Wright and A.G. Rand Jr.*

1136 Effect of hybrids and processing on the dimethyl sulfide potential of sweet corn—*M.P. Williams and P.E. Nelson*

1139 Food use of soybean 7S and 11S proteins. Extraction and functional properties of their fractions—*K. Saio, T. Watanabe and M. Kaji*

1145 Consistency of aqueous soybean-rice mixtures—*E.M. Ahmed, Y. Yoo and R.P. Bates*

1149 Polyphenol oxidase activity and browning of mango fruits induced by gamma irradiation—*P. Thomas and M.T. Janave*

1153 Use of limonate dehydrogenase of Arthrobacter globiformis for the prevention or removal of limonin bitterness in citrus products—*S. Hasegawa, L.C. Brewster and V.P. Maier*

1156 Effect of pH on the activity of Schizosaccharomyces pombe—*H.Y. Yang*

1158 Tocopherols in the unsaponifiable fraction of cocoa lipids—*J.A. Erickson, W. Weissberger and P.G. Keeney*

1162 Production of β-nitropropionic acid in foods—*T. Iwasaki and F.V. Kosikowski*

1166 Effect of dipicolinate on vegetative cells of Bacillus—*M.L. Fields*

1169 Rheological properties of hydrocolloids—*E. Balmaceda, C-K. Rha and F. Huang*

1174 Microscopic investigations of the freeze drying of volatile-containing model food solutions—*J.M. Flink, F. Gejl-Hansen and M. Karel*

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

A PUBLICATION OF THE INSTITUTE OF FOOD TECHNOLOGISTS

— CONTENTS (CONTINUED) —

1178 Structural functions of taste in the sugar series: Cyclohexane polyols as sweet analogues of the sugars—*G.G. Birch and M.G. Lindley*

1182 Effects of supersaturation and temperature on the growth of lactose crystals—*P. Jelen and S.T. Coulter*

1186 Effects of certain salts and other whey substances on the growth of lactose crystals—*P. Jelen and S.T. Coulter*

APPLIED SCIENCE and ENGINEERING

1190 Techniques for stunning channel catfish and their effects on product quality—*T.S. Boggess Jr., E.K. Heaton, A.L. Shewfelt and D.W. Parvin*

1194 Quality comparisons of albino and regular (gray) channel catfish—*E.K. Heaton, T.S. Boggess Jr., R.E. Worthington and T.K. Hill*

1197 Effect of packaging on shelf life of frozen silver salmon steaks—*T.C. Yu, R.O. Sinnhuber and D.L. Crawford*

1200 Phospholipid changes and lipid oxidation during cooking and frozen storage of raw ground beef—*J.D. Keller and J.E. Kinsella*

1205 Effects of end point and oven temperatures on beef roasts cooked in oven film bags and open pans—*T.A. Shaffer, D.L. Harrison and L.L. Anderson*

1211 Effect of varying the ratio of beef and textured vegetable protein nitrogen on protein nutritive value for humans—*C. Kies and H.M. Fox*

1214 Relationship of tenderness measurements made by the Armour tenderometer to certain objective, subjective and organoleptic properties of bovine muscle—*F.C. Parrish Jr., D.G. Olson, B.E. Miner, R.B. Young and R.L. Snell*

1220 ^{15}N tracer studies of nitrite added to a comminuted meat product—*J.G. Sebranek, R.G. Cassens, W.G. Hoekstra, W.C. Winder, E.V. Podebradsky and E.W. Kielsmeier*

1224 Measurement of sausage emulsion stability by electrical resistance—*A. Haq, N.B. Webb, J.K. Whitfield, A.J. Howell and B.C. Barbour*

1228 Changes in meat components during fermentation, heat processing and drying of a summer sausage—*F.B. Wardlaw, G.C. Skelley, M.G. Johnson and J.C. Acton*

1232 Chicken lipid changes during cooking in fresh and reused cooking oil—*W.T. Lee and L.E. Dawson*

1238 Use of γ-irradiation to prevent aflatoxin production in bread—*L.B. Bullerman, H.M. Barnhart and T.E. Hartung*

1241 Microbiology of a modified procedure for cooling pasteurized salt yolk—*K. Ijichi, J.A. Garibaldi, V.F. Kaufman, C.A. Hudson and H. Lineweaver*

1244 Method for estimating limonin content of citrus juices—*J.H. Tatum and R.E. Berry*

1247 Storage quality of bananas packaged in selected permeability films—*H. Daun, S.G. Gilbert, Y. Ashkenazi and Y. Henig*

RESEARCH NOTES

1251 Influence of freshness and color on pototo chip sensory preferences—*J.A. Maga*

1253 The possibility of recognizing irradiated and nonirradiated potatoes by their weight loss—*G. Magaudda*

1255 Monosodium glutamate ingestion and thirst production—*P.E. Araujo, D. Hourihan and J. Mayer*

1256 Hydrocyanic acid in canned sweet cherries—*G.S. Stoewsand and J.L. Anderson*

1257 Simple determination of phosphorus in pet foods—*T. Surles and J. Darby*

1258 Relation between shear force and tenderness of beef—*A.W. Khan, C.P. Lentz and L. van den Berg*

1260 Activity of antioxidants in fresh fish—*C.W. Sweet*

1262 A qualitative and quantitative study of sugar-alcohols in several foods—*J. Washüttl, P. Riederer and E. Bancher*

1264 Dependence of fruit-berry wines' stability on the content of mineral and nitrogenous components—*S. Gorinstein*

1267 Use of forceps in sterility testing: A possible source of contamination—*L.M. Corson, G.M. Evancho and D.H. Ashton*

ii Memo from the Scientific Editor

1269 Annual Indexes

November-December 1973
Volume 38 : Number 7

JOURNAL of FOOD SCIENCE

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Memo *FROM THE SCIENTIFIC EDITOR*

DURING 1973, seven full 192-page issues of the *Journal of Food Science* were needed to prevent a delay in publication of accepted manuscripts. This allowed publication of manuscripts in approximately 7 months following the date received in the Editor's office.

The number of manuscripts submitted to the *Journal of Food Science* has steadily increased over the past 3 years. The number submitted was as follows:

(a) October 1, 1970 to October 1, 1971 – 338
(b) October 1, 1971 to October 1, 1972 – 449
(c) October 1, 1972 to October 6, 1973 – 533

In fact, more manuscripts were accepted during the period October 1, 1972 to October 6, 1973 than were submitted during the same period in 1970–1971. This was true even with a rejection rate of over 20%. Six issues of the *Journal* containing 224 pages per issue are planned for 1974. This should maintain the publication schedule as desired. If the submission of manuscripts continues to increase, a switch to monthly publication may be necessary in the following year.

The publication of a symposium from the annual meeting was delayed and finally published without one of the manuscripts. I hope this problem can be avoided in the future. The June, 1973 IFT meeting had several excellent symposia. One will be chosen for publication in its entirety. Several excellent review papers from other symposia will also be published individually.

The quality of manuscripts continues to improve. Also authors, generally, were more responsive to deadlines for revision of manuscripts. The Editorial Board and the large number of other reviewers have been prompt and extremely helpful in suggestions on manuscripts sent to them for review. Errors in this year's issues of the *Journal* have been minimal. You can thank Mrs. Anna May Schenck for this accomplishment. Mrs. Schenck does the copy editing and many other detailed tasks involved in publishing the *Journal*. Also, I can't forget the great support job by the members of the Chicago IFT office. With excellent support like this, the Editor's job is a busy but pleasant one.

The Editorial Board was placed on a 3-year term basis this year. I wish to thank the members leaving the Board for their hard work and assistance in reviewing manuscripts. Welcome to the new members joining the Board.

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ABSTRACTS:

IN THIS ISSUE

BLUEING DISCOLORATION IN CANNED CRAB MEAT (Cancer magister). J.K. BABBITT, D.K. LAW & D.L. CRAWFORD. *J. Food Sci.* 38, 1101–1103 (1973)–TCA and acetone-extracted, dialyzed crab meat were used to investigate the blueing reaction. The extracted, dialyzed crab meat (EDCM) did not turn blue in the presence of iron or copper during retorting. When dopa (3,4-dihydroxyphenylalanine) was added to EDCM, particularly in the presence of iron or copper under alkaline conditions, the characteristic blue pigment was formed. Citric acid and ascorbic acid prevented the blueing that occurred when dopa and iron or copper were retorted with EDCM. The change in phenolic compounds in crab appears to have a significant role in causing the blueing observed in canned crab meat. Total phenolic content of freshly caught crab held at 2°C rose from 140.8 to 322.5 μg/g after 4 days with a change in pH from 6.65 to 7.65.

REACTION OF HYDROGEN PEROXIDE WITH MYOGLOBINS. K.S. MOREY, S.P. HANSEN & W.D. BROWN. *J. Food Sci.* 38, 1104–1107 (1973)–Both sperm whale and yellowfin tuna myoglobins reacted with approximately equimolar quantities of hydrogen peroxide to form red-brown pigments with somewhat different spectra. Following reduction with dithionite the whale pigment became green, while similar treatment of tuna myoglobin resulted in a brown colored product. Myoglobins from either source when reacted with peroxide and then denatured by sodium dodecyl sulfate produced green pigments. Cysteine or homocysteine were without effect on this reaction. The green pigment formed is different from that previously known to be obtained upon reaction of denatured tuna myoglobin, cysteine and trimethylamine oxide.

OXIDATION-REDUCTION POTENTIAL AND GROWTH OF Salmonella AND Pseudomonas fluorescens. J.L. OBLINGER & A.A. KRAFT. *J. Food Sci.* 38, 1108–1112 (1973)–Measurements were made of changes in Eh, pH and viable cell numbers of *Salmonella* and *Pseudomonas fluorescens* in pure and mixed culture at 15, 30 and 37°C. At initial Eh values of +485 mv (pH 7.0), *Salmonella* strains showed intense reducing capacity at 30 and 37°C; at 15°C, the lag phase was extended considerably before reducing conditions occurred. *P. fluorescens* demonstrated gradual reducing activity at 15 and 30°C. In most instances, greater growth occurred at 15°C than at 30 or 37°C. These findings may have application to associative growth of these types of bacteria on meat and poultry products.

FACTORS ASSOCIATED WITH POSTMORTEM INCREASE OF EXTRACTABLE Ca IN CHICKEN BREAST MUSCLE. R. NAKAMURA. *J. Food Sci.* 38, 1113–1114 (1973)–To investigate the factors associated with the release of Ca from chicken breast muscle during postmortem aging, the effects of both pH change and ATP disappearance were studied. The results indicate that the release of Ca and loss of ATP occurred concomitantly in muscle tissues during postmortem aging, and did not depend on postmortem glycolysis.

SUBSTRATE INHIBITION OF CHICKEN MUSCLE LACTATE DEHYDROGENASE AS A FUNCTION OF TEMPERATURE. J.D. EHMANN & H.O. HULTIN. *J. Food Sci.* 38, 1115–1118 (1973)–The lactate dehydrogenase of chicken breast muscle is subject to substrate inhibition. The type of inhibition observed with the soluble enzyme in the presence of NAD+ depends on the temperature, but there is little quantitative effect of temperature on the inhibition. Binding of the enzyme to a particulate fraction of the homogenized muscle significantly reduces the inhibition. In addition, while there is still a significant inhibition of the bound LDH at 23°, 16° and 4°C, no inhibition is observed at 40°C. The difficulties of using results obtained with chicken breast muscle LDH at one temperature to predict its behavior at a different temperature are pointed out.

TEMPERATURE DEPENDENCE OF THE MICHAELIS CONSTANT OF CHICKEN BREAST MUSCLE LACTATE DEHYDROGENASE. J.D. EHMANN & H.O. HULTIN. *J. Food Sci.* 38, 1119–1121 (1973)–A study was made of the effect of temperature on V_{Max} and K_M of chicken breast muscle lactate dehydrogenase (LDH) both in the soluble form and when bound to the particulate fraction of the homogenized tissue. The enzyme displayed typical temperature dependence under conditions of V_{Max} (saturating substrate concentrations) with an activation energy of approximately 9500 cal per deg mole. The value of K_M, however, decreased significantly with decreasing temperature. Since the concentration of pyruvate in situ is probably close to the K_M value of the enzyme, lowering the temperature of chicken breast muscle may not bring about a concomitant reduction in LDH activity. This was shown to be the case in vitro. Bound LDH had a lower V_{Max} and a slightly higher K_M than soluble LDH at all temperatures, but the effect of temperature on both forms of the enzyme was similar.

EFFECT OF COPPER BINDING ON THE AUTOXIDATION OF OXYMYOGLOBINS. M. BEMBERS, N.Y. ZACHARIAH, L.D. SATTERLEE & R.M. HILL. *J. Food Sci.* 38, 1122–1123 (1973)–The purpose of this study was to determine the effect Cu^{2+} ion has on the stability of porcine, ovine and bovine MbO_2. The large rate constants obtained after Cu^{2+} addition to a MbO_2 solution indicated that copper concentrations of 200 molar equivalents or greater are needed to significantly affect the autoxidation rate of MbO_2. Porcine MbO_2 was able to bind more Cu^{2+} ion than was either bovine or ovine MbO_2, yet its stability was least affected by the bound copper. Binding of Cu^{2+} ion was shown to alter the isoelectric point of porcine MetMb and MbO_2.

INFLUENCE OF EPINEPHRINE AND CALCIUM UPON GLYCOLYSIS, TENDERNESS AND SHORTENING OF SHEEP MUSCLE. A.M. PEARSON, W.A. CARSE, C.L. DAVEY, R.H. LOCKER, C.J. HAGYARD & A.H. KIRTON. *J. Food Sci.* 38, 1124–1127 (1973)–Grinding of the LD (longissimus) and BF (biceps femoris) muscles accelerated glycolysis. Addition of either $CaCl_2$ or epinephrine to ground muscle caused a further small but consistent acceleration of glycolysis, while a combination of $CaCl_2$ and epinephrine resulted in an even greater increase in the glycolytic rate. Although the levels of ATP, creatine phosphate and inorganic phosphate tended to account for the decline in pH, they were not always in the expected order. Acceleration of glycolysis did not appear to be related to activation of phosphorylase a. With intact muscle, $CaCl_2$ caused extensive shortening alone or in combination with either epinephrine or propranolol, while the latter two compounds did not significantly influence shortening. Tenderometer values were significantly higher for all muscles injected with $CaCl_2$, whereas, neither epinephrine nor propranolol influenced tenderness. These results support the view that Ca^{2+} ions play a key role in acceleration of glycolysis and in shortening of muscles excised immediately postmortem.

THE ASSOCIATION OF PROTEIN SOLUBILITY WITH PHYSICAL PROPERTIES IN A FERMENTED SAUSAGE. J.T. KLEMENT, R.G. CASSENS & O.R. FENNEMA. *J. Food Sci.* 38, 1128–1131 (1973)–Batches of summer sausage (a fermented semi-dry variety) were prepared under commercial conditions. Samples were withdrawn from the smokehouse at various time intervals and tested for pH, shear force and solubility of the sarcoplasmic and myofibrillar protein fractions. With increasing process time, pH declined and the solubility of the nitrogen-containing compounds in the myofibrillar fraction decreased markedly, whereas the solubility of the sarcoplasmic proteins decreased less and at a lower pH. Nonprotein nitrogen remained relatively constant except for an increase at the end of the process under conditions of high temperature and acidity. Shear force data showed that an increase in firmness developed simultaneously with decreasing pH; this occurred at the same time that solubility of the two protein fractions (mainly myofibrillar) decreased. Two complete experimental runs were conducted and the results were similar.

DIRECT ENZYMATIC CONVERSION OF LACTOSE TO ACID: LACTOSE DEHYDROGENASE. D.G. WRIGHT & A.G. RAND JR. *J. Food Sci.* **38**, 1132–1135 (1973)–Enzymatic conversion of lactose to an acid by lactose dehydrogenase was studied as a mechanism for the acidification of milk. The crude enzyme preparation exhibited the characteristics of an oxygenase, while the partially purified form could only utilize an artificial hydrogen acceptor. Under optimal conditions and the presence of a hydrogen acceptor, both forms of lactose dehydrogenase were able to convert lactose to lactobionic acid as demonstrated by a reduction in pH. The crude lactose dehydrogenase reduced the pH of buffered lactose solutions and skim milk with atmospheric oxygen as an acceptor. The addition of hydrogen peroxide and catalase to milk increased the initial rate of reaction, but the level of acidity produced by lactobionic acid was insufficient for milk coagulation. When lactase (beta-galactosidase) was incorporated into milk, along with hydrogen peroxide and catalase, the pH reduction catalyzed by crude lactose dehydrogenase was clearly adequate for curd formation.

EFFECT OF HYBRIDS AND PROCESSING ON THE DIMETHYL SULFIDE POTENTIAL OF SWEET CORN. M.P. WILLIAMS & P.E. NELSON. *J. Food Sci.* **38**, 1136–1138 (1973)–21 sweet corn hybrids were evaluated for their dimethyl sulfide (DMS) potential. The effect of blanching and of blending cut whole kernel sweet corn on the DMS potential was also investigated. A routine laboratory method was modified quantitatively to prepare sweet corn serum for DMS determinations using an equilibration method. Significant differences in the DMS potentials were found between the hybrids. Hybrids which were used for fresh market and had shorter maturation periods showed higher DMS potentials than those hybrids used only for processing. Blanching results in extreme losses of the DMS precursor and therefore the DMS potential of sweet corn. Blending of hybrids with different DMS potentials proved to be a significant method for selecting the final DMS concentration in thermally processed sweet corn.

FOOD USE OF SOYBEAN 7S AND 11S PROTEINS. Extraction and Functional Properties of Their Fractions. K. SAIO, T. WATANABE & M. KAJI. *J. Food Sci.* **38**, 1139–1144 (1973)–Calcium precipitation behavior of soybean 7S and 11S proteins were described and discussed. Results suggest that 11S protein precipitates more rapidly with less calcium than 7S protein. Based on the difference of precipitation behavior between the two proteins, the paper proposes a practical method to fractionate 7S and 11S protein rich fractions (7S PRF and 11S PRF), using a direct extraction from defatted meal with a dilute calcium chloride solution. The ratio of 11S to 7S in 11S PRF is 3:1 and that in 7S 1:4. Functional properties of the fractions were investigated, with prepared calcium gel, heat-induced gel, cheese-like food, *kamaboko*-like food (fish paste product) and a sausage-like food. Preliminary results attest existence of a remarkable difference between 7S PRF and 11S PRF.

CONSISTENCY OF AQUEOUS SOYBEAN-RICE MIXTURES. E.M. AHMED, Y. YOO & R.P. BATES. *J. Food Sci.* **38**, 1145–1148 (1973)–The influence of formulation, thermal processing and storage duration on the consistency of processed aqueous soybean-rice mixtures was studied. The mixtures were thermally processed achieving a sterilizing F_o value of 15 min. Soybean:rice ratio exerted a large influence on the consistency of processed and unprocessed mixtures. Increased viscometric constants ("n", "K") and apparent viscosities (U_a) were obtained as the mixtures contained larger amounts of soybeans. Addition of fish protein concentrate (FPC) to the aqueous mixtures resulted in increased thickening of the homogenates. Thermally processed homogenates exhibited much higher apparent viscosities than those obtained by the addition of FPC. Processed samples thickened upon storage. Homogenate prepared from 1:1 soybean-rice mixture with added FPC would be more suited as a weanling food than that prepared from 3:1 mixture.

POLYPHENOL OXIDASE ACTIVITY AND BROWNING OF MANGO FRUITS INDUCED BY GAMMA IRRADIATION. P. THOMAS & M.T. JANAVE. *J. Food Sci.* **38**, 1149–1152 (1973)–The browning of mango fruits gamma irradiated at doses exceeding 75 Krad in the preclimacteric state was due to a several-fold increase in polyphenol oxidase activity. Unirradiated fruits possessed little or no polyphenol oxidase activity. The increase in enzyme activity was dose-dependent and paralleled with external manifestation of radiation injury. Enzyme preparation from 100 and 200 Krad irradiated fruits catalyzed the oxidation of both mono- and diphenols and showed a wide substrate specificity. p-cresol and dopamine were the most reactive substrates among the mono- and diphenols, respectively. The enzyme exhibited a pH optimum of 6.5–7.0 and was inhibited by sodium diethyl dithiocarbamate. Visible damage was accompanied by accumulation of phenolics and decreased ascorbic acid levels. The possible correlation between radiation induced browning, enzyme activity and disappearance of ascorbic acid are discussed.

USE OF LIMONOATE DEHYDROGENASE OF Arthrobacter globiformis FOR THE PREVENTION OR REMOVAL OF LIMONIN BITTERNESS IN CITRUS PRODUCTS. S. HASEGAWA, L.C. BREWSTER & V.P. MAIER. *J. Food Sci.* **38**, 1153–1155 (1973)–Conditions for the effective use of limonoate dehydrogenase of *Arthrobacter globiformis* in limonin debittering of citrus products were determined. Treatment of fresh navel orange juice with the enzyme immediately after juice extraction converted limonoate A-ring lactone to nonbitter 17-dehydrolimonoate A-ring lactone thereby preventing its ultimate conversion to limonin. As a result limonin levels in the enzyme-treated juice were kept well below those of the untreated juice. With processed juice or lemon seed slurry, it was necessary to first treat the sample with alkali to hydrolyze the D-ring lactone of limonin before enzyme treatment. Addition of NAD to the reaction mixtures allowed more effective use of the enzyme, particularly at low pH levels. Although the enzyme has optimum activity at pH 9.5, it has been shown to have sufficient activity in citrus products at lower pH to have potential for commercial debittering.

EFFECT OF pH ON THE ACTIVITY OF Schizosaccharomyces pombe. H.Y. YANG. *J. Food Sci.* **38**, 1156–1157 (1973)–A grape must was adjusted with concentrated HCl to different pH values between 2.50 and 3.50. Inoculated with *Schizosaccharomyces pombe* and *Saccharomyces cerevisiae*, the fermentation rate was found to be in direct proportion to the initial pH. At pH 2.50, *Sch. pombe* was still active, but the fermentation rate was reduced to 0.04 $days^{-1}$. The activity of *Sac. cerevisiae* was greatly reduced when the pH was below 2.82 and ceased to be active at pH 2.50. Malic acid was fully utilized by *Sch. pombe* during the fermentation. *Sac. cerevisiae* utilized only about 5% of the malic acid and consequently resulted in wines of higher acidity and lower pH.

TOCOPHEROLS IN THE UNSAPONIFIABLE FRACTION OF COCOA LIPIDS. J.A. ERICKSON, W. WEISSBERGER & P.G. KEENEY. *J. Food Sci.* **38**, 1158–1161 (1973)–The tocopherols in cocoa beans and its products were investigated using gas chromatography and mass spectrometry. One GLC peak accounted for over 90% of total peak area from the unsaponifiable lipid fraction of shell-free matter. This was a mixture of β- and γ-tocopherol which could not be separated. α-Tocopherol was a minor component, except in shell lipid where it was the principal tocopherol. Cocoa beans contained 128–273 μg β-/γ-tocopherol/g lipid. Total tocopherol in shell lipid was 2–10 times greater. Roasting had little effect, but reduced levels were found in the lipids of cocoa powder and moldy beans and in cocoa butter after storage.

PRODUCTION OF β-NITROPROPIONIC ACID IN FOODS. T. IWASAKI & F.V. KOSIKOWSKI. *J. Food Sci.* **38**, 1162–1165 (1973)–Five *Aspergillus oryzae* strains among 18 different molds, produced β-nitropropionic acid (β-NPA) in Nakamura media. *Asp. oryzae* 12892 and *Asp. oryzae* Higati gave maximum levels of 1279 and 111 mg β-NPA/liter respectively. No β-nitropropionic acid was produced during the growth of seven *Penicillium roqueforti* and *Penicillium camemberti* strains. Maximum β-nitropropionic acid was attained in 5–7 days but after 20 days, the acid disappeared. Nitrate, but no nitrite, was detected in media cultured with *Asp. oryzae* ATCC 12892. Cheese curds, soybeans and peanuts in the order given provided the most suitable substrates for β-nitropropionic acid production by *Aspergillus* species. Maximum levels in cheese curds by *Asp. oryzae* ATCC 12892 and by *Asp. oryzae* Higati were 427 and 203 mg β-NPA/Kg appearing after 5 days. β-nitropropionic acid was not detected in commercial food grade fungal enzyme preparations and was detected in traces only on surfaces of old ripened cheese heavily infested with molds, yeast and bacteria.

ABSTRACTS:

IN THIS ISSUE

EFFECT OF DIPICOLINATE ON VEGETATIVE CELLS OF Bacillus. M.L. FIELDS. *J. Food Sci.* 38, 1166–1168 (1973)–47 strains of *Bacillus stearothermophilus* were inhibited by 20 mM dipicolinate (DPA) at pH 7.0. Only *Bacillus badius* was inhibited at 20 mM whereas only *Bacillus subtilis* and *Bacillus licheniformis* grew well at 53 mM DPA. *Bacillus lentus, Bacillus circulans* and *Bacillus megaterium* grew slightly at 53 mM DPA. Nine species failed to grow at 53 mM DPA. Loss of respiration, amino acids and sugars occurred when vegetative cells were exposed to DPA at 50.6 and 53 mM DPA. It is hypothesized that these damages to the cell were the cause of the inhibition.

RHEOLOGICAL PROPERTIES OF HYDROCOLLOIDS. E. BALMACEDA, C-K. RHA & F. HUANG. *J. Food Sci.* 38, 1169–1173 (1973)–Rheological properties of hydrocolloids used as binders in food products are presented. The power law constants and yield values were obtained from experimental data, using the general power law equation as a model. The effects of time, temperature and concentration on rheological behavior were also studied.

MICROSCOPIC INVESTIGATIONS OF THE FREEZE DRYING OF VOLATILE-CONTAINING MODEL FOOD SOLUTIONS. J.M. FLINK, F. GEJL-HANSEN & M. KAREL. *J. Food Sci.* 38, 1174–1178 (1973)–The behavior of n-butanol and n-hexanol during the freeze drying of aqueous carbohydrate solutions is investigated on a microscopic level. A specially constructed microscope stage permits observations of freezing and freeze-drying, as well as post-drying treatments, at high magnification. The freeze-drying microscope stage is fully instrumented to permit quantitative evaluation of the results. Results demonstrate that the volatile alcohols, soluble at the initial solution concentration, will be precipitated as droplets during the cooling of the solution. The influence of the growing ice crystals on the segregation of the droplets can be seen. The movement of the freeze-drying front through the sample is observed for its influence on the retention of the volatile. The results have permitted visualization of the kinetic factors which influence the retention of organic compounds by freeze-dried carbohydrate solutions.

STRUCTURAL FUNCTIONS OF TASTE IN THE SUGAR SERIES: CYCLOHEXANE POLYOLS AS SWEET ANALOGUES OF THE SUGARS. G.G. BIRCH & M.G. LINDLEY. *J. Food Sci.* 38, 1179–1181 (1973)–Paper reports the sensory properties of some cyclohexane polyols containing from one to six hydroxyl substituents in relation to their configuration, conformation and analogy with the sugars. Concludes that changes in configuration in polyhydroxyl cyclohexanes cause alteration in their sweetness values from those of the sweetest known sugars down to nothing. These changes cannot be explained simply on the basis of hydrogen bonding and imply changes in binding mode due to alterations in the lipophilic character of the molecules.

EFFECTS OF SUPERSATURATION AND TEMPERATURE ON THE GROWTH OF LACTOSE CRYSTALS. P. JELEN & S.T. COULTER. *J. Food Sci.* 38, 1182–1185 (1973)–Crystallization velocities of lactose were studied at 30, 50, 60 and 70°C. Large replicate single lactose crystals were grown in supersaturated lactose solutions of 10, 15, 20 and 25g lactose/100g water in excess of the solubility limits at each of these temperatures. Increasing temperature increased the growth rate of the crystals, measured as their average weight gains, within the whole experimental range in solutions of comparable supersaturation ratio (C/C_S). In solutions of the same excess supersaturation, no significant weight increase was observed above 50°C. The growth rate increased with 2–2.5 power of excess supersaturation, depending on temperature. Composite effects of temperature and excess supersaturation on the growth rate can be well described by a second power statistically significant polynomial.

EFFECTS OF CERTAIN SALTS AND OTHER WHEY SUBSTANCES ON THE GROWTH OF LACTOSE CRYSTALS. P. JELEN & S.T. COULTER. *J. Food Sci.* 38, 1186–1189 (1973)–The effects of various amounts of KCl, $CaCl_2$, NaH_2PO_4 and lactic acid on growth rate of replicate single lactose crystals were studied at 50°C with a moderately supersaturated model lactose solution. The most significant effect was exhibited by $CaCl_2$ which tripled the growth rate of the lactose crystals at the 10% impurity level. Similar effect was exhibited by NaH_2PO_4 at the 20% impurity level. A less significant rate increase was found with KCl up to the 5% impurity level; a retarding effect was observed at higher concentrations of KCl or with lactic acid. Identical growth rates were found in the model lactose-in-water solution and in deproteinated, clarified cheddar cheese whey of the same lactose/water concentration with no additives. The addition of NaH_2PO_4 to the clarified whey increased the growth rate by 30%.

TECHNIQUES FOR STUNNING CHANNEL CATFISH AND THEIR EFFECTS ON PRODUCT QUALITY. T.S. BOGGESS JR., E.K. HEATON, A.L. SHEWFELT & D.W. PARVIN. *J. Food Sci.* 38, 1190–1193 (1973)–Channel catfish were slaughtered by five methods to determine their effect on the quality of frozen, stored fish. The procedures were: (1) tail bleeding; (2) CO_2 gas; (3) a-c shock; (4) d-c shock; and (5) icing. The CO_2 gas and bleeding treatments generally produced more complete bleeding and a whiter-fleshed product, while the a-c stunning and icing produced a lower quality product. The incidence of hemorrhaging and blood retention down the middle vein was more noticeable in the a-c shock and the ice treatments for both the fresh and cooked fish. The ice-packed fish were harder to skin, and the distended gall bladder made it hard to remove the heads without rupturing it, thus staining the flesh around the collar bone. Aroma and flavor of the stored and cooked product were influenced more by the stunning procedures than were appearance, color and texture. The lower quality and reduced storage life were attributed largely to incomplete bleeding. The results suggest that revised slaughtering techniques should be considered for the improvement of product quality.

QUALITY COMPARISONS OF ALBINO AND REGULAR (GRAY) CHANNEL CATFISH. E.K. HEATON, T.S. BOGGESS JR., R.E. WORTHINGTON & T.K. HILL. *J. Food Sci.* 38, 1194–1196 (1973)–Differences in quality of albino and regular channel catfish were evaluated. Data on the percentage of edible carcass and of waste including head, viscera, visceral fat, skin and miscellaneous waste for albino and regular catfish are presented. Moisture and total fat content of the raw and cooked flesh and shear press and organoleptic data of the cooked fish are included. Regression analyses showed that differences in dressing percentage associated with fish size and harvest date and between albino and regular fish were nonsignificant. The proportions of head, skin and viscera decreased while visceral fat increased as fish size increased from 0.25 lb to 1.05 lb (live weight). Moisture of the raw and cooked flesh was significantly higher in the 0.75 lb fish than in the restocked fish. Total fat content of the raw fish was similar for all sizes, but in the cooked fish it increased significantly with size. Panels preferred the whiter color of the albino to the regular fish. The 0.50–0.75 lb (live weight) fish of both colors were preferred to other sizes. The albino and regular channel catfish differed significantly in content of a fatty acid tentatively identified as heptadecenoic (17:1).

EFFECT OF PACKAGING ON SHELF LIFE OF FROZEN SILVER SALMON STEAKS. T.C. YU, R.O. SINNHUBER & D.L. CRAWFORD. *J. Food Sci.* **38**, 1197–1199 (1973)–Silver salmon steaks were vacuum packed in low oxygen permeability pouches with or without the addition of antioxidants and were subjected to storage at both constant −18°C and fluctuating temperatures. The control fish were packed in 1 mil low density polyethylene bags. Results of sensory evaluation showed that the control sample became unacceptable at the end of 9 months storage at constant −18°C. The vacuum-packed samples were judged desirable and of good quality through 12 months storage. Storage temperature fluctuations were found to cause severe quality damage to the fish.

PHOSPHOLIPID CHANGES AND LIPID OXIDATION DURING COOKING AND FROZEN STORAGE OF RAW GROUND BEEF. J.D. KELLER & J.E. KINSELLA. *J. Food Sci.* **38**, 1200–1204 (1973)–The composition and changes in the concentration of lipids and phospholipid classes in three grades of commercial ground beef (hamburgers) were determined during cooking and frozen storage of raw patties at −18°C. Ground round, ground chuck and ground beef contained 9.9, 13.8 and 25.6g total lipid and 0.54, 0.46 and 0.33g phospholipid per 10g meat respectively. Phosphatidylcholine (Pc) (>55%) and phosphatidylethanolamine (Pe) (24%) were the major components of the phospholipids of all samples. The fatty acids of both of these classes were mostly unsaturated. Pe contained 39% arachidonic acid. During cooking the meat lost 30–40% of the fresh weight. The amount of lipid material lost in the drip increased with original fat content. Negligible amounts of PL were lost in the drip. Pc and Pe decreased in most cases during cooking and frozen storage of raw meat at −18°C. The lysophosphatides tended to increase during these treatments. Arachidonic acid showed the greatest decrease. Concurrent increases in TBA values and carbonyls indicated that lipid oxidation occurred during cooking and storage of raw patties. It is suggested that limited hydrolysis of meat lipids may also occur during cooking.

EFFECTS OF END POINT AND OVEN TEMPERATURES ON BEEF ROASTS COOKED IN OVEN FILM BAGS AND OPEN PANS. T.A. SHAFFER, D.L. HARRISON & L.L. ANDERSON. *J. Food Sci.* **38**, 1205–1210 (1973)–Top round roasts were cooked from the frozen state in oven film bags (moist heat) and in open pans (dry heat) at 177° or 205°C to an internal temperature of 60°, 70° or 80°C. Generally, palatability of meat was similar for roasts cooked by dry and moist heat to the same end-point temperature. Moist heat required less time than dry heat to cook meat to 80°C. Cooking by dry heat to 60°, 70° or 80°C produced less weight loss than cooking by moist heat. Beef cooked by moist heat appeared more well-done than beef cooked by dry heat to the same end point. Type of heat × end point temperature interactions indicated differences between moist and dry heat at specific end-point temperatures.

EFFECT OF VARYING THE RATIO OF BEEF AND TEXTURED VEGETABLE PROTEIN NITROGEN ON PROTEIN NUTRITIVE VALUE FOR HUMANS. C. KIES & H.M. FOX. *J. Food Sci.* **38**, 1211–1213 (1973)–The project objective was to obtain information on which to base protein equivalency curves on the replacement value of TVP protein for beef protein. During five randomly arranged periods of 5 days each, nitrogen intake from test sources was maintained constant at 4.0g nitrogen/subject/day supplied by the following ratios of beef to TVP nitrogen: 4/0, 3/1, 2/2, 1/3 and 0/4. Nitrogen balances of eight adult human subjects while receiving these diets were as follows: −0.44, −0.56, −0.75, −0.90 and −1.11g nitrogen per day, respectively. No mutual supplementation effect was demonstrated; hence, values were predictable on a simple ratio basis.

RELATIONSHIP OF TENDERNESS MEASUREMENTS MADE BY THE ARMOUR TENDEROMETER TO CERTAIN OBJECTIVE, SUBJECTIVE AND ORGANOLEPTIC PROPERTIES OF BOVINE MUSCLE. F.C. PARRISH JR., D.G. OLSON, B.E. MINER, R.B. YOUNG & R.L. SNELL. *J. Food Sci.* **38**, 1214–1219 (1973)–Three studies were carried out to determine the relationship of tenderness measurements made by Armour Tenderometer (AT) to certain objective and subjective measures of bovine muscle composition and palatability. In the first study, AT measurements were compared with Warner-Bratzler (W-B) shear, chemical analyses and organoleptic evaluation on steaks taken from wholesale ribs after 1, 3 or 7 days postmortem. Correlation coefficients for the three separate days ranged from −0.52 to −0.03 between AT and W-B, from −0.49 to −0.03 between AT and organoleptic tenderness and from −0.60 to −0.19 between W-B and organoleptic tenderness. Few significant correlations were found between AT and chemical values. In the second study, AT measurements and subjective estimates of quality made on the longissimus at the 12–13th rib after 24 hr postmortem were compared with W-B shear, and palatability measurements made on top round steaks that had been removed after 7–8 days postmortem, frozen and then thawed before measurement. This study used 211 A-maturity beef carcasses grading mostly U.S. Choice and Good. Most correlation coefficients between AT values and other measures were low; the significant relationships were between AT measurements and subjective and objective carcass values rather than between AT and organoleptic values. A significant difference for AT values between sides was noted. In study three, wholesale ribs were selected from carcasses that varied widely in AT values. Rib roasts and steaks were evaluated for firmness, palatability, cooking losses and W-B shear. Correlation coefficients between AT and several palatability values were significant. A significant positive correlation coefficient was found between AT values and subjective firmness scores of the longissimus. These three studies indicate that, with the exception of subjective firmness scores, AT values are not highly related to the objective and subjective measurements of beef quality used in this study. AT values also are not highly related to organoleptic properties of bovine muscle, but the AT shows some usefulness in predicting tenderness where wide differences in tenderness exist.

^{15}N TRACER STUDIES OF NITRITE ADDED TO A COMMINUTED MEAT PRODUCT. J.G. SEBRANEK, R.G. CASSENS, W.G. HOEKSTRA, W.C. WINDER, E.V. PODEBRADSKY & E.W. KIELSMEIER. *J. Food Sci.* **38**, 1220–1223 (1973)–The stable isotope of nitrogen was used to study the fate and distribution of nitrite in a cured meat product. The meat product was fractionated into water soluble, salt soluble (protein) and insoluble forms in order to conduct quantitative analysis for ^{15}N as a function of storage time up to 65 days and processing temperatures of 0, 71°C and 107°C. Residual nitrite, determined 2 days after processing, accounted for less than half of the label added in frozen samples and in samples processed at 71°C. Samples processed at 107°C were initially very low in residual nitrite compared to the other heat treatments. The amount of label as nitrite decreased during storage in all samples. As residual nitrite decreased, the amount of label found in the nonnitrite water-soluble fraction and in protein fractions (both soluble and insoluble) increased. About 5% of the label was lost as a gas during processing and 9–12% was present in the pigment fraction. Total recovery of label ranged from 72–86%.

MEASUREMENT OF SAUSAGE EMULSION STABILITY BY ELECTRICAL RESISTANCE. A. HAQ, N.B. WEBB, J.K. WHITFIELD, A.J. HOWELL & B.C. BARBOUR. *J. Food Sci.* **38**, 1224–1227 (1973)–A system was developed for continuously measuring the a-c impedance of sausage emulsions prepared with a laboratory model system. The results indicated that the a-c impedance measurement could be used successfully as an objective technique to evaluate the stability of sausage emulsions prepared in a prototype model system. It was concluded that the a-c impedance measurements reflected the changes in the structure of an emulsion during the emulsification process. Studies on various types of emulsions indicated that this technique could be of value for investigating various parameters relative to the stability of sausage emulsions. The measurement of sausage emulsion stability by d-c resistance was found to be ineffective in highly viscous systems.

CHANGES IN MEAT COMPONENTS DURING FERMENTATION, HEAT PROCESSING AND DRYING OF A SUMMER SAUSAGE. F.B. WARDLAW, G.C. SKELLEY, M.G. JOHNSON & J.C. ACTON. *J. Food Sci.* **38**, 1228–1231 (1973)–One style of summer sausage was examined for changes in pH, lactic acid content and nitrogen fractions during three phases of processing. The end product would be classified as a fermented-cooked sausage. During fermentation and heat processing, the pH significantly ($P < 0.05$) decreased as lactic acid content increased. Myofibrillar protein and sarcoplasmic protein nitrogen fractions significantly

ABSTRACTS:

IN THIS ISSUE

decreased while nonprotein nitrogen and the insoluble nitrogen fractions significantly increased during the sames phases. Increases of lactic acid, salt, nonprotein nitrogen, insoluble nitrogen and total nitrogen contents during drying of the product were all dependent on the decrease of moisture content. Heat processing of the fermented sausage mixture reduced lactic acid bacteria counts by 4.5 log cycles with one additional log reduction occurring during 60 days of drying. Product evaluated at 0, 10, 30 and 60 days of drying showed no difference in panel preference although shearing force and quantity of chemical components had significantly increased.

CHICKEN LIPID CHANGES DURING COOKING IN FRESH AND REUSED COOKING OIL. W.T. LEE & L.E. DAWSON. *J. Food Sci.* **38,** 1232–1237 (1973)–Chicken pieces were cooked in fresh corn oil and in corn oil previously heated for 24 and 42 hr. Fresh corn oil had a total unsaturation of 84%, predominantly linoleic acid (18:2) followed by oleic (18:1) and palmitic (16:0). Raw chicken muscle and skin contained more individual fatty acids than corn oil, predominantly oleic, linoleic and palmitic. During cooking in fresh corn oil, the unsaturated fatty acids increased in both muscle and skin, and in both neutral lipids and phospholipids. Cooking in reheated corn oil accentuated these differences. Other primary changes during cooking were increases in percentages of linoleic acid and decreases in oleic and arachidonic acids.

USE OF γ-IRRADIATION TO PREVENT AFLATOXIN PRODUCTION IN BREAD. L.B. BULLERMAN, H.M. BARNHART & T.E. HARTUNG. *J. Food Sci.* **38,** 1238–1240 (1973)–Irradiation doses of 100 and 200 Krad reduced the growth of *Aspergillus parasiticus* in bread stored for 10 days at 25°C. With bread stored longer than 2 wk, mold growth in irradiated samples tended to approach the amount which occurred in the non-irradiated controls. Mold strain NRRL 3000 seemed slightly more sensitive to irradiation than strain NRRL 2999. A 200 Krad dose prevented aflatoxin production by either strain at both high and low levels of inoculation with spores in bread stored for 10 days, except in one case where a very low level of aflatoxins was detected. At 100 Krad, bread inoculated with 10^2 spores/slice contained none to low concentrations of aflatoxins after storage for 10 days, but with 10^6 spores/slice higher amounts of toxins were detected. When the inoculated bread was stored for periods up to 6 wk, the 200 Krad treatment prevented aflatoxin production in all bread samples containing 10^2 spores/slice, and in almost all samples containing 10^6 spores/slice. The 100 Krad treatment also prevented aflatoxin production during 6 wk of storage in bread that contained 10^2 spores/slice. However, with 10^6 spores/slice both strains were capable of producing very high amounts of aflatoxins after irradiation at 100 Krad. At 1, 2 and 6 wk of storage, these amounts were greater than the unirradiated controls, suggesting possible stimulation of aflatoxin production.

MICROBIOLOGY OF A MODIFIED PROCEDURE FOR COOLING PASTEURIZED SALT YOLK. K. IJICHI, J.A. GARIBALDI, V.F. KAUFMAN, C.A. HUDSON & H. LINEWEAVER. *J. Food Sci.* **38,** 1241–1243 (1973)–A modified procedure that avoids the high pressures and pumping problems associated with the cooling of salt yolk to 18°C in plate pasteurizers has been tested for safety. Studies show that salt yolk from the regeneration section of the pasteurizer filled at 30°C into 30-lb containers can be cooled in less than 7 hr to 10°C by placing the containers in a –10 to –20°C room in a manner that allows free air circulation. Since neither salt-tolerant staphylococci nor adventitious contaminants show growth in salt yolk held for 2 days or longer even at the favorable temperature of 28.9°C the suggested procedure provides a wide margin of safety.

METHOD FOR ESTIMATING LIMONIN CONTENT OF CITRUS JUICES. J.H. TATUM & R.E. BERRY. *J. Food Sci.* 38, 1244–1246 (1973)–A simplified method for estimating limonin content in grapefruit and orange juice is presented. The untreated juice is applied directly and the limonin separated from the other components on thin-layer chromatographic plates. The concentration is estimated by comparing the density of the unknown spot to that of limonin standards by visual color and/or fluorescence under ultraviolet light. Using standards, unknowns and added limonin, the method was found reliable and reproducible and should provide a new quality control test for citrus products. The method, applied to a study of juice yield, indicated limonin content increased with higher yield.

STORAGE QUALITY OF BANANAS PACKAGED IN SELECTED PERMEABILITY FILMS. H. DAUN, S.G. GILBERT, Y. ASHKENAZI & Y. HENIG. *J. Food Sci.* **38,** 1247–1250 (1973)–Studies have been conducted on storage of bananas in retail-type packages with polymeric films of a wide range of gas permeabilities. The packages including unwrapped control were kept at 15°C. Gas chromatographic measurements of O_2 and CO_2 within the packages, color readings and sensory evaluation of bananas were conducted during storage time. The results show that a combination of ethylene prepackaging treatment followed by hermetic storage in selected permeability films effected a separation in time of the process of chlorophyll degradation, maintenance of yellow color, repression of skin browning and internal softening. After 30 days of storage in packages which contained 3.0% O_2 and 3.3% CO_2, the bananas were still excellent in color, odor, flavor and texture.

INFLUENCE OF FRESHNESS AND COLOR ON POTATO CHIP SENSORY PREFERENCES. J.A. MAGA. *J. Food Sci.* **38,** 1251–1252 (1973)–A study was designed to determine whether the difference between regular and dark-colored chips could be detected and which was preferred when the visual variable was eliminated; also, to follow chip sensory preferences for both regular and dark-colored chips as influenced by storage. No significant differences in sensory properties (α = 0.05) between regular and dark chips were noted for relatively fresh chips while the panel was blindfolded. As storage time increased, the blindfolded panel had a clear preference for the odor and flavor of the dark chips. Of samples frozen after each storage period, dark chips were preferred to regular color chips even though the dark chips were held at room temperature storage longer.

THE POSSIBILITY OF RECOGNIZING IRRADIATED AND NONIRRADIATED POTATOES BY THEIR WEIGHT LOSS. G. MAGAUDDA. *J. Food Sci.* **38,** 1253–1254 (1973)–Tubers of potatoes were irradiated in an industrial irradiator facility (^{60}Co source) at total exposures of 0-5-7, 5-10-12, 5-15-20-40-80 Krads; stored 150 days at 10°C, 70% RH; and samples withdrawn periodically for weighing. Potato "cores" (60/sample variable) were analyzed at 2-day intervals over a 24-day storage period at RT. Calculated weight losses of treated and nontreated controls were used to plot the regression line and its angular coefficient b for each experimental lot. These data show a good relationship between weight loss and gamma ray exposure in the range applied for antisprouting purposes, especially for "core" potatoes. This analysis might be useful for recognizing "a posteriori" whether or not potatoes have been irradiated.

MONOSODIUM GLUTAMATE INGESTION AND THIRST PRODUCTION. P.E. ARAUJO, D. HOURIHAN & J. MAYER. *J. Food Sci.* **38,** 1255 (1973)–Monosodium glutamate (MSG) was shown to have as great a thirst-producing effect as NaCl. Comparison among sodium, potassium and ammonium salts failed to demonstrate any peculiar effects of these glutamate salts on thirst induction. Hypothesis of an increased stimulation of drinking resulting from ingestion of foods seasoned with a small amount of MSG is shown to be false. No relationship of the thirst reported and the current controversy involving MSG use is made.

HYDROCYANIC ACID IN CANNED SWEET CHERRIES. G.S. STOEWSAND & J.L. ANDERSON. *J. Food Sci.* **38,** 1256 (1973)–Underprocessed sweet cherries contained 1.1–2.1 ppm of hydrocyanic acid after storing in cans from 1–50 wk at 38°C. This amount of cyanide, assumed toxicologically insignificant, occurred from the β-glucosidase hydrolytic activity of amygdalin in the intact kernels of the canned cherries. Heating at 100°C for 12 min inactivated this enzyme as only very trace amounts of cyanide were present. No cyanide was present in the cherries when the kernels were removed.

SIMPLE DETERMINATION OF PHOSPHORUS IN PET FOODS. T. SURLES & J. DARBY. *J. Food Sci.* **38,** 1257 (1973)–Various colorimetric methods are presently used to determine phosphorus in pet foods; all involve considerable reaction time, critical timing and/or preparation of unstable solutions. The method of Kennedy and Weetman was adapted for determining phosphorus in various pet foods and very good agreement was obtained between this method and conventional molybdate methods. In addition it has the advantage of being much simpler and less time consuming for doing many analyses simultaneously.

RELATION BETWEEN SHEAR FORCE AND TENDERNESS OF BEEF. A.W. KHAN, C.P. LENTZ & L. van den BERG. *J. Food Sci.* **38,** 1258–1259 (1973)–Comparison of shear force measurements with the results of taste panel tests using a pair comparison method showed that differences in shear force of 0.5 kg and greater between samples could readily be detected by the taste panel. These results were obtained using the same samples for both the shear press and taste panel tests, and using samples with similar levels of tenderness in each test. This technique permitted a direct comparison of the two methods by minimizing between-sample and within-muscle variability. The differences in shear force detected by the panel were independent of the level of tenderness (shear force varied from 1.7 to 7.3 kg) and method of cooking (in boiling water or by broiling). The panel was somewhat less sensitive in comparing samples from the same or similar muscles than different muscles.

ACTIVITY OF ANTIOXIDANTS IN FRESH FISH. C.W. SWEET. *J. Food Sci.* **38,** 1260–1261 (1973)–The relative potencies of a variety of authorized food antioxidants and chelators were studied in several types of marine and fresh-water fish. Thiobarbituric acid (TBA) test values were determined in ground fish flesh after storage at 5°C. The most potent inhibitors of oxidation were found to be combinations of either butylated hydroxyanisole (BHA) or tert-butylhydroquinone (TBHQ) with EDTA or citric acid. In view of the relatively high potencies of these stabilizer combinations, it was concluded that the storage stability of frozen fish might be improved if suitable application methods could be developed.

A QUALITATIVE AND QUANTITATIVE STUDY OF SUGAR-ALCOHOLS IN SEVERAL FOODS. J. WASHÜTTL, P. RIEDERER & E. BANCHER. *J. Food Sci.* **38,** 1262–1263 (1973)–Work reported shows that sugar-alcohols are found in a very wide variety of foods and beverages. Xylitol and occasionally arabitol was found in most vegetables and in a few fruits and fruit products. Sorbitol was found in many fruits and fruit products as was galactitol, but the latter only on rare occasions. Neither was found in vegetables except in trace amounts; the sugar-alcohol predominantly present was mannitol.

DEPENDENCE OF FRUIT-BERRY WINES' STABILITY ON THE CONTENT OF MINERAL AND NITROGENOUS COMPONENTS. S. GORINSTEIN. *J. Food Sci.* **38,** 1264–1266 (1973)–Inverse dependence between the content of protein nitrogen and iron in fruit-berry wines and their stability was shown. The investigations carried out enable recommendation to industry to decrease exciting rates of nitrogenous nourishment for a number of fruit-berry juices. This investigation of fruit-berry wines indicates that regularities found in the study of beer are common for both ethanol media.

USE OF FORCEPS IN STERILITY TESTING: A POSSIBLE SOURCE OF CONTAMINATION. L.M. CORSON, G.M. EVANCHO & D.H. ASHTON. *J. Food Sci.* **38,** 1267 (1973)–The use of forceps to transfer particulate food matter aseptically into subculture media in sterility testing was investigated. Forceps were sterilized by alcohol flaming and when used as purchased with the inside points file-cut, a contamination rate as high as 56% was encountered. When the same procedure was followed with the inside points filed smooth, contamination from this source was significantly reduced. Thorough cleaning of the file-cut tips was often difficult because food particles became "baked" into the file cuts. The results indicate that heat resistant forms are capable of surviving the alcohol flaming when embedded with food in the file cuts.

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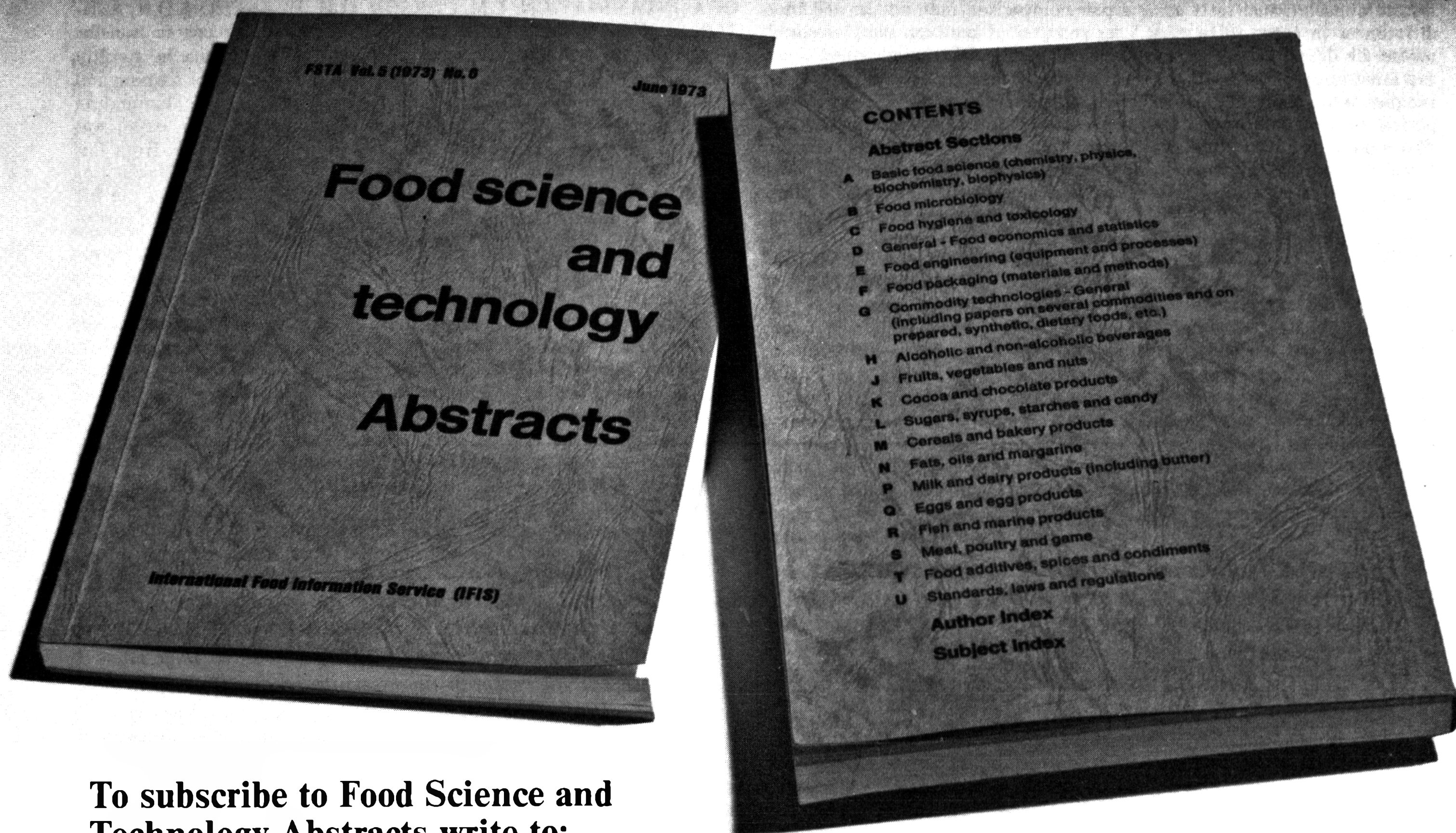

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BLUEING DISCOLORATION IN CANNED CRAB MEAT (Cancer magister)

INTRODUCTION

ALTHOUGH canned crab meat retains most of its fresh qualities when packed under ideal conditions, serious technical difficulties have been experienced in the retention of the natural color (Farber, 1953). The most common and least understood problem is a blueing discoloration that predominates on the surface of the meat and in the coagulated blood that is released from the meat. The discoloration can range from blue-gray to black and has been observed in king crab (Groninger and Dassow, 1964), blue crab (Waters, 1971), and Dungeness crab (Elliott and Harvey, 1951). It has been difficult to assign a simple chemical reaction to the blueing. Several plausible explanations have linked the blueing to the formation of copper-protein and buiret complexes (Groninger and Dassow, 1964), copper sulphide (Inoue and Motohiro, 1970b), and iron complexes (Waters, 1971).

Little is known about melanin formation in Dungeness crab, yet it may be the most serious problem in regard to discoloration. The blueing of crab meat has not been considered an enzymatic reaction since the temperatures used during retorting would destroy the enzymes. However, tyrosinases and phenol oxidases present in live crabs may initiate the oxidation of phenols to melanins (Pinhey, 1930; Summers, 1967). The subsequent oxidation and polymerization of these intermediate phenolic derivatives in canned crab meat may proceed nonenzymatically to form colored chromophores, particularly in the presence of metals (Mathew and Parpia, 1971). The blueing of whole cooked frozen crab and processed crabs not yet canned may also be explained by the formation of melanin-like compounds (Babbitt et al., 1973). The purpose of this study was to investigate the characteristics of the blueing reaction and the nature of the discolored product.

EXPERIMENTAL

Nature of blueing reaction

Crab meat was picked from locally caught, cleaned, uncooked Dungeness crab. One part picked meat was blended at low speed with two parts acetone in a Waring Blendor for one min. In addition, one part picked crab meat was blended with four parts of 5% trichloroacetic acid (TCA). The blended materials were centrifuged at 1,000 × G for 10 min and the liquid fraction decanted off. The crab tissue was then blended with 2 vol (wt/wt) of deionized water and the blended tissue was dialyzed against several changes of deionized water for 2 days at 4°C. 50g of this blended crab meat were mixed in 307 × 113 C-enamel cans with a final concentration of the following materials: NaCl (0.67%); L-dopa (3,4-dihydroxyphenylalanine) (15 μg/g); $FeSO_4 \cdot 7 H_2O$ (30 μg Fe^{+2}/g); $CuCl_2 \cdot 2 H_2O$ (30 μg Cu^{+2}/g); citric acid (0.12%); and ascorbic acid (0.13%). Deionized water was added to bring the weight of the total contents to 150g. In some instances 0.1M phosphate buffer (pH 8.0) was substituted for the deionized water. The cans were vacuum-sealed and retorted at 116°C for 55 min. After retorting, the cans were cooled in cold water and held at room temperature.

Analytical methods

Total phenolic content. Total phenols were determined colorimetrically by the Folin-Ciocalteau reagent (Phenol Reagent, Fisher Scientific Co.). 10g of sample material were blended with 40 ml of 5% TCA in a Virtis Model 45 homogenizer at high speed for 2 min. The blended material was filtered and 1 ml of the filtrate was mixed with 1 ml of phenol reagent (1 part phenol reagent:2 parts water). Then, 3 ml of a 14% Na_2CO_3 solution were added and the absorbance determined at 660 nm after 15 min with a Beckman DB spectrophotometer. Pyrocatechol (Eastman Organic Chemicals) was used to prepare standard solutions.

pH. A Corning Model 7 pH meter was used to determine the pH of a slurry of crab meat.

Copper analysis. Copper was determined by atomic absorption spectrophotometry using a Perkin-Elmer Model 403 after dry-ashing the sample (Baker, 1971).

Degree of blueing. A Gardner Automatic AC-2A Color Difference Meter was used to measure the degree of discoloration. The instrument was standardized with a white color standard: L = 94.9, a = −1.3, b = 3.0. The Gardner Color-Difference Meter values "a" and "b" indicated that crab exhibited a slight yellowish-green tint. Since the "a" and "b" values were similar for the various treatments, "L" values were used to measure the degree of blueing in crab meat caused by the addition of various substances. An "L" value of 55 and higher indicates that the crab meat was a desirable whitish color. An "L" value of 53 and lower begins to reflect a grayish-blue to blueish-black discoloration that is typical of the blueing in canned crab meat.

Handling and processing of crab

Live crab in good condition were purchased from local processors, covered with wet towels and held at 1–3°C. At daily intervals, changes in the phenolic content and the pH of five uncooked crab were determined. Lots of 15 crab were handled at 0, 2 and 4 days post extraction in a manner similar to a commercial canning operation. The crab were backed and cleaned, cooked in boiling water for 12 min, cooled with a spray of cold water and the meat hand picked from the shell. Then, 142g of this picked meat were packed with 40 ml of 2.5% brine (NaCl) in 307 × 113 C-enamel cans, sealed in a vacuum and retorted for 55 min at 116°C. After water cooling, the cans were held at 30°C. The cans of crab meat were opened and examined visually for blueing after 7 days. To prevent the inclusion of copper-bound hemocyanin found in crab blood with the crab meat, one lot of crab was pre-cooked at 60°C for 20 min prior to the cooking step.

RESULTS & DISCUSSION

EARLIER INVESTIGATIONS in our laboratory confirmed the presence of polyphenoloxidases in Dungeness crab meat, and the presence of tyrosine, dopa and other phenols were tentatively identified (Babbitt et al., 1973).

It is well documented that copper (Groninger and Dassow, 1964; Elliott and Harvey, 1951) and iron (Waters, 1971) are involved in the blueing of canned crab meat. To determine the substances reacting with these metals, crab meat was extracted with acetone and trichloroacetic acid (TCA) and dialyzed extensively against deionized water. Over 90% of the phenolic compounds could be removed by these treatments (Table 1). Copper content was reduced with TCA but increased with the acetone treatment. The substance or substances that interact with iron or copper to cause blueing in crab meat are removed by TCA and to a lesser degree by acetone-extraction and dialysis against deionized water (Table 2). Copper (Cu^{+1}, Cu^{+2}) and iron (Fe^{+2}, Fe^{+3}) were equally effective in causing blueing when added to canned crab meat. The addition of iron and copper to TCA-treated crab meat did not produce blueing. When a phenolic compound (dopa) was included, pronounced blueing occurred. This was also evident when untreated crab meat was used. The results illustrate that blueing is a complex system. The higher Gardner "L" values for the untreated crab meat indicate that certain constituents in the crab tissue may interfere or inhibit blueing. The acetone and TCA treatments may not only be removing the reactive constituents necessary for blueing but also the inhibitory constituents as well.

Apparently, iron and copper are reacting with the phenols still present in the acetone-treated crab meat particularly at pH 8.0 (Table 3). Dopa, particularly in the presence of iron or copper, polymerizes to form blueish-black chromophores. The dopa-iron complex was darker than dopa and visually the color resulting from the dopa-copper complex was similar to the dopa-iron complex.

Citric acid and ascorbic acid prevented the discoloration that occurred when dopa and iron or copper were mixed with the crab meat (Table 3). Citric acid (Waters. 1971) and ascorbic acid (Groninger and Dassow, 1964) have been reported to inhibit blueing. Citric acid has been used by industry for many years to reduce the incidence of blueing under the assumption that the acid blocks the color formation simply by lowering the pH of the medium. However, the results suggest that citric acid may block the reaction by chelating the metals and the antioxidant properties of ascorbic acid may retain the phenols (dopa) in a reduced state. The visual ratings of the degree of blueing corresponded closely to the Gardner Color-Difference Meter values (Table 3). In studies where accurate instrumental readings are difficult to obtain, a visual rating using color standards can be very useful.

From these model studies, the changes in total phenols and pH of live crab were followed during refrigerated storage (Table 4). The phenolic content in the crab meat increased steadily during the first 3 days of storage. After the third day, the phenolic content increased rapidly. The pH of the meat also increased steadily during storage. The pH value of 7.65 after 4 days indicates that the crab were in poor condition.

The total phenolic content of retorted crab meat from crab held up to 4 days was similar to retorted crab meat produced from freshly handled crab

Table 1–Effect of extraction and dialysis on phenolic and copper content of crab meat

Treatment	Total phenols[a] μg/g	Copper content[a] μg/g (dry wt)
Raw crab meat	245.8 ± 6.0	30.94 ± 1.67
Acetone		
extracted	172.8 ± 64.8	39.84 ± 0.49
dialyzed	24.6 ± 11.5	50.54 ± 0.42
TCA		
extracted	46.89 ± 3.2	19.78 ± 0.51
dialyzed	16.98 ± 0.1	17.94 ± 0.17

[a] Mean ± standard deviation of duplicate samples for two experiments

Table 2–Blueing observed in TCA- and acetone-extracted and dialyzed crab meat after retorting[a]

	Gardner "L" values[b] of blends of		
Treatment	Untreated crab meat	Acetone dialyzed	TCA dialyzed
Blend	73.6	66.0	68.6
+ dopa	67.2	58.6	54.4
+ dopa + Fe	53.4	39.4	46.9
+ dopa + Cu	52.5	46.4	50.6
+ Fe	66.0	45.9	61.5
+ Cu	57.8	55.0	66.1

[a] Data taken from three experiments
[b] "L" values ≥55 = desirable color; ≤53 = less than desirable

Table 3–Effect of various chemicals on the extent of blueing after retorting dialyzed, acetone-extracted crab meat

	Gardner color values[a]			
Treatment	L	a	b	Visual color evaluation
Dialyzed crab blend	67.5	−3.0	+6.0	White
+ dopa	58.6	−2.7	+4.2	Grayish-white
+ dopa at pH 8.0	29.7	−3.5	+3.7	Dark blue-gray
+ Cu	57.6	−3.5	+13.4	Yellowish-white
+ Cu at pH 8.0	23.5	−6.6	+14.5	Greenish-gray
+ Fe	43.8	−3.3	+5.3	Grayish-white
+ Fe at pH 8.0	37.2	−3.9	+3.7	Blueish-gray
+ dopa + Cu	42.9	−2.7	+8.3	Blueish-gray
+ dopa + Cu at pH 8.0	7.8	−4.3	+3.3	Dark brownish-black
+ dopa + Cu + citric acid	64.3	−2.7	+5.6	White
+ dopa + Cu + ascorbic acid	67.3	−1.1	+16.4	Yellowish-white
+ dopa + Fe	38.8	−3.3	+2.0	Blueish-gray
+ dopa + Fe at pH 8.0	25.7	−3.8	−0.1	Dark blue-gray
+ dopa + Fe + citric acid	57.9	−4.7	+10.0	Yellowish-white
+ dopa + Fe + ascorbic acid	49.5	−4.1	+6.4	Grayish-white

[a] "L" values of ≥55 = desirable color; ≤53 = less than desirable

(Table 5). However, blueing was much more pronounced. Either the phenols present in the aged crab were more reactive or the higher pH favored the formation of the blueing.

To prevent the inclusion of copper-bound hemocyanin of the crab blood with crab meat, Osakabe (1957) proposed precooking the crab at 60°C prior to cooking. Thus, the crab meat is coagulated while the hemocyanin can be washed free of the meat preventing graying in canned Kegani crab. Using this technique, the phenolic content and blueing was reduced in retorted crab meat produced from freshly handled crab (Table 6). When the crab were held for 4 days prior to processing the blueing of the retorted crab meat was much more pronounced that the retorted crab meat processed without being precooked. The degree of blueing may have resulted from the very high pH of the retorted precooked crab meat.

We and others (Inoue and Motohiro, 1970a) have found the copper content of "blue" crab meat to be higher than in the "white" crab meat. The results from Tables 2 and 3 indicate that copper is involved in the blueing reaction. However, the role of copper may be that of a catalyst since it can be freed from the blue-pigment after retorting by dialysis against deionized water (Table 7). This might explain why precooking the crab to remove the copper bound to the hemocyanin did not prevent blueing in crab held 4 days prior to processing (Table 6).

The change in phenolic compounds in crab appears to have a significant role in causing the blueing observed in retorted canned crab meat. The results indicate that once oxidation of phenols is enzymatically initiated, further oxidation and polymerization of phenols may proceed nonenzymatically particularly in the presence of metals and under alkaline conditions.

Table 4—Changes in phenolic content and pH of live crab held at 1–3°C

Days held at 1–3°C	Phenolic content[a] µg/g	pH[a]
0	140.8 ± 15.1	6.65 ± 0.30
1	169.5 ± 37.5	7.05 ± 0.07
2	188.3 ± 37.7	7.10 ± 0.28
3	194.9 ± 54.2	7.19 ± 0.01
4	322.5 ± 80.6	7.65 ± 0.21

[a] Mean ± standard deviation from five crab

Table 5—Phenolic content, pH and extent of blueing of retorted crab meat

Days crab held prior to processing	Phenolic content µg/g	pH	Visual evaluation of blueing
0	157.5	7.3	None-slight
2	163.0	7.5	Slight-moderate
4	161.0	7.8	Moderate-extensive

Table 6—Effect of precooking crab on pH, phenolic content and blueing of retorted crab meat

Days crab held prior to processing	Phenolic content µg/g	pH	Visual evaluation of blueing
0	131.58	7.4	None
2	156.35	7.8	Slight-moderate
4	159.23	8.0	Extensive

Table 7—Effect of dialysis of crab blends with added copper and dopa after retorting

Treatment	Copper content after dialysis[a] µg/g (dry wt)		Visual evaluation of color Dialysis	
	Sample 1	Sample 2	Before	After
Acetone dialyzed meat				
+ copper	713.95	589.69	White	White
+ dopa + copper	606.85	597.41	Blueish-Gray	Blueish-Gray
TCA dialyzed meat				
+ copper	759.98	761.85	White	White
+ dopa + copper	764.36	871.86	Blueish-Gray	Blueish-Gray

[a] Mean of duplicate samples

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REACTION OF HYDROGEN PEROXIDE WITH MYOGLOBINS

INTRODUCTION

THE FORMATION of undesirable green-brown-tan pigments in cooked tuna is collectively known as "greening." This problem has been reported in albacore (*Germo alalunga*), big-eye (*Thunnus obesus*), bluefin (*Thunnus orientalis*), yellowfin (*Thunnus albacares*) and skipjack (*Katsuwonus pelamis*) tuna (Tomlinson, 1966). It appears that there are several different types of greening in cooked tuna. Brown et al. (1958) suggested that one off-color is due to oxidation of ferrohemochromes, responsible for normal tuna color, to undesirable ferrihemochromes. Another type of off-color which occurs in cooked tuna involves trimethylamine oxide (TMAO) and cysteine which are present in tuna flesh. Sasano et al. (1961) and Sasano and Tawara (1962) were the first to observe this phenomenon. Koizumi and Hashimoto (1965a, b) identified the substance involved as TMAO and also demonstrated that addition of TMAO to raw meat induced greening on subsequent cooking of yellowfin and albacore tuna. Grosjean et al. (1969) observed that when TMAO, cysteine and yellowfin tuna myoglobin were heat denatured, a green pigment was formed. The green product could not be produced with mammalian myoglobins which contain no cysteine residues. Their results indicated that the sulfhydryl groups of both free cysteine and the cysteine residue in tuna myoglobin were involved in the greening reaction.

Hydrogen peroxide, acting as an oxidizing agent, is known to cause changes in the oxidation state of the iron in hemoproteins. George (1952) reported that the products of the interaction of ferricatalase (the prefixes "ferri" and "met" have been used interchangeably in this paper to denote the +3 oxidation state of the heme iron), ferriperoxidase, ferrihemoglobin and ferrimyoglobin with H_2O_2 have a ferryl, (+4), oxidation state. George and Irvine (1955, 1959) subsequently demonstrated the ferryl structure, which was also confirmed by King and Winfield (1963). Hydrogen peroxide interaction with hemoproteins may be of significance in irradiation of meats, because H_2O_2 is one of the known products of radiolysis of water (Giddings and Markakis, 1972). Its production is enhanced by the presence of oxygen in the irradiated aqueous medium, while it is minimal in an anaerobic medium. This suggests that ferryl myoglobin may be formed if an oxygen containing metmyoglobin solution is irradiated. Formation of ferryl myoglobin and subsequent products may affect the color and quality of the irradiated product.

The experiments reported herein were done to study the changes in color and spectra that occur during the interaction of H_2O_2 with tuna or sperm whale myoglobins in native and denatured states. The effects of cysteine and homocysteine on the intensity of green color produced by H_2O_2 were studied in order to compare this form of greening to the one produced by TMAO. The effect of the reducing agent dithionite on the green color and spectra of myoglobins was also studied.

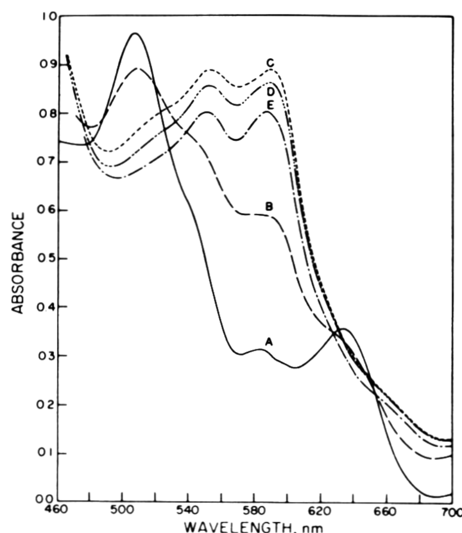

Fig. 1—Spectra of sperm whale myoglobin before and after its interaction with hydrogen peroxide. A, control, no peroxide; B, 1 µl H_2O_2; C, 5 µl H_2O_2; D, 10 µl H_2O_2; and E, 25 µl H_2O_2.

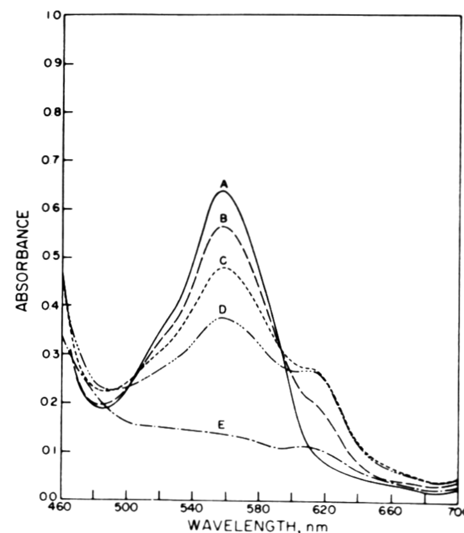

Fig. 2—Effect of dithionite addition on the spectra of sperm whale myoglobin treated with hydrogen peroxide. A, control, no peroxide; B, 1 µl H_2O_2; C, 5 µl H_2O_2; D, 10 µl H_2O_2; and E, 25 µl H_2O_2.

EXPERIMENTAL

CHEMICALS used were reagent grade or better. Deionized, glass distilled water was used throughout. Myoglobin from sperm whale skeletal muscle was obtained from Sigma and used without further purification. Yellowfin tuna myoglobin was isolated by ammonium sulfate fractionation (method of Dollar et al., 1959), followed by chromatography on Sephadex G-75 in 0.05M Tris-HCl buffer (pH 8.0), to remove residual hemoglobin. For the reaction with H_2O_2, the myoglobins and other chemicals, except dithionite, were dissolved in 0.1M phosphate buffer (pH 5.8). Fresh 1% H_2O_2 was prepared for each experiment by diluting a stock solution with water. In the model systems used, the final concentration of reactants/ml was as follows:

myoblobin	1.8 – 2.00 mg
1% H_2O_2	0.3 – 33 µl
sodium dodecyl sulfate (SDS)	10 mg
cysteine · HCl	0 – 2 mg
homocysteine	0 – 2 mg
dithionite	0.5 – 1 mg

Interaction of H_2O_2 with myoglobins was studied under the following conditions:

1. With undenatured (native) myoglobins with and without dithionite.
2. With denatured myoglobins (denatured with SDS) with and without dithionite.

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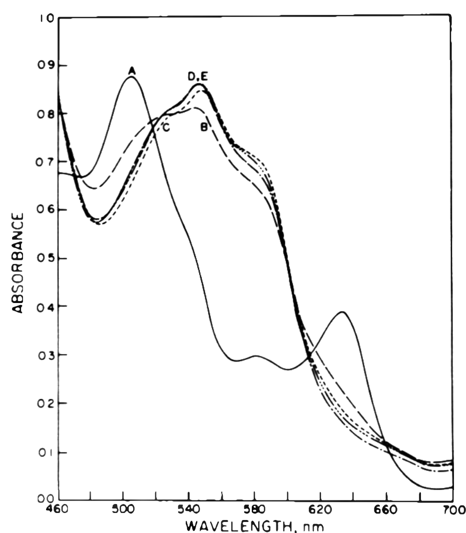

Fig. 3–Spectra of yellowfin tuna myoglobin before and after its interaction with hydrogen peroxide. A, control, no peroxide; B, 1 µl H_2O_2; C, 5 µl H_2O_2; D, 10 µl H_2O_2; and E, 25 µl H_2O_2.

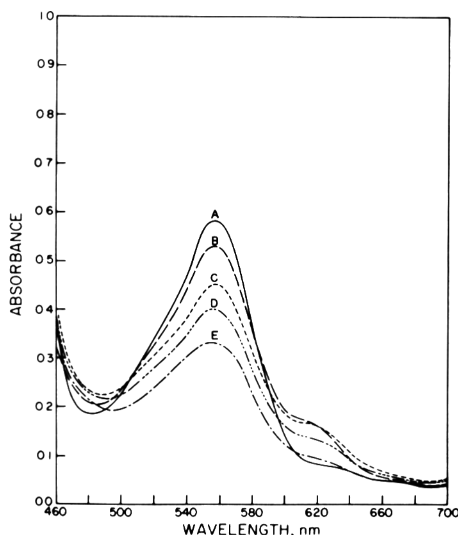

Fig. 4–Effect of dithionite addition on the spectra of yellowfin tuna myoglobin treated with hydrogen peroxide. A, control, no peroxide; B, 1 µl H_2O_2; C, 5 µl H_2O_2; D, 10 µl H_2O_2; and E, 25 µl H_2O_2.

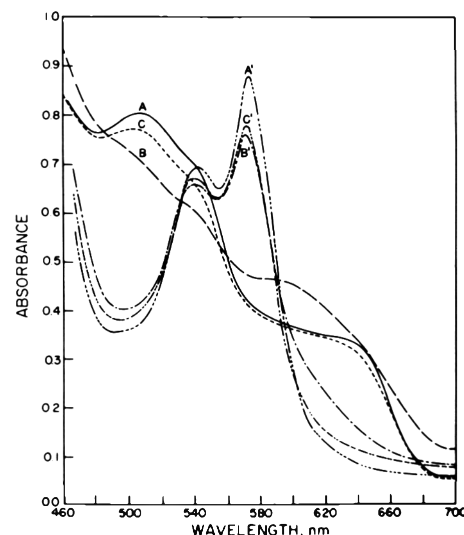

Fig. 5–Spectra of denatured yellowfin tuna myoglobin treated with 1 µl H_2O_2. Effects of dithionite addition and altering the sequence of denaturation are also shown. A, denatured control myoglobin; A', sample in curve A + dithionite; B, myoglobin treated with H_2O_2 prior to denaturation; B', sample in curve B + dithionite; C, myoglobin treated with H_2O_2 after its denaturation; and C', sample in curve C + dithionite.

3. With denatured myoglobins in the presence of cysteine and with or without dithionite.
4. With denatured myoglobins in the presence of homocysteine and with or without dithionite.
5. Experiments (2) and (4) were also done in a slightly modified manner, in which H_2O_2 was first reacted with myoglobin, and then the protein was denatured and other reagents added.

In addition, a limited number of studies were done with heat denatured myoglobin. In these studies 15 mg of myoglobin in 0.5 ml were added to 4.0 ml phosphate buffer, pH 5.7; also added was 1.0 ml of a 50 mg per ml solution of human serum albumin. When used, H_2O_2 was added as 10 µl of a 1% solution. Denaturation was carried out by placing tubes containing the mixture just described in 70°C water bath for 15 min. Colors of the resulting precipitates were noted.

All experiments were done at room temperature. Three or more minutes after addition of H_2O_2 to the system, absorbance spectra (visible range) were recorded in a Cary Model 15 spectrophotometer. Visual observation of the systems indicated that reaction was complete within the three minute period.

RESULTS & DISCUSSION

Reaction of native myoglobins with H_2O_2

Effect of dithionite. Control myoglobins from sperm whale and yellowfin tuna were brown and had spectra typical of myoglobin in the ferri- state.

Figure 1 shows the spectra of sperm whale myoglobin before and after its interaction with H_2O_2. Control sperm whale myoglobin (curve A) had absorbance maxima at 506, 584, and 633 nm. Immediately after addition of H_2O_2 the color turned red-brown. The color remained visibly unchanged at 3 min after H_2O_2 addition, when the spectra were recorded. As seen in Figure 1, the H_2O_2-reacted myoglobin showed peaks at 550 and 588 nm (curves B-E). The intensity of these peaks decreased with increasing H_2O_2 concentration above 5 µl (curves C-E). Control myoglobin had a Soret peak at 410 nm (not shown); with increasing concentrations of H_2O_2 the intensity of the Soret peak was greatly decreased and it shifted to 415 nm. Immediately after addition of dithionite to the H_2O_2-treated myoglobin the color of the reaction mixture turned green; the spectrum showed a major peak at 558 nm and a shoulder at about 610 nm as shown in Figure 2 (curves B-E). With increasing H_2O_2 concentration, the intensity of the peak at 558 nm decreased. Control sperm whale myoglobin, which was originally in the met form, gave a bright red color on addition of dithionite, and a spectrum showing a peak at 558 nm (indicative of reduced myoglobin), but no shoulder around 610 nm (curve A).

Figure 3 shows the spectra of yellowfin tuna myoglobin before and after interaction with H_2O_2. The control (curve A) was brown and had peaks at 506, 580, and 633 nm. On addition of increasing concentrations of H_2O_2 to the myoglobin, the color turned red-brown and the spectrum revealed a peak at 548 nm and a shoulder at 580 nm (curves B-E). The intensity of these peaks was not affected by increasing H_2O_2 concentrations. Control myoglobin had a Soret absorbance peak at 4.0 nm (not shown) whose intensity decreased with increasing H_2O_2 concentration; it shifted to 420 nm. On addition of dithionite the color turned brown to golden-brown, depending on H_2O_2 concentration unlike the green color obtained in case of sperm whale myoglobin. But as shown in Figure 4 its spectra also showed a major peak at 558 nm indicative of reduced myoglobin and a very slight shoulder at 620 nm (curves B-E). The intensity of the peak at 558 nm decreased with increasing H_2O_2 concentrations. Control yellowfin tuna myoglobin had a bright red color and a single peak at 558 nm, on addition of dithionite (curve A).

It appears from these results that H_2O_2 affects the two myoglobins in a different manner as suggested by the variations in color and spectra. The spectral differences seem rather trivial; however, colors of the reduced derivatives of the two myoglobins may be readily differentiated visually.

The spectrum of the compound obtained by reaction of H_2O_2 with sperm whale myoglobin is similar to that of horse heart metmyoglobin treated with H_2O_2 (King and Winfield, 1963). The whale derivative also resembles the pigment obtained by Satterlee et al. (1971)

on irradiation of bovine metmyoglobin. The latter pigment had absorbance peaks at 540 and 580 nm, a Soret peak at 412 nm, and a red color. On addition of dithionite, the color turned deep purple and the spectrum showed a peak at 560 nm. However, when the H_2O_2-reacted sperm whale myoglobin was treated with potassium ferricyanide (few drops of 1% solution in 0.1M phosphate, pH 5.8) it turned from deep red-brown to light greenish-yellow and the spectra was not like that of the similarly treated irradiated bovine metmyoglobin of Satterlee et al. (1971). This is not particularly surprising inasmuch as the pigments were prepared by entirely different methods.

Reaction of denatured myoglobins with H_2O_2

Effect of dithionite. Myoglobins were denatured with SDS and H_2O_2 was added in increasing amounts; color and spectra were recorded according to the conditions described above. In a variation of this experiment, myoglobins were first reacted with H_2O_2 and then denatured after which their spectra were recorded. The effect of dithionite on color (and spectra) was also studied in both sets of experiments.

Figure 5 shows the effects of H_2O_2 on the spectra of denatured yellow-fin tuna myoglobin. As shown in curve A, denatured control (no H_2O_2) yellowfin tuna myoglobin had an absorbance maximum at about 510 and a shoulder at 630 nm; it was a golden-brown color. On addition of dithionite, the color immediately turned bright red and the spectrum showed peaks at 540 and 572 nm indicative of oxymyoglobin (curve A′). Results of earlier experiments had indicated that in case of both yellowfin tuna and sperm whale myoglobins, denaturation caused a decrease in intensity and shift of the Soret peaks to lower and higher wavelengths respectively. On addition of 1 μl H_2O_2 to denatured myoglobin (curve C) the profile of the spectrum was like the control but the color turned reddish-brown. On addition of dithionite the color turned bright red and peaks were seen at 540 and 572 nm (curve C′), but with a slightly lower intensity than those in case of control. However, if myoglobin was first reacted with 1 μl H_2O_2 followed by denaturation, the color turned green and the spectrum (curve B) was quite nondescript. Addition of dithionite resulted in a reddish-brown color and peaks at 540 and 572 nm (curve B′).

At higher levels of H_2O_2 the intensities of peaks at 540 and 572 nm obtained on addition of dithionite were further reduced. Before addition of dithionite the spectra were quite nondescript although the spectral profile of myoglobin which was already denatured before H_2O_2 treatment was somewhat similar to the control.

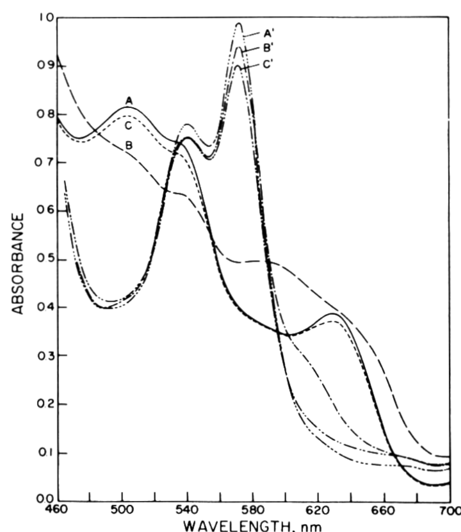

Fig. 6–Spectra of denaturated sperm whale myoglobin treated with 1 μl H_2O_2. Effects of dithionite addition and alteration of the sequence of denaturation are also shown. A, denatured control myoglobin; A′, sample in curve A + dithionite; B, myoglobin treated with H_2O_2 prior to denaturation; B′, sample in curve B + dithionite; C, myoglobin treated with H_2O_2 after its denaturation; and C′, sample in curve C + dithionite.

Figure 6 shows the effects of H_2O_2 on the spectra of denatured sperm whale myoglobin. Experimental conditions were similar to those in the case of yellowfin tuna myoglobin. As shown in Figure 6, denatured control sperm whale myoglobin (no H_2O_2) had a spectrum with absorbance maxima at 505 and 630 and a shoulder at 536 nm (curve A). It had a golden brown color. On addition of dithionite the color turned bright red and peaks appeared at 540 and 572 nm indicative of oxymyoglobin (curve A′). When 1 μl H_2O_2 was added to the previously denatured myoglobin the color turned reddish-brown but the spectral profile (curve C) was quite similar to the control. On addition of dithionite the color turned bright red and peaks at 540 and 572 nm appeared, although with a decreased intensity (curve C′). When 1 μl H_2O_2 was first reacted with myoglobin followed by denaturation the color turned green and the spectrum was quite nondescript (curve B). When dithionite was added the color turned deep brown and the peaks at 540 and 572 nm were seen with a shoulder around 620 nm (curve B′).

At higher H_2O_2 concentrations the intensities of peaks at 540 and 572 nm obtained after dithionite addition were further reduced and the shoulder around 620 nm (obtained when myoglobin had reacted with H_2O_2 prior to denaturation) became more prominent.

The sequence of denaturation of the protein is important for the development of green color. When H_2O_2 was allowed to react with myoglobin prior to its denaturation even 1 μl was sufficient to produce a stable green color. However, if the myoglobin was denatured before its reaction with H_2O_2, it took 25 μl H_2O_2 to produce a slight green tinge. The green color is stable at least 30 min after addition of H_2O_2. The peaks at 540 and 572 nm obtained on addition of dithionite resemble those of oxymyoglobin.

A limited number of parallel experiments using heat denaturation gave similar results. In these studies we were limited to systems in which H_2O_2 was added prior to denaturation as heat denaturation results in precipitation. In any case, both the tuna and whole myoglobins treated first with H_2O_2 then heat denatured yielded green, precipitates. Controls, i.e., no H_2O_2 added, gave reddish-brown precipitates.

It can be concluded that denaturation of the protein is essential for formation of green color with H_2O_2. The results indicate that H_2O_2 affects the two myoglobins in a slightly different manner, with whale myoglobin being somewhat more sensitive.

Reaction of denatured myoglobins with H_2O_2 in the presence of cysteine or homocysteine

Effect of dithionite. In these experiments cysteine-HCl or homocysteine was added in increasing amounts (0–2 mg/ml final concentration) to test its effect on the green color and spectrum of denatured sperm whale and yellowfin tuna myoglobins treated with a constant amount of H_2O_2 (5 μl/ml final concentration). In a variation of the above experiment, H_2O_2 was first allowed to react with the protein before it was denatured and other reagents added.

Denatured sperm whale myoglobin treated with H_2O_2 in the presence or absence of cysteine had no well-defined absorbance peaks in the visible range. Color was golden brown at all cysteine concentrations. After dithionite addition the color was deep red brown and the spectrum showed peaks at 540 and 572 nm. The intensity of these peaks diminished with increasing concentrations of cysteine. If H_2O_2 was first reacted with the protein and followed by denaturation, the color was green in the presence or absence of cysteine and the spectrum showed no peaks in the visible range. On addition of dithionite the color immediately turned light green and the spectra showed peaks at 527 and 570 nm and a shoulder at 610 nm which may indicate the presence of metmyoglobin. Qualitatively, the major peaks in both sets of

experiments were similar indicating formation of a compound like oxymyoglobin. Dithionite could not completely reverse the effects of H_2O_2 in the system where H_2O_2 had reacted with myoglobin prior to its denaturation.

Denatured yellowfin tuna myoglobin was also reacted with H_2O_2 in the presence or absence of cysteine and with or without dithionite. The conditions were the same as in the case of the sperm whale myoglobin experiment. The spectra were similar to those of sperm whale myoglobin. But the color differences dependent upon the order of denaturation of protein were not as marked as in the other case.

Similar experiments were done with homocysteine; in a variation of this experiment, H_2O_2 was first allowed to react with the proteins, then they were denatured and the effect of other reagents was tested.

Results in this experiment were similar to those obtained with cysteine. Denatured myoglobins from either source showed no definite peaks in the presence or absence of homocysteine before dithionite was added. The color at this stage was brown in the case of both myoglobins. After dithionite addition, in both cases, the color turned red brown in the absence of homocysteine and gold-brown in its presence. The spectrum showed peaks at 540 and 572 nm. The intensity of these peaks decreased with increasing concentrations of homocysteine.

The results suggest that neither cysteine nor homocysteine are involved in greening associated with H_2O_2. The green pigment formed by reaction of myoglobin (denatured before or after H_2O_2 addition) with H_2O_2 is spectrally different from the pigment obtained by heating a mixture of tuna myoglobin, cysteine and TMAO. Koizumi and Matsuura (1968) reported that the latter pigment, after dissolving in sodium dodecyl sulfate and of reduction with dithionite, gave a green color and a characteristic absorption maximum at 600–605 nm and one at 560 and a shoulder at 540 nm. The pigment obtained in the present study had no well-defined peak in the 600–605 nm range. Koizumi and Matsurra (1968) also observed that cysteine was most effective in causing greening with myoglobin and TMAO on heating. Our results indicate that neither cysteine nor homocysteine had any influence on the formation the green color with H_2O_2. Increasing concentrations of cysteine or homocysteine did not increase the intensity of green color. Grosjean et al. (1969) reported that free sulfhydryl groups are necessary for the TMAO-induced greening. TMAO and cysteine could form a green pigment with yellowfin tuna but not with sperm whale myoglobin apparently because the latter does not have cysteine residues. On the other hand, the H_2O_2 reaction can produce green pigments with myoglobins from both sources.

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OXIDATION-REDUCTION POTENTIAL AND GROWTH OF Salmonella AND Pseudomonas fluorescens

INTRODUCTION

AMONG ENVIRONMENTAL factors influencing bacterial growth, oxidation-reduction potential has received relatively little attention, probably because of difficulties in measurement. The association of decreasing oxidation-reduction potentials with the amount of bacterial growth has been established (Burrows, 1941). Usually the lowest electrode potential is reached during the log phase of growth when the metabolic activities of the bacteria are most intense (Hewitt, 1950).

The development of a suitable apparatus and method for the measurement of changes in Eh, pH and cell numbers (Tabatabai and Walker, 1970), facilitated studies of changes in these parameters. We believed that such studies using pure and mixed cultures of *Salmonella* and *Pseudomonas* might contribute to understanding of growth relationships of these organisms on meats and poultry, particularly with regard to vacuum and nonvacuum packaged products.

EXPERIMENTAL

THE APPARATUS used for the measurement of Eh, pH and bacterial numbers was essentially identical to that described by Tabatabai and Walker (1970) with slight modifications.

Apparatus

The apparatus for measuring Eh, pH and cell numbers was constructed from a 500 ml, three-neck, round-bottom distilling flask, further modified by the University glass shop (Fig. 1). A Pyrex tube and a screw-capped culture tube with the bottom removed were added onto the flask to provide openings for the salt bridge and inoculation (and sampling), respectively. The salt bridge was constructed from soft glass tubing (1.5 cm diam), drawn to capillary size at one end. Both the gas inlet and outlet tubes were constructed from Pyrex glass tubing (0.6 cm), the latter with two glass bulbs to serve as traps and connected by black latex rubber tubing to a flask of 0.1% Roccal (Hilton-Davis Chem. Co. Div., Cincinnati, Ohio) to accommodate any overflow of foam from the culture vessel. The gas inlet tube of the apparatus was connected to a gas manifold via: (1) latex rubber tubing; (2) capillary glass tubing (5 cm long, 0.1 cm bore); and (3) latex rubber tubing (5 cm long). The upper end of the inlet tube was plugged with cotton to prevent contamination. Fine adjustments of the bubbling rate were made by using a screw clamp on the rubber tubing between the gas manifold and the capillary tubing.

A flowmeter (size 11, Cole-Parmer Instrument & Equipment Co., Chicago, Ill.) was used for monitoring the flow of either compressed air or prepurified N_2 through the electrode vessels. A cotton-packed tube between the gas manifold and the flowmeter served as a filter.

The Eh was measured with a platinum inlay electrode (Corning 476060 or Beckman 39273) and a saturated calomel reference electrode (Corning 476002 or Sargent-Welch 30080). The pH was measured with a general-purpose glass electrode (Corning 476022 or Sargent-Welch 30080) and a saturated calomel reference electrode. For measurement of either Eh or pH, a pH meter (Beckman Expandomatic) was connected to a recorder (model VOM-7, Bausch and Lomb, Rochester, N. Y.).

Standardization of pH and Eh electrodes

The pH changes in cultures were measured in millivolts rather than pH units to avoid changing the zero setting on the pH meter. Calibration curves of pH versus millivolt readings were prepared for each set of pH electrodes at each experimental temperature by using standard buffers of the following pH: 4.01, 6.85, 6.99, 7.40 and 9.14 (Beckman Co., Fullerton, Calif.). To standardize the platinum electrodes, phthalate buffer, pH 4.00, saturated with quinhydrone was used at each of the experimental temperatures; e.g., at 30°C, this system has an Eh of +456 mv.

Sterilization of the apparatus

Except where noted, 350 ml of trypticase soy broth (Baltimore Biological Laboratory, Baltimore, Md.) was sterilized in the electrode vessel at 121°C for 15 min. The gas inlet and outlet assembly and the salt bridge were sterilized at 121°C for 25 min.

KCl agar was prepared by dissolving 3g of agar and 35g of KCl in 100 ml of distilled water and autoclaving at 121°C for 15 min. The salt bridge was prepared by pouring the hot solution of saturated KCl agar into the sterile salt bridge. Platinum and glass electrodes were sterilized by immersion in a solution containing 0.05% sodium hypochlorite for 15 min, rinsed four times in sterile distilled water and dried

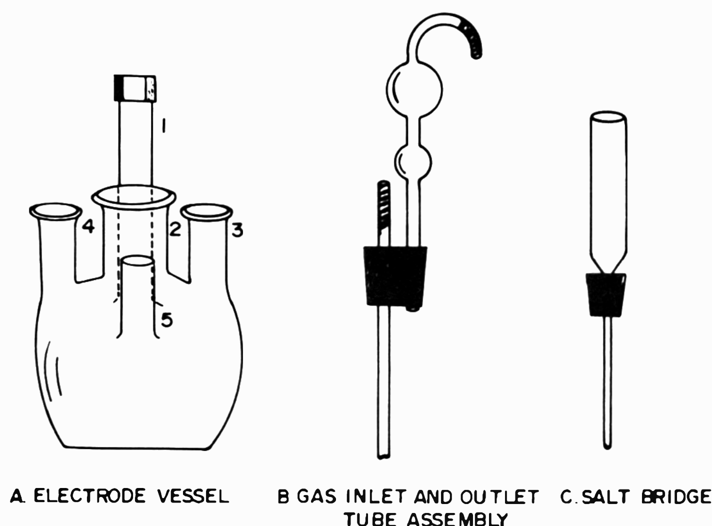

Fig. 1—Schematic drawing of electrode vessel for measuring Eh and pH: A = electrode vessel [1 = screw-cap tube for introduction and removal of samples; 2 = port for gas inlet and outlet assembly (B); 3 = port for glass electrode; 4 = port for platinum electrode; 5 = port for salt bridge (C)]; B = gas inlet and outlet assembly; C = salt bridge.

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with sterile cheesecloth before insertion into the electrode vessel.

To prevent contamination during assembly of the various components, all sterile materials were placed in a bacteriological hood equipped with a germicidal lamp which was left on for 2 hr before assembly of the vessels.

Equilibration of the medium

After assembly and before inoculation, the vessels were placed in the controlled temperature bath and connected to the gas manifold. The compressed air used for aeration was adjusted to a flow rate of 25 ml per min in each vessel and bubbled through the medium until a stable Eh was observed, 24–48 hr usually. When prepurified N_2 was used for gassing, usually at a flow rate of 75 ml per min per vessel, the vessels required 48–72 hr to attain a stable Eh.

Calculation of Eh_7

The Eh of a system at pH 7.0 is referred to symbolically as Eh_7 and was calculated by the formula adapted by Leistner and Mirna (1959). $Eh_7 = E + E_{ref} + 2.303\ (RT/F)\ (pH \times -7.0)$, where E = the measured potential; E_{ref} = the potential of the saturated calomel reference electrode at the particular experimental temperature; 2.303 (RT/F) = a constant at a given temperature; and (pH × −7.0) = the pH correction term.

This formula permits comparison of data of different experiments.

Organisms

Six serotypes of *Salmonella* and three strains of *P. fluorescens* were used. *Salmonella* serotypes included: *S. typhimurium, S. heidelberg, S. infantis, S. tennessee, S. enteritidis* and *S. thompson*. *P. fluorescens* strains were designated F21, F17 and 2. All organisms used in these studies were from the departmental stock collection (Dept. of Food Technology, Iowa State University, Ames) and previously had been isolated from poultry products.

In all instances, cultures were incubated at the temperature that was to be employed during the experiments (24 hr at 30 and 37°C; 96–120 hr at 15°C) in trypticase soy broth.

Inoculation and enumeration

For pure culture studies at 15, 30 and 37°C, three or more vessels were inoculated with the particular *Salmonella* or *Pseudomonas* strain to yield approximately 10^2–10^3 cells per ml. For mixed culture studies at 15 and 30°C, four vessels were inoculated with mixtures of selected *Salmonella* and *Pseudomonas* strains to yield varying initial ratios of *Salmonella* to *Pseudomonas*.

During the pure culture investigations, trypticase soy agar (BBL) was used for enumeration of both *Salmonella* and *Pseudomonas*. For mixed culture enumeration, surface plating was used with the agar described by King et al. (1954). This medium was evaluated along with trypticase soy agar and brilliant green agar (Difco); little variation was observed in counts with these media. Plates were incubated at either 15, 30 or 37°C, depending on the temperature used for cultivation.

Table 1—Eh_7 Values (mv)—Pure cultures of Salmonellae

	15°C				37°C			
Organism	Minimum	(hr)	Final	(hr)	Minimum	(hr)	Final	(hr)
S. typhimurium	−243	(173)	+63	(354)	−422	(15)	−3	(176)
S. heidelberg	−220	(206)	+20	(406)	−410	(18)	−33	(176)
S. infantis	−285	(150)	+155	(406)	−420	(14)	0	(176)
S. tennessee	+80	(230)	+175	(326)	−384	(19)	−7	(185)
S. enteritidis	−85	(238)	+135	(402)	−412	(17)	−15	(177)
S. thompson	−315	(180)	+107	(404)	−415	(11)	−25	(187)

Table 2—pH Values—Pure cultures of Salmonellae

	15°C				37°C			
Organism	Minimum	(hr)	Final	(hr)	Minimum	(hr)	Final	(hr)
S. typhimurium	6.24	(186)	7.85	(354)	5.73	(16)	8.23	(176)
S. heidelberg	6.45	(207)	7.90	(402)	5.95	(19)	8.25	(176)
S. infantis	6.25	(151)	8.70	(406)	5.85	(15)	8.20	(176)
S. tennessee	6.85	(234)	8.55	(326)	5.90	(20)	8.18	(185)
S. enteritidis	5.90	(240)	8.25	(404)	5.57	(21)	8.15	(177)
S. thompson	6.35	(181)	8.35	(402)	5.95	(12)	8.15	(187)

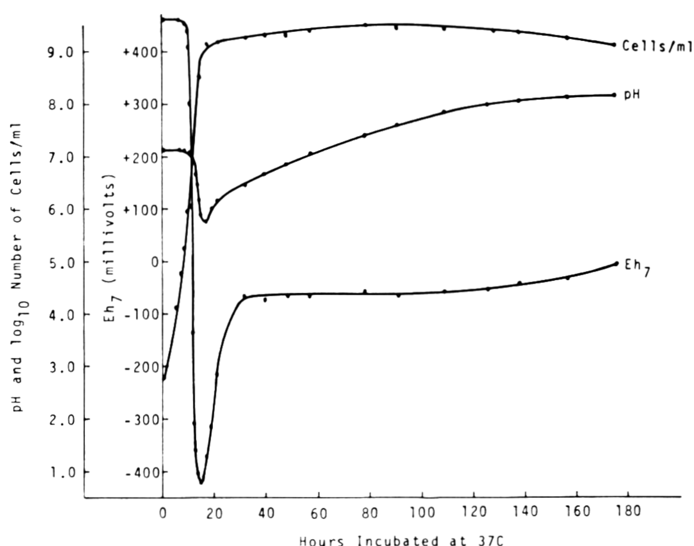

Fig. 2—Growth, Eh_7 and pH curves of S. typhimurium in TSB at 37°C.

RESULTS & DISCUSSION

Aerated conditions

Preliminary investigations were conducted using three different cultivation media to evaluate the poising effect of these media. Poising effects have been described by Hewitt (1950) and Clark (1960) as the capacity of systems (here, a sterile medium) to obstruct oxidation or reduction processes because the systems themselves have to be oxidized or reduced before the level of electrode potential can be altered appreciably. Nutrient broth (Difco), brain heart infusion broth (Difco), and trypticase soy broth (BBL) were examined.

Cultures in TSB showed the most marked effects in reduction in Eh, cell growth and maintenance of numbers, as well as pH changes. Additional work confirmed that NB had a greater poising effect than other media. Therefore, we decided to adopt TSB for future experiments. High (10^6 cells per ml) and low (10^2 cells per ml) levels of inoculum were also tested to determine wheter initial cell concentrations caused appreciable differences in the Eh, pH and later cell num-

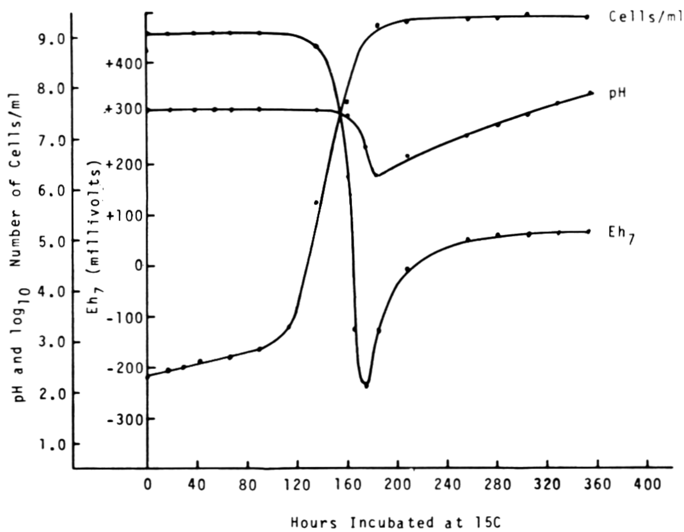

Fig. 3–Growth, Eh_7 and pH curves of S. typhimurium in TSB at 15°C.

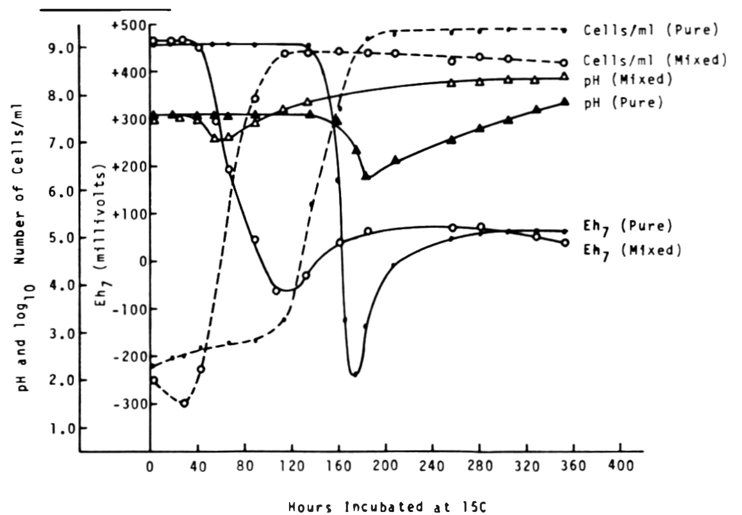

Fig. 5–Effect of P. fluorescens F21 on changes in Eh_7, pH and growth of S. typhimurium at an initial ratio of 30:1, respectively, in TSB at 15°C.

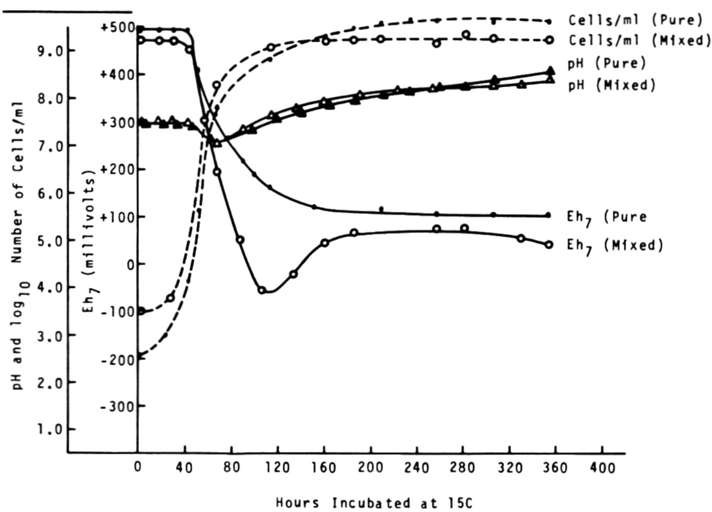

Fig. 4–Effect of S. typhimurium on changes in Eh_7, pH and growth of P. fluorescens F21 at an initial ratio of 1:30, respectively, in TSB at 15°C.

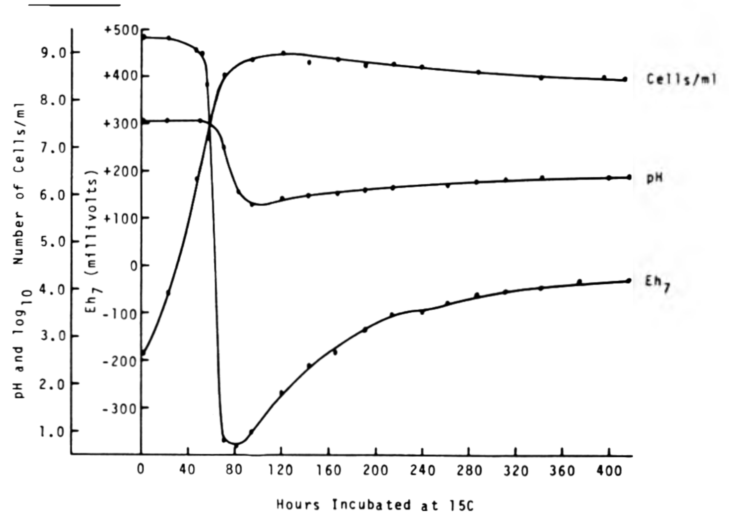

Fig. 6–Growth, Eh_7 and pH curves of S. heidelberg in TSB at 15°C with no aeration.

bers observed. With the exception that all the changes in the cultures occurred a bit sooner with the greater inoculum, the over-all patterns of the parameters, including maximal and minimal values, were similar. The lower level of inoculum was used to obtain a more characteristic growth curve and accompanying changes in Eh and pH.

Tables 1 and 2 show data obtained from the six pure cultures of *Salmonella* strains at 15 and 37°C. Table 1 gives minimum and final Eh_7 values, and Table 2 gives minimum and final pH values attained by these organisms. Figure 2 shows Eh, pH and growth curves for *S. typhimurium* at 37°C. Very nearly coincidentally with the establishment of minimal Eh, the pH reached its minimum value. Viable cell numbers also attained a near maximum shortly thereafter. After minimal Eh was observed, there was a sharp rise in Eh for the next 10–15 hr followed by a gradual increase to more positive values.

Figure 3 presents data obtained with *S. typhimurium* at 15°C. As would be expected, there was a considerable lag phase as compared with that at 37°C. After approximately 120 hr, however, changes in Eh and pH began to occur. There was a marked negative drift in Eh, although not to the degree observed at 37°C. The pH decreased rapidly. The fast recovery to higher Eh and pH values was obvious also. Attainment of stationary growth phase coincided with minimal Eh and pH values. Maximum and final cell concentrations were the same, 2.2×10^9 cells per ml, with no evident decrease in population.

The trends of Eh, pH and cell growth in TSB for the six salmonellae tested were very similar to those for *S. typhimurium*. In most instances, survival of *Salmonella*,

once in the stationary phase, was greater at the lower temperature (15°C). Attainment of maximal cell concentrations coincided with attainment of minimum Eh and pH values indicative of intense metabolic activity.

The reducing capacity of *P. fluorescens* F21 was much less marked than that observed with salmonellae. Rather than dropping abruptly, the Eh in *Pseudomonas* cultures continued to drift slowly downward during the entire experiment. The final Eh value was approximately 35 mv greater at 15°C than at 30°C. Essentially no change in pH occurred during the time that the Eh decreased most rapidly, but a gradual increase to more basic values was observed when the bacteria had reached the stationary phase.

Minimal and final Eh were identical for each strain of *P. fluorescens* examined. The reducing capacities of the pseudomonads were similar in that the cultures exhibited only a gradual negative drift in Eh. Changes in pH were slight as compared to the values observed with the salmonellae. Again, maximum populations of cells corresponded to the occurrence of minimal Eh and pH values. These observations are consistent with those of Hewitt (1950), Borromeo (1969) and Tabatabai and Walker (1970).

Limited investigations using mixed cultures of *S. typhimurium* and *P. fluorescens* were undertaken in an effort to understand the possible inhibition of one organism by another noted by Oblinger and Kraft (1970).

Figures 4 and 5 present data obtained from experiments done at 15°C in which *P. fluorescens* F21 initially outnumbered *S. typhimurium* by a ratio of 30:1. Figure 4 shows the effect of *S. typhimurium* on changes in Eh, pH and growth of *P. fluorescens* F21. The growth of the *Salmonella* had little or no effect on pH. The growth of the pseudomonads in mixed culture was somewhat less than that in pure culture even though these bacteria entered both log and stationary phases more rapidly in mixed culture. The maximal population in pure culture was 4.4×10^9 cells per ml vs. a maximum population of 2.0×10^9 cells per ml in mixed culture. Perhaps the effect of the *Salmonella* on the pseudomonad is most obvious from the Eh curve which shows a more intense reduction down to a level of about −60 mv at 112 hr and then a gradual rise to a final Eh of +45 mv.

Figure 5 shows the effect of the pseudomonad on *S. typhimurium* in regard to changes in Eh, pH and growth. The dominant influence of the pseudomonad is evident especially in the curve for the pH which, in these mixed cultures, was essentially that of the pure culture of *P. fluorescens*. The Eh curves show that the reducing activity in the mixed culture began at approximately the time that the pseudomonad would have begun its reduction in pure culture, about 140 hr before the pure *Salmonella* strain. In mixed culture, the reducing capacity of the *Salmonella* was not as great as in pure culture, yet the recovery of Eh towards the end of the experiment to a value of +63 mv is very near the final Eh value of +42 mv observed in pure culture. The *Salmonella* entered the logarithmic phase of growth much more quickly than when grown alone, by approximately 80 hr. It is entirely possible that the pseudomonad, through its metabolic reactions, provided the *Salmonella* with more readily available nutrients. Also it is conceivable that the highly aerobic metabolism of the *Pseudomonas* strain consumed the available oxygen and thereby encouraged the growth of the *Salmonella*. Results that tend to support this second conclusion will be presented later. Numbers of *Salmonella* in mixed culture reached 1.0×10^9 cells per ml and exhibited a decrease in numbers over 354 hr, possibly due to some form of inhibition by *Pseudomonas*. The *Pseudomonas* did not show a decrease in numbers even though these organisms did not attain as high a population as they did in pure culture.

Nonaerated conditions

Experiments also were conducted where there was no aeration of the culture with compressed air. The vessels were assembled and treated as if the cultures were to be aerated, but the air supply was not used. It has been reported (Clark, 1960) that there is a layering of Eh that affects the accuracy of the measurements recorded.

Absence of added aeration had little effect on early phases of Eh, pH, and growth curves of the six salmonellae strains investigated. Layering of Eh was not considered a significant problem since measurements were made on the unagitated cultures and then compared with readings taken after slight swirling of the cultures. Readings varied in the range of ±5 mv. Figure 6 shows results typical of nonaerated experiments. In our investigations, *S. heidelberg* was tested at 15°C. The characteristic recovery of both Eh and pH to higher values was greatly retarded without aeration. Growth and subsequent changes in Eh and pH at 15°C occurred faster than they did in pure culture at 15°C under aeration. This may help to explain the early initiation of growth by *S. typhimurium* in mixed culture with *P. fluorescens*; it is possible that *P. fluorescens* lessened the air content of the medium, thereby making it easier for the *Salmonella* to grow. By referring to the pure culture observations made with aeration (Tables 1 and 2), comparisons of minimal and final Eh and pH values are possible.

Inert gassing

Experiments were performed using prepurified nitrogen (PPN_2) as a means of lowering the initial Eh of sterile media and then observing the presence or absence of growth by particular organisms after inoculation. Results were compared with those obtained when compressed air was used. Tests of this type were designed to try to determine if there were limiting initial Eh values below which proliferation would not take place or would be greatly hindered. Various levels of Eh could be obtained by varying the flow rate of PPN_2 into the cultures.

When PPN_2 was used to lower the Eh of the medium, the *P. fluorescens* strains used in these studies did not grow at Eh levels below +80 mv. *Salmonella* strains were not able to initiate growth at Eh levels lower than +30 mv.

Generalizations

Through the use of the apparatus and techniques described, observations on Eh, pH and growth of *Salmonella* and *P. fluorescens* have been readily reproducible. Similarities within genera of *Salmonella* and *Pseudomonas* are evident, but at the same time there were slight but consistent differences between species. This observation is compatible with that made by Burrows and Jordan (1935) and tends to support the close metabolic relationship between these organisms. Zador (1961) suggested that redox potential in bacterial cultures could be an important aid to the classification of bacteria.

In instances when very intense reducing conditions were established (e.g., for the salmonellae examined) the oxidizing effect of molecular oxygen was essentially undetectable.

There seem to be definite values of Eh that determine if a given organism can initiate growth and/or survive. Values of this type have been reported for the anaerobes, particularly the clostridia (Barnes and Ingram, 1956; Reed and Orr, 1943).

The potentiometric method of Eh measurement may be less sensitive to changes in culture Eh than has been suggested by others. In our experiments, concentrations of organisms approached 10^5 to 10^6 cells per ml before detectable changes in Eh were observed.

Measurements of Eh, pH, and growth at 15°C showed that, once the salmonellae investigated had adapted to the somewhat adverse environment, they possessed the capacity to proliferate actively and attain large populations. Moreover, the salmonellae and the *Pseudomonas* persisted for a longer time at 15°C than at 30 and 37°C.

In the mixed culture studies, fewer cells per ml were produced by both the salmonellae and *Pseudomonas* strains than in pure culture. The pseudomonads

may have stimulated the salmonellae initially, but produced an inhibitory effect toward the end of the experiments. Eh curves obtained from mixed cultures show a blending of the Eh trends of both organisms. On the other hand, the pH values obtained in mixed show the preponderant influence of the *Pseudomonas*. Other effects may be revealed if studies with other initial *Salmonella:Pseudomonas* ratios are conducted.

Work of this nature may have application to inhibition of growth on meats and poultry under various packaging conditions. Investigations of this type also are an aid in understanding the relationships of *Salmonella* and typical spoilage organisms of these products.

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FACTORS ASSOCIATED WITH POSTMORTEM INCREASE OF EXTRACTABLE Ca IN CHICKEN BREAST MUSCLE

INTRODUCTION

IN LIVING MUSCLES, Ca ion is known as a regulating agent of the contractile system (Ebashi and Endo, 1968). Recently it has been suggested that Ca ion has an important role in the meat tenderization phenomenon during postmortem aging; Ca ion removes specially the Z-lines from muscle fibers (Davey and Gilbert, 1969; Busch et al., 1972a), weakens the strength of muscle fibers (Nakamura, 1972a, b; Busch et al., 1972b) and the extractable Ca from muscle tissues increases gradually during postmortem aging (Arnold et al., 1956; Nakamura, 1973). As the tenderness of meat varies with various sorts of pre- or postmortem treatments of muscles, it seems necessary to know the details of Ca release from muscle tissues. The cause of postmortem release of Ca, however, has not yet been clarified.

In this work, to investigate the factors associated with the postmortem increase of extractable Ca, experiments were done about the poultry breast muscle which was treated previously to change the postmortem glycolysis and the rate of ATP breakdown.

EXPERIMENTAL

CHICKEN (12–14 month old) of the White Leghorn (female) strain were used in this experiment. Muscle samples were obtained from Pectoralis major muscles. All birds were killed by cutting the jugular vein and caratid arteries, skinned without scalding and eviscerated. The carcass was placed in a plastic bag and aged in drained crushed ice.

Postmortem glycolysis in muscle tissues was controlled either by injecting epinephrine (an intramuscular dose of 4 mg/kg body weight) before slaughter (Khan and Nakamura, 1970) or by aging minced muscle tissues from untreated birds. Monoiodoacetate treatment was selected as the condition of postmortem treatment; muscles were minced immediately after slaughter, each 5-g portion was homogenized with 50 ml ice cold distilled water, sodium monoiodoacetate (2 mg%) added and kept at 0°C for the desired length of time, up to 48 hr.

Tests were also made on prerigor frozen muscles in order to obtain conditions known to cause a more rapid than normal rate of ATP loss (de Fremery and Pool, 1960). In these tests muscles were removed from the carcass immediately after slaughter, placed in a plastic bag and frozen in a dry ice-acetone bath. After keeping for 30 min at this temperature, muscles were thawed in running tap water and kept at 0°C for the desired length of time, up to 48 hr. To minimize the effect of bird-to-bird variability within the treated and untreated groups, comparisons were made between the left and right muscles from one bird, one muscle being frozen and thawed, and the other as a control muscle.

Extractable Ca was determined in water extracts by the atomic absorption method as described previously (Nakamura, 1973). 5g of minced muscles was extracted by homogenizing with 30 ml ice cold distilled water for 5 min in a Waring Blendor, with motor speed about 2,000 rpm. To inhibit postmortem glycolysis during extraction, sodium monoiodoacetate (2 mg%) was added to the water used for the extraction. The homogenate was made up to 50 ml with water and centrifuged for 10 min at 4°C and 10,000G. A definite amount of the supernatant was ashed by the wet combustion method using HNO_3-$HClO_4$ and concentrated until the Ca concentration became as high as 0.1 mM or more. Before Ca determination the extracts were neutralized and 1% of EDTA-Na_2 was added.

ATP content of muscles was analyzed by thin layer chromatography (Randerath and Randerath, 1964) after extraction with perchloric acid according to Davidek and Khan (1967). To follow the disappearance of ATP, an absorbance ratio between 258μ and 250μ was measured in the perchloric acid extracts of muscles (Khan and Frey, 1971). pH of muscles was measured directly with a needle-type glass electrode using a Hitachi-Horiba pH meter (Hitachi Co. Ltd., Tokyo).

RESULTS & DISCUSSION

pH OF THE MUSCLE samples used as controls dropped from 6.8 to the ultimate pH, 5.8 until 8 hr after slaughter but the release of Ca increased gradually until 24 hr after slaughter (Table 1). The pH of iodoacetate-treated samples and samples obtained from epinephrine-treated birds had a constant pH, 6.8 and 7.0, respectively and remained the same during the postmortem aging period. The postmortem changes in their extractable Ca content, however, were largely different; that of iodoacetate-treated samples was low at the initial period of aging and increased gradually until 0.13 μmoles/g muscles, while that of the latter was as high as that of the aged control muscles at the initial period of aging and did not change during further storage. The extractable Ca content of these two kinds of muscles at 48 hr after slaughter was almost the same as that of control muscles at the same aging period. Although postmortem pH change is often considered to be the cause of the release of Ca from muscle tissues (for example, Lawrie, 1966), the result in Table 1 clearly shows that postmortem pH change and the release of Ca from muscle tissues does not occur concomitantly. On the other hand, release of Ca and loss of ATP occurred concomitantly in both control and epinephrine-injected muscles (Fig. 1).

Tests made on prerigor frozen and thawed muscles also confirmed that release of Ca and loss of ATP occurred concomitantly (Fig. 2). In this experiment, an easier method to follow the disappearance of ATP was needed rather than the time-consuming measurement of ATP, since a large number of samples had

Table 1—Effects of monoiodoacetate treatment and epinephrine injection on the extractable Ca content of chicken breast muscle. Values are averages of five birds[a]

Treatment		Aging time (hr) 0	8	24	48
None	pH	6.8 ± 0.1	5.8 ± 0.1	5.8 ± 0.1	5.8 ± 0.1
	Ca (μmoles/g muscle)	0.031 ± 0.006	0.08 ± 0.01	0.13 ± 0.03	0.13 ± 0.03
Monoiodoacetate treatment	pH	6.8 ± 0.1	6.8 ± 0.1	6.8 ± 0.1	6.8 ± 0.1
	Ca (μmoles/g muscle)	0.028 ± 0.003	0.08 ± 0.01	0.11 ± 0.02	0.12 ± 0.02
Epinephrine injection	pH	7.0 ± 0.2	7.0 ± 0.2	7.0 ± 0.2	7.0 ± 0.2
	Ca (μmoles/g muscle)	0.13 ± 0.01	0.13 ± 0.02	0.13 ± 0.02	0.13 ± 0.02

[a] Mean ± SD

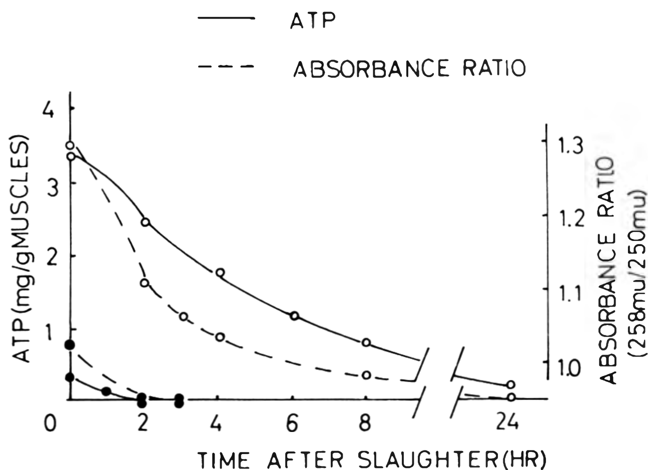

Fig. 1–The postmortem changes in ATP and absorbance ratio of control (○) and epinephrine-injected (●) samples.

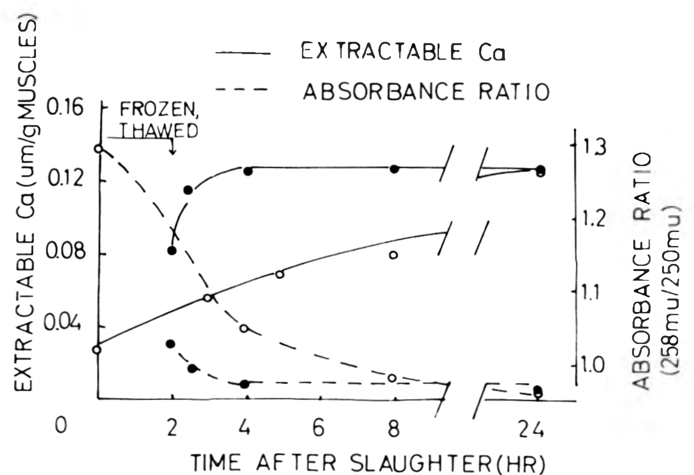

Fig. 2–Postmortem changes in an absorbance ratio and extractable Ca content of control (○) and frozen-thawed (●) samples.

to be analyzed to investigate the relation between the extractable Ca and the ATP content of muscles. For this purpose, an absorbance ratio according to Khan and Frey (1971) was selected after the comparison of this value and the amount of ATP measured in muscles of control and epinephrine injected birds (Fig. 1). In frozen-thawed muscles, extractable Ca increased very rapidly and reached a constant value, 0.13 μmoles/g muscles, at 3 hr after thawing (Fig. 2). The absorbance ratio of the same muscles decreased rapidly and reached a constant value, 0.98, at the same time. This value of absorbance ratio was almost equal to that of control muscles aged for 24 hr, of which extractable Ca content was the same as that of frozen-thawed muscles, 0.13 μmoles/g muscles.

Relation between the increase of the extractable Ca and the loss of ATP during postmortem aging was further ascertained by plotting all the experimental data of both the control muscles and the frozen-thawed muscles (Fig. 3); extractable Ca increased gradually until an absorbance ratio of about 1.0 was reached which corresponded to the 70–80% loss of ATP (judged from the result of Fig. 1), and increased rapidly below this value of absorbance ratio. All results in the present work clearly show that Ca is released from muscle tissues during postmortem aging as the loss of ATP proceeds.

Since sarcoplasmic reticulum membranes have a specific Ca accumulating ability and easily release Ca under physiological condition, the increase of extractable Ca during postmortem aging seems to be due to the release of bound Ca from it. In this case, ATP is necessary for the Ca accumulation of sarcoplasmic reticulum membranes (Ebashi and Endo, 1968). So, it is most probable that the loss of ATP reduces the Ca accumulating ability of sarcoplasmic reticulum membranes. The Ca accumulating ability of sarcoplasmic reticulum membranes is shown to decrease gradually during postmortem aging and the cause of it is suggested as the decrease of pH (Greaser et al., 1967, 1969; Goll et al., 1971). Both the decrease of pH and ATP, however, often occur concomitantly under normal aging condition and the effect of ATP loss on the Ca accumulating ability of sarcoplasmic reticulum membranes has not been studied fully. So, the present result does not exclude the possibility that sarcoplasmic reticulum membranes release the bound Ca with the loss of ATP and then change their properties with some other causes, for example, decrease of pH or the action of proteolytic enzyme (Goll et al., 1971).

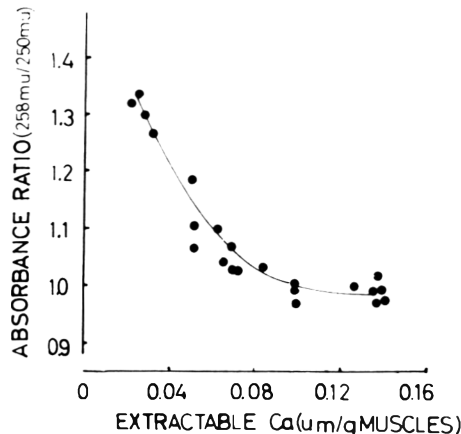

Fig. 3–Relation between the absorbance ratio and extractable Ca content of both control and frozen-thawed samples.

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SUBSTRATE INHIBITION OF CHICKEN MUSCLE LACTATE DEHYDROGENASE AS A FUNCTION OF TEMPERATURE

INTRODUCTION

THE RATE OF glycolysis in postmortem muscle tissue has been implicated in various quality attributes of muscle foods such as tenderness, the pale soft exudative (PSE) condition, protein solubility and water-holding capacity. An elucidation of the factors controlling the activities of the glycolytic enzymes postmortem is of significance, therefore, in understanding the biochemical changes, and thus the quality changes, occurring in muscle tissues postmortem.

Lactate dehydrogenase (LDH) catalyzes the final step in muscle glycolysis, i.e., the reduction of pyruvate to lactate with the oxidation of NADH to NAD^+. LDH is a tetrameric enzyme with a molecular weight of approximately 135,000. Two different subunits give a potential for five isoenzymic forms. In chicken breast muscle, one species predominates which contains four muscle-type subunits (lactate dehydrogenase isoenzyme 5). We had previously shown that this isoenzyme is capable of binding to the particulate fraction of homogenized chicken breast muscle and that such binding influences the kinetic properties of the enzyme (Hultin et al., 1972).

Lactate dehydrogenase is an enzyme which is inhibited by its substrate. This is most likely due to the formation of an abortive ternary complex of lactate dehydrogenase:NAD^+:pyruvate (Fromm, 1961; Stambaugh and Post, 1966). It has been suggested that the formation of this ternary complex and the subsequent inhibition of the enzyme is a regulatory mechanism in the metabolic control of LDH (Kaplan et al., 1968; Cahn et al., 1962).

In this paper we present results of a study to determine the effect of temperature on inhibition of LDH by substrate and also how this inhibition is affected when the enzyme is bound to the subcellular particulate fraction. The temperatures studied represent those encountered during the postmortem cooling of muscle tissue where glycolysis may be particularly active. As postmortem time progresses, the pH of the tissue decreases, making it more likely that the lactate dehydrogenase will be bound to the particulate structures of the cell (Hultin and Westort, 1966).

MATERIALS & METHODS

FEMALE CHICKENS of mixed breeds were obtained from the Dept. of Veterinary & Animal Sciences at the University of Massachusetts. The birds were fed a commercial pelleted diet ad libitum.

NADH and NAD^+ were purchased from P-L Biochemicals Inc. Sodium pyruvate and chlortetracycline were products of Nutritional Biochemicals Corp. All other chemicals were the purest available commercially.

Methods

The details of the preparation of enzymes, the assay techniques, and control measures have been described in detail previously (Ehmann and Hultin, 1973). Major points of the procedure will be briefly described here.

Preparation of enzymes. A particulate fraction rich in lactate dehydrogenase activity was prepared by homogenization of chicken breast muscle in water followed by centrifugation and further washing with deionized distilled water. Soluble enzyme was prepared from this particulate lactate dehydrogenase by solubilization with a salt solution followed by dialysis. A muscle particulate fraction essentially free of lactate dehydrogenase activity was prepared by washing the LDH-rich fraction several times with 0.15 M sodium chloride followed by several water washes. This enzyme-poor particulate fraction was used to adjust the enzyme-rich particulate fraction to the desired ratio of insoluble protein content to enzyme activity.

Assay conditions. The assay medium for the stopped-flow assay contained imidazole buffer, 10 mM, pH 6.5; NADH, 0.1 mM; and various concentrations of pyruvate as indicated in the Results section. These conditions were chosen to maintain most of the lactate dehydrogenase bound to the particulate fraction during the assay. In all cases, less than 2% of the total enzyme in the bound enzyme preparation was solubilized by the assay medium. This amount of soluble enzyme had a negligible effect on

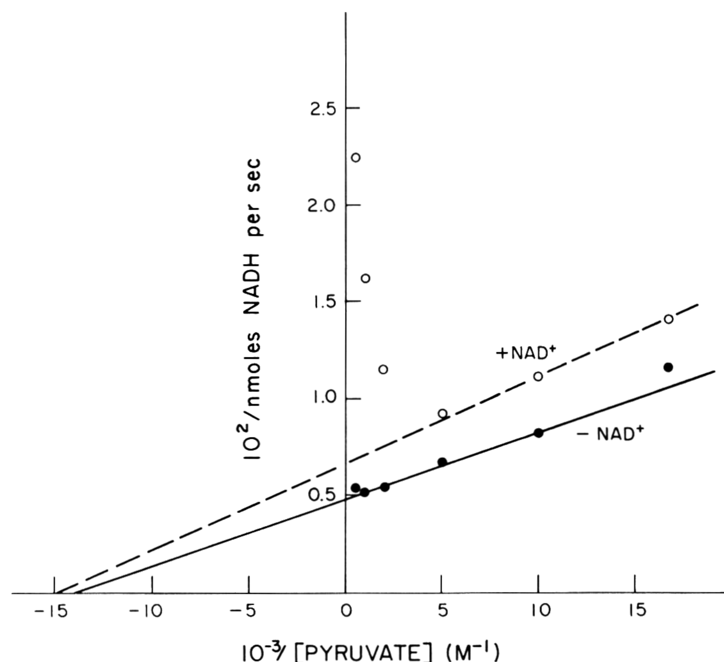

Fig. 1—Reciprocal plots of initial velocity vs. pyruvate concentration of soluble LDH at 23°C with (---○---○---) and without (———●———●———) preincubation with NAD^+. The enzyme concentration was 10^{-7}M, the pH was 6.5 and the concentration of NADH was 0.1 mM. When used, the concentration of NAD^+ was 0.1 mM and incubation time was 15 min. A stopped-flow assay was used. The lines were obtained by computer calculation of the least-square straight line fit, ignoring data in the non-linear portions of the curves.

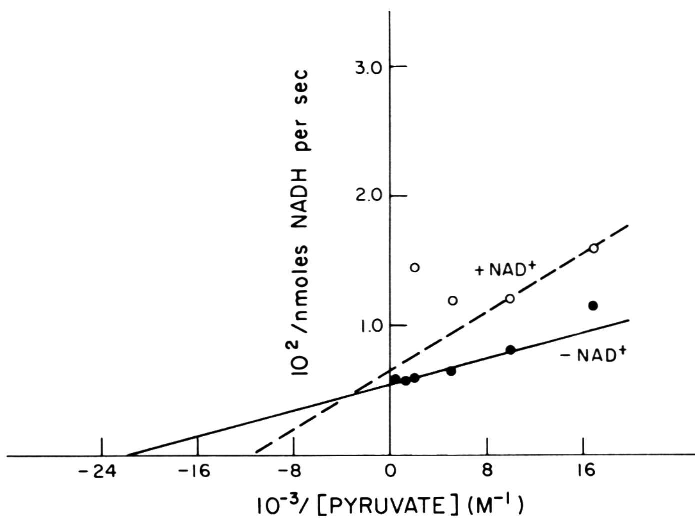

Fig. 2–Reciprocal plots of initial velocity vs. pyruvate concentration of soluble LDH at 16°C with (---○--○---) and without (———●——●———) preincubation with NAD^+. The conditions were the same as for Figure 1 except that the incubation time of enzyme with NAD^+ was 30 min.

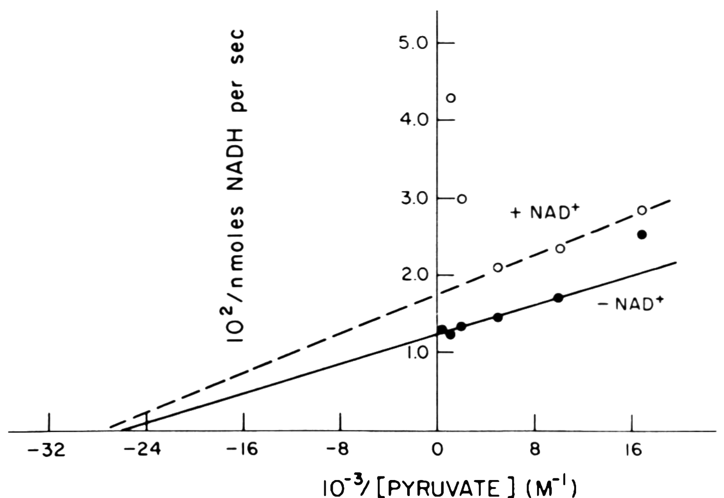

Fig. 3–Reciprocal plots of initial velocity vs. pyruvate concentration of soluble LDH at 4°C with (---○--○---) and without (———●——●———) preincubation with NAD^+. The conditions were the same as for Figure 1 except that the incubation time of enzyme with NAD^+ was 60 min.

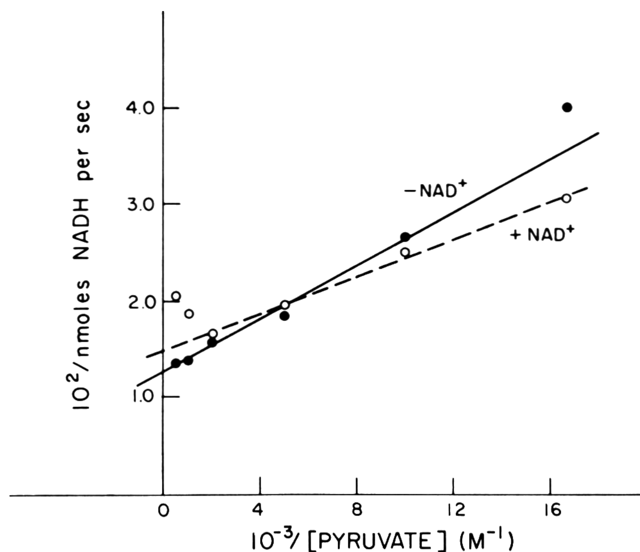

Fig. 4–Reciprocal plots of initial velocity vs. pyruvate concentration of bound LDH at 23°C with (---○--○---) and without (———●——●———) preincubation with NAD^+. The conditions were the same as described for Figure 1. The bound enzyme preparation contained approximately 10 mg of insoluble protein per ml in the final assay medium. Preincubation time was 15 min.

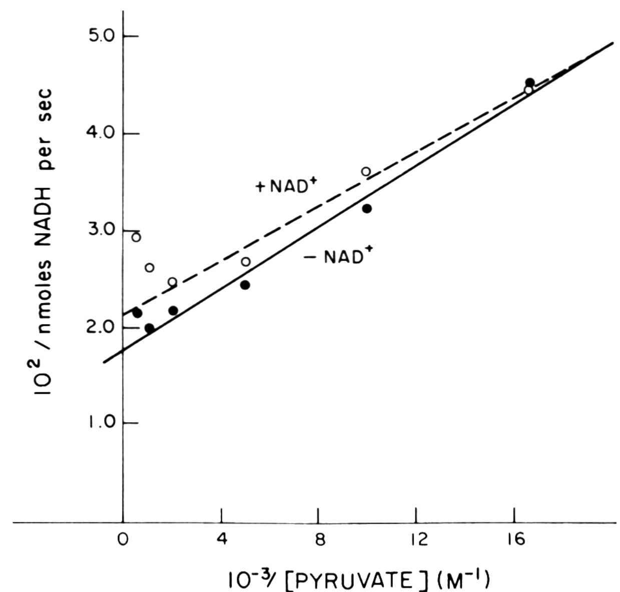

Fig. 5–Reciprocal plots of initial velocity vs. pyruvate concentration of bound LDH at 16°C with (---○--○---) and without (———●——●———) preincubation with NAD^+. The conditions were the same as described for Figure 4. Preincubation time was 30 min.

evaluation of the results. Solubilization studies were performed at 4, 16 and 23°C.

Adjustment of enzyme concentrations. The enzyme concentrations in the bound and soluble preparations were adjusted to equal values by determining the lactate dehydrogenase activity under conditions of pH (7.5) and ionic strength (0.050) where all of the enzyme in the bound preparation would be solubilized. Thus, a direct comparison could be made between the bound and soluble preparations. V_{Max} was determined by extrapolation of double reciprocal plots of activity vs. substrate concentrations. Enzyme concentrations were then calculated based on the turnover number of Pesce et al. (1964) and adjusted to 10^{-7}M for the stopped-flow assays.

Stopped-flow assay. The soluble and bound lactate dehydrogenase preparations were assayed in a stopped-flow apparatus, and the change in transmission at 340 nm was recorded on a storage oscilloscope. Temperatures of reaction were maintained by a circulating water bath. The final concentration of insoluble protein in the bound preparation was approximately 10 mg per ml. The final concentrations of the reagents in the assay were as described above.

Assay in the presence of NAD^+. To study the effect of product inhibition, NAD^+ was in-

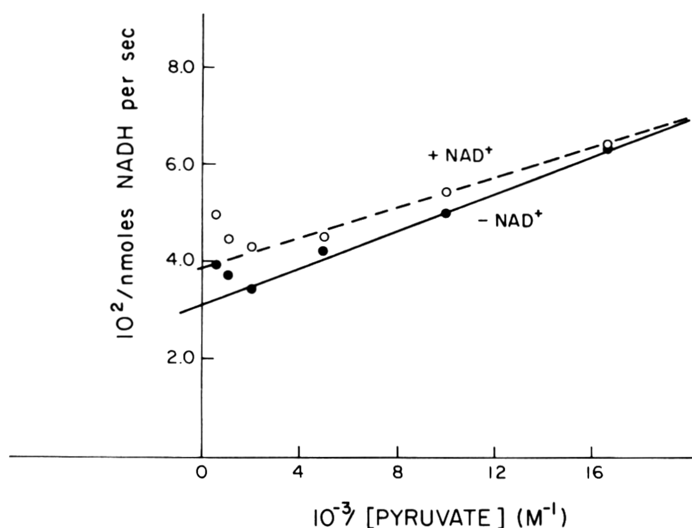

Fig. 6–Reciprocal plots of initial velocity vs. pyruvate concentration of bound LDH at 4°C with (---o---o---) and without (———•——•———) preincubation with NAD^+. The conditions were the same as described for Figure 4. Preincubation time was 60 min.

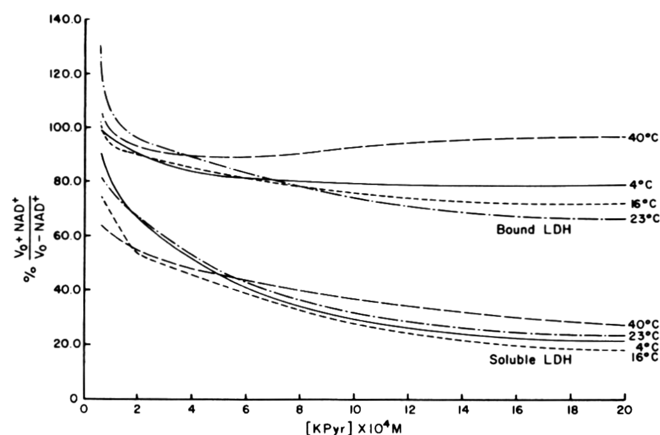

Fig. 7–Percentage of initial velocity of LDH in the presence of NAD^+ compared to that in its absence as a function of pyruvate concentration. The data for 4, 16 and 23°C are taken from Fig. 1–6 and those for 40°C from our earlier work (Ehmann and Hultin, 1973).

cubated with the enzyme for a period of time which varied with the temperature. These preincubation times were 15 min at 23°, 30 min at 16° and 60 min at 4°C. These times were predetermined as those necessary to give maximal inhibition. In most studies of substrate inhibition of LDH, the NAD^+ is generated during the reaction. Pre-incubation of the enzyme with the NAD^+ should more nearly duplicate conditions in situ.

There was no binding of NAD^+ or NADH to the particulate fraction of the muscle, and there was no oxidation of NADH in the absence of pyruvate.

Determination of protein. The protein contents of the enzyme-rich and enzyme-poor particles were determined by a biuret procedure (Gornall et al., 1949).

RESULTS

DOUBLE RECIPROCAL plots of initial velocity versus substrate concentration for soluble enzyme in the presence and absence of NAD^+ are shown for 23°, 16° and 4°C in Figures 1 through 3. It is clear that there is a significant amount of inhibition of the soluble enzyme by NAD+ in the presence of pyruvate. Although it is difficult to evaluate the kinetics in great detail because of the high degree of inhibition at high substrate concentrations which results in a very small straight-line portion of the curves of the inhibited enzyme, it does appear that the type of inhibition is a function of temperature. The inhibition discussed here is product inhibition and not substrate inhibition since the data points at high pyruvate concentrations have been excluded from the plots. The effect at all temperatures is typical of mixed inhibition, but the data at 4° and 23°C indicate an almost noncompetitive situation, that is, where the NAD^+ does not interfere with the binding of substrate but does interfere with the breakdown of the enzyme-substrate-inhibitor complex. At 16°C a mixed type of inhibition is seen, which indicates that both the binding of substrate is reduced and the breakdown of the enzyme:substrate complex is inhibited. These data do not deviate greatly from a competitive type of inhibition. We had earlier shown that at the physiological temperature (40°C), the inhibition was not greatly different from an uncompetitive type of inhibition (Ehmann and Hultin, 1973). Uncompetitive inhibition means that the inhibitor promotes the binding of the substrate but does not lead to a complex that will break down to give the end product. It appears that the inhibition kinetics of LDH by NAD^+ and pyruvate are very complex and that there are some very significant temperature effects.

In Figures 4 through 6 are shown the reciprocal plots of velocity versus substrate concentration for the bound enzyme at 23, 16 and 4°C. These results indicate that there is considerably less inhibition of the bound enzyme at these temperatures than for the soluble enzyme. The small amount of soluble enzyme in the bound preparation makes any further evaluation of these data tenuous. Previous work at 40°C indicated that there was no inhibition at the physiological temperature (Ehmann and Hultin, 1973).

The data in Figures 1–3 indicate that in the absence of NAD^+, the activity of soluble LDH is nonlinear at the lowest pyruvate concentration in the double reciprocal plots. The very low activities observed at the low pyruvate concentration at all temperatures suggest cooperativity for the soluble LDH. We had observed such a phenomenon previously for soluble LDH at 1.5×10^{-7}M and 6.9×10^{-9}M utilizing NADPH as cofactor at pH 6.7 (Hultin et al., 1972), but cooperativity was not seen with either bound LDH under the same conditions or with soluble LDH at 10^{-10}M with NADH as cofactor. In this study, no cooperativity was observed with bound LDH at 4° or 16°C, but there did appear to be some at 23°C.

The percentage of the initial velocity of lactate dehydrogenase in the presence of NAD^+ to that in its absence as a function of pyruvate concentration at 4°, 16°, 23° and 40°C is presented in Figure 7. The last temperature is included here to compare results at the physiological temperature with those in this study. It appears that two major points can be made concerning the data. First, the soluble enzyme is obviously more sensitive to inhibition by NAD^+ than is bound LDH. The second point is that whereas the ratio of activity of soluble LDH in the presence and absence of NAD^+ is not a function of temperature, there appears to be a definite dependency on temperature in the case of the bound enzyme. At 40°C there is little or no inhibition at any substrate level; however, at the lower temperatures of 4, 16 and 23°C, there is a measurable amount of inhibition at the higher substrate concentration.

DISCUSSION

IT IS OBVIOUS from the results presented that the inhibition kinetics of lactate dehydrogenase of chicken breast muscle are very complex, being affected both by temperature and by whether the enzyme is bound to the particulate fraction of the homogenized tissue. The varying effect of temperature on substrate inhibition may explain the differing results that people have observed in evaluation of LDH activities as a function of temperature (Vesell, 1965). If the studies are carried out in whole homogenates, the possibility of interaction of enzyme with the particulate structures may also occur, further affecting the kinetic properties of the enzyme and making interpretation of the results more complex. The low inhibition of bound LDH may be due to preservation of the tetrameric integrity of the enzyme due to the binding (Ehmann and Hultin, 1973) since it has been suggested that the ternary complex cannot form unless the LDH first dissociates into its monmeric subunits (Griffin and Criddle, 1970). If this is true, then the corollary to this would be that under the conditions studied in this report, soluble LDH must dissociate. Whether conditions in situ are such as to allow for dissociation of soluble LDH in the cytoplasm is not known. However, if dissociation to monomers is required for the ternary complex to form, then inhibition by NAD^+ and pyruvate in situ could only occur if the conditions in the cytoplasm allowed dissociation.

Our results point out the importance of examining the kinetic properties of this enzyme, and possibly others, over the range of temperatures used in food storage. It may not be always possible to simply extrapolate inhibition data from one temperature to another, expecting only slight quantitative changes due to lowering of the temperature. There may be qualitative differences as well.

In a previous paper (Hultin et al., 1972) we had suggested a rationale for the phenomenon of binding-solubilization of lactate dehydrogenase to the particulate fraction of the skeletal muscle cell. This hypothesis was based on the lower activity of the enzyme when bound compared to when it was in a soluble form. This, along with the effect of NADH in solubilizing the enzyme, was suggested to be a control mechanism in situ. The data presented in this report fit in with this hypothesis. The additional feature now which would have to be added to the hypothesis is that the bound enzyme gives a low but relatively steady activity even under conditions of high NAD^+ concentrations since it is not strongly subject to inhibition by NAD^+. On the other hand, even though the activity of the soluble enzyme can be very high, it is also very susceptible to control by NAD^+ and pyruvate concentrations. Thus the bound enzyme gives a low but steady rate of activity, whereas the soluble enzyme has the potential for having a high activity but this activity is subject to a fine control.

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TEMPERATURE DEPENDENCE OF THE MICHAELIS CONSTANT OF CHICKEN BREAST MUSCLE LACTATE DEHYDROGENASE

INTRODUCTION

ALTHOUGH temperature-dependent changes in the kinetic properties of enzymes in foods would be extremely important in understanding changes which occur in foods postmortem or post-harvest, surprisingly little attention has been given to this facet of enzyme research. It is generally assumed that there is a decrease in enzymic activity with a decrease in temperature which can be expressed by the Arrhenius relationship between reaction rate and reciprocal of temperature. The rates are determined in dilute enzyme solutions and with concentrations of substrate in large excess. The possibility must be considered, however, that the kinetic characteristics of enzymes may be subject to changes brought about by subtle conformational changes due to changes in temperature. Conformational changes in enzymes due to a variety of influences are well documented.

We report here the results of a study on the effect of temperature on some kinetic properties of chicken breast muscle lactate dehydrogenase (LDH). This enzyme was chosen because it is a tetrameric enzyme, and subtle changes would more likely be apparent in an enzyme with subunit structure than in one consisting of a simple polypetide chain. The LDH from chicken breast muscle is comprised of four muscle-type subunits. Results of our study indicate that important changes occur in this enzyme with a reduction of temperature which are manifested by a change in the kinetic properties of the enzyme. The principal change is that the enzyme becomes more efficient catalytically at low substrate concentrations as the temperature is lowered to 4°C. This means that under conditions found in the cell, the enzyme retains much more of its activity than would be predicted on the basis of the Arrhenius relationship observed at high substrate concentrations. If this phenomenon proves to be widespread, it would have important implications in the control of enzymic activity in foods during storage.

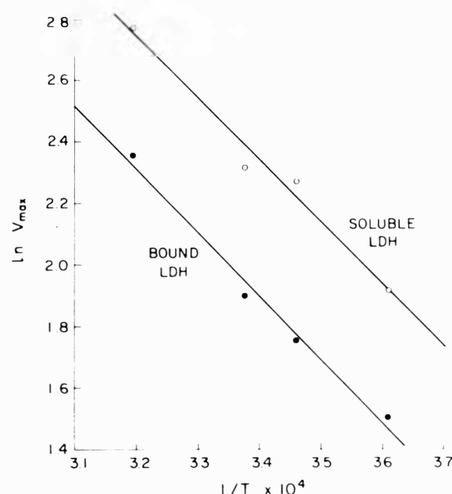

Fig. 1—Arrhenius plots of soluble and bound LDH. The conditions of assay are described in the text.

MATERIALS & METHODS

BREAST MUSCLE of female domestic chickens was used as the test material for preparation of the soluble and bound enzymes and the particulate fractions. NADH was purchased from P-L Biochemicals, Inc. Potassium pyruvate was obtained from Sigma, and chlortetracycline was a product of Nutritional Biochemicals Corp. All other chemicals were the purest available commercially.

Preparation of enzymes

Bound LDH was prepared by homogenizing chicken breast muscle in deionized distilled water followed by centrifuging and further washings in water. Soluble LDH was prepared by solubilization of the bound enzyme with 0.15M sodium chloride. A particulate fraction washed free of enzyme was used to adjust the ratio of enzymic activity and insoluble protein in the bound enzyme preparation. The details of these procedures have been described previously (Ehmann and Hultin, 1973).

Assay conditions

The stopped-flow assays were carried out in imidazole buffer, 10 mM, pH 6.5; NADH, 0.1 mM; and various concentrations of pyruvate as indicated in the Results section. The enzyme concentration was 10^{-7}M for both the bound and soluble enzyme. Enzyme concentrations were determined by assaying the enzyme under conditions where all of the LDH in the bound preparation was solubilized, i.e., at pH 7.5 and high ionic strength. The solubilization observed under the conditions used in the stopped-flow assay was minimal, generally being less than 1%. Determinations of solubilization were carried out at all the temperatures used in the assays, i.e., 4, 16, 23 and 40°C. The final concentration of insoluble protein of the bound preparation in the assay medium was approximately 10 mg per ml. Details of the procedure were described previously (Ehmann and Hultin, 1973).

The stopped-flow assay

A stopped-flow assay was carried out by placing in one syringe the imidazole buffer and NADH and pyruvate (at twice the desired final concentrations). The other syringe contained buffer and either the soluble or bound enzyme. Changes in transmission at 340 nm were recorded on a storage oscilloscope. Temperature in the chamber was maintained by circulating water from a controlled temperature bath. Details of the procedure have been described by us (Ehmann and Hultin, 1973).

Determination of protein content

Protein content of the bound enzyme was determined by a biuret procedure (Gornall et al., 1949).

RESULTS

INITIAL VELOCITIES were measured as a function of pyruvate concentration for both bound and soluble LDH at 40, 23, 16 and 4°C. Double reciprocal plots of initial velocity versus substrate concentration were made, and maximal velocities (V_{Max}) and Michaelis constants (K_M) were calculated by standard procedures. When the natural logarithms of the maximal velocity were plotted against the reciprocal of temperature in the usual Arrhenius-type plot, two straight lines were obtained (Fig. 1). The slopes of the lines are related to the function called the activation energy, which is a measure of how the reaction velocity changes as a function of temperature. Although the activity of the bound LDH was always less than that of the soluble, the slopes of the lines are very similar. The activation

energy was calculated to be 9400 calories per degree mole for the soluble and 9500 calories per degree mole for the bound enzyme. Within experimental error, these are equivalent. The response, therefore, of both the soluble and the bound enzyme to temperature is essentially the same although the absolute rates are considerably different.

In Figure 2 are shown the data when the Michaelis constant is plotted against temperature for both the bound and soluble enzyme. There is a slight increase in the Michaelis constant of the bound enzyme compared with the soluble. This was true for all the temperatures examined even though the differences were not very large. We suggest that perhaps binding of the LDH to the subcellular particulate fraction creates a diffusion layer around the enzyme. This diffusion layer necessitates a higher concentration of substrate in the bulk phase for the substrate to be at the same concentration at the site of the bound enzyme as is attained in solution for soluble LDH. Another possibility might be that binding of the enzyme per se produces a conformational change in the enzyme which results in lower efficiency of the bound enzyme.

The effect of temperature on the K_M values of both bound and soluble LDH is, however, similar, and there is a very significant decrease of K_M with decrease in temperature. The major change occurs in the range between 40 and 23°C. The decrease in Michaelis constant with decreasing temperature means that the enzyme retains a greater proportion of its maximal activity at low substrate concentrations as the temperature is lowered. There is about a fivefold difference in the Michaelis constant for the enzymes between 4 and 40°C. This could amount to a very significant effect on enzymic activity at low substrate concentrations.

This latter point is illustrated in Figures 3 and 4 where we show a plot of initial velocities versus substrate concentrations at 4 and 40°C for the soluble and bound enzymes. Although the absolute values of activities for the enzyme in the two phases are significantly different, the same pattern is observed as a function of temperature. It can be seen that as the pyruvate concentration is lowered, the relative activity of the enzyme at 40° compared to 4° becomes less.

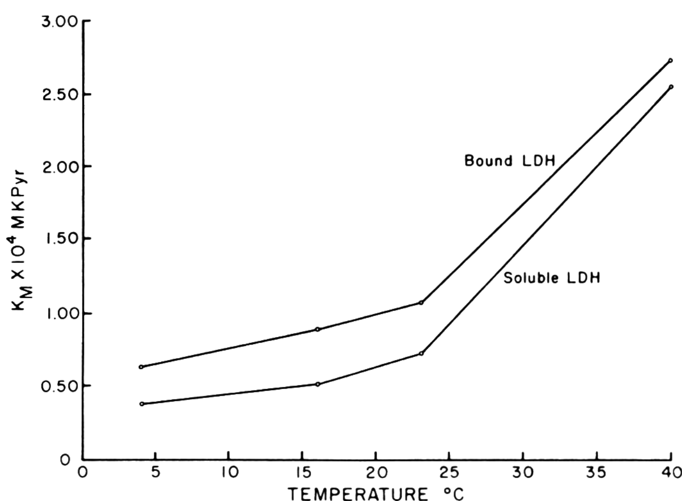

Fig. 2–Change in Michaelis constant (K_M) as a function of temperature.

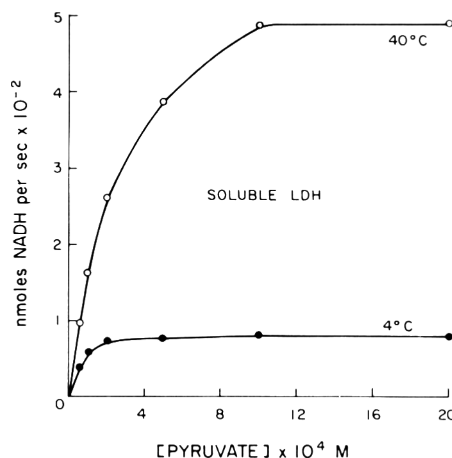

Fig. 3–Relationship of initial velocity of soluble LDH to pyruvate concentration at 4° (–●–) and 40°C (–○–). The conditions of assay are described in the text.

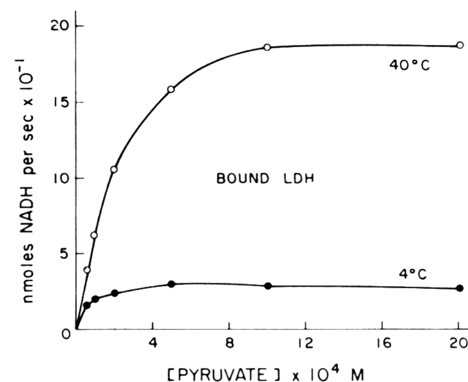

Fig. 4–Relationship of initial velocity of bound LDH to pyruvate concentration at 4° (–●–) and 40°C (–○–). The conditions of assay are described in the text.

DISCUSSION

THE RESULTS reported in this study demonstrate a very significant decrease in the K_M of LDH of chicken breast muscle with decreasing temperature. A decrease of K_M with decreasing temperature has been shown for several enzymes of poikilotherms and has been suggested to relate to temperature acclimation of the organism (Hochachka and Lewis, 1971; Hebb et al., 1972). However, the results with LDH from chicken breast muscle might indicate that this phenomenon is more widespread than had been supposed and might apply to many types of tissues.

The molecular changes leading to this decrease in K_M are not known. It is possible, however, that there is a change in the association of the subunits of the chicken muscle LDH with temperature. Jaenicke et al. (1971) have shown that dissociation of LDH is a function of temperature. These authors showed that ionic strength and pH were involved in the dissociation as well. Kastenschmidt et al. (1968) showed that there was a shift in the dimer-tetramer forms of phosphorylase b from rabbit skeletal muscle as a function of temperature. These phenomena may be related to something more fundamental, such as a change in the structure of water with temperature as suggested by Lumry and Rajender (1970).

Reactions in foods with a Q_{10} of one or less are known, including enzyme-catalyzed reactions (McWeeny, 1968). Postmortem shortening of skeletal muscle is minimal in the range 14–19°C and increases at both lower and higher temperatures (Lawrie, 1966; Smith et al., 1969). The occurrence of a minimum or maximum at an intermediate temperature is usually considered to be due to effects on two or more enzymes which have differ-

ent temperature dependencies. Therefore, one enzyme may be relatively more important at the higher temperature, while another becomes relatively more important at the lower temperature, thus causing the reversal in the rate of the overall reaction as the temperature is lowered.

We suggest that this is not necessarily the only explanation for this type of phenomenon. The same effect can be observed if there is a sufficient change in K_M as a function of temperature. It is not likely that the extent of the change of chicken muscle LDH could account by itself for a phenomenon such as the reversal of cold shortening with decreasing temperature, since at no substrate concentration does the activity at the lower temperature exceed that at the higher temperature (see Fig. 3 and 4).

The fundamental question that is related to the situation in situ concerns the substrate concentration that exists in the cell. If the substrate concentration for a particular enzyme is saturating, then one should look at V_{Max} data to compare results at high and low temperatures. On the other hand, if the substrate concentration in the cell is near or below the K_M value, then the values of K_M would be more important in making comparisons. It has been reported that the pyruvate concentration in muscle cells is of the order of 0.1 mM (Scopes and Newbold, 1968), which is at the lower end of the scale shown in Figures 3 and 4. Thus, in the case of chicken breast muscle LDH, it would be much better to compare K_M values rather than V_{Max} values in evaluating temperature effects. It is probable that substrate concentrations in situ are near or below the value of the K_M for most enzymes (Sols and Marco, 1970).

This means that evaluation of an enzyme at V_{Max}, or optimal conditions, as is usually done, may not tell very much about what is going on in biological tissue. A better rule of thumb might be that if one wants to find out what is actually happening in a food tissue, it is better to work at a substrate concentration approaching the K_M. This is especially important if some factor such as temperature (as shown in this report), pH, or water activity markedly effects the K_M of the enzyme being studied. Our results indicate that lowering of the temperature most likely does not lead to the decrease in activity of chicken breast muscle LDH in situ, that would be expected based on the Arrhenius relationship observed under conditions of maximal velocity.

Determination of K_M has not been a very popular approach in studying enzymes in foods, due perhaps to the significantly greater effort that is involved in determining K_M as opposed to V_{Max} for most enzymes. We hope this paper, however, points out a warning that unless this is done, very misleading results may be obtained.

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EFFECT OF COPPER BINDING ON THE AUTOXIDATION OF OXYMYOGLOBINS

INTRODUCTION

THE AUTOXIDATION RATES of oxymyoglobin (MbO_2) have been studied because of their direct relationship to the stability of meat color. It is known that partial pressure of oxygen, pH and temperature affect the autoxidation rate of oxymyoglobin (George and Stratmann, 1952a, b; 1954). Changes in autoxidation rates are also caused by changes in buffer strength, freezing and thawing cycles and degree of purification of MbO_2 (Brown and Dolev, 1963a, b). Snyder and Ayres (1961) demonstrated that sodium hydrosulfite concentration and temperature affected the autoxidation rates of MbO_2. Snyder and Skrdlant (1966) found autoxidation rates of purified MbO_2 to be extremely variable. They concluded that this variability was partly due to trace metal ion contamination. Copper addition in equimolar amounts to the MbO_2 concentration resulted in a 25-fold increase in autoxidation rate constant.

Banazak et al. (1965) characterized the sites of cupric and zinc ion binding to the sperm whale Mb through x-ray diffraction analysis. Hartzell et al. (1967) indicated that the copper binding sites were found on the outer shell of Mb molecule and could exist in an equilibrium with hydrogen ions in the solvent.

This study was designed to use the latest methods of oxymyoglobin purification and characterization to determine the exact effect of copper ions on the stability of the oxymyoglobin molecule. This study utilized purified oxymyoglobin from bovine, ovine and porcine tissue.

EXPERIMENTAL

Isolation and purification

The purification procedure used for bovine myoglobin (Mb) was that of Satterlee et al. (1969). Porcine and ovine Mb purification was accomplished using the procedure of Satterlee and Zachariah (1972). Oxymyoglobin was prepared according to the procedure of Brown and Mebine (1969) with the modifications of Satterlee and Zachariah (1972).

Autoxidation of MbO_2

All autoxidation rates were determined by incubating the MbO_2 at 30°C in 10 mM phosphate buffer pH 6.0. Each trial was performed in duplicate. Three trials were completed for each of the three oxymyoglobins studied. The copper solutions used in the autoxidation experiments were prepared from analytical grade $CuCl_2 \cdot 2H_2O$, serially diluted from 100 μM to 1 μM. Each copper solution was added to freshly prepared MbO_2 just prior to initiating an autoxidation experiment.

Atomic absorption analysis for copper ion

After completion of autoxidation experiment, the myoglobin samples were frozen. Upon thawing, each sample was dialyzed for 48 hr against two changes of distilled water. Final dialysis was against 1 liter of double distilled water for 24 hr. Upon completion of the dialysis, concentrated HCl was added to the samples to obtain a final HCl concentration of 15%. The samples were then heated in a 90°C water bath for 1 hr. A Perkin Elmer Model 303 Atomic Absorption Spectrophotometer with the copper lamp at a wavelength 3247A was used for copper ion detection. Copper standards were prepared by the method of AOAC (1965).

Isoelectric focusing

All isoelectric points were determined using the procedure of Satterlee and Snyder (1969). A pH 6–8 ampholyte was used to establish the pH gradient. The pH was measured at 23°C with the aid of a Corning Model pH meter.

RESULTS & DISCUSSION

THE AUTOXIDATION rate constants, as shown in Figure 1, were not greatly influenced by added copper ion (Cu^{2+}) until a concentration of 200 molar equivalents was present along with the MbO_2. Copper ion in solution had the greatest

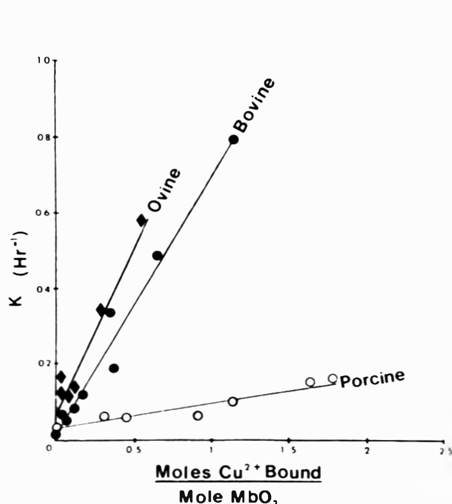

Fig. 1—The effect of copper ion (Cu^{2+}) in solution upon the autoxidation rate constants of bovine, ovine and porcine MbO_2.

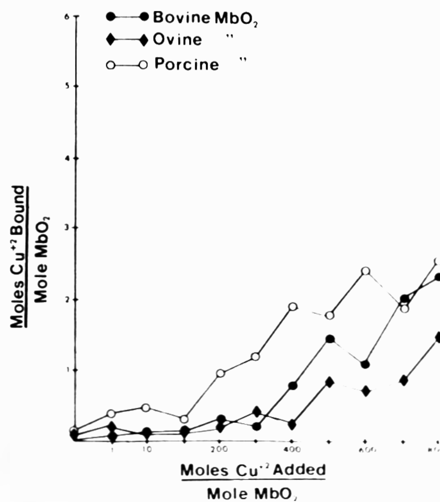

Fig. 2—The ability of bovine, ovine and porcine MbO_2 to bind copper ion, dependent upon the copper ion concentration of the solution.

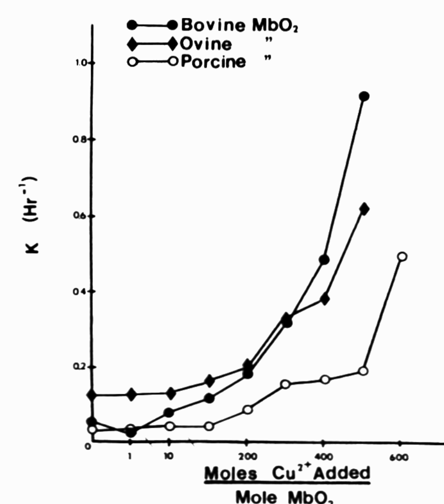

Fig. 3—The effect of bound copper ion on the autoxidation rate constants of bovine, ovine and porcine MbO_2.

Table 1—The effect of sodium hydrosulfite and copper on autoxidation rate constants

Moles Cu^{2+} added / mole MbO_2	$Na_2S_2O_4$ conc ($\mu g/ml$)	K (Hr^{-1}) x 10^{-2}
0.0	0.00	2.5
0.0	0.32	5.3
0.0	3.20	6.3
0.5	0.00	4.5
0.5	0.32	4.0
0.5	3.20	4.0
1.0	0.00	3.6
1.0	0.32	9.1
1.0	3.20	10.1

Table 2—The isoelectric points (pI) for porcine myoglobin and with copper ion addition

Porcine myoglobin solution	pI	Avg dev from mean	No. of trials
MetMb	6.49	0.01	4
MbO_2	5.78	0.01	4
*MetMb + Cu^{2+}	6.55	0.01	2
*MbO_2 + Cu^{2+}	5.87	0.00	2

* Moles Cu^{2+}/mole MbO_2 = 900/1

effect on bovine MbO_2, a lesser effect on ovine MbO_2 and the least effect on porcine MbO_2. The effect of copper ion on the autoxidation of sperm whale oxymyoglobin was also observed to be very similar to the effect seen with both bovine and ovine MbO_2.

These results do differ from those reported by Snyder and Skrdlant (1966) where they found that the presence of equal molar concentrations of bovine and sperm whale MbO_2 and Cu^{2+} ion caused a 25-fold increase in autoxidation rate constant.

This discrepancy in findings could be explained by the possible contamination of the bovine and sperm whale MbO_2 Synder and Skrdlant, 1966) with a small amount of sodium hydrosulfite ($Na_2S_2O_4$) that was not removed during CM cellulose column chromatography. It was noted in this laboratory, and previously described by Snyder and Ayres (1961) and Brown and Mebine (1969), that trace amounts of sodium hydrosulfite will enhance the autoxidation of MbO_2. Experience in this laboratory also indicates that all excess hydrosulfite can easily be removed from freshly prepared MbO_2 by using the mixed bed ion exchange resin, described by Brown and Mebine (1969).

To determine the significance of trace amounts of hydrosulfite oxidation products, studies were conducted to detect changes in autoxidation rate constants of MbO_2 in the presence of Cu^{2+} ion and sodium hydrosulfite. After MbO_2 was prepared, known amounts of sodium hydrosulfite and copper were added. Table 1 shows the increase in autoxidation rate constants in the presence of hydrosulfite and low copper ion concentrations. Autoxidation rates were determined at 30°C in pH 6.0 10 mM phosphate buffer.

Figure 2 shows the ability of each MbO_2 to bind copper ion. Porcine MbO_2 has the greatest affinity for Cu^{2+} binding, bovine having moderate affinity, with ovine having the least affinity for Cu^{2+} binding. In all cases the copper concentration in solution had to exceed the MbO_2 concentration by 100-fold before any appreciable amount of Cu^{2+} ion was bound.

Figure 3 illustrates the effect of bound copper ion on the autoxidation. Previous Figures (1 and 2) indicated that bovine and ovine MbO_2 bound the least amount of copper ion, but their autoxidation rates were affected the greatest by copper ion in solution. Figure 3 shows that both bovine and ovine MbO_2 have their autoxidation stabilities significantly altered by copper ion binding. Whereas, porcine MbO_2 has the ability to bind a greater amount of copper ion, but then to have its autoxidation rate only slightly altered by bound copper.

Table 2 gives the isoelectric points (pI) of porcine MetMb as 6.49 and porcine MbO_2 as 5.78. After the addition of 900 molar equivalents of copper ion to the porcine MbO_2. The pI of porcine MetMb is slightly lower than previously reported by Satterlee and Zachariah (1972). The pI for natural porcine MbO_2 was found to be the same as that for artificially prepared porcine MbO_2 (prepared using sodium hydrosulfite).

CONCLUSIONS

THE PRIMARY CONCERN during this study was to determine the effect of copper ion on the autoxidation rate constants for porcine, ovine and bovine MbO_2. The larger rate constants obtained after copper addition indicated that copper concentrations of 200 molar equivalents or greater are needed to significantly affect the autoxidation rates of any of the three MbO_2 studied. Porcine MbO_2 also differs from bovine and ovine MbO_2 in its capability to bind to porcine myoglobin alter the isoelectric point of both the oxy and met forms, but have only a slight effect on the autoxidation stability of the oxy form. Bovine and ovine bind less copper ion, but the copper which is bound drastically affects the stability of the oxy forms.

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INFLUENCE OF EPINEPHRINE AND CALCIUM UPON GLYCOLYSIS, TENDERNESS AND SHORTENING OF SHEEP MUSCLE

INTRODUCTION

RESISTANCE to frozen New Zealand lamb on the American market has been shown to be associated with toughening occurring as a consequence of cold shortening (Locker and Hagyard, 1963). However, the cold-induced toughness can be prevented by holding at elevated temperatures in special conditioning rooms until the carcasses pass into rigor mortis (about 15°C for 16–20 hr) before freezing (Marsh and Leet, 1966; McCrae et al., 1971). Since this process slows down the slaughter line and requires special handling, it is considered expensive. Thus, New Zealand freezing works are interested in practical procedures for accelerating rigor development, which would allow earlier freezing and thereby speed up processing.

Earlier work in our laboratory (Pearson et al., 1973) has shown that antemortem injection of epinephrine into sheep failed to increase immediate postmortem muscle glycolysis, although theoretically epinephrine should trigger glycolysis by stimulation of cyclic AMP (Robison et al., 1968; Haschke et al., 1970; Pastan and Perlman, 1971). The role of calcium in the regulation of phosphorylase activity has been outlined by Haschke et al. (1970) and by Brostrom et al. (1971). Their results suggest that calcium could be a limiting factor in the initiation of glycolysis since the sarcoplasmic reticulum is intimately involved in the binding of calcium and probably controls not only initiation of muscle contraction but also the onset of rigor mortis (Nauss and Davies, 1966; Newbold, 1966). Nauss and Davies (1966) have suggested that the sarcoplasmic reticulum gradually loses its ability to bind calcium postmortem and that the efflux initiates glycolysis.

The present study was undertaken to ascertain the influence of epinephrine and calcium upon glycolysis, tenderness and muscle shortening. Ground muscle was used for measurement of pH, for ATP, creatine phosphate (CP) and inorganic phosphate (Pi) levels as well as for total phosphorylase and phosphorylase a activity. Tenderness and shortening were followed with intact muscle.

EXPERIMENTAL

Ground muscle

The muscles used in this investigation came from seven aged Romney ewes that were slaughtered as described earlier by Pearson et al. (1973). As soon as possible after pelting and evisceration (approximately 10–30 min after death), the LD (longissimus) and BF (biceps femoris) muscles were excised and all dissectable fat was removed. A sample was taken at once for pH readings; a portion was held as an unground control and the remainder was ground at once through a 3-mm plate of an electric meat grinder. The ground muscle was then divided into four approximately equal portions. The samples were then labelled and treated as follows:

(1) LD–C and BF–C, intact controls;
(2) LD–4 and BF–4, ground controls;
(3) LD–2 and BF–2, 1 ml of 0.1M $CaCl_2$ was added per 100g of muscle;
(4) LD–3 and BF–3, 10 µl of a 1 in 1,000 solution of epinephrine was added per 100g of muscle;
(5) LD–5 and BF–5, 1 ml of 0.1M $CaCl_2$ and 10 µl of a 1 in 1,000 solution of epinephrine was added per 100g of muscle.

The samples were either reground a second time in order to thoroughly mix with the various additives or else a mortar and pestle was used to thoroughly incorporate the additives. All samples were placed in beakers and held at room temperature (approximately 18–22°C). Aliquots were removed for pH readings, for analysis of ATP, CP and Pi levels and for measurement of total and phosphorylase a activity at regular intervals of time. Prior to removing aliquots, each sample was thoroughly mixed to be representative of the entire mass. The re-

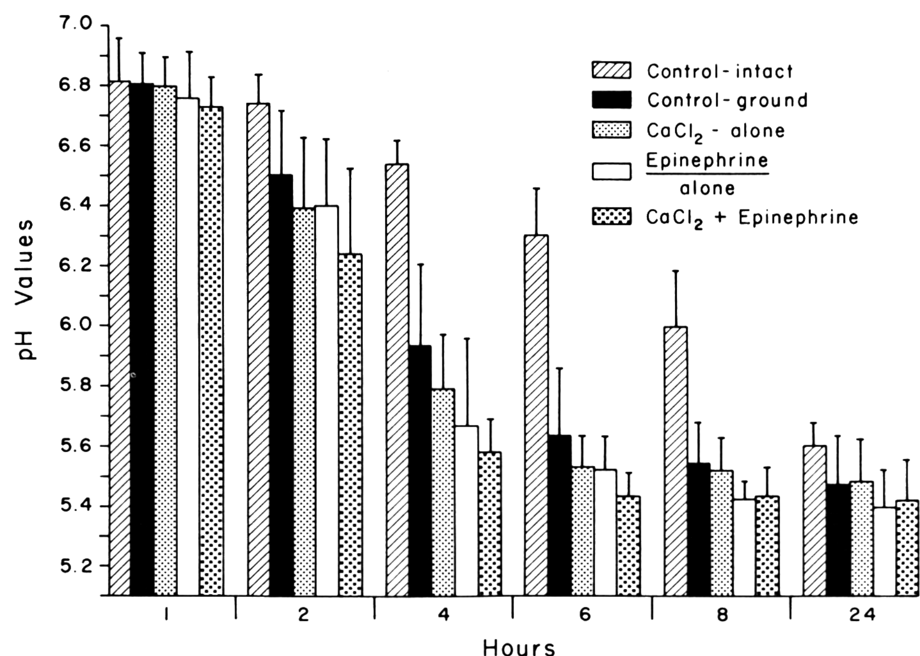

Fig. 1—Changes in the pH of the LD muscle as a result of grinding and of adding epinephrine and $CaCl_2$ alone and in combination together. The bar above each mean value depicts one standard deviation. Initial pH readings are not shown.

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mainder was then made into a uniform closely packed mass and the beaker was covered with polythene to prevent excessive evaporation. Times of removal of each sample were recorded and used for plotting so that all values could be compared at any given time postmortem.

The levels of epinephrine and $CaCl_2$ used in this study were those shown to cause maximum stimulation of glycolysis in preliminary studies. Although the original samples were removed over a period of 10–30 min following death, the range in sampling time is justified by the relatively slow glycolytic rate of untreated sheep muscle (Pearson et al., 1973) and by plotting all values to give a curve back to zero time. Thus, all values shown herein are reported from the plotted data.

Intact muscle

Both ST (semitendinosus) muscles were removed from the same carcasses or from other similar sheep. In some cases, the paired intact muscles were used for comparison of treatments, whereas, sometimes the individual ST muscles were split longitudinally into either two or four sections to permit more comparisons. The intact muscles or muscle strips were injected to give the same total levels used in ground muscle with either $CaCl_2$, epinephrine or propranolol-HCl (10 µl of a 1 mg/ml solution/100g of muscle) alone or in various combinations together, using isotonic KCl as the solvent. The total dosage was administered as several (4–6) micro-injections in both the deep and superficial tissues. Length measurements were made through 24 hr at 15°C and are expressed as percent shortening.

All samples were then labelled, wrapped, frozen and stored at −18°C until removed for shear readings. In no case did the period of freezer-storage exceed 90 days. Shear force readings were obtained on samples cooked 60 min in an 80°C water bath using the MIRINZ tenderometer (Macfarlane and Marer, 1966) as outlined by McCrae et al. (1971).

pH values

pH measurements were made on small samples (1–3g) of muscle as soon as possible after death (usually within 10–30 min), immediately after grinding and addition of the various additives and at 2 hr intervals until 8 hr postmortem. A final reading was taken between 23 and 24 hr postmortem. All samples were immediately homogenized in 12–15 ml of 0.005M iodoacetate solution. Values for pH were plotted against time postmortem so that the values could be compared at any given time. Initial pH readings are not given herein as there was little change in pH until approximately 1 hr after addition of the various additives.

ATP, CP and Pi analysis

Samples were removed at regular time intervals over the first 8 hr and analyzed for ATP, CP and Pi according to the procedures of Eggleton and Eggleton (1929) as outlined by Bendall (1951) and by Bendall and Davey (1957).

Total phosphorylase and phosphorylase a activity

Small samples (2–5g) were placed in plastic bags and immediately immersed and frozen in liquid nitrogen. The samples were then stored in a walk-in freezer at approximately −29°C until removed for measurement of total and phosphorylase a activity. Total phosphorylase activity was measured as units of inorganic phosphate liberated from G-1-P (glucose-1-phosphate) in the presence of AMP while phosphorylase a was determined in the absence of AMP according to the procedures of Cori and Illingworth (1956) as described by Stull and Mayer (1971). The ratio between total phosphorylase and phosphorylase a (Stull and Mayer, 1971) was calculated as follows:

$$\text{Ratio} = \frac{\text{units of phosphorylase a}}{\text{units of total phosphorylase}} \times 100$$

RESULTS & DISCUSSION

Rate of pH fall

Figure 1 graphically shows the changes occurring in pH of the LD muscle as a consequence of the various treatments. The rate of pH fall for the intact controls was much slower than any other treatment with the final pH being achieved somewhere between 8 and 24 hr postmortem. Although pH readings were not taken during the period between 8 and 24 hr, previous work (Pearson et al., 1973) has shown that the final pH of intact sheep muscle is generally not reached until about 16 hr postmortem.

Grinding alone caused a rapid decline in pH with an average of 5.63 being achieved within 6 hr postmortem (Fig. 1). The samples treated with $CaCl_2$ alone and epinephrine alone (Fig. 1) were on average consistently, but not significantly, lower in pH than the ground control for the first 6 hr. $CaCl_2$ plus epinephrine caused an even greater acceleration in pH fall with an average value of 5.58 being reached within 4 hr, which was significantly lower than any other treatment (Figure 1).

Several investigators (Briskey, 1964; Bodwell et al., 1965) have demonstrated that postmortem pH values are good indicators of the rate of muscle glycolysis. This being true, the present study verifies the findings of Newbold and Lee (1965) that grinding alone accelerates glycolysis. Although neither $CaCl_2$ alone nor adrenaline alone significantly accelerated gly-

Table 1—Effect of treatment upon the pH of the BF muscle

	Mean pH values[a]					
	Times postmortem (hr)					
Treatments[b]	1	2	4	6	8	24
Control – intact	7.03	6.94	6.72	6.46	6.22	5.62[c]
Control – ground	6.82[d]	6.37[d]	5.76[d]	5.56[d]	5.49[d]	5.52[c]
$CaCl_2$ alone	6.81[d]	6.32[d]	5.76[d]	5.58[d]	5.52[d]	5.54[c]
Epinephrine alone	6.81[d]	6.39[d]	5.75[d]	5.53[d]	5.47[d]	5.48[c]
$CaCl_2$ + epinephrine	6.80[d]	6.16[c]	5.62[e]	5.49[d]	5.48[d]	5.52[c]

[a] Mean ph values are averages representing seven different sheep. Initial pH readings are not shown.
[b] Significant differences in treatment effects are indicated by different letter superscripts in the same column, i.e., all values that are significantly different are indicated by different letters.

Table 2—Levels of ATP, inorganic phosphate and creatine phosphate in LD muscle at different time intervals following addition of $CaCl_2$ and epinephrine alone and in combination together[a]

	ATP-P[b] (µg/g)					Creatine phosphate (µg/g tissue)					Inorganic phosphate (µg/g tissue)				
Treatment	1 hr	2 hr	4 hr	8 hr	24 hr	1 hr	2 hr	4 hr	8 hr	24 hr	1 hr	2 hr	4 hr	8 hr	24 hr
Intact control	369[a]	316[a]	147[a]	54[a]	4[a]	102[a]	93[a]	67[a]	26[a]	5[a]	684[a]	800[a,b]	950[a]	1079[a]	1268[a]
Ground control	274[a]	207[a]	145[a]	0[a]	0[a]	90[a,b]	76[a,b]	48[a]	10[a]	0[a]	850[c]	910[b]	1032[a]	1282[a]	1315[a]
$CaCl_2$	362[a]	297[a]	185[a]	48[a]	2[a]	85[a,b]	77[a,b]	59[a]	21[a]	1[a]	678[a]	729[a]	848[a]	1008[a]	1104[a]
Epinephrine	372[a]	307[a]	189[a]	29[a]	5[a]	82[a,b]	73[a,b]	52[a]	21[a]	3[a]	753[b]	833[a,b]	973[a]	1134[a]	1224[a]
$CaCl_2$ + Epinephrine	230[a]	203[a]	145[a]	19[a]	0[a]	65[b]	59[b]	45[a]	13[a]	4[a]	899[c]	952[b]	1060[a]	1208[a]	1300[a]

[a] All values in the same column not followed by same superscript are significantly different.
[b] ATP-P = ATP-phosphate expressed as µg/g of tissue.

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THE ASSOCIATION OF PROTEIN SOLUBILITY WITH PHYSICAL PROPERTIES IN A FERMENTED SAUSAGE

INTRODUCTION

THE PHYSICAL PROPERTIES, especially firmness, of fermented sausage impart not only identifying characteristics to this particular type of sausage but are essential for sales. Variation in firmness of product is a problem experienced by many manufacturers (Klement and Cassens, 1972). Such variation cannot be controlled unless the mechanism responsible for the production of firmness is understood.

In most processed sausage products it is essential that a portion of the contractile (salt soluble) muscle proteins be solubilized with sodium chloride. The same technique of salt solubilization is employed in the manufacture of fermented sausage but because of the low temperature (30–37°C) during processing, the solubilized proteins are exposed to minimal alteration by heat compared to other processed sausages.

The extractability of protein from different muscles as a function of time postmortem and of physiological conditions has been studied by a number of investigators (Scopes, 1964; Sayre and Briskey, 1963; van Eerd, 1972). In general, extractability of muscle proteins is decreased if low pH and high temperature combinations are realized in the early postmortem period. Pale, soft and exudative pork (PSE), which results from a rapid glycolysis at high temperature, decreases the solubility of muscle proteins (Bendall and Wismer-Pedersen, 1962; Wismer-Pedersen, 1964; Briskey and Wismer-Pederson, 1961). Other studies on protein solubility relate to conditions imposed on muscle postmortem, such as freezing in liquid nitrogen (Borchert and Briskey, 1965) and heat alterations (Hamm and Deatherage, 1960; Paul et al., 1966).

Extractable protein of various meats was investigated by Saffle and Galbreath (1964) in a study of least cost formulations in sausage. They reported that the amount of salt-soluble protein extracted was greater for pre-rigor than post-rigor meat. Also, freezing of post-rigor meat decreased protein extractability compared to the refrigerated controls. Trautman (1964) developed a system to study the effect of pH on muscle protein extracts. He found that the effect of decreasing pH was linear on the solubility of both water soluble and salt soluble proteins.

The effect of fermentation at various temperatures (22, 30 and 37°C) on lactic acid concentration, pH, flavor and water-holding capacity of summer sausage has been studied by Acton et al. (1972). The interrelationships of the parameters measured are discussed. Sajber et al. (1971) found that enzymes from the meat and from the bacterial starter cultures alter proteins in fermented sausage production, and they related this to the increase of free amino acid, thus showing a decrease in the protein nitrogen. It was also suggested that the changing pH in the sausage could influence the activity of the proteolytic enzymes.

Sokolov and Tchekhovskaya (1971) investigated the development of structure during drying of a fermented sausage. They found an aggregation of myofibrillar proteins accompanied with the appearance of electrostatic, hydrogen and disulfide bonds. The aggregation was greater in the periphery of the sausage possibly being due to the acid compounds in smoke which aided in the charge formation of the proteins. The objective of this study was to determine the changes in solubility of various classes of muscle proteins during processing of a summer sausage (semi-dry fermented meat product) and to relate these results to changes in pH and firmness of the product.

MATERIALS & METHODS

Sausage preparation

Coarse ground pre-salted beef (70% lean) and fine ground pre-salted beef trim (50% lean) were chopped in a silent cutter to a desired consistency at which time seasoning ingredients were added followed by starter culture (AC-1 Hanson Lab., Milwaukee, Wisc.). The mix was then stuffed into No. 2 fibrous casings (Union Carbide, Chicago, stuffing diameter 62 mm) fermented in an air-conditioned smokehouse at 37°C and then heated up to 55°C internal temperature. Sample 1 was taken immediately after stuffing and was at a temperature of approximately 6–8°C. Sample 7 was the finished product at a temperature of 55°C. Samples 3 through 6 were withdrawn from the smokehouse at intervals of 3–6 hr during the fermentation and ranged between 34–37°C in temperature. All samples withdrawn from the smokehouse were chilled down to 20°C in a cold water shower before they were placed in a cooler at 3°C. All processing took place under commercial conditions. A typical proximate analysis of the sausage was 27% fat, 50% moisture and 17% protein.

Two complete experimental runs were conducted at an interval of 45 days and are referred to as Experiment I and Experiment II; the formulations and procedures were indentical. Three different batches were manufactured for each experiment as follows: batch A had 2.5% NaCl and starter culture; batch B had 3.0% NaCl and starter culture; and batch C had 3.0% salt but no starter (control).

Sample preparation

The samples were cooled to an internal temperature of 3°C and were then shipped under refrigeration to the University of Wisconsin facilities where further tests were conducted. The samples were held an additional day at 3°C before firmness analyses were conducted.

A sample with standardized dimensions was obtained as follows: The sausage was chilled in an ice bath and then a 2.54 cm slice was obtained by slicing with a sharp knife in a template device. A carefully sharpened 5 cm diam stainless steel tube was used to core a standardized diameter sample from the 2.54 cm thick slice so that the surface layer could be discarded. The 5 cm diam core was used for shear analysis. This method was designed to yield uniformly sized samples and to circumvent the firmness effect due to case hardening of the surface during thermal processing, which would not be the same firmness that resulted from the constant low temperature fermentation.

A paired sample was frozen in liquid nitrogen (Liq-N_2) to prevent changes in protein extractability that might occur during storage from the time of firmness analysis until analysis of protein extractability which took place during the following 3 wk.

Firmness analysis

The previously described standardized samples were sheared at a rate of 30 sec down stroke with a L.E.E. Kramer shear press. Five replicates of each of the 21 samples per experiment were sheared and the average was used as the firmness value. The shearing apparatus was located at room temperature, but all the samples were stored in an ice bath at 0°C before shearing. All samples for each experiment were run on the same day and at the scale setting of 200, except for samples 7 which were run on the 1,000 scale.

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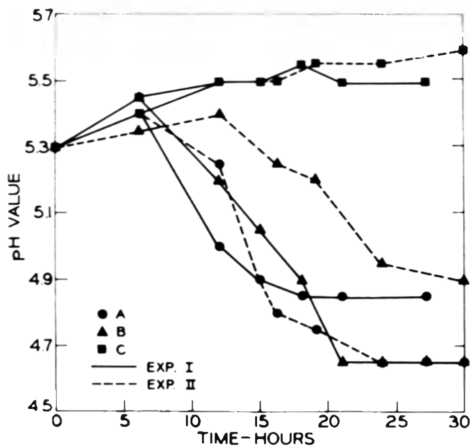

Fig. 1–pH values of the sausage during processing. A contains 2.5% NaCl and starter culture; B contains 3.0% NaCl and starter culture; C contains 3.0% NaCl.

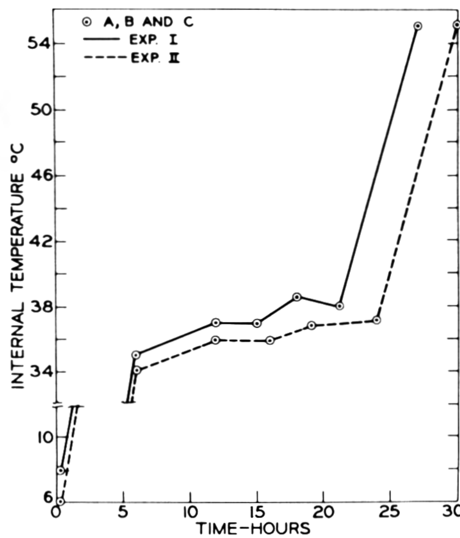

Fig. 2–Internal temperature of the sausage during processing. Batches representing salt levels and starter culture combinations are pooled.

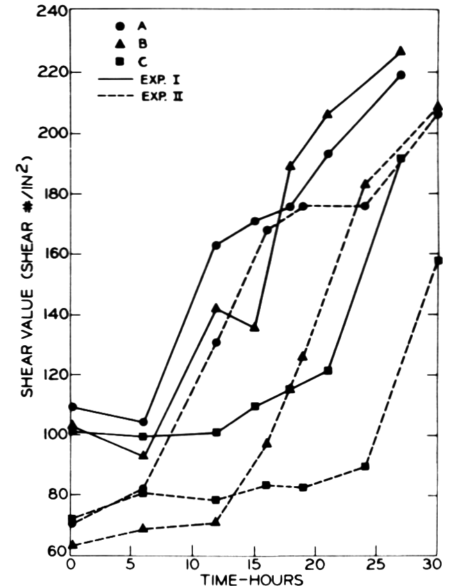

Fig. 3–Shear press values of the sausage during processing. A contains 2.5% NaCl and starter culture; B contains 3.0% NaCl and starter culture; C contains 3.0% NaCl.

Extractable protein

Samples were removed from Liq-N_2 storage and powdered in a previously cooled aluminum blender cup (Waring) at top speed according to the procedure of Westenbrink and Krabbe (1936). The samples were extracted at 2°C according to the modified method of Helander (1957). Sarcoplasmic protein was extracted with 0.03N potassium phosphate, pH 7.4 and total soluble protein was extracted with 1.1N potassium iodide in 0.1N potassium phosphate, pH 7.4. Nonprotein nitrogen (NPN) was measured after tricholoroacetic acid (20% w/v) precipitation. Triplicate extractions were performed on each of the 42 samples (21 per experiment). With duplicate analyses for nitrogen, this yielded six values for solubility per sample which were averaged. Myofibrillar protein was calculated as the difference between the amounts of total soluble protein and sarcoplasmic protein (including NPN). Sarcoplasmic protein was calculated on the basis of nitrogen difference between the 0.03N potassium phosphate extract and its TCA precipitation filtrate. Nitrogen quantity was determined on the original sausages in triplicate by macro-Kjeldahl method. The triplicate extracts and NPN quantities each were determined in duplicate by the micro-Kjeldahl method (AOAC, 1960).

The following modifications were made to the Helander (1957) method: (1) 2-g samples were used instead of 1-g because of smaller quantities of soluble protein; (2) centrifugation was conducted at 10,000G instead of 1,500G; (3) a third 2-hr extraction was added to the 0.03N potassium phosphate fraction instead of just two 3-hr extractions.

Myoglobin extractability

2g of sample was extracted with 20 ml of 0.03N potassium phosphate (pH 7.4) for 3 hr. The resulting mixture was centrifuged at 10,000G for 20 min and visual estimate of the supernatant was used as an observation of myoglobin extractability.

pH measurements

5g of sample was homogenized in 45 ml of distilled water. The subsequent mixture was filtered and the pH of the filtrate was measured with a combination glass-reference electrode.

Statistical analysis

All data were subjected to an Analysis of Variance and Duncan's Multiple Range Test for significant differences as described by Steel and Torrie (1960).

RESULTS & DISCUSSION

pH value and internal temperature

The pH values for sausage from both experiments at time intervals throughout processing are shown in Figure 1. The results from the two experiments were similar. pH, in general, declined to a final value of 4.6 to 4.9. An interesting pattern was established which showed that batch A had a more rapid pH decline than batch B. This could perhaps be explained by the fact that batch A had a lower salt level; salt is inhibitory to growth of the starter culture which is responsible for acid production. The control batch (C) which did not include starter culture did not show a pH decline, and, in fact, the pH actually rose from 5.3 to 5.6 during processing. Other investigators have reported the same phenomena of increasing pH during the heating of meat (Hamm and Deatherage, 1960; Kauffman et al., 1964; Paul et al., 1966). Fox et al. (1966) found a pH increase from 5.4 to 5.9 in the processing of frankfurters. A possible explanation of this increase is the loss of free acidic groups by the formation of new stable cross linkages (Hamm and Deatherage, 1960).

The internal temperature of the sausage during processing is shown in Figure 2. As the product was moved into the smokehouse for fermentation the temperature increased to the range of 34–37°C and remained at that approximate level until it was finished at 55°C.

Firmness

The results for the shear analysis are given in Figure 3. The controls showed little firmness development until they were subjected to the heating change from the 34–37°C plateau to 55°C. In both experiments, sample 7 of the controls was significantly firmer ($P \leqslant 0.01$) than sample 6; there were no significant differences from samples 1 through 6. We interpret these results as demonstrating no formation of a characteristic summer sausage structure during the slight increase in pH that occurred in the control. Firmness development was more rapid in the A batches than the B batches, which is probably a reflection of the faster development of acid in the A batches (see Fig. 1). Although firmness development was more rapid in the A batches than the B, the final firmness values were not significantly different. This points out that either 2.5% or 3.0% salt level is adequate for satisfactory development of firmness. All samples that had a pH below 5.0 were significantly different ($P \leqslant 0.01$) from the control sample at that same time of

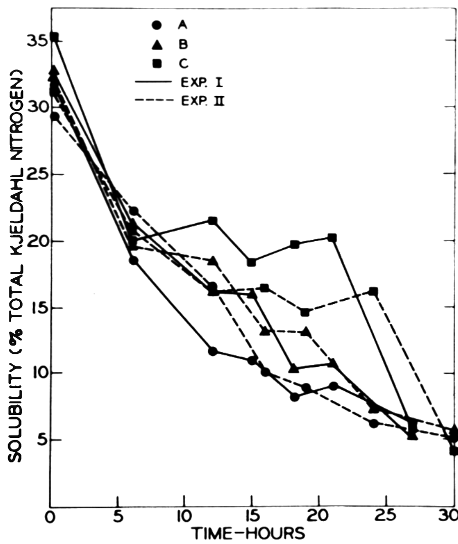

Fig. 4–Solubility of myofibrillar proteins in the sausage during processing. Results are expressed as a percentage to total protein nitrogen. A contains 2.5% NaCl and starter culture; B contains 3.0% NaCl and starter culture; C contains 3.0% NaCl.

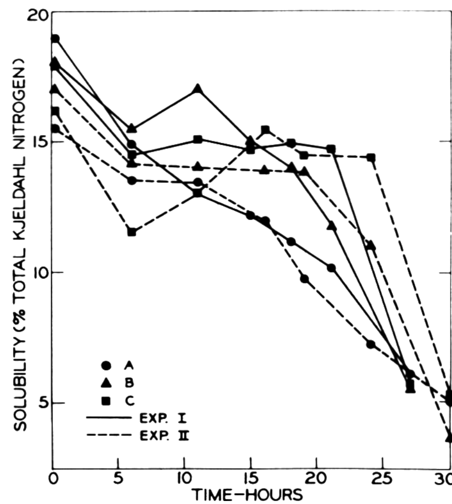

Fig. 5–Solubility of sarcoplasmic proteins in the sausage during processing. Results are expressed as a percentage of total protein nitrogen. A contains 2.5% NaCl and starter culture; B contains 3.0% NaCl and starter culture; C contains 3.0% NaCl.

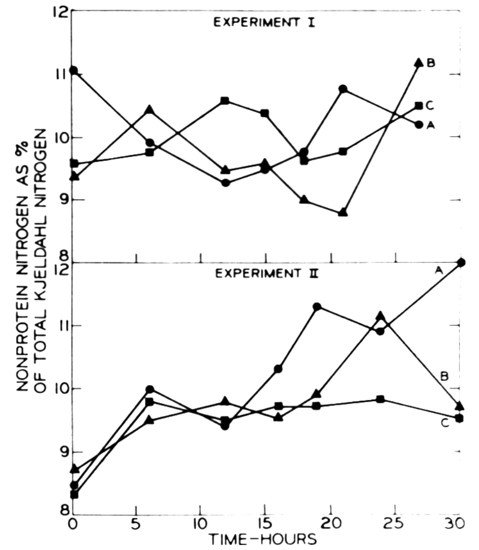

Fig. 6–Nonprotein nitrogen levels in the sausage during processing. Results are expressed as a percentage of total protein nitrogen. A contains 2.5% NaCl and starter culture; B contains 3.0% NaCl and starter culture; C contains 3.0% NaCl. All were heated to 55°C except A in Experiment 11 which was heated to 60°C.

incubation. From the general shape of the curves, it is clear that the sausage became firmer as the pH of the sausage dropped. For example, from Experiment 1, sample 3 from batch A (pH = 5.0) was significantly different ($P \leqslant 0.01$) from sample 3 of the control (pH = 5.4) in shear value. It should be noted that the three batches of sausage from an experiment were in the same smokehouse for exactly the same amount of time. The difference between values from the two experiments probably represent actual differences in firmness.

Myofibrillar protein extractability

The change in solubility of the myofibrillar proteins during processing is shown in Figure 4. There were no significant differences ($P \leqslant 0.05$) for A, B and C batches for either experiments in the sample taken immediately after chopping. The values of 29.2% to 35.5% extractability for both experiments are for post-rigor meat and are within the range reported by Sayre and Briskey (1963). They found an extractability of myofibrillar proteins of 10% to 40% (35% average for the six conditions they studied) for the myofibrillar proteins in pork muscle 24 hr postmortem; the percent extractability was dependent upon temperature and pH at the onset of rigor mortis. They also postulated that under conditions of high temperature and medium or low pH (5.3–5.6), loss of solubility was more severe for the myofibrillar proteins than it was for the sarcoplasmic fraction which also agrees with the findings of this experiment (see Fig. 5 and discussion).

The significant ($P \leqslant 0.01$) drop of solubility for all samples from the first to the second sampling time demonstrates the effect of the heat change from 6°C to about 37°C on the solubility of the myofibrillar proteins. Paul et al. (1966) found a decrease of about 40% in solubility of myofibrillar protein by the heat treatment of 40°C for 10 hr. We found a decrease in extractability of from 35% to 42% as temperature was raised from about 6°C to 34–37°C. There was a 60% reduction in solubility in samples held 10 hr at 50°C (Paul et al., 1966), whereas in this experiment there was a reduction of 80% by the time samples had reached 55°C, even in the samples where no pH drop occurred. These results agree rather well, even though different extraction solutions were used. Paul et al. employed a KI-borate buffer, I = 0.6, pH = 7.5, to remove myofibrillar proteins, whereas we used 1.1N KI in 0.1N K phosphate, pH = 7.4.

It should be noted also that the final pH of the samples did not change the amount of extractable myofibrillar protein in sample 7. If the pH was 5.6 or 4.6, the amount that was extractable was not significantly different, pointing out the heat susceptibility of this type of protein, regardless of pH influence. Although the amount of extractable protein was the same, these samples possessed a difference in shear value that was significant, showing the importance of acid development in the fermentation that adds to the final firmness of this type of sausage.

The change in solubility of myofibrillar proteins during the fermentation when pH is declining but temperature is constant, is interesting. The percent decrease in protein solubility was calculated as:

$$\frac{\text{\% sol. sample 2} - \text{\% sol. sample 6}}{\text{\% solubility sample 2}}$$

In Experiment I, the percent decrease was 52% for batch A and 50% for batch B while in Experiment II it was 62% for A and 62% for B. The control batches failed to show any reduction in solubility of myofibrillar protein even though the mix was exposed to a pH of 5.3–5.5 and a constant temperature of 34–37°C. In contrast, batches A and B for both experiments showed a marked decrease in solubility; these samples were exposed to a decreasing pH at a temperature of 34–37°C. The results indicate that the declining pH at constant temperature decreases the solubility of myofibrillar proteins and in turn increases the shear value (firmness) of the sausage. The difference between experiments (a decrease of about 50% in Experiment I compared to 60% in Experiment II) may have been due to differences in pH and temperature between the two experiments. The percent decrease in solubility of the myofibrillar

proteins is greater in this type of sausage than that of the sarcoplasmic proteins (see Fig. 5).

Sarcoplasmic protein extractability

The change in solubility of the sarcoplasmic proteins during processing is presented in Figure 5. Sample 1 for all batches ranged from 16.1% to 18.8% soluble sarcoplasmic protein as a percentage of total protein nitrogen. There was no significant difference among samples. Sayre and Briskey (1963) worked with 24 hr postmortem pork muscle pH of 5.3–5.6 with different temperatures at onset of rigor and found an extractability of the sarcoplasmic protein of 18–23%. This previous finding generally agrees with the results of this paper, because some further loss in solubility of the sausage sample could have taken place due to handling of the meat at temperatures that could induce damage to the proteins before manufacture; the history of the meat used in our experiments was unknown before it entered the establishment of manufacture.

Paul et al. (1966) revealed a 20% decrease in solubility of sarcoplasmic proteins of rabbit on exposure to 40°C for 10 hr at constant pH. Our work revealed a 16–22% decrease in solubility of sarcoplasmic protein in samples heated from 6°C to about 37°C for 4 hr.

Although there was some fluctuation, the control batches showed little variation between samples 2 and 6.

Batches A and B, from samples 2 to 6, both show decreases in solubility which were significantly different from the control batch. For example, in Experiment II, batch A was significantly different from the control sample at sample 4 ($P \leq 0.05$) and samples 5 and 6 ($P \leq 0.01$) and batch B was different at sample 6 ($P \leq 0.05$). Also, in Experiment I, both batches A and B were significantly different from the control at sample 6 ($P \leq 0.01$). The decrease in solubility of these sarcoplasmic proteins was not as drastic as experienced with the myofibrillar proteins, but rather showed a gradual decrease to sample 6. This suggests that the sarcoplasmic proteins were less susceptible to insolubilization at pH 5.2–5.0 than were the myofibrillar proteins which showed significant decreases in solubility ($P \leq 0.01$) at 5.2–5.0 (see sample 3 for batch A of Experiment I).

The solubility at sample 7 was significantly different ($P \leq 0.01$) from sample 6 in all cases except in Experiment II, batch A, where the pH of 4.65 and temperature had already lowered the solubility level to 7.2% so that the heat treatment to 55°C did not further decrease solubility.

The percent decrease in solubility of sarcoplasmic proteins during the constant temperature fermentation period was also calculated. In Experiment I, batch A decreased 36% and batch B decreased 24% while in Experiment II, batch A decreased 47% and batch B decreased 21%. There was little decrease in the control batches.

We concluded that the myofibrillar proteins are more important than the sarcoplasmic proteins in development of the firmness that is characteristic of summer sausage.

Nonprotein nitrogen

The results of the nonprotein nitrogen (NPN) determination on the various extracts is presented in Figure 6. The higher nonprotein nitrogen in batch A of both experiments could be due to a high acid-high temperature condition altering protein structure, because in both experiments the acidity developed more rapidly in batch A than in batch B. In Experiment I there were no significant differences except sample 1 of batch A and sample 7 of batch B, which were different ($P \leq 0.05$).

In Experiment II, batch A showed the same trend of a significant increase in sample 7. In the low-acid control batches, a significant increase was not noted. This indicates that acid at high temperatures may increase NPN; an exception occurred in Experiment II batch B, however. Hamm and Deatherage (1960) reported a slight increase in NPN with increasing temperature at slightly rising pH conditions. They offered the suggestion that at higher temperatures smaller nitrogen containing molecules (e.g., nucleotides, ammonia) bound by proteins are released. Paul et al. (1966) also found significant increases in NPN by heating muscle protein systems for several hours at 60°C. They attribute this increase to enzymatic activity naturally present in the meat tissue that could change solubility. Sajber et al. (1971) found an increase of NPN due to an increase of certain amino acids during the fermentation of "Stajer" sausages. Results from our work resemble all of these findings although the heat treatment was not as drastic as the Paul et al. (1966) example. In their lower heated samples (40°C) they found little change of NPN level.

Myoglobin solubility

The extractability of myoglobin decreased as the fermentation process proceeded and the pH declined.

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DIRECT ENZYMATIC CONVERSION OF LACTOSE TO ACID: LACTOSE DEHYDROGENASE

INTRODUCTION

PRESENT METHODS for the formation of acid in milk involve production of lactic acid by the action of bacteria on lactose. The direct enzymatic conversion of lactose to lactobionic acid might also produce similar changes in pH, while eliminating some of the variability in the manufacturing process. Rand (1965, 1972) studied methods to utilize the lactose already present in skim milk through enzymatic conversion of the lactose. Substantial amounts of acid were obtained following the hydrolysis of lactose by lactase (beta-galactosidase) (EC 3.2.1.23), and the subsequent enzymatic oxidation of glucose and galactose by glucose oxidase (EC 1.1.3.4) and hexose oxidase (EC 1.1.3.5). While this study indicated that enzymatic conversion of the hydrolyzed sugar in milk could be utilized to acidify milk, the results with hexose oxidase for the direct oxidation of lactose were inconclusive. A single enzyme system which could convert lactose directly to an acid would further simplify the process.

The ability of a bacterium to directly oxidize disaccharides was first reported by Stodola and Lockwood (1947). They demonstrated that *Pseudomonas graveolens* was capable of oxidizing lactose and maltose directly to their corresponding aldobionic acids. Kluyver et al. (1951) obtained similar results using *Pseudomonas calco-acetica, Pseudomonas guercito-pyrogallica* and *Pseudomonas aromatica.* Bently and Slechta (1960) obtained a preparation from *Ps. guercito-pyrogallica* which directly oxidized lactose, maltose and other aldoses to their corresponding aldobionic acids. Their report indicated that the enzymes which catalyze direct oxidation of aldoses are intracellular, and usually are found in subcellular particles. Nishizuka et al. (1960) reported that a lactose dehydrogenase (EC 1.1.99) could be isolated from *Ps. graveolens.* The enzyme was also located in the subcellular particles and could be solubilized by a sodium desoxycholate treatment.

Nishizuka and Hayaishi (1962) purified both lactose dehydrogenase and lactonase (EC 3.1.1.17) from disrupted cells of lactose adapted *Ps. graveolens.* The crude form of lactose dehydrogenase reacted directly with oxygen and 2,6-dichlorophenolindophenol (DIP) and exhibited optimal activity at pH 5.8 when assayed manometrically. Following partial purification, lactose dehydrogenase utilized only DIP or methylene blue as hydrogen acceptors, and did not react with oxygen or pyridine nucleotides. When assayed spectrophotometrically with DIP, the purified lactose dehydrogenase had maximum activity at pH 5.6 for the conversion of lactose to lactobionic-delta-lactone. The lactone produced was hydrolyzed to lactobionic acid by the enzyme lactonase.

The object of this work was to study the direct enzymatic conversion of lactose to lactobionic-delta-lactone and determine if the lactobionic acid, which resulted from hydrolysis of this lactone, could be utilized for acidification of milk. The lactose specific enzyme *Ps. graveolens* was chosen to study this reaction.

EXPERIMENTAL

Materials

Lactase (beta-galactosidase), an enzyme preparation produced from yeast, was provided by Enzyme Development Corp., New York, N.Y. A portion of the dry product was suspended in water prior to use. A sample of purified catalase containing 1600 units/ml was provided by Fermco Labs., Chicago, Ill. Hydrogen peroxide was 50% and stabilized. It was stored in a polyethylene bottle at −15°C. Low-heat nonfat dried milk (NDM) was obtained from Land O'Lakes Co., Minneapolis, Minn. The 2,6-dichloroindophenol sodium salt (DIP) was from Eastman Organic Chemicals, Rochester, N.Y. Sodium desoxycholate was purchased from Fisher Scientific Co., Fair Lawn, N.J. All other reagents were reagent grade. Distilled water was used throughout.

Methods

Culture methods. *Ps. graveolens* (ATCC-4683) was grown according to the culture methods of Hayaishi et al. (1961) and Nishizuka and Hayaishi (1962), except that the concentration of yeast extract was increased to 0.2% and the dibasic potassium phosphate reduced to 0.1% to improve total cell yield (Wright, 1969).

Enzyme assay. The procedure for detecting lactose dehydrogenase by the reduction of DIP developed by Nishizuka and Hayaishi (1962) was employed in this study. The only change was a slight increase in the dye concentration to 0.3 micromoles of DIP, in order to attain a desired initial absorbance of 0.8.

Enzyme purification. Lactose dehydrogenase was separated into purification fractions according to the procedure reported by Nishizuka and Hayaishi (1962). As a result of enzyme stability studies (Wright, 1969), all work was done at 2°C and a pH of 5.5, except where noted.

(1) **Particulate fraction.** Disrupted cells of *Ps. graveolens* were centrifuged at 10,000 × G in a Sorvall RC-2B high speed refrigerated centrifuge for 30 min to remove cell debris. The supernatant (120 ml) was centrifuged at 40,000 × G for 1 hr. The precipitate of subcellular particles was washed once with 100 ml of 0.02M phosphate buffer at pH 5.5, centrifuged at 40,000 × G for 1 hr and suspended in 20 ml of 0.02M phosphate buffer at pH 5.5.

(2) **Desoxycholate fraction.** The particulate fraction was constantly mixed on a magnetic stirrer, and 8 ml of 2% sodium desoxycholate at pH 7.5 was added, followed by 2 ml of n-butanol. The solution was homogenized for 5 min in a Thomas tissue grinder and centrifuged at 25,000 × G for 30 min. The clear supernatant was removed with a glass syringe to prevent contamination by the loose precipitate.

(3) **Chloroform fraction.** An equal volume of cold chloroform (20°C) was slowly added to the desoxycholate fraction while the mixture was agitated on a magnetic stirrer. The solution was centrifuged at 25,000 × G for 30 min and formed a bottom chloroform layer, a solid middle layer and brownish-yellow supernatant on top. The aqueous supernatant was again recovered with a syringe and dialyzed against 0.02M phosphate buffer at pH 5.5 for 3 hr at 2°C.

The enzyme isolated by this procedure exhibited definite dehydrogenase activity in the presence of DIP and lactose. The Michaelis constant obtained for the chloroform enzyme fraction was 1.38×10^{-2} M (Wright, 1969), which is comparable to the value of 1.1×10^{-2}M reported by Nishizuka and Hayaishi (1962).

Enzymatic acidification. The ability of lactose dehydrogenase to convert lactose to lactobionic-delta-lactone, with subsequent hydrolysis to lactobionic acid, was studied under a variety of conditions. The standard reaction mixture contained 3.3% lactose and 30 units of enzyme in a volume of 30 ml, except when noted. When the partially purified form of lac-

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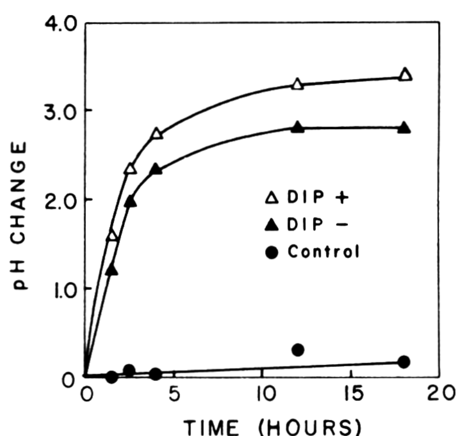

Fig. 1–The conversion of lactose to lactobionic acid at 25°C by the particulate fraction of lactose dehydrogenase. Substrate: nonbuffered 3.3% lactose at pH 6.5; DIP: 1.5 micromoles.

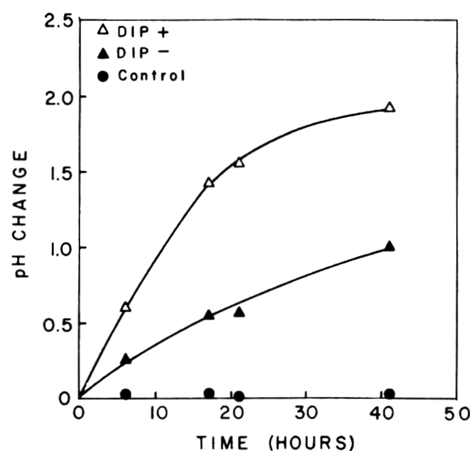

Fig. 2–Effect of desoxycholate-butanol purification step on the conversion of lactose to lactobionic acid by lactose dehydrogenase at 25°C. Substrate: nonbuffered 3.3% lactose at pH 6.5; DIP: 1.5 micromoles.

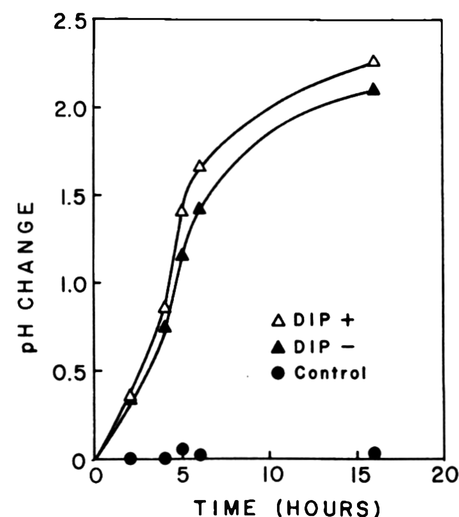

Fig. 3–The conversion of lactose to lactobionic acid at 25°C catalyzed by a recombination of the soluble and loose-precipitate portions of the desoxycholate-butanol fractionation for lactose dehydrogenase. Substrate: nonbuffered 3.3% lactose at pH 6.5; DIP: 1.5 micromoles.

tose dehydrogenase was used, varying concentrations of DIP were also incorporated into the reaction mixture. The general procedure was to mix the required aliquots of lactose and DIP, prepared in the desired solvent of either distilled water or 0.04M phosphate buffer at pH 6.5, and this mixture was tempered to 25°C in a water bath. Lactose dehydrogenase was stored at pH 5.5, in 0.02M phosphate buffer at 2°C and the enzyme solution was assayed prior to use. The volume of enzyme which contained the desired number of enzyme units was added to each mixture to start the reaction. Sufficient distilled water or 0.04M phophate buffer at pH 6.5 was also added to reaction mixtures, when required, to adjust the final volume to 30 ml. In nonbuffered systems, the pH was immediately adjusted to 6.5 with the addition of 0.02M monobasic or dibasic potassium phosphate. The pH of 6.5 was then recorded as the zero time reading. A control consisting of all components in the reaction mixture except lactose dehydrogenase was maintained for each experiment.

The acidification reaction in milk was studied with 30 ml of pasteurized reconstituted skim milk containing 9% nonfat dry milk solids (NDM) and a crystal of thymol. The reaction was started with the addition of 300 units of lactose dehydrogenase. Reaction samples with additives contained lactase at a concentration equivalent to 5% of the estimated lactose concentration, while the total catalase concentration was 100 units and hydrogen peroxide was 0.1%. Water was added to adjust all samples to the same volume. All reaction mixtures were held at 25°C and evaluated against a milk control.

The enzymatic acidification reaction was followed by pH measurements at regular intervals employing a Corning Model 12 Research pH meter equipped with an expanded scale and temperature compensator. All results were expressed as the change in pH from the zero time reading, and are usually typical of several trials.

RESULTS

Enzyme fraction requirements for lactose conversion

Direct conversion of lactose to an acid was studied initially with nonbuffered solutions at pH 6.5 to evaluate the lactose dehydrogenase purification fractions. The enzymatic acidification reaction with lactose dehydrogenase in the particulate fraction is demonstrated in Figure 1. The reaction proceeds in the presence or absence of DIP, in agreement with results of Hayaishi et al. (1961), indicating that the particulate fraction can utilize oxygen directly. There was some difference in rate, and the presence of dye did accelerate the reaction and increased the amount of lactobionic acid produced.

The solubilization of lactose dehydrogenase from the cell particles in the desoxycholate fraction appeared to isolate the enzyme from a complex system. This can be shown in Figure 2, since the enzyme did not utilize oxygen to any great degree, and required the addition of DIP to acidify lactose solutions. Although some acidification did occur in the absence of DIP, this was probably due to incomplete isolation of the soluble portion from the particles. This hypothesis was substantiated by a recombination of the supernatant with the loose precipitate obtained in this fractionation step. The results presented in Figure 3 demonstrate that the enzyme reverts back to the characteristics of the particulate fraction.

The acidification reaction catalyzed by the chloroform fraction of lactose dehydrogenase was almost completely dependent on the presence of the artificial hydrogen acceptor, DIP. This can be demonstrated by a comparison of the particulate and chloroform enzyme fractions as presented in Figure 4. The ability of the particulate fraction to utilize oxygen was again evident, while the chloroform fraction did not react unless DIP was present. The amount of acid produced and the rate of the reaction catalyzed by the chloroform fraction increased in proportion to the concentration of added dye. These results indicate that both enzyme fractions, when combined with their respective hydrogen acceptors in sufficient quantities, can catalyze the conversion of lactose to lactobionic acid and lower the pH of a nonbuffered 3.3% lactose solution from pH 6.5 to at least pH 5.0. A possible explanation for the difference in acid production, not related to the concentration of the hydrogen acceptor, could be a change in the rate of hydrolysis of the lactone to lactobionic acid due to lactonase contamination of the particulate fraction. Nishizuka and Hayaishi (1962) also purified lactonase from the soluble fraction obtained by centrifugation of the crude extract of lactose dehydrogenase at 40,000 x G.

The experimental assay procedure of Bently and Slechta (1960) was employed to establish whether lactonase was present in the particulate and chloroform fractions, as well as the 40,000 x G supernatant. Aliquots of 1 ml from each solution were added separately to 29 ml of a 3.3% glucono-delta-lactone solution at 25°C. A 3.3% lactone solution, without additives, hydrolyzed to gluconic acid

at a standard rate upon solution in water and was used as the control. The gluconic acid formation was measured with a pH meter as the change in pH at 30 sec intervals during a 3 min reaction period. An increase in the rate of lactone hydrolysis over the control was interpreted as an indication of lactonase presence. Figure 5 shows that the addition of all three solutions increased the rate of lactone hydrolysis, with a change of 0.4–0.6 pH units over the control at the end of 3 min. The most significant change in the rate of lactone hydrolysis occurred with the particulate fraction, indicating the presence of a higher concentration of lactonase/ml as compared with the chloroform fraction.

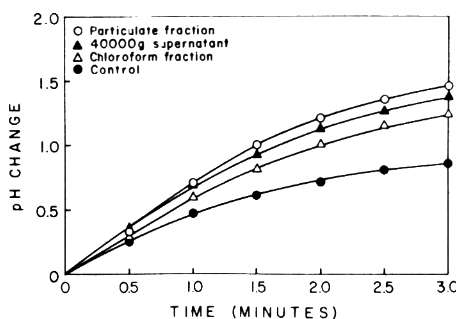

Fig. 5–Lactonase assay of the 40,000 × G supernatant, and the particulate and chloroform fractions of lactose dehydrogenase at 25°C. Substrate: 3.3% glucono-delta-lactone.

Lactose conversion for acidification

The effect of lactose dehydrogenase in converting lactose directly to lactobionic acid for acidification was evaluated with buffered lactose solutions. The particulate fraction was added at a concentration of 1 unit/ml to solutions of 1.25%, 2.50%, 3.75% and 5.00% lactose, dissolved in 30 ml of 0.04M phosphate buffer at pH 6.5. The maximum pH change using the above concentrations occurred with 3.75% lactose. However, this was only a decrease in pH of 0.81 units in 24 hr. Due to the slow reaction during this period of time, the enzyme concentration was increased to 3 units/ml, and the results are shown in Figure 6. A rapid decrease in pH occurred with all levels of lactose, producing a pH change of at least 2.0 units within 5–10 hr. There was a change in rate of about three times that found with 1 unit/ml of the same enzyme. The optimum substrate concentration was 3.75% lactose for 3 units/ml of the particulate lactose dehydrogenase fraction, in agreement with the initial experiment using 1 unit/ml.

The acidification reaction was repeated utilizing 4 units/ml of the chloroform fraction and the optimum substrate concentration of 3.75% lactose dissolved in 0.04M phosphate buffer at pH 6.5. The reaction was studied between 0 and 7.5 micromoles of DIP in an effort to establish the optimum concentration of hydrogen acceptor for maximum lactose conversion. There was essentially no change in pH with this range of dye concentration during an 8 hr observation period. However, a loss of blue color in each solution indicated that the reaction was taking place. Apparently, the concentration of DIP was insufficient to permit conversion of enough lactose to change the pH of buffered solutions. The alkaline characteristics of the dye made it impractical to employ higher concentrations of DIP.

Enzymatic acidification of milk

The particulate fraction of lactose dehydrogenase was examined for its effect on skim milk. The reaction was studied with both lactose and glucose and galactose available as substrates, as well as in the presence of an oxygen source from hydrogen peroxide and catalase. The results are presented in Figure 7. The reaction with lactose alone exhibited a long lag period and did not show acid development until after 12 hr, followed by a slow increase in acidity. When lactase was added, the lag period was reduced to only 6 hr and the rate of sugar conversion to acid increased. The introduction of catalase and hydrogen peroxide into the system stimulated the reaction in both cases. However, sufficient acid for coagulation and gel formation in milk occurred only when glucose and galactose were both available as substrates. Coagulation was noted in the sample containing lactase and catalase-hydrogen peroxide after 25 hr, and a smooth uniform gel which could be cut was formed after 30 hr.

DISCUSSION

LACTOSE DEHYDROGENASE, extracted from lactose adapted cells of *Ps. graveolens*, can catalyze the conversion of lactose to acid for milk acidification un-

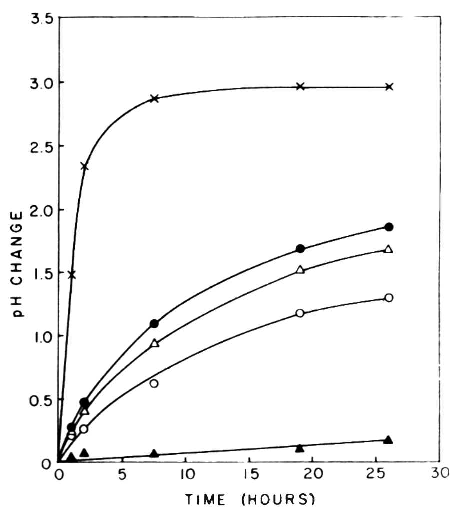

Fig. 4–Comparison of lactobionic acid formation at 25°C by the particulate and chloroform fractions of lactose dehydrogenase. Substrate: nonbuffered 3.3% lactose at pH 6.5; particulate fraction: × 0.0 micromoles of DIP; chloroform fraction: ▲ 0.0, ○ 1.5, Δ 3.0, ● 4.5 micromoles of DIP.

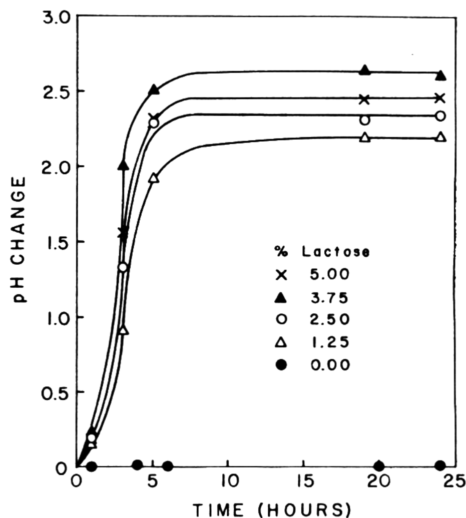

Fig. 6–Effect of lactose concentration on the formation of lactobionic acid at 25°C in 0.04M phosphate buffer at pH 6.5 by the particulate lactose dehydrogenase (enzyme conc 3 units/ml).

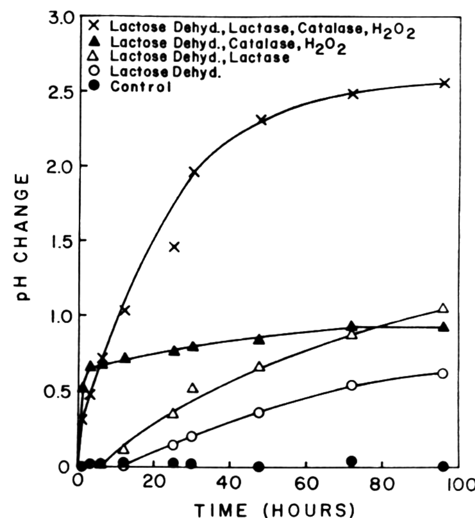

Fig. 7–Formation of acid in skim milk at 25°C by the particulate fraction of lactose dehydrogenase (enzyme conc 9 units/ml).

der certain conditions. The initial purification step, the particulate fraction, has additional characteristics of an oxygenase and rapidly acidified nonbuffered lactose solutions utilizing either atmospheric oxygen or DIP as hydrogen acceptors. Subsequent purification apparently isolated the enzyme from a complex system, since the acidification reaction was dependent on the addition of an artificial hydrogen acceptor. The particulate fraction exhibited a higher rate of reaction than the chloroform fraction, which was at least partially due to the relative amounts of lactonase in each enzyme preparation. The particulate fraction also rapidly acidified lactose solutions buffered at pH 6.5, and the reaction was proportional to the enzyme concentration. While the optimum substrate concentration appeared to be about 3.75%, the reaction rate was only slightly lower in 5% lactose, demonstrating that the enzyme could effectively operate at the concentrations expected in milk.

The direct enzymatic conversion of lactose to lactobionic acid in a buffered system indicated that sufficient acidity could be produced for milk coagulation. However, when the particulate fraction of lactose dehydrogenase was added to milk, the lactobionic acid formed was unable to overcome the buffer capacity of the food and produce the necessary pH change of 1.5/2.0 units required for gel formation. This substantiates the results reported for direct oxidation of lactose by hexose oxidase (Rand, 1972). The acidification reaction in milk increased significantly when lactase was present, both in terms of rate and acid formation. A partial explanation can be attributed to the substrate specificity of the particulate fraction of lactose dehydrogenase, which Nishizuka and Hayaishi (1962) demonstrated with a spectrophotometric assay was 4/5 times greater with glucose and galactose than with lactose. The reaction of lactose dehydrogenase in the presence of lactase would also indicate that gluconic and galactonic acids are more effective acidogens for milk acidification than lactobionic acid. The reactivity of the particulate fraction was stimulated by the addition of catalase and hydrogen peroxide, confirming the capability to function as an oxygenase in the crude form. The distribution of oxygen throughout milk was an essential factor for uniform curd formation, as previously reported for glucose oxidase and hexose oxidase (Rand, 1972).

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EFFECT OF HYBRIDS AND PROCESSING ON THE DIMETHYL SULFIDE POTENTIAL OF SWEET CORN

INTRODUCTION

LIMITED DATA are available on the dimethyl sulfide (DMS) content of frozen or thermally processed sweet corn. Bills and Keenan (1968) reported levels of DMS in processed sweet corn of 5.7–14.2 ppm, while frozen sweet corn, when heated 10 min in an autoclave, produced only 0.3–6.8 ppm. Williams et al. (1972) found commercially canned sweet corn to contain 10.1–16.0 ppm DMS. These concentrations greatly exceed the reported flavor threshold value of 12 ppb (Patton et al., 1956) and odor threshold value of 0.33 ppb (Guadagni et al., 1963) for DMS in water. However, no statistical correlations have been reported between the DMS content of processed sweet corn and flavor.

The DMS precursor in sweet corn has been identified as a methylmethionine sulfonium salt (Bills and Keenan, 1968). They also found that the DMS concentrations in commercially canned sweet corn did not increase with additional heat processing. This suggested that the DMS precursor was totally decomposed in the recommended thermal process or that the maximum DMS potential had been reached. Therefore, if the DMS concentrations in whole-kernel processed sweet corn were to be altered, it would have to be accomplished by selection of hybrids with different DMS precursor levels (Williams et al., 1972) or by altering a unit operation. The purpose of this investigation was to determine the variation of the DMS potential among several sweet corn hybrids and to observe the effects of blanching and blending of hybrids on their DMS potential.

Table 1—DMS potential of sweet corn hybrids

Hybrid	Field Rep. 1 (ppm)	Field Rep. 2 (ppm)	Mean	Duncan Test (1%)
Monarch Advance	11.0[a]	10.4[a]	10.7	
Midway	14.1	9.73	11.9	
Buttersweet	18.0	9.73	13.8	
Golden Charm	16.1	13.4	14.8	
Merit	15.8	14.3	15.0	
Sweet Tennessee	20.4	11.4	15.9	
Capitan	17.6	15.6	16.6	
Stylepack	18.2	19.1	18.6	
Triumphant 11	19.1	19.8	19.4	
Golden Queen	18.1	21.3	19.7	
Golden Rod	22.9	17.3	20.1	
Silver Queen	15.4	26.2	20.8	
NK-199	21.8	21.7	21.8	
Jubilee	16.2	29.0	22.6	
Ballerina	26.7	19.9	23.3	
Sunshine State	27.8	31.5	29.6	
Gold Crown	30.9	32.2	31.6	
Yukon	29.1	34.2	31.6	
Golden Security	26.6	36.9	31.8	
Gold Cup	20.7	34.3	32.5	
Seneca Warrior 225	38.9	41.8	40.4	

[a] Mean of four analytical replications

EXPERIMENTAL

Survey of sweet corn hybrids for DMS potential

Sweet corn hybrids were grown at the Purdue University O'Neall Farm in two field replications. They were harvested at their optimum maturity from July 29, 1972 through August 28, 1972. The totai yield of each hybrid within a replication, 25–50 ears, was harvested from each replication on the same day and analyzed separately. 21 hybrids were harvested and delivered during the mornings to the laboratory where sample preparation began immediately. 25 ears from each field replication were randomly selected for evaluation.

Preparation of sample

25 ears of sweet corn were husked and the kernels cut from the cobs. The kernels were thoroughly mixed in a large stainless steel pan (mixture A). Mixture A was the source for further subsampling. 500g of mixture A were transferred to a Waring Blendor jar and diluted with an equal weight of distilled water. A homogenate was prepared by blending the contents for 90 sec. Approximately 250 ml were then centrifuged at 0°C for 10 min at 9,000 rpm. 100 ml of the serum supernatant were diluted with an equal volume of distilled water. The resulting solution was filtered through Whatman No. 1 filter paper and once again through Whatman No. 42 filter paper. 11.5 ml of the filtrate were pipetted into each of five Teflon-lined screw-cap culture tubes (16 × 100 mm). One tube served as a control while the other four tubes were heated. A sample was prepared for each of the 21 hybrids from each field replication.

Production of DMS

The samples were heated in an autoclave for 45 min to assure total conversion of the DMS precursor to its complete DMS potential. At the end of the process, the steam pressure of the autoclave was quickly released and the tubes cooled to 0°C in an ice-water bath. At 0–4°C, 0.11 ml of ethylene glycol was added to the solution of each tube and the samples brought to room temperature (24°C ± 1°).

Heating of the diluted sweet corn serum caused the soluble starches present in solution to gelatinize. The solutions were clarified by centrifugation for 10 min at 3,000 rpm. A soft pellet was formed in the bottom of the tubes with a volume of approximately 1 ml.

Determination of DMS produced

The method of Williams et al. (1972) was modified to determine the concentration of DMS. After adding a small drop of G.E. antifoam 60, 10 ml of helium were added to each

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Table 2–Effect of blanching on the DMS potential and °Brix of cut whole kernel sweet corn

		Blanch time (min)[a]					
		0		10		20	
Hybrid	Field replication	DMS[b] (ppm)	°Brix	DMS[b] (ppm)	°Brix	DMS[b] (ppm)	°Brix
Yukon	1	29.1	24.1	8.88[c]	12.7	4.56[c]	12.6
Yukon	2	34.2	23.2	8.99	12.4	6.10	11.6
Gold Crown	1	30.9	21.0	7.93	10.9	3.59	10.2
Gold Crown	2	32.2	19.8	7.98	10.8	2.41	9.70
Stylepack	1	18.2	25.5	7.30	11.6	3.42	12.5
Stylepack	2	19.1	25.6	6.86	11.8	3.36	10.5

[a] Minutes in 100°C water
[b] Mean of four analytical replications
[c] DMS potential which remained after blanching

Table 3–Effect of blending raw sweet corn hybrids on the DMS potential

	DMS potential		
Hybrid	Individual (ppm)	Mixture (50:50) (ppm)	% of Expected
NK-199	22.0[a]	17.5[a]	94.6
Merit	15.0		
Seneca Star	48.0	62.7	100.0
E.B. 11-C	77.4		

[a] Mean of two analytical replications on each of three cans

10 ml sample in a syringe. After 3 min equilibration a known gaseous volume was injected into a gas chromatograph for analysis. The concentration of DMS in the solutions was determined from a standard curve prepared by equilibrating 10-ml samples of DMS standard solutions. Since 0.11 ml of ethylene glycol had been added to 11.5 ml of solution, the ppm DMS obtained for each sample was divided by 0.9905. This corrected DMS concentration was then multiplied by a factor of 4 since the initial weight of sweet corn had been diluted four times. The ppm DMS determined using this method of evaluation was reported on a fresh weight basis.

Effect of blanching on DMS potential

Samples of Yukon, Gold Crown and Stylepack hybrids from each of the two field replications were blanched and analyzed separately. The raw hybrids were used as the controls.

The samples were prepared by placing two 600-g samples from mixture A in cheesecloth bags. One 600-g sample was blanched in boiling water for 10 min and the other for 20 min. After blanching, the samples were cooled, drained and then placed in plastic bags for freezing. The samples were stored at –10°C until further analysis.

The frozen samples were later thawed at room temperature and drained on an 8 mesh screen for 5 min. 500g were analyzed for the remaining DMS potential as previously described.

Blending of sweet corn hybrids

Four hybrids (NK-199, Merit, Seneca Star and E.B. 11-C) were husked and a mixture A prepared for each. Three 303 x 406, C-enamel–lined tin cans were filled with 290g of mixture A from each hybrid and covered with 190 ml of boiling 0.6% NaCl brine. The contents of the cans were exhausted to a center temperature of 80–82°C in boiling water and a closing machine with steam exhaust closure was used to seal the cans. The cans were given a thermal process of 25 min at 121°C in a stationary retort and then immediately water cooled. The procedure was repeated by combining equal weights of two hybrids, NK-199:Merit and Seneca Star:E.B. 11-C. Duplicate determinations of the DMS potential in each of three cans were determined.

RESULTS & DISCUSSION

DMS POTENTIALS of the 21 sweet corn hybrids are summarized by field replication in Table 1. No DMS was detected in the raw controls. There was no significant difference in the DMS potential of the hybrids between the two field replications at the 5.0% significance level; however, there was a significant difference between the DMS potentials of the 21 hybrids at the 0.5% significance level. The mean DMS potential of the two field replications was determined and the 21 hybrids were ranked and grouped by the Duncan Multiple Range Test (Li, 1968). The mean DMS potentials ranged from 10.7 ppm to 40.4 ppm on a fresh weight basis. The mean DMS potentials of Monarch Advance, Midway, Buttersweet, Golden Charm and Merit hybrids were significantly lower than those of Sunshine State, Gold Crown, Yukon, Golden Security, Gold Cup and Seneca Warrior 225 at the 1% significance level. The other 10 hybrids could not be grouped independently of the above hybrids on the basis of the available data.

It is interesting to note that Seneca Warrior 225, Gold Cup, Golden Security and Yukon had high DMS potentials and are hybrids intended for fresh market sales. With the exception of Golden Security, these hybrids were also the first to mature. Monarch Advance, Midway and Golden Charm hybrids had low DMS potentials and are used primarily as processing hybrids. The other hybrids are either new releases or are used for both fresh market and processing. Primary criteria for selecting hybrids for processing are yield and quality factors such as uniformity of maturation, color, sweetness and pericarp tenderness (Nelson and Steinberg, 1970). Perhaps the DMS potential of new hybrids should also be considered in the list of quality criteria. Genetic selection could be used to alter the level of the methionine analog and, therefore, the DMS levels resulting from its thermal decomposition.

The °Brix of each hybrid was determined to see if there was correlation between the soluble solids levels and the DMS potentials. A correlation coefficient of –0.4563 was found which indicated that the °Brix was not a good indicator of the DMS precursor concentration. The significance of the soluble solids level and precursor level was further investigated with blanching experiments.

The blanching times used were extreme; however, it pointed out what dramatic effect blanching has on both the soluble solids and the DMS potential. The concentration of DMS and °Brix are summarized in Table 2. After 10 min of blanching, the °Brix had dropped to approximately 50% of the level found in the samples with no blanching. The 50% level was maintained even after 20 min of blanching. This was probably due to the coagulation of the starches which aided in retaining the remaining soluble solids.

The effect of blanching on the DMS potential was even more dramatic. After 10 min the DMS potential was reduced by approximately 60–75% and after 20 min, 81–92% reduction had occurred. From kinetic studies of sweet corn serum (Williams, 1973), it was found that 60–66% of the DMS precursor was decomposed after 20 min at 99°C. This means that the DMS produced would be driven off and the remaining 21–26% loss must be due to leaching of the DMS precursor into the blanch water. The leaching losses at 10 min would be ex-

pected to be less than after 20 min of blanching; however, the leaching rate apparently decreases with time. From the kinetic studies, one can assume a DMS precursor decomposition of approximately 30% after 10 min at 99°C. Leaching would then account for 30–45% of the DMS potential which was lost.

The results indicate that the blanching operation should be controlled in order to obtain desired DMS levels. Blanching on the cob, steam blanching, microwave blanching and other methods of blanching should be evaluated when attempting to optimize DMS levels.

The DMS precursor is exhausted during the recommended thermal process of 25 min at 121°C for 303 × 406 tin cans (Williams, 1973). The selection of optimum DMS levels in thermally processed sweet corn requires a knowledge of the DMS potentials possessed by each hybrid used for processing. A blend of hybrids could also be made to attain the desired DMS level. Table 3 shows that if hybrids are combined 1:1, then one can expect the DMS content to be equal to the mean of their individual DMS potentials after thermal processing. This would apply to any combination of hybrids and be dependent upon their individual DMS potentials.

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FOOD USE OF SOYBEAN 7S AND 11S PROTEINS
Extraction and Functional Properties of Their Fractions

INTRODUCTION

THE PHYSICAL properties of calcium gel (gel prepared by coagulation with calcium salt like *Tofu*) prepared from 7S or 11S protein respectively, were remarkably different (Saio et al., 1969; 1971); namely, the gel from 11S protein was harder and more elastic than that from 7S protein. And the difference in food processing between the two was recognized not only in calcium gel but also in surface induced film of soybean milk (*Yuba*) (Okamoto et al., 1973). While it is presumed that 7S protein and 11S protein will show characteristic functional properties in every variety of soybean foods, it is worth trying to fractionate both proteins on a practical scale.

The present paper consists of an investigation and proposal of a conventional method to fractionate 7S and 11S proteins, based on their different precipitation behaviors with calcium salt. The phenomenon that 11S protein selectively precipitated from the supernatant after removing the cold insoluble fraction in the presence of calcium salt was recognized in earlier studies (Vaintraub, 1965; Wolf and Sly, 1965; Koshiyama, 1965; Mitsuda et al., 1965; Eldridge and Wolf, 1967; Fukushima, 1968) and was also confirmed in this paper.

The crude protein fractions obtained by the proposed method were submitted to the preparation of several soybean foods to ascertain the possibilities for food use.

MATERIALS & METHODS

Materials

Hawkeye, U.S. variety, was used to investigate the reactability of purified 7S and 11S proteins with calcium. Soybean was ground and defatted with n-hexane. The purification of 11S protein was performed by the method of Eldridge and Wolf (1967) and 7S protein was prepared by ultrafiltration of crude 7S protein (Koshiyama, 1965) with a Diaflo membrane (UX-10). The defatted meal used for fractional extraction of soybean proteins was a commercial product, Golden SL, produced by Yoshihara Oil Mill Co., Ltd.

Calcium requirements of proteins for precipitation

Protein solutions containing 1.56 mg N of 7S or 11S protein were added with calcium chloride to adjust the final concentration to 0–2 mM calcium, kept at room temperature for 30 min and centrifuged at 800 × G for 15 min. The transmittance of the supernatants after centrifugation was examined at 400 nm with a spectrophotometer. The transmittance of the supernatants decreased with an increase of calcium concentration, because of the resulting protein turbidity which does not precipitate by centrifugation, but increased again because of precipitation.

The same experiments were carried out in the presence of different concentrations of sodium chloride. Where calcium concentration transmittance almost reached 100; namely, where protein precipitated almost completely with calcium after a centrifugation, was described as the calcium requirement of protein for precipitation.

Calcium precipitation behavior by means of Particle Size Analyzer

10 ml of protein solution containing 5 mg N of 7S and 11S protein was added with 0.1 ml of 0.5M calcium chloride solution. The mixture was stirred for 1 min and then added with 30 ml of 2% formalin and 5 ml of 1M sodium chloride to fix the precipitated particles. The mixture was stirred constantly on a magnetic stirrer and a portion was transferred into the cuvette of the Particle Size Analyzer (Hitachi PSA-1). The protein solution in the cuvette was mixed with a spatula after a standard method and submitted to analysis. The time from stop of stir-

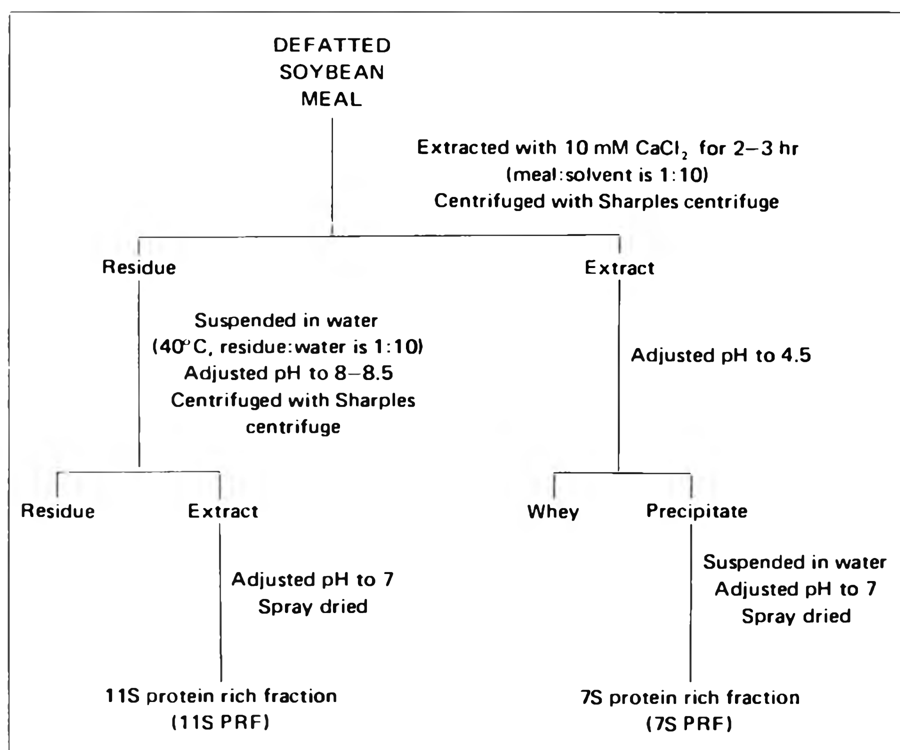


Fig. 1—Flow sheet of fractional extraction of 7S and 11S protein rich fractions.

ring to starting the measurement was accurately 4 min. Although the Particle Size Analyzer is usually used to calculate the distribution of particle size in the solution after Stoke's formula, we used it to obtain the precipitation curves proceeding with time after addition with calcium as shown in this paper.

Fractional extraction of 7S and 11S proteins

The flow sheet from the defatted meal is shown in Figure 1. To 25 liters of 10 mM calcium chloride solution, 2.5 kg of defatted meal were added with stirring. The mixture was stirred for 2–3 hr and centrifuged with a Sharples centrifuge. The extract was adjusted to pH 4.5 and centrifuged to remove whey. The precipitated protein was suspended in water, adjusted to pH 7 and then spray dried (7S PRF).

The residue was suspended with warm water (40°C) of 10 times the weight of the residue. The suspension was adjusted to pH 8–8.5, stirred for 30 min and centrifuged with a Sharples centrifuge. The extract was adjusted to pH 7 and spray dried (11S PRF). pH adjustment was made with dilute NaOH and HCl.

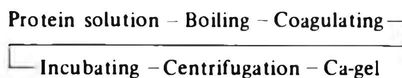
Prior to the experiment using 2.5 kg of defatted meal, the experimental conditions were tested using 50g of defatted meal. Calcium concentrations for extraction from meal were 10, 12.5 and 15 mM at room temperature and 12.5 mM at 0–5°C. For the purpose of preparing high quality 11S/7S in adequate amounts, 11S PRF protein solutions prepared in different conditions were submitted to ultracentrifugal analysis and nitrogen determination by the Kjeldahl method.

Ultracentrifugal analysis

Protein solutions in standard buffer (Wolf and Sly, 1965) (about 5 mg of protein fraction) were submited to ultracentrifugal analysis with a Hitachi UCA-centrifuge. Ultracentrifugation was carried out at 51,200 rpm and photographs were taken every 9 min.

Preparation of calcium gels

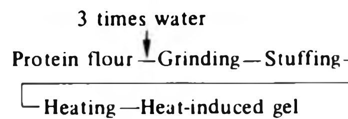
The flow sheet of calcium gel preparation is as follows:



That is, the solution of 7S or 11S protein rich fraction (7S PRF or 11S PRF respectively) containing 5 mg/ml was heated for 3 min after boiling. Calcium sulfate (7S PRF requires a larger amount of calcium sulfate to prepare gel than 11S PRF) was placed in a 50 ml centrifuge tube, suspended in 2 ml of water and warmed at 70°C beforehand in a water bath. While constantly stirring the calcium sulfate suspension on a magnetic stirrer, 25 ml of 7S or 11S PRF solution cooled to 70°C was poured into each tube. The tubes were incubated in a water bath to keep their contents at 70°C for 10 min. The contents were centrifuged for 10 min at 600 × G. The prepared calcium gel remaining at the bottom of the tubes was carefully removed with the aid of a spatula. The textural properties of calcium gel were evaluated by a Texturometer (General Foods Corp.). A No. 1 (17 mm, lucite) plunger was used in the Texturometer and the clearance between the plunger and plate was adjusted to 2 mm. To give the samples an accurate measure of physical shape, calcium gel prepared in the round bottom of a centrifuge tube (30 mm diam) was cut parallel to the top with a razor to make a slice precisely 15 mm in height. The Texturometer profile curve was obtained by two chewings of a standardized sample at 10-volt input. The mechanical parameters were recorded and qualified from the profile curves. Hardness was measured from the profile as the height of the first chew and all values were normalized to a 1-volt input (H). Cohesiveness was measured as the ratio of the area comprising the second peak to the area comprising the first peak (A_2/A_1). Cohesiveness is considered to be a direct function of the work needed to overcome the internal bonds of the materials. The other Texturometer parameters (springiness, adhesiveness and fracturability of both calcium gels) differed insignificantly.

Preparation of heat-induced gels

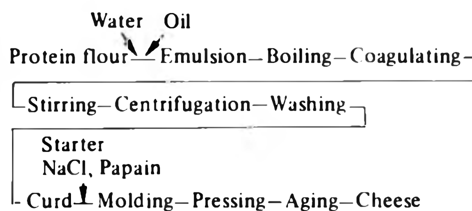
The flow sheet of heat-induced gel is as follows:



That is, 30g of 7S or 11S PRF were added with 90 ml of water, kneaded for 10 min in an electric mortar and stuffed into a film bag (30 mm diam) with care to avoid air bubbles. The stuffed protein paste was heated in a boiling water bath for 30 min and cooled with tap water. After refrigerating overnight, the textural properties of the heat-induced gel were evaluated by the Texturometer. The heat-induced gel was removed from the film bag, cut into a slice precisely 15 mm in height, and the Texturometer profile curve taken the same as indicated for the calcium gel, except at 2-volt input.

Preparation of cheese-like food

The method for preparation was approximately that of Obara et al. (1970). The flow sheet of cheese-like food is as follows:



That is, 60g of 7S or 11S PRF were dissolved in 1,500 ml of water. The protein solution was heated for 3 min after boiling, cooled up to 80°C with tap water and added with calcium chloride solution to 15 mM in the final concentration. The mixture was constantly sitrred for 30 sec, kept in room temperature for 30 min and the introduced curd separated with a basket-type centrifuge (Fuji Denki Co., Ltd.). The curd was wrapped in several layers of gauze, soaked in hot water at 80°C for 5 min and squeezed. The washing process was repeated three times. Finally, the curd was centrifuged again and then mixed with 30 ml of starter, 0.25g of papain and 3g of sodium chloride. The inoculum size of starter was 3% in defatted cow's milk and the acidity after incubation at 30°C for 17 hr was about 1. The mixture was molded and aged in an incubator at 17°C for 2–4 wk. In the case of adding oil, 50g of 7S or 11S PRF was dissolved in 1,250 ml of water and the protein solution was emulsified with 45 ml of soybean oil (salad oil not fortified lecithine) with Minisonic emulsifier (Ultrasonic Ltd.). Moisture of the curd before aging was measured by heating at 105°C for 1 hr in vacuum. Nitrogen and oil content of the curd before aging was carried out by the Kjeldahl and

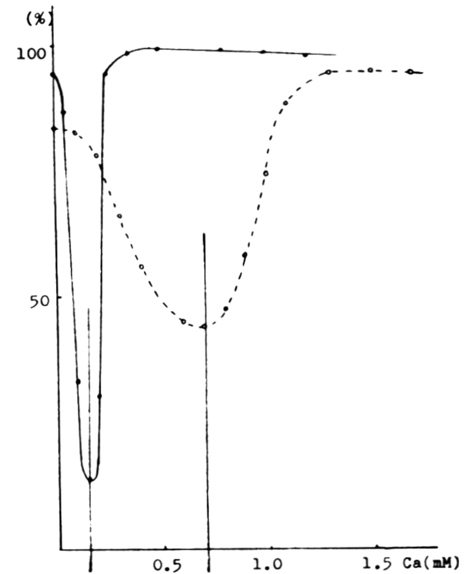


Fig. 2—Precipitation of 7S and 11S protein with calcium: ●—● 11S protein; ○----○ 7S protein. (Protein solution containing 1.56 mg N was filled up to 10 ml by adding different concentrations of $CaCl_2$ solution, kept for 30 min and centrifuged. The transmittance of supernatant at 500 nm is shown on the vertical axis. Vertical lines show calcium requirement of each protein for precipitation.)

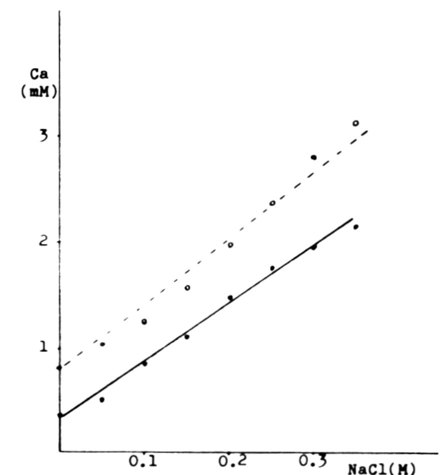


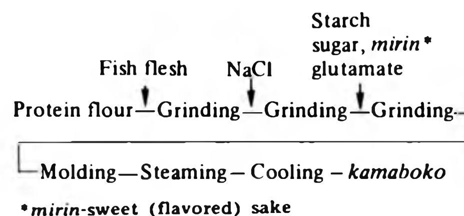
Fig. 3—Calcium requirement of 7S or 11S protein in the presence of NaCl: ●—● 11S protein: ○----○ 7S protein. (Calcium requirement of each protein for precipitation in the presence of NaCl, shown by vertical lines in Fig. 2 was plotted on the vertical axis.)

Rose-Gottlieb methods respectively. The textural properties of the curd before aging were evaluated by the Texturometer. The curd was cut into a block 30 mm × 15 mm and the Texturometer curves taken at 0.5-volt input. Springiness (the rate at which a deformed material goes back to its undeformed condition after the deforming force is removed) was estimated from the curve as the difference between the distance C–B, where C is the measurement made on a completely inelastic material such as clay and B is the distance from the initial contact on the first chew to that on the second chew.

Fig. 4–Calcium precipitation behavior of native or heated 7S and 11S proteins examined with a Particle Size Analyzer. [10 ml of 7S or 11S protein solution (5 mg N) was added with 0.1 ml of 0.5M $CaCl_2$ solution, stirred and added to formalin and NaCl. The mixture was stirred and submitted to analysis. Numbers described on right shoulder of curves show the time after starting measurement.]

Preparation of kamaboko-like food

The flow sheet for preparation of *kamaboko* (fish paste product) is as follows:

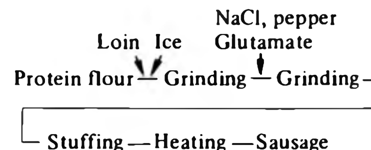


**mirin*-sweet (flavored) sake

Super A grade *surimi* (a fish paste produced from fish-like cod, grau mullet, etc. and usually marketed in frozen condition is classified as Super A, A, B and C grades depending upon quality) and soybean flour were ground with ice in a mortar. In the mix of *surimi* 80 and soybean flour 20, 800g of *surimi*, 50g of 7S or 11S PRF and 150g of ice were mixed for about 5 min, ground with 13g of sodium chloride for 5 min and again ground with 40g of starch, 10g of sugar, 40g of *mirin* and 10g of sodium glutamate for 5 min. The mixture was molded and steamed at 50°C for 40 min and successively at 80°C for 20 min. The prepared *kamaboko*-like food was cooled with tap water. The textural properties of the *kamaboko*-like food were evaluated by the Texturometer and Tensiron UTM-II. The gel was cut into a block 30 mm × 30 mm × 15 mm and the standardized sample submitted to Texturometer measurements at 1-volt input. The gel was also cut into a small slice 5 mm wide × 1.5 mm height for Tensiron measurements. Shear strength was recorded with 4–5 samples as the force of a 1.0 mm thick plunger to shear the 5 mm × 1.5 mm sample and normalized to kg/cm^2. Tensile stress was recorded with 4–5 samples as the force to tensile up to shear and normalized to kg/cm^2. The length of the tensiled part was 20 mm. Sensory properties were evaluated by nine testers. They were asked to describe the order of their liking and brightness of four samples. The sum of the order was divided by nine, the number of testers.

Preparation of sausage-like food

A flow sheet for preparation of a sausage-like food is as follows:



That is, 50g of 7S or 11S PRF were mixed with 125g of loin and 75g of ice and ground for 5 min in an electric mortar. Adding 5g sodium chloride, 7.5g pepper and 5g glutamate, the mixture was stuffed in a film bag and heated in a water bath at 72–73°C for 60 min. The prepared sausage-like food was cooled with tap water. The 11S PRF curd is the same material as the curd used for preparation of the cheese-like food: namely, the curd was prepared from 11S PRF protein solution by heating, coagulating with calcium chloride and washing with hot water at 80°C repeatedly. Textural and sensory properties were evaluated the same way as *kamaboko*-like food.

RESULTS & DISCUSSION

Difference of calcium precipitation behavior between 7S and 11S proteins

In the past decade, calcium salt has frequently been used as a step in the fractionation of 7S and 11S proteins (Vaintraub, 1965; Eldridge and Wolf, 1967; Fukushima, 1968; Koshiyama, 1965; Mitsuda et al., 1965; Wolf and Sly, 1965). The outline of the method (Wolf and Sly, 1965) is as follows: the entire water extract from defatted soybean meal is kept in an ice bath for more than 3 hr and centrifuged in the cold to prepare the cold-insoluble fraction that is rich in 11S protein. Calcium salt to 12.5 mM is added to the supernatant, after centrifugation, and kept in the cold overnight. As the supernatant in this process has little 11S protein, 7S protein can be prepared by acid precipitation. The last process shows clearly that 11S protein is easier to precipitate with calcium salt than 7S protein.

The difference of precipitation behavior between 7S and 11S proteins with calcium salt was confirmed quantitatively and qualitatively: the quantitative difference of calcium requirement for precipitation and the qualitative difference of the state in precipitation.

Figure 2 shows that 11S protein precipitated in lower calcium concentration than 7S protein. Although the calcium concentration sufficiency for precipitation is changeable with protein concentration, in the protein solution containing 1.56 mg N/10 ml (Fig. 2), 11S protein precipitated at 0.4 mM, while 7S precipitated at about 1.1 mM.

The calcium concentration sufficiency to precipitate was also affected by ionic strength. Figure 3 shows the relation between the concentration of sodium chloride and calcium concentration where protein precipitated completely and the transmittance of the supernatant after centrifugation showed 100%. Both proteins were difficult to precipitate with calcium as sodium chloride concentration increased, but as shown in Figure 3, the calcium concentration sufficiency for precipitation of 11S protein was lower than 7S protein at every concentration of sodium chloride. Decrease in easiness to precipitate with calcium in the presence of sodium chloride is perhaps caused by the binding of soluble sodium ions with reacting sites of protein molecules for calcium ions. The fact that calcium concentration was affected by ionic strength also explains the contradiction of different calcium concentrations to precipitate protein between a water extract of soybean meal and purified soybean protein, the former being 12.5 mM and the latter 1–5 mM. The latter may contain less salts compared with the former.

Even when sufficient calcium was added to the protein solution, the induced calcium precipitates were different in physical state between 7S and 11S proteins. As shown in Figure 4, native 11S protein began to precipitate at 3 min after starting the measurement and finished at almost 20 min, while native 7S protein did not begin to precipitate even at 20 min. In the case of heated proteins, heated 11S-protein precipitated more rapidly than native 11S protein. Sketches of the precipitation behaviors of the two proteins are shown in Figure 5.

The calcium precipitate of 7S protein was finer, sedimented slowly and resulted in a larger volume of sediment, whereas that of 11S protein was floccus, sedimented quickly and resulted in a more dense sediment of smaller volume.

Experimental production of 7S and 11S proteins

The cold precipitation method by Wolf and Sly (1965) to fractionate 7S and 11S proteins was developed from an interesting phenomenon but may be time-consuming and low in yield for practical application. This prompted consideration of fractionating 7S and 11S proteins by direct extraction from defatted meal with a dilute calcium salt solution,

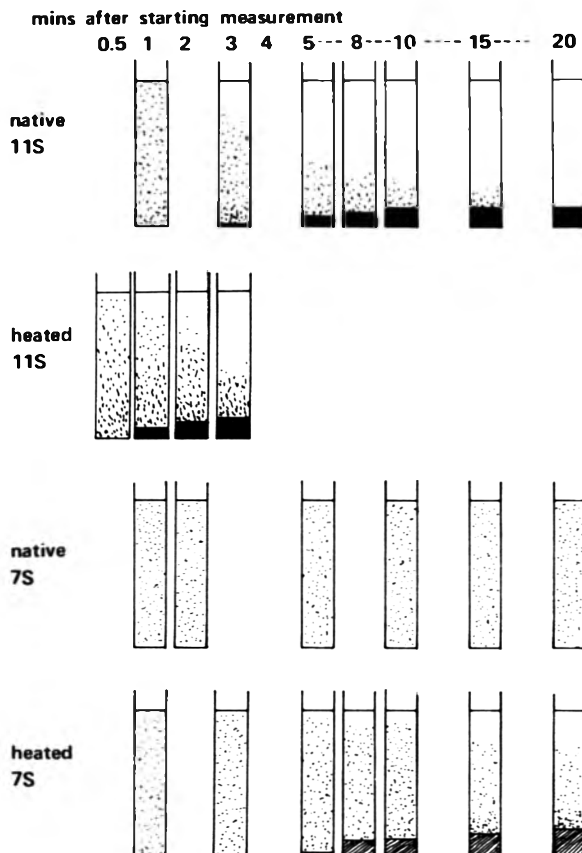
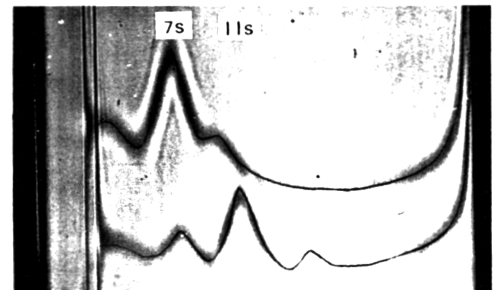


Fig. 5–Sketches of calcium precipitation behavior of native or heated 7S and 11S proteins. (From data in Fig. 4)



	7S Rich Fraction	11S Rich Fraction
2S	14.7	7.1
7S	68.0	21.1
11S	17.4	61.8
15S	–	9.5
11S : 7S	1 : 3.9	3.0 : 1
11S+15S : 7S	–	3.4 : 1

Fig. 6–Protein composition of 7S PRF and 11S PRF. (Photograph was taken after 36 min of centrifugation at 51,200 rpm, 20°C; protein fractions were dissolved in standard buffer. Upper curve shows 7S PRF; bottom curve shows 11S PRF.)

Table 1—Protein composition and yield of 11S PRF under different conditions of extraction from meal

Solvent $CaCl_2$ (mM)	2S	7S	11S	15S	11S/7S	Yield of 11S PRF (% to total protein)
	(%)					
			Room temperature			
10	9.9	20.0	61.4	9.5	3.1	34.5
12.5	5.8	22.5	62.7	9.6	2.8	38.0
15	3.8	31.0	61.4	3.8	2.0	43.1
			In cold			
12.5	18.3	21.7	52.8	7.2	2.5	24.8

Table 2—Average yield in each process[a]

	Total N	% to total protein
Defatted meal (2,500g)	199.0g	100
Extract with $CaCl_2$	72.4	36.4
Whey	19.9	10.0
Precipitate	50.5	25.4
7S PRF	42.6	21.4
Residue with $CaCl_2$	126.6	63.6
Extract with dil.alkaline soln	89.9	45.2
Residue with dil.alkaline soln	23.5	11.8
11S PRF	77.6	39.0

[a] Results are mean values of 3–4 experiments using 2,500g meal.

Table 3—Properties of cheese-like food from 7S or 11S PRF

	Protein only		Protein and Oil				Texturometer Unit[d]		
	Curd wt (g)	Moisture[b] %	Curd[a] wt (g)	Moisture %	Protein[c] %	Oil[c] %	Hardness (H)	Cohesiveness (A_2/A_1)	Springiness (S)
11SPRF	133	66	198	56	22	17	6.8	0.23	1.37
7SPRF	192	77	297	60	18	18	4.0	0.21	0.85

[a] Curd weight is the weight before aging.
[b] Moisture was measured by heating at 105°C for 1 hr in vacuum.
[c] Protein was estimated by the Kjeldahl method and oil by the Rose Gottlieb method.
[d] Texturometer evaluation: sample size, 30 mm X 30 mm X 15 mm; plunger, 17 mm lucite; clearance, 2 mm; voltage input, 0.5 volt.

based on the differences of precipitation behavior with calcium.

The authors propose the following method (see Fig. 1) as a conventional method to fractionate 7S and 11S proteins, where 7S and 11S proteins are termed the protein-rich fractions (7S PRF and 11S PRF, respectively).

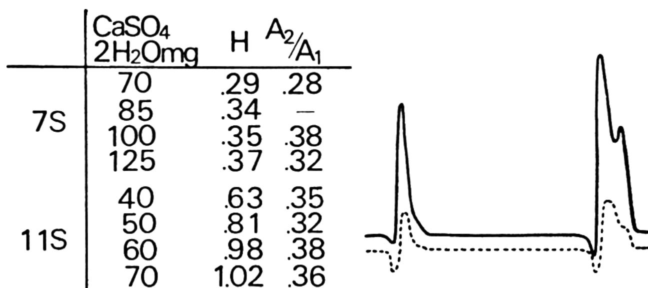
The optimum calcium concentration for extraction from meal was preliminarily determined by using 10, 12.5 and 15 mM at room temperature and 12.5 mM in the cold. The protein composition and yield of 11S PRF are shown in Table 1. As far as this experiment was concerned, 10 mM calcium concentration at room temperature resulted in the highest ratio (3.1) of 11S to 7S proteins. The cold extraction was expected to be the higher ratio of 11S protein because 11S protein is easier to precipitate in the cold than 7S protein; however, the result showed the low ratio (2.5) as well as the low efficiency for extraction. As a result of these preliminary experiments, 10 mM of calcium chloride solution at room temperature was used for the larger scale of experiment using 2.5 kg of meal.

In order to extract effectively the protein remaining in the residue which was separated from the extract, the pH of the suspension of the residue was adjusted to about 8–8.5 with a dilute NaOH solution and the extraction temperature was raised up to 40°C by adding hot water. After centrifugation and neutralization with a dilute HCl solution, the extract was spray dried directly (11S PRF).

The extract from the meal was spray dried directly at first, but as it was very hygroscopic (because of sugar and low molecular substances) it was finally spray dried after acid precipitation, redissolution and neutralization (7S PRF).

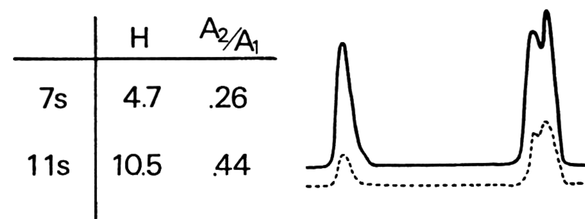
The average yield in each process is shown in Table 2: the yield of 11S PRF was almost 40% and that of 7S PRF 20% to total protein.

The typical ultracentrifugal patterns of both fractions and the average of protein compositions are shown in Figure 6. In the course of our investigation, the variety which had the highest ratio of 11S protein to total protein was Shirotsuru-



	$CaSO_4$ $2H_2O$ mg	H	A_2/A_1
7S	70	.29	.28
	85	.34	–
	100	.35	.38
	125	.37	.32
11S	40	.63	.35
	50	.81	.32
	60	.98	.38
	70	1.02	.36

Fig. 7—Textural properties of calcium gel from 7S or 11S PRF. (Sample size: 30 mm diam × 15 mm high; plunger, 17 mm lucite; clearance, 2 mm; voltage input, 10 volt.) (—— 11S PRF, ----- 7S PRF)



	H	A_2/A_1
7s	4.7	.26
11s	10.5	.44

Fig. 8—Textural properties of heat-induced gel from 7S or 11S PRF. (Sample size: 30 mm diam × 15 mm high; plunger, 17 mm lucite; clearance, 2 mm; voltage input, 2 volt.) (—— 11S PRF, ----- 7S PRF)

Table 4–Properties of a Kamaboko-like food from 7S or 11S PRF

Ratio wt on dry basis	Texturometer[a] Hardness (H)	Texturometer[a] Cohesiveness (A_2/A_1)	Tensionmeter[b] Tensile stress kg/cm²	Tensionmeter[b] Shear strength kg/cm²	Color[c] Order	Liking[c] Order
Surimi 100	13.2	0.39	1.38	0.42	1.2	1.1
Surimi 80 11S PRF 20	6.2	0.51	0.73	0.30	2.0	1.8
Surimi 80 7S PRF 20	6.2	0.57	0.77	0.24	2.8	3
Surimi 60 11S PRF 40	3.6	0.46	0.29	0.13	4	4

[a] Texturometer evaluation: same as Table 3 except input 1 volt.
[b] Tensionmeter evaluation: sample size 5 mm X 15 mm X 20 mm for tensile stress and 5 mm X 15 mm X 1 mm for shear strength.
[c] Color and liking were evaluated by nine testers. Values are the mean of order by testers, pale-to-dark and high-to-low in their liking.

Table 5–Properties of sausage-like food from 11S PRF

Ratio wt on dry basis	Texturometer[a] Hardness (H)	Texturometer[a] Cohesiveness (A_2/A_1)	Springiness (S)	Liking[b]
Loin 100	6.2	0.39	1.10	1.2
Loin 50 11S PRF 50	3.7	0.27	0.72	3
Loin 50 11S PRF curd	13.3	0.53	1.13	1.9

[a] Texturometer evaluation: same as Table 3 except input 1 volt.
[b] Liking was evaluated by nine testers, same as Kamaboko-like food.

noko (11S:7S is 1.8:1) and that of 7S protein was Hakuho (11S:7S is 1:1.3) (Saio et al., 1969). Therefore, the ratios shown in both fractions in Figure 6 cannot be expected from the usual acid-precipitated protein of soybean meal.

The 7S PRF and 11S PRF contain 92–94% protein on a dry basis and may be classified into sorts of protein isolate.

Possibility for food use of 7S PRF and 11S PRF

Figure 7 shows the physical properties of calcium gels from 7S PRF and 11S PRF measured with the Texturometer. Calcium requirement for coagulation was higher in 7S PRF than 11S PRF, as suggested by the difference in calcium requirement between the two purified proteins. Generally speaking, the hardness of calcium gel increases with the increase of calcium salts added (Saio et al., 1969); the results in Figure 7 confirm this and also show that calcium gels from 11S PRF were much harder than those from 7S PRF.

Figure 8 shows the physical properties of gels induced by heating at 100°C for 30 min. As far as this experiment was concerned, the gel from 11S PRF was harder and higher in cohesiveness than that from 7S PRF. The textural properties of the two fractions as measured with Tensiron UTM-II were also found to be different and these detailed results will be discussed in a subsequent paper.

Table 3 shows the weight of curd, composition and physical properties of a cheese-like food from 7S PRF or 11S PRF. During the process of washing curd with hot water, the curd decreased its water retention capacity and changed into a more elastic and gummy gel. The trend was observed more clearly in the curd from 11S PRF than that from 7S PRF and resulted in less weight of curd as shown in Table 3. When comparing the physical properties of both curds before aging, the curd from 11S PRF was harder and higher in cohesiveness and springiness than that from 7S PRF. Previous papers (Matsuoka et al., 1968; Kawaguchi, 1973) reported that the short point of soybean protein in preparing a cheese-like food was its high water retention, that is, it is difficult to make a low-moisture curd as in the case of casein. However, by using 11S PRF, a curd having 50% or less moisture can easily be prepared after mechanical pressing. Moreover, the color of the curd from 11S PRF was fairly yellowish even after aging. After aging, the overall qualities of a cheese-like food from 11S PRF seemed to be superior to that from 7S PRF; however, since both curds were contaminated after 1–2 wk, the textural properties and a sensual evaluation were not taken.

Table 4 shows the physical properties and sensual evaluation of *kamaboko*, a fish paste product prepared by adding 7S PRF or 11S PRF. *Kamaboko* with added 7S or 11S PRF was lower in hardness, tensile stress and shear strength but slightly higher in cohesiveness, compared with fish flesh only. The brightness in color and testers' liking showed that the order bright-to-dark or high-to-low in sensual evaluation was fish flesh only, the product with 20% added 11S PRF, one with 20% 7S PRF and one with 40% 11S PRF. However, it must be considered that both fractions were added to super A grade *surimi* and in larger amount. Generally speaking, 3–5% of soybean flour is added to C grade *surimi* to prepare low quality *kamaboko* in a commercial plant.

Table 5 shows the results of 11S PRF in a sausage-like food. Sausage with 11S PRF added in the state of flour was lower in hardness, cohesiveness and springiness and also poorly evaluated, whereas the product with added 11S PRF curd was higher in hardness, cohesiveness and springiness and rather highly evaluated.

It is well recognized that for the preparation of elastic gel from soybean protein, the protein has to be heated once at more than 90°C, while with fish or meat protein heating at 70–80°C is sufficient. The 7S and 11S PRF were not heated during the process of fractional extraction. The unsuccessful results in fish or meat product were partly attributed to insufficient heating.

From these preliminary experiments we may conclude that there exists a remarkable difference between 7S and 11S PRF and that it is worthwhile to try to find effective ways of utilizing them for food use which can be put into practice.

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CONSISTENCY OF AQUEOUS SOYBEAN-RICE MIXTURES

INTRODUCTION

THE MAJORITY of the infants in rice eating countries are breast-fed. Under conditions of insufficient production or unavailability of breast milk, breast feeding is replaced by rice products which are protein-poor in terms of quality and quantity. Soybean products are rich in protein and are popular foods in Far Eastern countries. Soybean-rice formulas were developed in dry powder form, as protein-rich and low-cost products for weaned infants and children (Huang et al., 1967; Cheigh and Kwon, 1970). However, the reconstituted products exhibited poor rehydration and textural qualities. Weaning foods should have consistencies suitable for easy swallowing by infants but without excessive fluidity.

The majority of non-Newtonian semisolid foods are considered to be pseudoplastic bodies in which the apparent viscosity (the ratio of shear stress to shear rate) decreases with increasing shear rates (Harper, 1960; Holdsworth, 1969, 1971; Saravacos and Moyer, 1967). The logarithmic plot of shear stress versus shear rate is linear over a certain range of shear rates. This relationship could be expressed mathematically by the simple power law equation of $\tau = K\Upsilon^n$, where τ = shear stress (dynes/cm^2) Υ = shear rate (sec^{-1}); K = consistency coefficient (dynes-cm sec^{-2}); and n = flow behavior index. Two parameters "K" and "n" are required to characterize the flow behavior of pseudoplastic materials (Harper, 1960; Holdsworth, 1969, 1971; Watson, 1968). These parameters are subject to change by various thermal, chemical and mechanical actions. The consistency coefficient "K" decreased as the temperature of the foodstuff increased, while the flow behavior index "n" remained practically unchanged (Harper, 1960; Harper and Leberman, 1962; Saravacos, 1970; Watson, 1968). The coefficient "K" increased while the index "n" decreased with increasing solid contents of foodstuff (Harper and Leberman, 1962; Holdsworth, 1969, 1971; Saravacos and Moyer, 1967; Watson, 1968). Values for "K" decreased as the pectic materials in apricot purees were enzymatically degraded (Watson, 1968). Reduction in particle diameter in applesauce resulted in lower "K" and higher "n" values.

The purpose of the present study was to investigate the influence of formulation, method of thermal processing and storage duration on the flow behavior of processed aqueous soybean:rice mixtures.

EXPERIMENTAL

Materials

Soybean seeds (cv. Bragg) and long grain rice (trademark, Mahatma) were obtained from a local market. Fish protein concentrate (EFP90) was obtained from Astra-Nutrition, New York. Proximate analysis showed that these materials contained proteins in the proportions of 40, 7.4 and 93%, respectively. Canned puddings and junior baby food were obtained from a local market.

Sample preparation

Soybeans were washed, dried at 75°C for 1.5 hr in a forced-air oven and cracked in a Waring Blendor at low speed. The cracked soybeans were dehulled by a forced air stream, arranged in 0.4-in. thick layers and steamed at 5.0 psi for 30 min. Rice was steamed by exposing rice:water mixture (1:2, w/w), as a 4.0-in. thick layer, to steam at 5.0 psi for 30 min. The steamed materials were homogenized in a commercial Waring Blendor and strained through the 0.02-in. screen of a paddle pulper. In formula 1 a mixture of 46% steamed soybeans, 46% steamed rice and 8% fish protein concentrate was mixed with 2 parts of water (w/w). Formula 2 was composed of 3 parts steamed soybean, 1 part steamed rice and 10 parts water (w/w). The protein contents of both formulas, by calculation, were 9.1 and 9.2g per 100g homogenate, respectively and the moisture contents were about 80%.

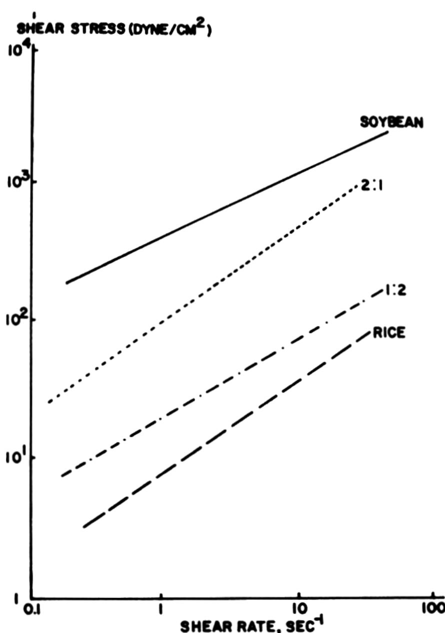

Fig. 1—Flow behavior at 25°C of unprocessed soybean, rice and soybean-rice aqueous mixtures.

Thermal processing

Still retorts were used for the thermal processing of all samples. Calculations of process times were based on the graphic method with F_0 of 15 min (Brody, 1971).

Pouch processing. Flexible pouches, 7.0 × 8.0 × 1.0 in. with capacities of 21.0 oz each were manufactured from polyester-aluminum foil-nylon laminate film supplied by Reynolds Metal Co., Richmond, Va. The pouches were filled with the preheated homogenate (60°C), sealed under vacuum and placed in racks with shelves spaced 1 in. apart. In order to prevent pouch rupturing during heating or cooling, steam and air were used alternately to maintain total retort pressure at 25 psi and temperature at 250°F. The pressure of 25 psi was maintained with air during cooling to a temperature of 120°F, after which it was slowly released to atmospheric pressure. Average process time was about 40 min.

Glass jar processing. Sterilized Mason jars (8.0 oz capacity) were filled with preheated homogenate (60°C), capped with lid and placed in 60°C water inside the retort for 20 min. The top metal band was screwed down by hand. Steam was slowly introduced, and a steam pressure controller was utilized to maintain retort temperature at 121°C. Air replaced steam during cooling to a temperature of 49°C, after which the pressure was slowly released to atmospheric pressure. Average process time was 65 min.

Metal can processing. Samples in the 303 × 406 enameled cans (15 oz capacity) were prepared similarly as for the glass jars. The cans were sealed and thermally processed in the conventional manner at 121°C for 75 min and cooled to 43°C.

Storage

The thermally processed samples were stored at 37°C for periods of 2 and 4 months prior to consistency measurements. The stored samples were kept for 2 days at room temperature prior to consistency measurements. Portions of the processed samples were used for consistency measurement immediately following processing (0 storage).

Consistency measurements

Consistencies of the homogenates were de-

termined at 25° ± 0.01°C using a Brookfield Synchro-Lectric model RVT viscometer (Brookfield Engineering Laboratories, Stoughton, Mass.) equipped with the Colora model NB circulating water bath. Measurements were made utilizing cylindrical spindles and 8 speeds. The contents of each sample were mixed thoroughly prior to measurement. Samples were placed in the water bath for temperature equilibration. Readings were taken 30 sec after the instrument was turned on. Spindle lengths were corrected for end effects (Brookfield Engineering Laboratories data sheet 66-0112). The procedure described by Garcia-Borras (1965) for the calculation of flow behavior constante ("K" and "n") and apparent viscosity (Ua) of non-Newtonian fluids was used. The data were treated by the analysis of variance and Duncan Multiple Range Test according to accepted statistical methods. Results are presented as the average of three replicates.

RESULTS & DISCUSSION

ABSOLUTE VALUES of the shear stress-shear rate relationships and power law constants of food materials could be directly determined by the use of narrow-gap coaxial cylinder viscometers (Charm, 1962a; Charm, 1963a, b; Harper and Leberman, 1962). The Brookfield viscometer, under the conditions used in the present study, is a wide gap viscometer. Hence, all results obtained in the present study should be considered as relative values expressing the comparative effects of formulation, processing and storage variables on the consistency of aqueous soybean-rice mixtures.

Unprocessed pureed and steamed soybeans, rice or soybean-rice mixtures were non-Newtonial pseudoplastic bodies as indicated by the value of "n" which ranged from 0.39–0.71 and with their increased shear stresses and decreased apparent viscosities with increasing shear rates (Fig. 1 and Table 1). Their flow behavior was similar to those found for other foods: apricot, peach, pear, plum and tomato purees and applesauce (Charm, 1962b; Charm, 1963a; Harper, 1965; Harper and Leberman, 1962; Saravacos, 1968; Saravacos and Moyer, 1967; Watson, 1968) and apple and orange juice concentrates (Charm, 1962b; Saravacos, 1970). Considerable decreases in "K" values were found as the amounts of rice in the mixture increased (Table 1). No definite trends were observed for the "n" values. The influence of soybean:rice ratios on changes in viscometric constants and apparent viscosities was similar for the processed and unprocessed mixtures, with the exception that processed samples exhibited higher "K" and apparent viscosity values than those for the unprocessed samples. Moisture contents for the steamed soybean and rice were 14.8% and 70.0%, respectively. Mixtures of the steamed soybean and rice contained variable contents of moisture depending on the amount of rice in the mixture, ranging from 26.0% for the 4:1 ratio to 51.6% for the 1:2 ratio. Decreases in viscometric constants and apparent viscosities paralleled the increases in rice or moisture contents of the mixtures. These results were in general agreement with those shown for other foodstuffs with different solids contents (Charm, 1962b; Harper, 1965; Harper and Leberman, 1962; Watson, 1968). Huang et al. (1967) stated that as the amount of soy flour was decreased and rice flour was increased, the liquid formula was too thick to be fed easily. The variance between these results and those reported in the present study may be partly due to the differences in sample preparation: dry soybean and rice flours versus steamed soybean and rice.

Commercial puddings showed smaller "n" values than those for the processed soybean-rice mixtures, but the apparent viscosities of the processed soybean-rice samples of 1:1 and 2:1 ratios were similar to that of the commercial puddings (Table 2). Sensory evaluation indicated that the experimental and the commercial products possessed similar consistencies.

Mixtures containing FPC showed higher apparent viscosities than those without FPC (Fig. 2), probably due to the increased gelation by the added animal protein. The pseudoplastic characteristic of soybean-rice mixture was also maintained for samples containing FPC.

Thermal processing of the steamed soybean-rice mixtures resulted in products with decreased "n" values and higher values for the consistency coefficient "K"

Table 1—Viscometric constants and apparent viscosities (poises) at 25°C at various shear rates (sec^{-1}) of processed and unprocessed soybean, rice and their mixtures.

	Constants		Apparent viscosity (poises)		
Product	"n"	"K"	2 sec^{-1}	5 sec^{-1}	10 sec^{-1}
Unprocessed					
Soybean only	0.46	458.0	316.2	193.7	133.7
4:1[a]	0.58	362.0	270.3	183.8	137.2
3:1	0.45	213.8	146.2	88.5	60.5
2:1	0.71	102.1	83.4	63.7	52.0
1:1	0.44	105.1	71.4	42.8	29.1
1:2	0.58	22.4	16.7	11.4	8.5
Rice only	0.81	7.5	6.6	5.5	4.8
Processed (303 × 406)					
Soybean only	–[b]	–	–	–	–
4:1	–	–	–	–	–
3:1	0.52	1192.3	853.2	548.2	392.3
2:1	0.39	651.5	426.9	244.2	156.0
1:1	0.41	401.1	265.9	154.5	102.4
1:2	0.45	72.1	49.4	29.9	20.5
Rice only	0.50	73.1	51.6	32.5	23.0

[a] The steamed soybean to the steamed rice ratio
[b] Off-scale

Table 2—Viscometric constants and apparent viscosities (poises) at 25°C at various shear rates (sec^{-1}) of commercial puddings and processed soybean:rice mixtures

	Constants		Apparent viscosity (poises)		
Product	"n"	"K"	2 sec^{-1}	5 sec^{-1}	10 sec^{-1}
Commercial puddings					
Banana[a]	0.24	675.6	397.9	197.7	116.4
Chocolate[b]	0.29	377.9	231.7	121.4	74.4
Rice[b]	0.22	514.0	300.7	147.9	86.5
Soybean:rice					
2:1	0.39	651.5	426.9	244.2	156.0
1:1	0.41	401.1	265.9	154.5	102.4
1:2	0.45	72.1	49.4	29.9	20.5

[a] Junior baby food, manufactured by Gerber Products Co.
[b] Canned puddings, manufactured by General Mills, Inc.

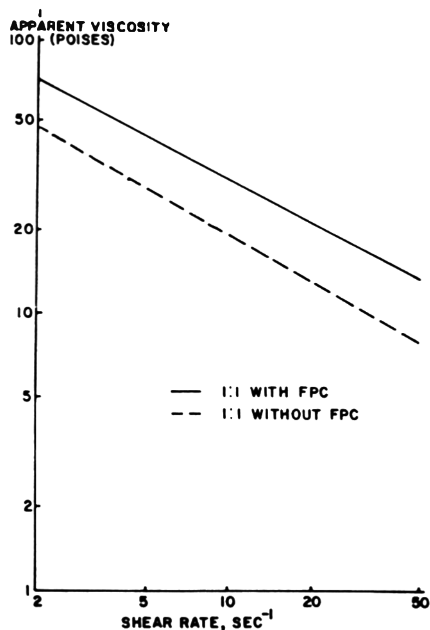

Fig. 2—Influence of FPC on the apparent viscosities (poises) at 25°C of the unprocessed 1:1 soybean:rice aqueous mixture at various shear rates (sec⁻¹).

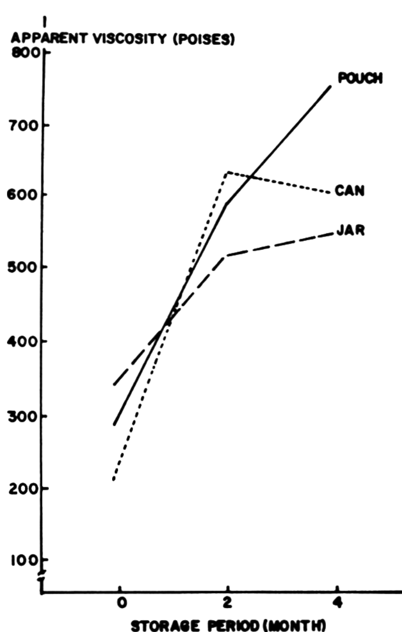

Fig. 3—Influence of storage duration on the apparent viscosities (poises) at the shear rate of 5.0 sec⁻¹ (25°C) of processed 1:1 soybean-rice aqueous mixture with FPC.

Table 3—Influence of method of thermal processing on the viscometric behavior (at 25°C) of 1:1 + FPC and 13:1 soybean:rice mixture.

Product	Constants		Apparent viscosity (poises)		
	"n"	"K"	2 sec⁻¹	5 sec⁻¹	10 sec⁻¹
1:1 + FPC mixture					
No processing	0.47	178.0	123.7	76.4	53.1
Jar	0.48	782.6	584.7	343.4	241.1
Can	0.43	529.2	357.5	212.9	143.8
Pouch	0.46	702.9	484.5	296.5	204.6
3:1 mixture					
No processing	0.53	141.8	102.3	66.5	48.0
Jar	0.35	1255.2	802.7	444.6	284.3
Can	0.38	1131.1	738.2	419.9	274.0
Pouch	0.34	1427.2	903.9	494.2	313.0

Table 4—Consistency index "K", of soybean:rice mixtures[a] at 25°C as influenced by storage duration, formulation and method of processing

Process Method	Storage period						
	0 month		2 months		4 months		
	F_1[a]	F_2[a]	F_1	F_2	F_1	F_2	Mean[b]
Jar	782.6	1248.7	1069.1	1912.7	1245.7	1928.1	1364.5a
Can	528.6	1130.5	1402.4	1715.6	1382.2	1790.6	1325.1a
Pouch	702.9	1425.4	1309.8	1831.5	1773.5	2128.4	1528.6a
Storage mean	967.8x		1540.4y		1708.1y		
Formula mean		1133.1r (F_1)		1679.0s (F_2)			

[a] F_1 = Formula 1 (1:1 + FPC); F_2 = Formula 2(3:1)
[b] Means, within groups, followed by the same letter are not statistically different at the 95% confidence level.

and apparent viscosity (Table 3). Changes in these values were much greater for formula 2 than formula 1. The interaction formula × processing method was significant at the 95% confidence level. Although there were little differences in the "K" and "Ua" values between the two formulas before processing, the difference became markedly larger after processing. Decreases in "n" values due to processing were greater for formula 2 than for formula 1. Formula 1 processed in the pouch showed lower "K" and "Ua" values than those processed in the glass jar (Table 3), while formula 2 showed the highest "n" value. The thickening effect of thermal processing of aqueous soybean-rice mixtures may be due to the denaturation of protein (Circle et al., 1964) and gelatinization of starch (Meyer, 1960) to form a 3-dimensional network throughout the samples. Increased apparent viscosities were evident as the amount of soybeans or solids in the mixture increased (Tables 1, 2 and 3). Saravacos (1970) found the apparent viscosities of fruit purees and pulps depend primarily on concentration and size and shape of their suspended particles but to much lesser extents on temperature. Soybean-rice mixtures contain higher amounts of starch and protein than the fruit purees used by Saravacos (1970) and this may account for the thickening of the thermally processed mixtures.

The influences of formulation, method of processing and storage duration on the values of the consistency index "K" are shown in Table 4. Statistical analysis of the data indicated that the main effects of formulation and storage duration significantly influenced the index. Higher "K" values were found for mixtures containing increased amounts of soybeans or stored for 2 or 4 months at 37°C. No differences were found due to processing method. Changes in "n" values followed patterns similar to those of "K" values. Soybean-rice mixtures processed by the three different methods showed similar consistencies, suggesting that all samples received equal heat treatments (F_o of 15 min) although exterior portions received considerably longer heating times in the jar and the can than that in the pouch. In all cases, heating and cooling curves were dictated by container geometry. Differences in processing times among the different processing methods were due to corresponding differences in sample thickness in the container. Sample thicknesses were 1.0, 2.5 and 3.0 in. for the pouch, glass jar and enameled can, respectively. Corresponding processing times were 40, 65 and 75 min.

Storage duration influenced the consistency of soybean-rice-FPC mixtures (Fig. 3). Increases in apparent viscosity values were evident after 2 months of

storage for all the processing methods. Mixtures packed in the pouch exhibited continued increases in apparent viscosity by lengthening the storage period from 2 to 4 months. Those packed in the glass jar or enameled can showed smaller changes in their apparent viscosities with prolonged storage. The effect of storage duration on changes of apparent viscosities of formula 2 showed patterns similar to those of formula 1. Luh et al. (1969) attributed the increased consistencies during storage of strained carrot purees to gel formation by hydrogen bonding of the polymers. Increased gelation could be one reason for the elevated apparent viscosities during storage of soybean-rice mixtures. The crystallization of solvated molecules is an important phenomenon affecting the consistency of starch gels (Matz, 1962). Temperature and shear rate influence the apparent viscosities of pseudoplastic foodstuffs. Heat transfer through these products could be improved by the use of high shear rates. This could be accomplished by the use of agitated heat exchangers (Harper, 1960), scraped vessels (Saravacos and Moyer, 1967) or pumping through small diameter tubes or film evaporators at high flow rates (Saravacos, 1970). Results obtained in the present study indicate that formula 1 was less viscous than formula 2 (Tables 3 and 4), probably due to the presence of smaller amounts of soybeans, thus less power requirements are needed to pump the slurry through the unit operations of the manufacturing process.

The homogenate prepared from either formula contains about 9% protein; thus a 200-g portion of the homogenate supplies the minimum daily requirements of protein for an infant weighing 20 lb (Food & Nutrition Board, NAS-NRC, 1968). Several investigators have established that addition of FPC to grains and legumes results in products with higher protein efficiency ratios and higher amounts of sulfur containing amino acids; thus it is expected that formula 1 is a better quality protein source than formula 2 and it should be more suited for infant and child feeding than formula 2. However, additional quality improvement and economic studies are needed to determine the feasibility of development of such a product for the consuming populations. If it is economically feasible to develop such a product, then a step is achieved toward the alleviation of protein malnutrition in Far Eastern countries.

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POLYPHENOL OXIDASE ACTIVITY AND BROWNING OF MANGO FRUITS INDUCED BY GAMMA IRRADIATION

INTRODUCTION

IN AN EARLIER STUDY on shelf-life extension by delaying the ripening processes and senescence in mangoes, it was observed that the exposure of the fruits to doses above 75 Krad of gamma rays resulted in radiation injury as manifested by browning of the skin and pulp tissues (Dharkar et al., 1966). It is known that discoloration of fruits and vegetables as a consequence of cutting, bruising, mechanical injury or infection is catalysed by the enzyme polyphenol oxidase (E.C. 1.10.3.10–diphenol:O_2 oxidoreductase) (Joslyn and Ponting, 1951; Farkas and Kiraly, 1962). [The terminology used in this paper: polyphenol oxidase, the enzyme which catalyses the oxidation of both mono- (cresolase activity) and diphenols (catecholase activity).] In banana fruits subjected to gamma irradiation, skin browning was observed to be due to an activation of polyphenol oxidase (Thomas and Nair, 1971). However, the presence of this enzyme in mango fruits has not so far been reported. It was of interest, therefore, to examine whether the radiation induced browning in mangoes was associated with alterations in the activity of polyphenol oxidase.

In this report, evidence is presented to show the presence of polyphenol oxidase in mango fruits and changes in this enzyme induced by gamma irradiation.

MATERIALS & METHODS

MANGOES of the variety Totapuri (syno: Bangalora) were used for these studies. Fully mature, preclimacteric fruits, 2 days from harvest, were gamma irradiated at a dose rate of 4.0 Krad per min to doses of 15, 25, 35, 50, 75, 100 and 200 Krad. For each dose 20 fruits were used. Irradiation was carried out in a package irradiator (Kumta and Sreenivasan, 1966) in air at an ambient temperature of 25–26°C. Dosimetry was performed either by the ferrous sulphate or the ceric sulphate aqueous dosimeter. Irradiated fruits along with controls were stored under ambient conditions (25–30°C, RH 80–85% in cardboard cartons).

Extraction of the enzyme

Only pulp tissues were chosen for the present studies. Diced pulp tissues were quick frozen in liquid nitrogen and stored at −30°C until used. Frozen tissue (10g) was thoroughly powdered in a pre-chilled (at −30°C) porcelain pestle and mortar and extracted with 20 ml of cold 0.02M K phosphate buffer, pH 7.0, containing 1% Tween 80. In a few experiments with control fruits, tannin complexing agents like polyvinylpyrrolidone or casein were added into the extraction medium. The mixture was ground for 10 min and the homogenate was passed through two layers of cheesecloth and centrifuged at 20,000G for 20 min. The supernatant, which showed negligible endogenous activity, was used as the enzyme source for all comparative studies.

In order to ascertain whether the extraction procedure released particulate associated activity, the centrifuge sediments from control tissue homogenates after washing with buffer and recentrifuging at 20,000G for 15 min were incubated with excess 4-methylcatechol in 0.02M K phosphate buffer, pH 7.0 for 30 to 60 min at 30°C and observed at 40x magnification with a binocular microscope.

In some experiments, the enzyme extracts were prepared from acetone powders of frozen tissues. The acetone powder was suspended in cold 0.02M K phosphate buffer, pH 7.0 (1g/20 ml) and the slurry was mechanically stirred at 0–2°C for 30 min before centrifuging for 25 min at 20,000G. The clear supernatant was used as the enzyme source.

Assay of the enzyme activity

Enzyme activities were determined by measurement of O_2 consumption with various mono- and diphenols as substrates using a Clark-type oxygen electrode fitted into one side of a water-jacketed (25°C) 3.0 ml cell equipped with a magnetic stirrer. The reaction was started by adding 0.5 mM of substrate to the reaction cell filled with air saturated K phosphate buffer, pH 7.0, containing an appropriate quantity of the enzyme (500–800 μg protein) in a final volume of 3.0 ml. The enzyme activity was calculated from the initial slope of the curve in case of catecholase and from the slope after the end of lag period in the case of cresolase (Thomas and Nair, 1971)

The ortho-dihydroxyphenols formed from p-cresol as substrate was determined as follows. To a reaction mixture consisting of 0.95 ml air saturated, 0.02M K phosphate buffer, pH 7.0 and 0.15 ml of enzyme solution (approximately 300 μg protein), 0.6 mM of p-cresol was added. The mixture was incubated at 25°C for different time intervals, then trichloroacetic acid (0.25 ml of a 30% w/v solution) added and the precipitate removed by centrifugation. An aliquot (1 ml) of the supernatant solution was used for the estimation of dihydroxyphenols formed by the procedure of Nair and Vaidyanathan (1964). The protein was estimated by Biuret method (Gornell et al., 1949).

Irradiation of crude extract

Extracts prepared from control fruits were irradiated to different doses ranging from 15–200 Krad at 0°C by keeping the extracts in ice during irradiation which was carried out in a gamma chamber 900 at a dose rate of 0.4 Mrad/hr.

Total phenolic constituents

The tissue (5g) was blended with 40 ml of 70% ethyl alcohol in a Sorvall Omnimixer for 5 min and centrifuged. Total phenols in the supernatant were determined by Folin-Dennis reagent according to Swain and Hillis (1959).

Ascorbic acid

10g tissue was blended with 100 ml of 3% metaphosphoric acid-acetic acid reagent in a Sorvall Omnimixer for 5 min and centrifuged. Ascorbic acid in the supernatant was estimated by visual titration, against 2,4-dichlorophenol indophenol dye (AOAC, 1960).

pH of extracts

The pH of the enzyme extracts were determined using a Beckman pH meter.

RESULTS

Browning phenomenon induced by gamma irradiation

It was observed that gamma irradiation at doses above 75 Krad resulted in discoloration of both skin and pulp tissues. The intensity of discoloration increased with increasing dose and with advancing storage period. In 200 Krad-dosed fruits, external discoloration was visible from the 6th day onwards as pale brown patches on the skin and by the 14th day the entire fruit tissue including the pulp had turned dark brown to black. However, such visible changes in the tissue were not observed uniformly in all fruits. Similar browning phenomena was noticed in five other varieties of mangoes as well.

Polyphenol oxidase activity in irradiated fruits

Initial experiments were carried out to assess the level of enzyme activity in pulp tissues of unirradiated preclimacteric and ripe fruits. Very little or no activity was detectable in these fruits. The pH of the pulp tissues ranged from 3.0–3.2 in the preclimacteric fruit and from 4.2–4.7 in the ripe fruits. The addition of KOH during extraction to neutralize the acids liberated or inclusion of 1–3% polyvinylpyrrolidone or 1% casein in the buffer to remove tannins and other phenolic substances which are known to inactivate enzymes (Jones et al., 1965; Haard and Tobin, 1971; Loomis and Battaile, 1966)

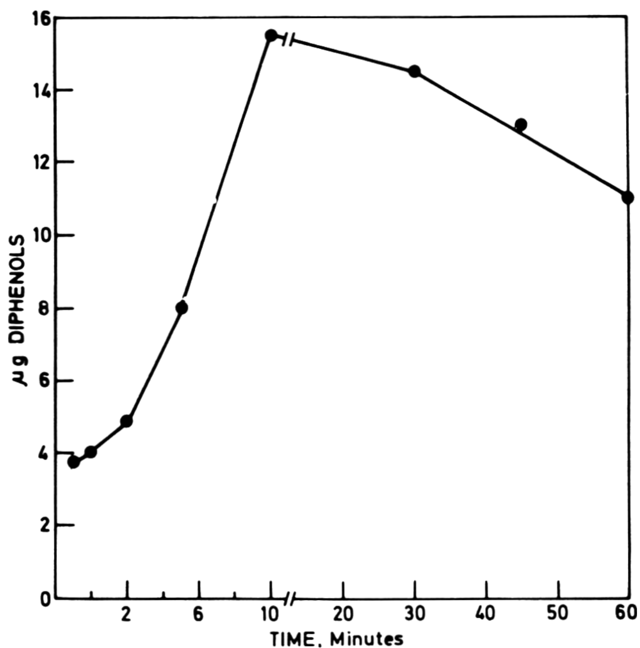

Fig. 1–Formation of o-dihydroxyphenol from p-cresol catalyzed by mango polyphenol oxidase. Enzyme extracted from pulp tissues of 200 Krad irradiated mangoes stored for 14 days. The reaction was carried out employing a reaction mixture as described under Materials & Methods.

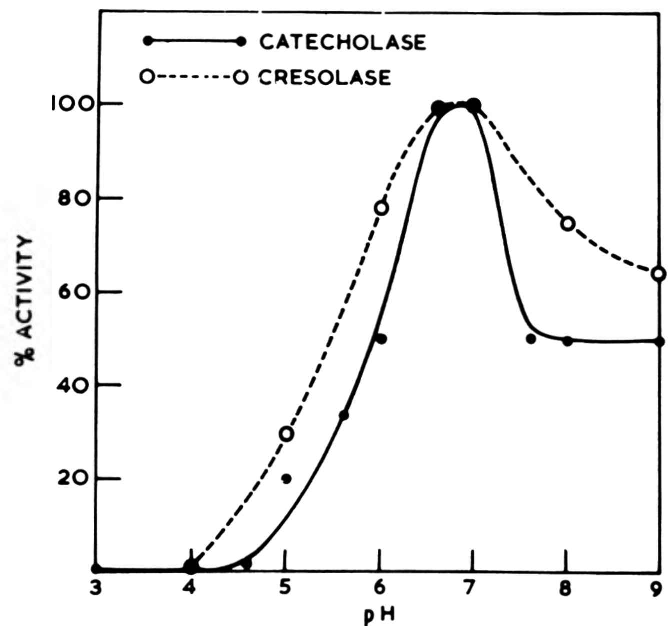

Fig. 2–The change of mango polyphenol oxidase activity with pH. Enzyme extracted from pulp tissues of 200 Krad irradiated mangoes stored for 14 days. The following buffer solution was used 0.1M sodium citrate-sodium phosphate (pH 3.0–7.0), 0.05M Tris-HCl (pH 7.5–9.0).

did not result in preparations with increased activity. Histochemical analysis of centrifuge pellets obtained from control fruits employing the above extraction media did not show measurable enzyme activity. Likewise incubation of tissue sections from control fruits with substrate failed to reveal enzyme activity whereas sections from 9-day stored 200 Krad irradiated fruits showed localized areas of red deposits indicative of enzyme activity. These observations suggest that tannin inhibition of enzyme activity was not occurring during homogenization of control fruits. Acetone powder extracts also showed little or no activity. Enzyme activity could not be detected even after fractionation of the crude extracts obtained from frozen pulp or acetone powder by cold acetone (1.6 volumes) or ammonium sulphate (50–70% saturation).

Table 1 shows the relative enzyme activity of extracts obtained from mango fruits subjected to different levels of gamma irradiation and stored for 9 days. It can be seen that irradiation resulted in a dose dependent increase in enzyme activity which was marginal up to 100 Krad. However, at 200 Krad, a dose which results in definite skin and pulp discoloration, several-fold increase in both mono- and diphenol oxidase activities was observed. The enzyme extracts from control and fruits irradiated up to 75 Krad were active only towards diphenols whereas extracts of fruits irradiated at 100 and 200 Krad exhibited both cresolase and catecholase activities. The enzyme activity in fruits exposed to 200 Krad of gamma rays determined at different time intervals after irradiation is given in Table 2. No increase in enzyme activity was detected until the fourth day. Increased activity was observed from the ninth day onwards and maximum activity was noticed on the fourteenth day when the experiments were terminated. It was interesting to note that the increase in enzyme activity paralleled with external manifestation of radiation injury. Fruits showing greater visible damage possessed enhanced enzyme activity and vice versa.

Irradiation of crude extracts prepared from control fruits did not show any effect on the level of enzyme activity.

Substrate specificity

As stated earlier, the enzyme preparation obtained from 100 and 200 Krad irradiated fruits showed both cresolase and catecholase activities. The specificity towards various mono- and diphenolic substrates is shown in Table 3. Of the various diphenols tested, dopamine showed more reactivity and, among the monophenols, p-cresol was the most reactive substrate. p-phenylene diamine, hydroquinone and vanillin were not at all oxidized, suggesting the absence of laccase activity in the preparation.

Figure 1 shows the rate of formation of orthodihydroxyphenol from p-cresol catalyzed by the mango enzyme. The data provides additional evidence to the fact that mango polyphenol oxidase could catalyze the oxidation of monophenol to give the corresponding diphenol.

Table 1–Effect of gamma irradiation on polyphenol oxidase activity of mangoes[a]

Dose in Krad	Specific activity μM O_2 consumed/mg protein/min	
	Cresolase	Catecholase
Control	Nil	9.0
15	Nil	14.0
25	Nil	21.0
50	Nil	40.0
75	Nil	41.0
100	10.0	80.0
200	69.0	275.0

[a] Enzyme extracted from pulp tissues of control and irradiated fruits stored for 9 days under ambient conditions (25–30°C, RH 80–85%). The cresolase and catecholase activities were estimated using p cresol and dopamine HCl, respectively. Buffer extracts of control and irradiated fruits did not show appreciable change in the protein content except in 200 Krads samples which showed approximately twice the quantity.

Table 2—Polyphenol oxidase activity in mango fruits assayed at different time intervals after irradiation to 200 Krad[a]

Time after irradiation (days)	Fruit color	pH of extract	$\mu M\ O_2$ consumed/mg protein/min	
			Cresolase	Catecholase
0	Green	3.23	Nil	Nil
1	Green	3.14	Nil	Nil
4	Green	3.37	Nil	Nil
6	Pale brown patches	3.36	Nil	25.0
9	Brown	3.34	43.0	227.0
12	Deep brown	3.35	67.0	291.0
14	Deep brown to black	3.54	110.0	446.0

[a] Cresolase and catecholase activities were estimated using p-cresol and dopamine HCl, respectively. Buffer extracts of fruits stored for 9 days and above had higher protein content compared to earlier samples.

pH optima

Using p-cresol and dopamine as substrates, the effect of varying pH values on polyphenol oxidase activity of mangoes is illustrated in Figure 2. Maximum activity was observed between pH 6.5–7.0. Only 20–30% of the original activity was observed at pH 5.0 whereas at pH 4.0 and below complete inhibition occurred.

Inhibitor studies

Inhibition of enzyme activity was observed by addition of sodium diethyl dithiocarbamate and complete inhibition occurred at concentrations of 66–100 μM.

Table 3—Substrate specificity of mango polyphenol oxidase[a]

Substrate	Specific activity $\mu M\ O_2$ consumed/ mg protein/min
Diphenols	
Dopamine HCl	446
4-Methyl catechol	439
Caffeic acid	420
Pyrocatechol	390
D-catechin	360
Chlorogenic acid	300
DL-arterenol HCl	270
L-dopa	186
DL-dopa	172
Monophenols	
p-Cresol	110
Tyramine HCl	72
L-tyrosine	7
DL-tyrosine	9

[a] Enzyme extract prepared from pulp tissues of 200 Krad irradiated mangoes stored for 14 days.

Changes in total phenols and ascorbic acid

A significant increase in the content of total phenolic constituents and a decrease in ascorbic acid was observed in 200 Krad irradiated fruits as a function of time. Total phenols increased by 7–8 fold on the fourteenth day post-irradiation, whereas ascorbic acid level dropped to 20% of the original value by that day (Table 4).

DISCUSSION

PHYSIOLOGICAL EFFECTS of gamma irradiation may be immediate or somewhat delayed. In an earlier study, we had reported an immediate activation of polyphenol oxidase in banana fruits upon irradiation (Thomas and Nair, 1971). Riov et al. (1968) observed an immediate increase in phenylalanine ammonia lyase activity in external layers of citrus fruit peel soon after irradiation at 200 Krad of gamma radiation. In contrast, the enhancement of polyphenol oxidase activity in mango fruits appears to be a delayed effect of gamma irradiation similar to that reported by Monselise and Kahan (1966, 1968) for peroxidase and catalase activation of flavedo of citrus fruit. Ogawa and Uritani (1970) demonstrated that gamma irradiation induced tissue browning of potato tubers was accompanied by a marked increase in peroxidase activity and a transient increase in o-diphenol oxidase activity.

Table 4—Changes in total phenolic compounds and ascorbic acid in control and 200 Krad irradiated mangoes during storage.

Days in storage	Total phenolics[a] mg/100g pulp	Ascorbic acid[a] mg/100g pulp
Control		
0 (Initial)	117	33
14	127	24
Irradiated		
0 (Initial)	107	21
11	416	7
14	772	4

[a] Average of triplicate estimations

The activation of polyphenol oxidase in mango fruits upon irradiation could be caused in many ways. It can be due to an activation of a pro-enzyme (Fox et al., 1962; McGuire, 1970; Ashida, 1971) or latent enzyme (Kenten, 1957, 1958; Deverall, 1961; Robb et al., 1964; Swain et al., 1966) or due to conformational changes in the enzyme (Lerner et al., 1972). Activation of latent polyphenol oxidase in beans as a consequence of fungus infection has been reported by Deverall (1961). Our unpublished observations show very high polyphenol oxidase activity in Alphonso mango fruits infected by the fungus *Botryodiplodia theobromae*. The fact that irradiation of extracts of control fruits did not produce increased enzyme activity suggests that the increase in activity in irradiated fruits was probably not due to conformational changes or activation of pro-enzyme or latent enzyme. Based on the development of enzyme activity in 200 Krad irradiated fruits, it would seem that a *de novo* synthesis of the enzyme is induced as a consequence of irradiation.

The accumulation of phenolic compounds in irradiated fruits would implicate the possible activation of phenylalanine ammonia lyase as observed in citrus peel tissues by Riov et al. (1968). Such suggestion, however, needs confirmation. Irradiation induced accumulation of phenolic substances has been reported in bananas (Thomas et al., 1971) and potatoes (Ogawa and Uritani, 1970).

It is interesting to note that the external manifestation of radiation damage in 200 Krad irradiated fruits was accompanied by a parallel decrease in the content of ascorbic acid. It would appear that, in the initial stages after irradiation, the orthoquinone compounds formed by the activated polyphenol oxidase are reduced back to the orthophenolic forms by the ascorbic acid present in the tissue, thereby preventing browning. When the ascorbic acid content of the tissue is exhausted, the enzymatically produced quinones, free of the reducing action of ascorbic acid, are then able to polymerize and form dark colored products (Reyes and Luh, 1962). A good correlation between browning of cut apples and the oxidation of ascorbic acid has been reported (Bauernfeind and Pinkert, 1970).

Dialyzed enzyme preparations from control fruits did not exhibit measurable enzyme activity which indicated that endogenous ascorbate was not responsible

for latent polyphenol oxidase in unirradiated fruit extracts. The results of our studies clearly show a good correlation between tissue browning and polyphenol oxidase activity in irradiated mango fruits.

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USE OF LIMONOATE DEHYDROGENASE OF Arthrobacter globiformis FOR THE PREVENTION OR REMOVAL OF LIMONIN BITTERNESS IN CITRUS PRODUCTS

INTRODUCTION

CERTAIN CITRUS FRUITS often are unsuitable for processing because of the development of limonin bitterness (Higby, 1938). A convenient solution to the bitterness problem would be a debittering enzyme that could be added directly to juice or other fruit tissues. Such an enzyme could be used alone or in conjunction with the presently available metabolic debittering treatment of the fruit which reduces the amount of limonin precursor entering the juice and subsequently the amount of limonin formed after processing (Maier et al., 1973).

We have recently reported the isolation of a limonoate dehydrogenase (LD; limonoate:NAD oxidoreductase) from *Arthrobacter globiformis* which converts the limonin precursor, limonoate A-ring lactone (LARL) (Maier and Margileth, 1969), to a nonbitter compound, 17-dehydrolimonoate A-ring lactone (17-DLARL) (Hasegawa et al., 1972) (Fig. 1). Prior conversion to 17-DLARL would prevent the ultimate conversion of LARL to bitter limonin in the juice. This enzyme is produced intracellularly by *A. globiformis* which has been grown on a medium containing limonoate as a single carbon source. LD requires Zn ions, sulfhydryl groups and the cofactor NAD for its catalytic action. LD attacks only limonoids which have the furan ring, epoxide, and open D-ring in their molecules.

The success of preliminary tests prompted further investigation of the use of LD to prevent or remove limonin bitterness in citrus juices and other products.

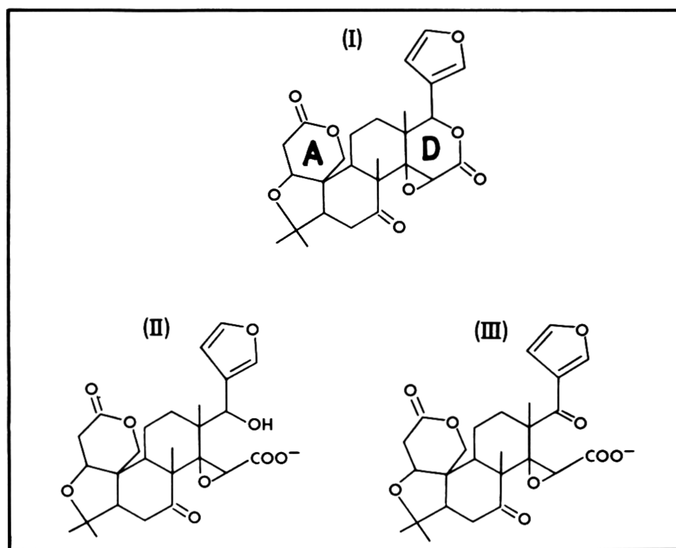

Fig. 1—Structures of limonin (I), limonoate A-ring lactone (II), and 17-dehydrolimonoate A-ring lactone (III).

EXPERIMENTAL

DUE TO THE unavailability of fresh navel orange juice during a major portion of the year, navel orange juice concentrate (65.2° Brix), diluted to 13° Brix, was used for most of our experiments. It was adjusted to pH 8 with NaOH and either heated for 15 min on a steam bath or held several hours to open the D-ring of limonin (the A-ring also opens). The D-ring lactone could also be hydrolyzed with limonin D-ring lactone hydrolase at pH 7.0 or higher (Maier et al., 1969). This juice was brought to room temperature before use. When fresh navel oranges became available, freshly prepared navel orange juice was used as is, and after adjustment to the desired pH with NaOH.

Navel orange peel was prepared for treatment by blending with 3 volumes of water for 2 min to achieve a slurry.

Lemon seeds were prepared by soaking overnight in 30 volumes of water, then grinding with a Polytron homogenizer for 3 min. Large particles were removed by screening through two layers of cheesecloth yielding a fine slurry which remained dispersed overnight. One portion of the slurry was adjusted to pH 7.5 with NaOH, another left unadjusted (pH 6.1), and both were held refrigerated overnight.

Limonoate dehydrogenase was partially purified from cell-free extracts of *A. globiformis* by $(NH_4)_2SO_4$ precipitation followed by separation on a DEAE cellulose column (Hasegawa et al., 1972). In one experiment the enzyme was used without separation on a DEAE column. The $(NH_4)_2SO_4$ precipitate was dissolved in 0.1M phosphate buffer (pH 7.5) and dialyzed against 0.01M phosphate buffer (pH 7.5) for 2 hr. One unit of enzyme is defined as the amount which catalyzes the formation of 1 μmole of NADH per min under the conditions used (Hasegawa et al., 1972).

Samples of 5–20g were taken from large volumes of the juice, peel slurry or seed slurry. Each sample was used as is or was adjusted to the desired pH with HCl or NaOH just prior to enzyme addition. The enzyme and NAD were then added to each sample. Samples were held at 24°C, loosely covered, and stirred occasionally during the incubation period.

After incubation, the reaction mixtures were acidified to pH 2 with HCl to convert any remaining LARL into limonin. Juice and seed samples were then boiled for 10 min. The standard limonin procedure of Maier and Grant (1970) was followed, analyzing for total juice limonin. Peel samples were not boiled and were analyzed by the modified peel method (Maier et al., 1973). Since the residual LARL of the reaction mixtures and controls was converted to limonin before analysis, the difference in total limonin content of untreated and LD-treated samples is a measure of the amount of LARL converted to 17-DLARL. Percent conversion is calculated on the basis of initial LARL content.

The reaction product (17-DLARL) in fresh navel orange juice was identified by extraction of the reaction mixtures after LD treatment and TLC comparison of the product and its methyl ester with known standards (Hasegawa et al., 1972).

RESULTS

TABLE 1 shows the reduction in limonin content of fresh navel orange juice (conversion of limonoate A-ring lactone to 17-dehydrolimonoate A-ring lactone) after treatment with limonoate dehydrogenase of *Arthrobacter globiformis*. Treatment of the juice with 190 munit

Table 1–Reduction of ultimate limonin content of fresh navel orange juice by limonoate dehydrogenase of Arthrobacter globiformis

Treatment[a]			
NAD added (μmole/ml)	pH[b]	Limonin (ppm)	Conversion (%)
Control	4.3	15.8	0
0.06	4.3	6.7	58
0.06	5.0	5.4	66
0.06	5.5	4.2	73
0	5.5	7.2	54
0.06	6.0	1.7	89
0.06	9.0	0.6	96
0	9.0	6.9	56

[a] Incubated with 190 munit enzyme/ml juice for 2 hr at 24°C; Control contained NAD and heat-inactivated enzyme.
[b] Natural pH of the juice was 4.3; NaOH was added to achieve the higher pH levels.

Table 3–Effect of incubation time on limonin debittering of reconstituted navel orange juice concentrate

Treatment[a]				
enzyme (munit/ml)	NAD added (μmole/ml)	Time of incubation	Limonin (ppm)	Conversion (%)
Control[b]	0	17 hr	16.25	0
6.5	0.025	20 min	11.25	31
6.5	0.025	40 min	8.75	49
6.5	0	40 min	11.25	31
6.8	0.025	17 hr	0.50	98

[a] Incubated at pH 9.5 and 24°C
[b] No enzyme added

Table 2–Effect of added NAD on the limonin debittering of reconstituted navel orange juice concentrate at pH 6.5[a]

NAD added (μmole/ml)	Limonin (ppm)	Conversion (%)
Control	18.0	0
0.1	2.0	89
0.05	3.0	83
0.025	4.0	78
0	11.0	39

[a] Incubated with 35 munit enzyme/ml juice for 17 hr at 24°C; Control contained NAD and heat-inactivated enzyme.

Table 4–Reduction in ultimate limonin content of navel orange peel slurry with limonoate dehydrogenase of A. globiformis

Treatment[a]			
NAD added (μmole/g)	pH[b]	Limonin (ppm)	Conversion (%)
Control	5.6	52.1	0
1.0	5.6	37.2	29
1.0	8.0[b]	21.9	58
0	8.0[b]	26.6	49

[a] Incubated with 120 munit enzyme/g slurry for 1 hr at 24°C; Control contained NAD and heat-inactivated enzyme.
[b] pH adjusted with NaOH

Table 5–Reduction in ultimate limonin content of lemon seed slurry by limonoate dehydrogenase of A. globiformis

Treatment[a]			
NAD added (μmole/g)	pH	Limonin (ppm)	Conversion (%)
Control	6.1	89.9	0
2.5	6.1	83.3	7.0
0	6.1	86.7	3.5
Control	7.5[b]	93.3	0
2.5	7.5[b]	33.3	64
0	7.5[b]	80.0	14

[a] Incubated with 310 munit enzyme/g slurry for 1 hr at 24°C; Control contained NAD and heat-inactivated enzyme.
[b] pH adjusted with NaOH

enzyme/ml juice and 0.06 μmole of added NAD/ml juice for 2 hr resulted in sufficient conversion to reduce the limonin content of the juice to near or below the bitterness threshold range (4–6 ppm) (Guadagni et al., 1973). The conversion was more extensive at higher pH and was essentially complete near the pH optimum of the enzyme. Substantial conversion also occurred without the addition of NAD. This finding suggests that NAD would not necessarily have to be added for commercial juice debittering. This experiment also demonstrated that the enzyme could be used without DEAE column purification, in fact, the crude preparation exhibited greater stability in acidic systems than did the purified preparation. The conversion to 17-DLARL was confirmed by TLC of the conversion product and its methyl ester against known standards (Hasegawa et al., 1972). The known substrate specificity of LD and the identification of 17-DLARL as the reaction product reconfirms the fact that LARL is present in the juice immediately after expression from the fruit (Maier and Beverly, 1968; and Maier and Margileth, 1969).

Table 2 shows the effect of added NAD on limonin debittering of reconstituted navel orange juice concentrate. With 35 munit enzyme/ml juice, addition of 0.025 μmole NAD/ml juice was sufficient to reduce the limonin level of an 18.0 ppm juice to below the reported bitterness threshold. At this enzyme level and pH, limonin conversion with 0.025 μmole NAD/ml juice was double that without added NAD.

The rate of limonin debittering was not linear with time of incubation (Table 3). With 6.5 munit enzyme/ml juice and 0.025 μmole added NAD/ml juice, approximately 31% of the conversion occurred in the first 20 min while only 18% occurred in the second 20 min. Addition of NAD increased the rate of the reaction markedly; conversion was 50% greater during the first 40 min with the addition of 0.025 μmole NAD/ml juice.

Table 4 shows the reduction in ultimate limonin content of a navel orange peel slurry. A 1-hr incubation at pH 5.6 (unadjusted pH of the slurry) and pH 8.0 resulted in 14.9 and 30.2 ppm decreases in limonin, respectively. At pH 8.0 conversion proceeded at approximately the same rate with or without the addition of 1.0 μmole NAD/g slurry.

Table 5 shows the LD catalyzed conversion of LARL to 17-DLARL in a lemon seed slurry. The limonin content decreased 64% during a 1-hr incubation at pH 7.5 with 310 munit enzyme/g slurry and 2.5 μmole NAD/g slurry. Seeds contain appreciable amounts of the limonin D-ring lactone hydrolase, which catalyzes the reaction: limonin⇌limonoate A-ring lactone. The pH dependence of the equilibrium favors the closed D-ring at pH 6.1 and the open D-ring at pH 7.5 (Maier et

al., 1969). Adjustment to pH 7.5 permitted limonin hydrolysis to occur before LD treatment.

DISCUSSION

THE ULTIMATE LIMONIN content of navel orange juice was greatly reduced by direct enzymic treatment of the juice. The rate of limonin debittering increases with increasing pH, and NAD and enzyme concentration. In the case of fresh navel orange juice, the juice was treated with LD immediately after it was extracted from the fruit. Relatively large amounts of enzyme were used to rapidly convert LARL to 17-DLARL thereby preventing its conversion to limonin. In the case of stored navel orange juice or juice concentrate (wherein the LARL has already been converted to limonin) it was necessary to first treat the juice with alkali to hydrolyze the D-ring of limonin before LD treatment.

At neutral or higher pH, the addition of NAD is not necessary to achieve substantial reduction in ultimate limonin levels. The addition of NAD does, however, allow more efficient use of the enzyme. At acidic pH where the enzyme is less active, the effect of added NAD is more pronounced, although substantial conversion does occur with only the natural NAD present in the juice. In the navel orange peel system, the addition of NAD enhanced activity slightly, whereas in the lemon seed system, addition of NAD was necessary to achieve significant conversion in 1 hr.

With regard to the commercial use of the enzyme, in the preferred practice, LD would be applied to the juice without delay or pH adjustment immediately after extraction from the fruit (Hasegawa and Brewster, 1972). For juice that has been stored, pasteurized or concentrated, hydrolysis of the D-ring of limonin with alkali or limonin D-ring hydrolase would first be necessary. After the LD debittering treatment the pH would be readjusted with food grade organic acids to avoid the saltiness which results when inorganic acids are used. While the need for pH adjustment in the latter cases would preclude the use of this process for pure orange juice, the process would be useful for other orange juice products and other citrus products. Also, the enzyme might be used to debitter citrus juices through application of immobilized-enzyme technology. Additionally, the activity of the enzyme in the lower pH range might be enhanced by modification of the enzyme protein through derivatization reactions.

LD successfully catalyzed a substantial reduction in the ultimate limonin content in slurries of other citrus tissues such as navel orange peel and lemon seeds. Debittering might allow more extensive use of these tissues in products and by-products such as flavoring, coloring and clouding agents, comminuted whole fruit or peel products, etc.

Since the objective of this work was to demonstrate that LD will function in a variety of citrus product systems, the limonin content was not always reduced to levels below the reported bitterness threshold in every treatment reported here. However, the combined data indicate that in those instances appropriately lower limonin levels could be achieved by adjustments in the enzyme concentration, the NAD concentration, and/or the time of treatment.

Since LD of *A. globiformis* has a high pH optimum and has comparatively low activity in acidic solutions, we have continued our survey of microorganisms in search of other enzymes. We recently isolated a new bacterium, No. 321-18 (Hasegawa, unpublished data). This organism produces an LD which differs significantly from that of *A. globiformis* and has a lower pH optimum. Studies on the application of this new dehydrogenase for limonin debittering of citrus products are underway.

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EFFECT OF pH ON THE ACTIVITY OF Schizosaccharomyces pombe

INTRODUCTION

GRAPES growing in many parts of U.S. and Canada frequently have acidity too high and pH too low for making good wines. It does not happen every season, but when the late summer and early fall weather is cold or rainy, the grapes may not ripen sufficiently, and high acidity develops.

Chemical neutralization is not desirable, as it may impair the delicate flavor of the wine. Another method of deacidification is malo-lactic fermentation by *Leuconostoc oenos*, which converts malic acid (a dicarboxylic acid) to lactic acid (a monocarboxylic acid) thus reducing the acidity of the wine. Malic acid may constitute as much as 40% of the acid of grapes. However, malo-lactic fermentation does not develop when the pH is below 3.0 (Peynaud, 1956).

Schizosaccharomyces species has been shown to metabolize both glucose and malic acid into ethanol and carbon dioxide (Peynaud and Sudraud, 1964; Benda and Schmitt, 1966), and has been investigated as a deacidification agent in wine making (Minarik and Navara, 1967; Yang, 1973). Since the pH of grapes varies widely, this investigation was undertaken to determine the effect of pH on *Sch. pombe* during fermentation. For comparison, *Saccharomyces cerevisiae* was used to ferment duplicate lots.

EXPERIMENTAL

Must preparation

Pinot blanc grape was used for this experiment and was harvested on Sept. 21, 1972, from the University vineyard at the Southern Oregon Experiment Station in Medford. The grapes were processed through a crusher-and-stemmer, and pressed immediately with the screw-type basket press. 100 ppm of sulfur dioxide, in the form of potassium meta-bisulfite, was added to the must. The must was divided into seven lots of 3 liters each in glass containers stoppered with cotton plugs.

pH adjustment

The pH of the must was adjusted with concentrated HCl. To determine the amounts of acid needed to obtain desired changes in pH, various volumes of concentrated HCl were added to several 100-ml samples of must. After 1 hr, the pH's of these test samples were measured. From this information were calculated the volumes of concentrated HCl required to adjust the six lots of must from pH 2.50 to 3.37, at intervals of approximately 0.15 units. The seventh lot of must was held at its original pH of 3.50. 1 hr after the addition of HCl, each lot was assayed for pH and titratable acidity.

Vinification

The pH-adjusted musts were allowed to stand with SO_2 overnight. The following morning, each lot was divided into two equal parts of 1.5 liters each; one was inoculated with a 3% (v/v) culture of *Sac. cerevisiae* and the other with a 3% culture of *Sch. pombe*. The cultures were 48 hr old, and the fermentation was carried out at 21°C.

Table 1—Fermentation rates by Saccharomyces cerevisiae and Schizosaccharomyces pombe

Initial pH of must[a] (adj with HCl)	Titratable acidity (as g tartaric acid/100 ml)	Time for completion of fermentation (days)		Fermentation rate ($days^{-1}$)	
		Sac.	Sch.	Sac.	Sch.
2.50	1.21	–	25	–	0.04
2.67	1.17	23	10	0.04	0.10
2.82	1.11	21	9	0.05	0.11
3.00	1.06	11	7	0.09	0.14
3.17	1.02	10	6	0.10	0.17
3.37	0.97	7	5	0.14	0.20
3.50	0.93	7	5	0.14	0.20

[a] °Brix = 21.9; tartaric acid = 5.4 mg/ml; L-malic acid = 3.9 mg/ml

Table 2—pH of wines fermented by Saccharomyces cerevisiae and Schizosaccharomyces pombe

Initial pH of must (adj with HCl)	pH of wine		Changes in pH		Differences in Δ pH
	Sac.	Sch.	Sac.	Sch.	
2.50	–	2.44	–	-0.06	–
2.67	2.68	2.84	0.01	0.17	0.16
2.82	2.86	3.05	0.04	0.23	0.19
3.00	3.10	3.32	0.10	0.32	0.22
3.17	3.22	3.50	0.05	0.33	0.28
3.37	3.50	3.78	0.13	0.41	0.28
3.50	3.61	3.91	0.11	0.41	0.30

Table 3—Titratable acidity of wines fermented by Saccharomyces cerevisiae and Schizosaccharomyces pombe

Initial pH of must (adj with HCl)	Titratable acidity of must (as g tartaric acid/100 ml)	Titratable acidity of wine (g/100 ml)		Changes in titratable acidity (%)	
		Sac.	Sch.	Sac.	Sch.
2.50	1.21	–	0.88	–	−27.3
2.67	1.17	1.20	0.84	+2.9	−28.0
2.82	1.11	1.14	0.76	+3.0	−31.3
3.00	1.06	1.02	0.70	−3.6	−33.8
3.17	1.02	0.97	0.66	−5.3	−35.5
3.37	0.97	0.94	0.60	−3.2	−38.2
3.50	0.93	0.89	0.56	−3.9	−39.5

Table 4—Malic acid utilization by Saccharomyces cerevisiae and Schizosaccharomyces pombe

Initial pH of must (adj with HCl)	L-Malic acid[a] (mg/ml)		Utilization (%)	
	Sac.	Sch.	Sac.	Sch.
2.50	–	1.2	–	69.2
2.67	3.9	1.1	0.0	71.8
2.82	3.8	0.7	2.8	82.1
3.00	3.7	0.0	5.5	100.0
3.17	3.7	0.1	5.5	97.4
3.37	3.8	0.0	2.8	100.0
3.50	3.8	0.0	2.8	100.0

[a] Malic acid in must = 3.9 mg/ml

Yeast cultures

The *Sac. cerevisiae* culture was the *Montrachet* strain, obtained from the Dept. of Viticulture and Enology, University of California, Davis. The *Sch. pombe* culture was obtained from Bayerische Landesanstalt fur Wein-, Obst- und Gartenbau, Wurzburg, Germany. Both yeasts were grown on agar slants, scraped, diluted with glucose-peptone media and added to the must.

Chemical analyses

The Brix readings were determined by the hydrometer procedure (Amerine, 1965). Titratable acidity (as tartaric acid g/100 ml) was determined by direct titration with standardized NaOH (Guymon and Ough, 1962). The pH was measured with a Corning pH meter, model 7, standardized at pH 3.56 with a saturated solution of potassium bitartrate (Lingane, 1947). Tartaric acid was determined by the vanadate method (Amerine, 1965). L-malic acid was determined enzymatically with Calbiochem malic dehydrogenase (Amerine, 1965). It was also detected qualitatively during fermentation by ascending paper chromatography employing n-butanol, formic acid, water and bromcresol green as the carrier solvent system (Kunkee, 1968).

RESULTS & DISCUSSION

Must

Table 1 shows the pH and titratable acidity of the seven lots of musts. The lowest pH of 2.50 was selected to account for rare grapes with very low pH, which was observed from time to time.

Grapes with a Brix reading of 22 and titratable acidity of 0.90 g/100 ml are ideal for table wine production. *Pinot blanc* used in this experiment had a Brix reading of 21.9 and titratable acidity of 0.93 g/100 ml. With this grape, it was possible to adjust the acidity of the must upward to a range where grapes of high acidity were found. The white grape also made it possible to ferment the juice instead of the pulp and skin as the red grapes normally require. The juice provided homogeneous samples and facilitated accurate chemical determinations without interferences from grape skin and seeds.

Hydrochloric acid was chosen for pH adjustment instead of organic acids in order to minimize the variation in organic content. Grapes of varying maturity and/or different varieties would also provide variations in initial pH; but that would also introduce variations in the complement of organic acids, sugars and other constituents in the must.

Fermentation rate

Table 1 also shows the time required for completion of fermentation in wines inoculated with *Sac. cerevisiae* and *Sch. pombe* at various pH. Since no sign of fermentation was observed at pH 2.50 in a must inoculated with *Sac. cerevisiae* after 60 days, this sample was discarded. The fermentation rate is expressed in 1/time, in days, for completion of fermentation. The fermentation was judged complete when the °Brix reached −1.5. The fermentation rate increased with the increase in pH for both yeasts. *Sch. pombe* fermented at a faster rate than *Sac. cerevisiae*, and also fermented at a lower pH. At pH 2.50, the activity of *Sch. pombe* was comparable to that of *Sac. cerevisiae* at pH 2.67. The acidity affected *Sac. cerevisiae* more than *Sch. pombe*; the former was greatly inhibited when the pH was below 2.82 and ceased to be active at pH 2.50. The effect of residual SO_2 should be negligible, since its presence is in such a low concentration.

pH Changes

Table 2 shows the changes in pH caused by fermentation. All wines showed higher pH than the initial with the exception of the one fermented with *Sch. pombe* at pH 2.50. The discrepancy was within the range of experimental errors. The data also show the effect of the initial pH on the extent of final pH of wine. The pH increase in wines fermented with *Sch. pombe* was higher than those fermented with *Sac. cerevisiae*. Increase in pH was the greatest when the initial pH of the must was at the highest level tested (pH 3.50).

Changes in titratable acidity

Table 3 shows the titratable acidity in wines fermented with *Sac. cerevisiae* and *Sch. pombe*. Wines fermented with *Sac. cerevisiae* had but small changes in acidity, ranging from +2.9 to −3.9%. Those fermented with *Sch. pombe* had much greater changes, from −27.3 The paper chromatography confirmed this due to the metabolism of malic acid by *Sch. pombe*.

Malic acid utilization

Table 4 shows the utilization of malic acid by *Sac. cerevisiae* and *Sch. pombe*. Only a small quantity of malic acid was utilized by *Sac. cerevisiae* as has been reported by Rankine (1966). *Sch. pombe*, however, actively metabolized malic acid. The *Sch. pombe* utilized all the malic acid present when the pH of the must was 3.00 or above. Below pH 3.00, malic acid was not completely utilized, although approximately 70% was utilized even at pH as low as 2.50.

CONCLUSIONS

ALTHOUGH pH at the lower range affects the activity of *Sch. pombe* somewhat, it is safe to say that the *Sch.* yeast will function within the pH range of grapes. The activity of *Sch. pombe* may be curtailed to a certain degree with grapes of extremely low pH when harvested in some unusually cool seasons. However, even grape musts with pH as low as 2.50 can expect a malic acid utilization by *Sch. pombe* of approximately 70%.

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TOCOPHEROLS IN THE UNSAPONIFIABLE FRACTION OF COCOA LIPIDS

INTRODUCTION

ALTHOUGH chocolate flavor is of non-lipid origin primarily, cocoa fat, because of its unique crystallization behavior in confectionery products, is of great economic importance. Fatty acid composition of cocoa fat is 26% palmitate, 34% stearate, 35% oleate, 3% linoleate, 1% arachadic and trace amounts of several other acids (Bracco et al., 1970). Phospholipids vary between 0.1% and 0.9% depending on recovery method (Parsons et al., 1970), and free fatty acid content is usually less than 1%. The unsaponifiable fraction, approximately 0.3%, is dominated by sterols (Bracco et al., 1970).

Many constituents of cocoa fat have been studied extensively, but little attention has been given to the tocopherols. In a survey of fats and oils, by colorimetric analysis, Herting and Drury (1963) reported 136 μg to 173 μg tocopherol/g lipid in three cocoa butter samples. They indicated that α-tocopherol is not the major tocopherol in cocoa fat.

The limited amount of available data on the tocopherols of cocoa fat prompted the investigation reported herein.

EXPERIMENTAL

Sample preparation

Samples of Ghana, Bahia (Brazil), Arriba (Ecuador), Sanchez (Dominican Republic) and Trinidad cocoa beans were obtained from several member companies of the Chocolate Manufacturer's Association of the USA. Cocoa powders, cocoa butters and nibs, also supplied, made possible comparison between dutched and undutched nibs, and cocoa butter and its corresponding cocoa powder.

Most analyses were carried out on unroasted cocoa beans. The effect of the roasting process on tocopherol concentration was ascertained by analyses before and after roasting a batch of each variety in a forced-air oven at 150°C for 30 min. For the tocopherol analysis of shell lipids, a thin, metal spatula was used to separate shell from nib on samples of unroasted Bahia, Ghana and Sanchez beans. Cocoa butter, nibs and cocoa powder were analyzed as they were received.

Extraction of lipid

Shell, whole-bean and nib were prepared for solvent extraction by pulverization to a homogeneous mass in a Waring Blendor with an equal weight (35g) of Celite 545. This mixture, in a 60 × 3 cm glass column, was extracted by eluting with 500 ml of diethyl ether. Compared to Soxhlet extraction 75% of the cocoa bean lipids were recovered.

Percolation of solvent through cocoa powder-Celite columns was excessively slow. Consequently, lipids were extracted from 100g cocoa with 200 ml of diethyl ether by mixing on a magnetic stirrer for 5 min and filtering under vacuum through a layer of Celite 545. A second extraction using 150 ml of ether was carried out. Lipid recovery was about 75% compared to Soxhlet extraction.

Solvent was removed from lipids by evaporation over a steam bath under a continuous stream of nitrogen gas. Except in the storage study, lipid samples were analyzed within 2 days of extraction.

Preparation of tocopherol-rich fraction

Of the several methods evaluated for tocopherol recovery (Eisner et al., 1966; Chow et al., 1969; Slover et al., 1969; Low and Dunkley, 1971), Slover's procedure was found most adaptable to cocoa beans and was faithfully followed with only minor modification. With this method precautions are taken to minimize tocopherol oxidation during extraction and includes the addition of pyrogallol as an oxygen scavenger.

Lipid samples, 2 ± 0.1g, were reacted with aqueous KOH and an unsaponifiable fraction was recovered according to Slover et al. (1969). This involved extraction of the refluxed alkaline reaction mixture with petroleum ether and steps to assure removal of water to yield a benzene solution of tocopherols ready for thin layer chromatography (TLC).

The benzene solution of tocopherols was applied across the bottom of the TLC plate as quantitatively as possible with solutions of α-tocopherol and δ-tocopherol being spotted along the side of the plate as reference standards.

Thin-layer plates (20 cm × 20 cm) had been coated 250μ thick with Supelcosil 12C (Supelco, Inc., Bellefonte, Pa.) which contained a fluorescing indicator. Plates were developed in benzene-methanol (98:2). When viewed under UV light, the tocopherols appeared as dark, fluorescence-quenching spots or streaks. After development, the tocopherol band between the α- and δ-standards was scraped from the plate into a 25 × 1 cm glass column and eluted with 20 ml diethyl ether. Solvent was removed under N_2 and derivatization and GLC was carried out within 24 hr.

Before derivatization, 1 ml β-sitosterol solu-

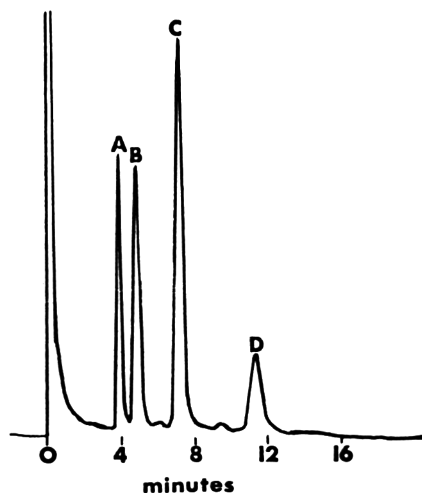

Fig. 1—GLC of TMSi derivatives of tocopherol standards. Key: (A) δ-tocopherol; (B) β-/γ-tocopherols; (C) α-tocopherol; (D) internal standard, β-sitosterol.

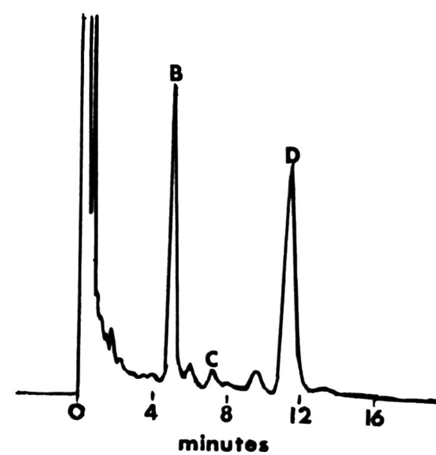

Fig. 2—GLC of TMSi derivatives of tocopherol fraction from typical cocoa bean lipids. Key: (B) β-/γ-tocopherols; (C) α-tocopherol; (D) internal standard, β-sitosterol.

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Table 1—Mass spectral data[a] for TMSi tocopherol standards and TMSi tocopherol isolates

m/e	α-standard	α-isolate	m/e	δ-standard	m/e	δ-isolate	m/e	β-standard	β-/γ-isolate	γ-standard
43	19.2	10.2	43	28.2	41	23.0	43	22.4	18.1	20.3
57	17.4	7.2	55	13.2	69	100.0	57	26.0	13.0	13.8
73	57.1	53.6	57	18.3	73	89.3	73	63.4	81.2	69.0
236	32.1	27.6	73	69.5	75	18.5	222	56.2	34.8	50.4
237	66.5	56.5	208	45.6	81	47.7	223	58.0	79.7	82.2
238	15.2	12.3	209	49.3	135	46.2	224	14.2	17.4	21.9
502	100.0	100.0	210	20.3	197	26.2	488	100.0	100.0	100.0
503	42.0	40.0	249	13.2	281	43.2	489	41.0	40.6	42.4
504	12.5	11.6	474	100.0			490	12.4	10.1	10.6
			475	39.1						
			476	10.8						

[a] Values indicate percent of base peak.

tion (35.5 mg/100 ml chloroform) was added as an internal standard. Solvent was removed under a stream of N_2, 150 μl N,O-bis-(trimethylsilyl)-acetamide (BSA, Pierce Chemical Co., Rockford, Ill.) was added, and after 15 min at 60°C, the mixture was gas chromatographed.

Gas chromatography

Several nonpolar columns were used effectively including SE-30, OV-225 and OV-17, but none could separate the β/γ-isomers. The column primarily employed was a 6-ft., 1/8 in. o.d., stainless steel column packed with 2% OV-17 on acid-washed, dimethyldichlorosilane-treated, 80-100 mesh Chromosorb G. Operating conditions for the Hewlett-Packard Model 5750 chromatograph with hydrogen flamed detector were: injection port temperature 310°C, detector temperatures 320°C, column oven temperature 290°C and nitrogen carrier gas flow at 10 ml/min. Hydrogen and air flow rates to the detector were 40 and 450 ml/min, respectively.

Identification of tocopherols

TMSi derivatives of tocopherol standards, α, β, γ and δ, were prepared and retention times determined for the standard GLC procedure. In addition, mass spectra were obtained using an LKB 9000 combined gas chromatograph-mass spectrometer. A 6-ft., 1/4-in. o.d., glass column containing 1% OV-17 on 80-100 mesh Supelcoport was used. Ion source and separator temperatures were 310°C and 280°C, respectively; column temperature was 275°C; and carrier gas was helium at 25 ml/min.

Quantitative measurement

A standard curve was developed for quantitation of the tocopherols through the addition of known amounts of α-tocopherol standard to cocoa butter which was then subjected to the recovery and analysis procedures described previously. Since the same cocoa butter was used in all trials involving the addition of α-tocopherol standard, the ratio of naturally occurring α to β/δ-tocopherol was a constant. This made it possible to determine the contribution of naturally occurring α-tocopherol to total peak area, which was considered in the construction of the standard curve.

It was assumed that detector response was approximately the same for the various tocopherols and that the standard curve based on α could be used for the other tocopherols as well. The available samples of β- and δγ-tocopherol were too small to permit construction of individual standard curves.

RESULTS & DISCUSSION

Separation and identification

A chromatogram of the TMSi derivatives of the four tocopherol standards and the internal standard is presented in Figure 1. Unfortunately, β- and γ-tocopherols had identical retention times and quantitative data for this peak had to be treated as a mixture of the two compounds.

Figure 2 shows a chromatogram of the TMSi derivatives of the tocopherol fraction of a typical cocoa butter in which the β-/γ-peak contributes over 90% of the total tocopherol peak area. α-Tocopherol was present in all samples at a level of about 5% of the total except in shell lipid where it was much higher. Although small peaks were often present with retention times similar to δ-tocopherol, none could be identified as such.

Identification of the cocoa peaks was accomplished by comparison of retention times to standards and by mass spectrometry. Mass spectral data (Table 1) for the standards and cocoa isolates agree quite well for α-, β- and γ-tocopherol. Conversely, the mass spectrum of the compound with a retention time equal to δ-tocopherol did not show similarity to the standard and, obviously, was something other than a tocopherol.

Two methods were applied in an attempt to resolve the identity of the compound co-chromatographing with β-/γ-tocopherols. In both methods, the regular analysis procedure was followed through the collection of the tocopherol fraction from the thin-layer plate.

The procedure of Stowe (1963) should result in the separation of β- and γ-tocopherols by TLC which when sprayed with antimony pentachloride yields brown and green colors respectively. Good separation could not be obtained, but the developed brown color suggested β-tocopherol as the primary tocopherol in cocoa lipid.

However, the method of Lehman (1955) yielded different results. The procedure involves the diazotized-o-dianisidine coupling reaction in which γ- and δ-tocopherols produce color complexes but α- and β-tocopherols do not. Color development in the tocopherol fraction of cocoa lipids, measured spectrophotometrically and compared to a standard curve for δ-tocopherol, indicated that the fraction was 80% γ-tocopherol.

On the basis of these two experiments, it would appear that both β- and γ-tocopherol exist in cocoa butters, but no judgment concerning the proportions of each can be made. A possible explanation for the disparity may involve difficulty in discerning the colors produced by Stowe's (1963) method. Also, γ- and/or δ-tocotrienols, if present in cocoa lipid, could contribute to color development through the coupling reaction. However, GLC did not suggest the presence of these tocotrienols, at least at concentrations sufficient to affect colorimetric results.

Other small peaks sometimes occurred but identification of these was not attempted. Large peaks were present in the TMSi tocopherol fraction from shell lipid, including one with a retention time equal to δ-tocopherol. As was the case with whole bean lipids, the mass spectrum of this compound did not conform to any known tocopherol.

Quantitative determination of the tocopherols

Blank determinations, involving the entire extraction procedure but without fat, showed that impurities in reagents and materials did not produce GLC peaks. Elution of tocopherols from the TLC adsorbent was checked for completeness by the spot test method of Nair and Magar (1954). No tocopherol appeared after collection of the first 10 ml of diethyl ether from the column. By collecting 20 ml of eluate, it was concluded that recovery from the adsorbent was quantitative.

Elapsed time between lipid extraction

and tocopherol analysis never exceeded 2 days, except, of course, those aliquots taken from the extracted fat sample during the storage study. Because of the easily oxidizable nature of the tocopherol compounds, care was exercised to carry the analysis to completion in as short a time as was reasonably possible. Speed was considered essential for good precision, since the interruption of a partially completed analysis overnight generally resulted in poor duplication. Average deviation from the mean was 7.3% calculated for 72 determinations. Over 70% of the individual values were below 10% variation and was considered acceptable in light of the highly reactive nature of the tocopherols.

Since α-tocopherol was present in small quantity relative to the β-/γ-component, its quantitation was not attempted, except in shell lipids where α-tocopherol was present in high concentration. Quantitative data were obtained for the β-/γ-tocopherol compound in all samples.

Raw and roasted cocoa beans

As revealed in Table 2, tocopherol quantities varied considerably among beans of different geographic origins. Sanchez beans from the Dominican Republic are generally considered in commercial circles to be poorly-fermented; Ghana, Bahia and Trinidad are usually well-fermented; while Arriba beans are between these extremes. These data do not indicate a predictable correlation between tocopherol level and fermentation period. Analysis of lots of each bean variety from several suppliers would have been desirable to obtain averaged values.

As indicated in Table 2, roasting did not materially affect tocopherol concentrations. Since heat accelerates tocopherol oxidation (Bunnell, 1971), a decrease in tocopherol concentration might have been expected when beans were roasted. A possible protective mechanism for the tocopherols might involve the increase in reducing substances brought about by Maillard browning during roasting. More study in this area should be conducted.

Shell lipids

Shell material from unroasted cocoa bean was less than 4% by weight lipid and relatively large amounts (50g) had to be collected in order to obtain enough lipid for tocopherol analysis. As revealed in Figure 3, the tocopherol fraction of shell lipid was more complex than that from whole beans, and α-tocopherol was the dominant peak. This is in marked contrast to whole bean lipids which contained only trace amounts of α-tocopherol.

Of the three shell samples analyzed, tocopherol concentration, especially α, was highest in Sanchez beans which generally are poorly-fermented. This suggests a relationship between fermentation and shell tocopherols and is worthy of further study.

Table 2–β-/γ-tocopherol concentrations[a] in the lipids of cocoa beans of different origins before and after roasting

Variety	Raw	Roasted
Arriba	273	256
Bahia	128	158
Ghana	221	181
Trinidad	270	281
Sanchez	206	205

[a] Values indicate μg tocopherol per g lipid.

Table 3–Tocopherol concentrations[a] and percent lipid in cocoa bean shells

Variety	Percent lipid	α-Tocopherol	β-/γ-Tocopherols
Ghana	2.06	587	491
Bahia	3.97	235	200
Sanchez	2.28	1024	511

[a] μg tocopherol per g lipid.

The high α and β-/γ-tocopherol contents of shell lipid (Table 3) might suggest tocopherol analysis for differentiating commercial cocoa butters pressed from shell-free nibs and whole beans. However, calculations show that shell fat would contribute less than 1% of the butter pressed from whole beans and would not be enough to affect tocopherol concentration significantly.

Cocoa powder

To produce cocoa powder, nibs are ground in a milling device, during which the mechanical energy expended is absorbed, cocoa lipid melts, flows and becomes the continuous phase surrounding the nonfat particles. This product, chocolate liquor, can be heated and hydraulically pressed to squeeze out a portion of the lipid known as cocoa butter. The cake left from the pressing operation, when pulverized, becomes cocoa powder which has a multitude of uses. Depending on conditions employed during pressing, the fat content of cocoa powder may vary between 10 and 25%.

To determine the effect of the heating, pressing and grinding operations on the tocopherol concentrations, samples of Ghana cocoa butter and cocoa powder from the butter's press cake (12% fat) were analyzed. Tocopherol content of the cocoa powder was 29% less than that of the butter: Cocoa butter: 162 μg β-/γ-tocopherol per g lipid; Cocoa powder: 115 μg β-/γ-tocopherol per g lipid. This decrease could result from grinding which would expose a very high surface area to oxygen and tocopherol oxidation.

It is interesting to note that the tocopherol content of butter commercially pressed from Ghana beans was about 30% lower than the value obtained in this investigation for solvent-extracted Ghana beans. Perhaps pressing routinely destroys some of the tocopherols, or does not extract them as quantitatively as solvent.

Effect of dutching

Two samples of medium-roast Ghana nibs, identical except that one had been treated with 3.0% potassium carbonate (Dutch process), were analyzed for tocopherols. Dutching is an alkali treatment which modifies color and flavor, and reduces the natural acidity of cocoa

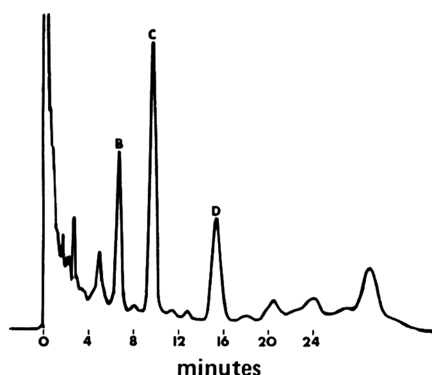

Fig. 3–GLC of TMSi derivatives of tocopherol fraction from Sanchez shell lipids. Key: (B) β-/γ-tocopherols; (C) α-tocopherol; (D) internal standard, β-sitosterol.

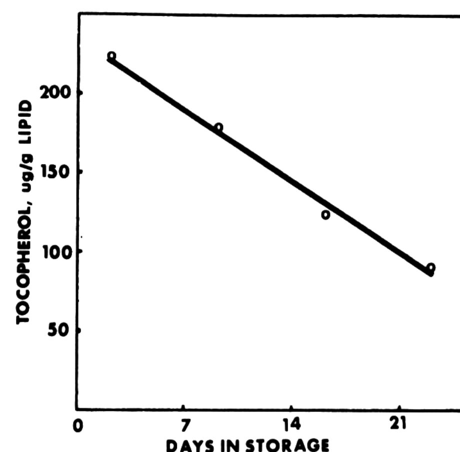

Fig. 4–Effect of storage on the tocopherol content of unroasted Ghana bean lipids.

(Kempf, 1964). Tocopherol concentrations were essentially the same in dutched and undutched nibs: Undutched: 158 μg β-/γ-tocopherol per g lipid; Dutched: 152 μg β-/γ-tocopherol per g lipid.

Alkali is a catalyst for tocopherol oxidation (Bunnell, 1971), and a decrease in tocopherol concentration could have been predicted. Since oxygen is a prerequisite for oxidation, the tocopherols may have been protected stucturally within the intact nib. Also, reducing substances produced during roasting may increase resistance to oxidation.

Effect of mold

Mold proliferation is an intermittent problem associated with the handling of cocoa beans, especially at their point of origin. If mold penetrates the shell, the lipids in the cotyledon are especially affected as shown by relatively large increases in carbonyl compounds (Hansen and Keeney, 1970) and free fatty acids (Kavanagh et al., 1970; Hansen and Shen, 1972).

To determine if tocopherols are altered by moldiness, Ghana cocoa beans were subjected to high humidity conditions for 6 wk to allow pronounced moldiness with mold penetration of the cotyledon. The beans were badly deteriorated and would not be acceptable for processing.

A 30% reduction in β-/γ-tocopherol content occurred during this period: Nonmoldy Ghana: 221 μg β-/γ-tocopherol per g lipid; Moldy Ghana: 155 μg β-/γ-tocopherol per g lipid. Although this may appear to be a significant change, it is unlikely that tocopherol analysis could serve as a practical gauge of moldiness, considering the deteriorated state of the beans from which the data were derived.

Effect of storage

Generally, processing and storage result in decreases in the tocopherol content of vegetable oils (Herting and Drury, 1963; Slover, 1971). Therefore, it seemed appropriate to determine if cocoa lipids followed this pattern.

Solvent extracted lipid (35g), in a clear-glass Erlenmeyer flask held at room temperature with no protection from light and air, was analyzed at intervals over a 3-wk period.

Figure 4 relates the decrease in tocopherol content to storage time. Reduction was essentially linear with a 50% decrease occurring over a 22-day storage period: from 221 μg β-/γ-tocopherol per g lipid to 97 μg β-/γ-tocopherol per g lipid. These data demonstrate the oxidative susceptibility of the cocoa tocopherols.

Although tocopherol oxidation would be much less in large blocks of cocoa butter wrapped or boxed to exclude light, change of undetermined magnitude, nevertheless, would be expected. This precludes attaching too much significance to tocopherol values for cocoa butters unless the complete history of the product can be traced.

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PRODUCTION OF β-NITROPROPIONIC ACID IN FOODS

INTRODUCTION

FOOD RESEARCH concerning toxic metabolites from molds generally has centered on aflatoxin from *Aspergillus flavus.* Recently Wilson (1966) indicated that other metabolites [oxalic acid, kojic acid, aspergillic acid and β-nitropropionic acid (β-NPA)] also presented a potential public health problem. β-nitropropionic acid was first isolated from the root bark of *Hiptage* by Gorter (1920) but since it has been reported by Bush et al. (1951), Marshall and Alexander (1961), Nakamura and Shimoda (1954) Raistrick and Stossl (1958) and Anzai and Suzuki (1959) as metabolites of *Penicillium* and *Streptomyces* species. Its bio-synthesis mechanisms using *Penicillium atrovenetum* G have been described by Hylin and Matsumoto (1961), Raistrick and Stossl (1958) and Shaw and Wang (1964).

Kinoshita et al. (1968) isolated 37 strains of fungi from 24 Japanese fermented foods and found 21 strains produced toxic culture filtrates without aflatoxin but with kojic acid and β-nitropropionic acid which in short term animal studies led to marked pathological changes, including liver necrosis. An examination of 70 strains of Japanese industrial seed mold by Yokotsuka et al. (1969) showed some strains producing β-nitropropionic acid in liquid cultures but the amounts produced in soybean and wheat were small and considered insignificant to food safety.

The degree of β-nitropropionic acid production in growth media by food producing molds, the presence of β-nitropropionic acid in selected foods, and the possible formation of nitrates or nitrites from its degradation were the subjects of the present study.

MATERIALS & METHODS

Medium

Media was that by Nakamura and Shimoda (1954) with 50g sucrose, 20g peptone, 5g KH_2PO_4, 2.5g $CaHPO_4$ and 2.5g $MgSO_4 \cdot 7H_2O$ brought to 1 liter distilled water at pH 6.4.

Molds

Aspergillus, Penicillium and Rhizopium mold strains from the American Type Culture Collection (ATCC), the Northern Utilization R & D Division (NRRL), and from Cornell University were transferred periodically on Czapek-Dox agar slants.

Preparation of solid protein materials

The procedure for pasteurized milk Cheddar cheese was followed as outlined by Kosikowski (1970). Fresh curds, after milling but before salting to assure growth of the mold, were diced and 30-g lots placed in Petri dishes. The soy-

Table 1—Production of β-nitropropionic acid by molds in Nakamura's medium[a]

No.	Mold	β-NPA (mg/liter culture)	Final pH of culture[b]
1[c]	*Penicillium roqueforti*-PI	ND[e]	6.50
2[d]	*Penicillium roqueforti*-P4	ND	6.35
3	*Penicillium roqueforti* ATCC 6987	ND	6.30
4	*Penicillium roqueforti* ATCC 9295	ND	7.10
5	*Penicillium roqueforti* ATCC 10110	ND	5.70
6	*Penicillium camemberti* ATCC 4845	ND	6.20
7	*Penicillium camemberti* ATCC 6985	ND	6.25
8	*Aspergillus oryzae* ATCC 11494	1	6.30
9	*Aspergillus oryzae* ATCC 14895	ND	5.00
10	*Aspergillus oryzae* ATCC 12892	1279	5.85
11	*Aspergillus oryzae* ATCC 9362	ND	5.30
12	*Aspergillus oryzae* ATCC 7252	40	5.30
13	*Aspergillus oryzae* Higati	111	6.25
14	*Aspergillus soyae*	ND	5.85
15	*Aspergillus flavus (oryzae)* ATCC 11500	17	5.60
16	*Aspergillus niger* NRRL 67	ND	3.30
17	*Rhizopium oryzae* NRRL 395	ND	7.75
18	*Rhizopium oligosporus* NRRL 2710	ND	7.55

[a] Incubated 6 days at 32°C
[b] pH of cultures incubated 6 days at 32°C
[c] Isolated from mature Danish Blue Cheese
[d] Isolated from mature Roquefort Cheese
[e] ND—Not detectable

Table 2—Influence of various treatments on β-NPA concentration in cultures

Treatment	Asp. oryzae ATCC 12892 mg β-NPA/150 ml	pH	Asp. oryzae Higati mg β-NPA/150 ml	pH
A. Control lot – a 5 day culture held 5 days at 32°C	195	5.50	22	5.90
B. Mold cells removed from A and cell-free culture incubated additional 7 days at 32°C	182	6.25	23	5.50
C. Mold cells removed from A and cell-free culture heated to 80°C, 5 min then incubated additional 7 days at 32°C	167	5.45	22	5.90
D. Mold cells removed from A were transferred to new medium and incubated additional 7 days at 32°C	7	7.55	ND[a]	6.70

[a] ND—Not detectable

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beans and peanuts were soaked in tap water for 3 hr then 30-g lots of each were heated in Petri dishes for 30 min at 100°C. All test samples were inoculated individually with *Aspergillus* mold from a fresh agar slant and incubated at 28°C.

Approximately 1.0 cm^2 portions of mold-contaminated surface from thawed ripened cheeses imported from Europe for other experiments and stored for 2 yr at −10°C were removed with a spatula.

Protease, lactase and lipase food grade fungal enzyme preparations were obtained from Miles Laboratories, Marschall Div., Elkhart, Ind. and Wallerstein Co., Morton Grove, Ill.

β-nitropropionic acid determination

The method of Matsumoto et al. (1961) was used on 3 ml of extract prepared as follows:

Incubated mold cultures. 150 ml of incubated mold culture was filtered through Whatman no. 1 paper and the filtrate acidified to pH 1.6 with 6N HCl and extracted four times with 150 ml of ethyl ether in a separatory funnel. This extract was evaporated to dryness in a rotary evaporator at 40°C and its residue dissolved in 25 ml distilled water.

Cheese, soybean and peanut. 30g cheese curd, 30g soybean and 30g peanut with active surface mold growth of almost equal thickness and 50-g lots microbial surface material from the aged, ripened cheese were individually extracted for 2 days with ethyl ether in extra large size Soxhlet extractors. The residues after ether evaporation were brought to 25 ml with distilled water.

Commercial food-grade enzyme preparations. 5 or 10g of fungal enzyme preparation powder was extracted in the Soxhlet extractors. The residues after evaporation of the ether were brought to 25 ml with distilled water.

Nitrates and nitrites

Nitrates and nitrites were determined by analytical methods of the Div. of Agricultural Chemistry, Tokyo University (1961) on 5 ml of filtrate prepared as follows: Approximately 1.0 cm^2 layers of moldy cheese surfaces were weighed to 50g and homogenized with 100 ml distilled water for three minutes in a Waring Blendor, then centrifuged 10 minutes at 10,000 rpm. The supernatant was treated with 50% trichloroacetic acid, heated to 95°C for 2 min and the precipitated protein suspension was then filtered through Whatman no. 3 paper. Sensitivity for nitrates was 0.02–2 ppm and for nitrites 0.01–0.6 ppm.

Thin-layer chromatography (TLC) for propionic acid was conducted by the method of Antonini and Adda (1969).

A Beckman Zeromatic glass electrode potentiometer was used for pH determination.

RESULTS

β-NITROPROPIONIC ACID production among 18 *Aspergillus*, *Penicillium* and *Rhizopium* species was limited to *Aspergillus* strains producing 1 to 1279 mg per liter of Nakamura media at 32°C, Table 1. Maximum levels were attained in 6 days at pH 5.1–5.3 by *Asp. oryzae* ATCC 12892 and 4 days by *Asp. oryzae* Higati at pH 5.8. β-nitropropionic acid decreased to zero on the 20th day, Figure 1.

No significant change in β-nitropropionic acid concentration was observed when mycelia-free filtrates from 5-day cultured media containing maximum β-nitropropionic acid levels were incubated an additional 7 days at 32°C. This indicated the intracellular nature of the β-nitropropionic acid splitting enzymes, Table 2. When mold cells, characterized as maximum β-nitropropionic acid producers, were transferred to another lot of new media and incubated an additional 7 days at 32°C, little or no β-nitropropionic acid production occurred indicating a loss of synthesizing activity and a retention of splitting characteristics. It showed, too, that β-NPA splitting factors are also extracellular (Table 2). The addition of pure β-nitropropionic acid to mold cultures did not induce more of this compound in the system (Table 3).

Nitrate and nitrite were not observed in two non-β-nitropropionic acid-producing mold cultures between 6 and 30 days at 32°C but with *Asp. oryzae* ATCC 12892 culture, a strong producer, nitrate appeared in the medium but no nitrite (Table 4). The pH of this culture during this period increased from 6.25 to 8.45.

TLC of filtrates from two β-nitropropionic acid-positive mold strains incubated 2–20 days at 32°C are shown in Figure 2. Propionic acid was not detected during any stage of the incubation but unknown substances fluorescing under ultraviolet were observed at the same Rf shown by the propionic acid standard. β-nitropropionic acid, as a pure compound, was undetectable by this method. Area B and C compounds were not identified. The former may have originated from the yeast extract of Nakamura's medium as TLC analysis of the extract produced compounds with similar values as Area B compounds.

Table 3—Inducement of β-NPA in Nakamura's medium by molds through prior addition of β-NPA

Molds[a]	β-NPA added to culture	Days Incubated at 32°C 2	4	6	15
		Increase in β-NPA – mg/50 ml			
1. *Asp. oryzae* ATCC 12892 – Control	None	25	69	67	37
2. *Asp. oryzae* ATCC 12892	70	15	27	57	0
3. *Asp. oryzae* ATCC 14895	70	0	0	0	0
4. *Rhizopium oryzae* NRRL 395	70	0	0	0	0
		pH			
1. *Asp. oryzae* ATCC 12892 – Control	None	6.00	5.10	6.20	7.95
2. *Asp. oryzae* ATCC 12892	70	5.65	4.95	5.80	7.75
3. *Asp. oryzae* ATCC 14895	70	5.60	5.00	4.95	8.40
4. *Rhizopium oryzae* NRRL 395	70	6.30	7.50	7.70	8.20

[a] Molds 1 and 2 are natural producers of β NPA; molds 3 and 4 originally nonproducers

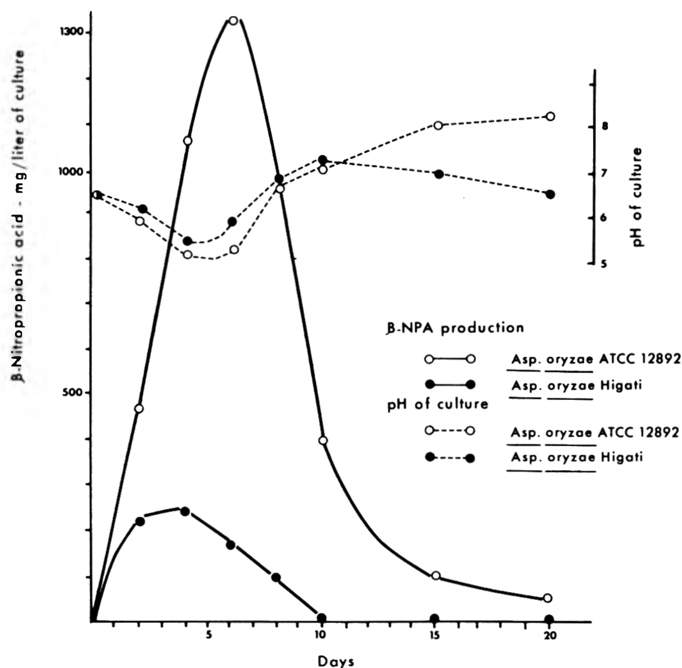

Fig. 1—Effect of time on β-nitropropionic acid production in Nakamura's medium by Asp. oryzae mold.

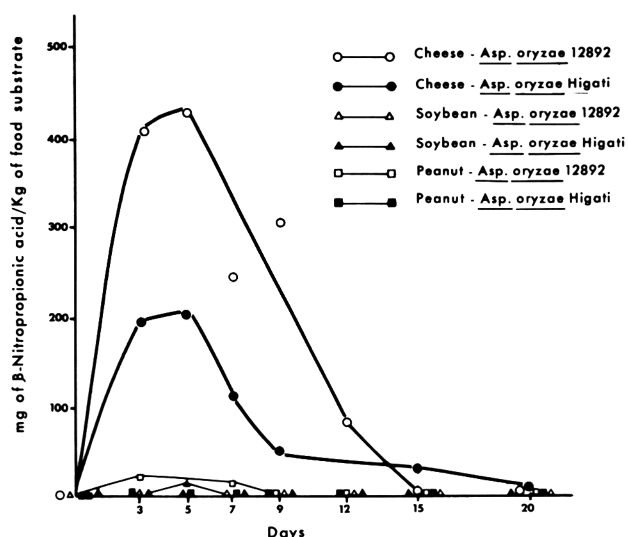

Fig. 2–Thin-layer chromatogram of ether extracts from Nakamura medium cultured by Asp. oryzae species. [Plate: cellulose (Eastman Chromagram Sheet 6064 without fluorescent indicator); Solvent: water-saturated butanol rendered alkaline with 0.1N ethylamine; Color reagent: 0.02% chlorophenol red in 95% ethanol]

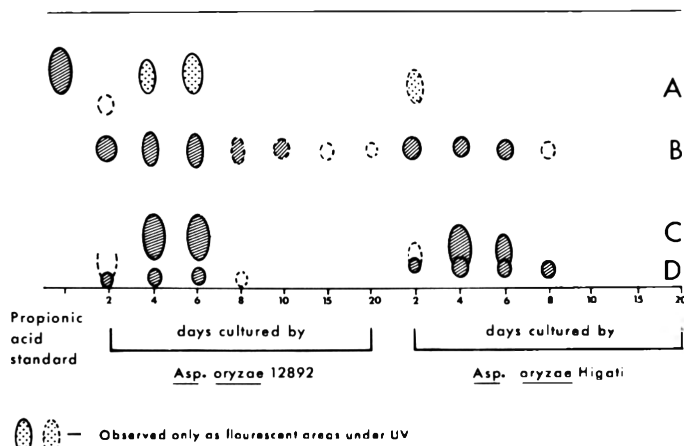

Fig. 3–β-nitropropionic acid production in cheese curd, soybean and peanut.

Cheese curds, soybean and peanut

During 20 days at 28°C, *Asp. oryzae* grew uniformly well on cheese curds, soybean and peanut. After the first 5 days, cheese curds inoculated with *Asp. oryzaes*, ATCC 12892 contained 427 mg β-NPA/Kg and with *Asp. oryzae* Higati 203 mg β-NPA/Kg. Thereafter, the metabolite decreased, virtually disappearing in 15 to 20 days (Fig. 3). Soybean and peanut inoculated with *Asp. oryzae* ATCC 12892 displayed a maximum 12–15 mg β-NPA/Kg which disappeared in 7 to 9 days and when inoculated with *Asp. oryzae* Higati developed no β-nitropropionic acid.

Low temperature storage of cheese curds slowed the decomposition of β-nitropropionic acid. At 5°C, after 30 days, an 80% reduction from maximum levels occurred and at 28°C after only 15 days, a 100% reduction occurred using *Asp. oryzae* ATCC 12892 (Figure 3, Table 5).

Commercial food grade enzyme preparation

Eight commercial food grade fungal enzyme preparations produced from *Asp. oryzae* or *Asp. niger* contained no β-nitropropionic acid.

Molded cheese

Surfaces of 18 aged ripened cheeses characterized by extensive yellow, green, black and white microbial surfaces showed no measurable β-nitropropionic acid, nitrate or nitrite (Table 6). Traces of β-nitropropionic acid were observed on five cheese surfaces and traces of nitrate on two (Table 6).

DISCUSSION

β-NITROPROPIONIC ACID synthesis is influenced by a number of factors. Raistrick and Stossl (1958), Hylin and Matsumoto (1961) and Shaw and Wang (1964) studied ammonium, nitrate and aspartic acid effects and attributed the differences in β-nitropropionic acid concentration of Raulin-Thom and Czapek-Dox solutions to the distribution of various nitrogen sources. When sodium nitrate was substituted for ammonium tartrate in the Raulin-Thom solution, its

Table 4–β-Nitropropionic acid, nitrate and nitrite production by Aspergillus oryzae (ATCC 12892)

Days at 32°C	β-NPA	NO_3^- mg/150 ml	NO_2^-	pH of final culture[a]
6	33.0	ND[b]	ND	6.25
9	25.0	1.5	ND	7.10
12	ND	6.2	ND	7.90
15	ND	12.9	ND	8.15
20	ND	10.5	ND	8.30
30	ND	25.1	ND	8.45

[a] pH of culture incubated 6 days at 32°C
[b] ND–Not detectable

Table 5–Retention of β-nitropropionic acid in high protein foods during storage

Mold	Food	Incubated[a] 5–7 days at 28°C	Incubated 5–7 days at 28°C then stored 30 days at 5°C
		mg β-NPA/kg	
Asp. oryzae ATCC 12892	Cheese	248	43
	Soybean	12	ND[b]
	Peanut	15	ND
Asp. oryzae Higati	Cheese	111	52
	Soybean	ND	ND
	Peanut	ND	ND

[a] Cheese incubated for 7 days; soybean and peanut for 5 days
[b] ND–Not detectable

Table 6—β-NPA, nitrate and nitrite from aged ripened cheeses with extensive moldy and discolored surfaces

Cheese	Surface characteristics[a]	β-NPA	NO_3^- mg/50g cheese	NO_2^-
1. Vasterbottom Graddost	blue molds	ND[b]	ND	ND
2. Fontina	blue molds and yeast	Trace	Trace	ND
3. Geitmelshe Kaas	yellowish molds and yeast	Trace	ND	ND
4. Cantal	blue molds and yeast	ND	ND	ND
5. Comté Gruyere	whitish and blue mold	ND	Trace	ND
6. Galbamino	black mold	ND	ND	ND
7. Valencianno	yellowish mold	ND	ND	ND
8. Fumiccitti	yellowish	ND	ND	ND
9. Le Sanglier	bad smell	Trace	ND	ND
10. Herve	yellowish	ND	ND	ND
11. Campama	dark green mold	ND	ND	ND
12. Fontinelli	dark yellow mold	ND	ND	ND
13. Olivet Cendre	dark green mold	ND	ND	ND
14. Pave De Moyan	yellowish	Trace	ND	ND
15. Fontinelli	yellowish brown	Trace	ND	ND
16. Robbiole Introbie	whitish	ND	ND	ND
17. Bel Paese	dark green mold	ND	ND	ND
18. Stilton	brown	ND	ND	ND

[a] Not usually normal to cheese, resulting from contamination and long hold
[b] ND—Not detectable

capacity to produce β-nitropropionic acid was reduced to that of the sodium nitrate-containing Czapek-Dox solution. Aspartic acid alone did not promote β-nitropropionic acid synthesis but required the presence of ammonium ions. The reason for the unsalted cheese curds' ability to synthesize much more β-nitropropionic acid than either soybean or peanut when *Asp. oryzae* was grown on these foods is not clear, but the quality of the nitrogen sources undoubtedly is important. Cheeses are not likely to be carriers of β-nitropropionic acid for many of the same reasons expressed by Shih and Marth (1969) concerning the absence of aflatoxins in commercial cheese. The *Aspergilli* species are not normally associated with cheeses or cheesemaking and the *Penicillium* species used in cheese have not been shown to produce β-nitropropionic acid, although the recent isolation of a mycotoxin with molecular formula $C_{17}H_{20}O_6$, from *Penicillium roqueforti* was reported by Wei et al. (1973). However, gross contamination of cheese surfaces by ubiquitous molds cannot be excluded as a possible source of β-nitropropionic acid. Studies on salted curds and different cheese varieties as substrate for β-nitropropionic acid development are underway. Our observations on the low capability of soybeans to produce β-nitropropionic acid from the growth of positive β-nitropropionic acid *Aspergilli* species agree with those by Yokotsuka et al. (1969). These results indicate soybean derived Japanese fermented foods like miso and shoyu are not likely to contain high levels of β-nitropropionic acid.

Neither propionic acid nor nitrite were observed after incubation of the *Aspergillus* mold in Nakamura's media or on cheese curds, soybeans or peanuts. Nitrate was not produced in media or foods cultivated by non-β-nitropropionic acid-producing mold cultures but was observed in the filtrates from strongly positive β-nitropropionic acid-producing mold cultures. Aleem and Lees (1964) demonstrated that cell-free extracts of *Aspergillus flavus*, *Aspergillus wentii*, and *Penicillium atrovenetum* oxidized ammonium to nitrate via hydroxylamine and nitrite. In the present study the cultured media initially shifted from acid to alkaline, pH 8.5, in some instances. Hirsch et al. (1961) reported that nitrate accumulation was inhibited at low pH because of the inability of the fungi to oxidize nitrogen in its most reduced form.

β-nitropropionic acid synthesizing and splitting enzymes apparently are not inducible but the presence of an enzyme system which decomposes β-nitropropionic acid is interesting. Similar β-nitropropionic acid breakdown has been observed by Marshall and Alexander (1961) but the pathway of the degradation and the nature and significance of the degradation products have not been resolved. Because of the possible toxicity of condensation products as outlined by Wolff and Wasserman (1972), further inquiry into this area including the specific nature of biosynthesis pathways and the influence of pH is underway.

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EFFECT OF DIPICOLINATE ON VEGETATIVE CELLS OF Bacillus

INTRODUCTION

BECAUSE of its heat resistance, the bacterial spore is of interest to the canning industry. Of particular interest are the spores of *Bacillus stearothermophilus* which are extremely heat resistant. *B. stearothermophilus* causes flat-sour spoilage in low acid, canned foods.

Since Powell isolated dipicolinic acid (DPA, pyridine-2,6-dicarboxylic acid) in 1953, food microbiologists have studied this compound in relation to its role in heat resistance (Murrell, 1969). It is a unique compound not found in the vegetative cells of bacteria. Although this compound is released from the spore when it germinates, DPA caused spores of strain M of *B. stearothermophilus* to germinate under specific conditions (Fields and Frank, 1969) but DPA inhibited spore outgrowth and vegetative growth (Fields and Frank, 1973). DPA is a chelating agent which forms strong chelates with cobalt, copper, iron and zinc with magnesium weakly chelated and calcium and manganese intermediately chelated (Riemann, 1963).

Because of the fact that dipicolinic acid forms different chelates, different effects upon the bacterial cell may be expected due to influence on cations in the bacterial cell. For example, Hachisuka et al. (1965) showed that DPA inhibited anaerobic oxidation of glucose by a cell-free extract from vegetative cells of *Bacillus subtilis*; Okabayaski and Ide (1970) also showed that DPA inhibited the phosphodiesterase of *Brevibacterium liquefaciens*. From these studies one might infer that the growth of the cell may be prevented by the loss of enzyme activity due to lack of a metal coenzyme. Therefore, one of the objectives of this research was to test for loss of respiration in vegetative cells treated with DPA. In addition, the possibility of the DPA influencing metals in the cellular membrane was also studied. This was done by determining the loss of sugars and amino acids from treated and control cells. A limited survey was made also to determine if other species of *Bacillus* were inhibited by DPA when added to agar media.

MATERIALS & METHODS

Respiration studies

Warburg flasks were calibrated for oxygen uptake at 40°C, according to the procedure of Clark (1964). DPA solution was prepared by dissolving reagent grade dipicolinic acid (Aldrich Chemical Co., Milwaukee, Wisc.) in 0.1N NaOH and adjusting the pH of the solution to 7 with HCl. The final concentration of DPA in the Warburg flask was 50.6 mM. Bacterial cells were suspended in 68 mM phosphate buffer (pH 7). Solutions of 60 mM $CaCl_2$ were prepared to be added to selected DPA solutions to limit the inhibitory effect of the DPA. Substrates were prepared at the following concentrations: glucose, 7.2 mM; sodium acetate, 31.7 mM; alpha-ketoglutaric acid, 8.9 mM; sodium pyruvate, 5.9 mM; and L-malic acid, 4.8 mM.

Cells of *B. stearothermophilus* strain M for the respiration studies were grown on tryptic soy agar for 24 hr, harvested and washed in distilled water. The cells were subjected to the substrates and DPA as follows: (1) no DPA added (control); (2) cells, substrate, DPA mixed at the same time; (3) cells exposed to the DPA 1 hr before mixing with substrate; (4) cells exposed to DPA-$CaCl_2$ 1 hr before mixing with substrates.

0.2 ml of 10% KOH were added to the center well of the Warburg flask. The main vessel contained 2 ml of substrate and the side arm contained 0.5 ml of cells and 0.6 ml of DPA (water for the control) to make a final volume of 3.1 ml. Manometer readings were taken every 5 min and oxygen uptake was calculated as μl per dry weight of cells. Data are presented as the % inhibition which was calculated by substracting the μl of oxygen per mg dry weight of cells of the DPA flask at 60 min exposure from the μl of oxygen per mg dry weight of control cells and dividing by the μl of oxygen per mg dry weight of cells of the control.

Leakage studies

Vegetative cells for the leakage studies were produced on tryptic soy agar at 55°C for 24 hr. The cells were washed from flasks with sterile, distilled water by rubbing the surface of the agar with a bent glass rod. The cells were pooled into a sterile Erlenmeyer flask and mixed well. The cells were centrifuged and

Table 1—Inhibitory effect of DPA on respiration of vegetative cells of Bacillus stearothermophilus in phosphate buffer (68 mM, pH 7.0)

	% Inhibition[a]		
Substrate	DPA[b]	DPA—1 hr[c]	DPA-Ca—1 hr[d]
Glucose	12	44	17
L-malate	15	33	9
Sodium acetate	33	64	4
Alpha-keto-glutarate	55	92	71
Sodium pyruvate	76	91	40

[a] The percent inhibition was calculated by subtracting the μl of oxygen per mg dry weight of cells of the DPA flask at 60 min exposure from the μl of oxygen per mg of dry weight of cells of the control flask and dividing by the μl of oxygen per mg dry weight of cells of control flask X 100. Mean of duplicate determinations. DPA concentration was 50.6 mM.
[b] DPA and substrates mixed immediately with cells.
[c] Cells in contact with DPA for 1 hr then mixed with substrates.
[d] Cells in contact with DPA and $CaCl_2$ (60 mM) for 1 hr then mixed with substrates.

Table 2—Leakage of amino acids from cells of strain M B. stearothermophilus

	μg of amino acids per 100 mg dry cells[a]		
Amino acid	Cells treated with DPA[b]	Cells not treated with DPA	% Increase due to DPA
Threonine	1.7	1.4	21.4
Serine	1.7	1.0	70.0
Glutamic acid	4.6	3.7	24.3
Alanine	2.9	2.2	31.8
Isoleucine	2.5	1.4	78.6
Leucine	3.9	1.5	60.0
Histidine	1.3	1.6	00.0
Arginine	1.0	0.6	66.6
All amino acids, mean	2.5	1.7	47.1

[a] Mean of duplicate analyses
[b] Concentration of 53 mM DPA

washed six times in sterile, distilled water. The cells were suspended in phosphate buffer (pH 5.5, 120 mM), and the suspension divided into two 40 ml portions. To one portion, 10 ml of DPA was added making the final DPA concentration 53 mM. 10 ml of distilled water was added to the control. The suspensions were prepared in duplicate but on different days.

The suspensions were incubated at 55°C for 72 hr before centrifuging. Cells were transferred to crucibles and dried at 105°C for 24 hr, cooled and weighed. The supernatant was analyzed for amino acids and sugars as evidence of leakage of these substances from the cells. 10 ml of a 10% trichloroacetic acid solution was added, and the supernatant was filtered.

Quantitative analyses were made by column chromotography by a slight modification of the method of Benson and Patterson (1971). The conditions were: a single column was run with a resin bed height of 56 cm (aminex A-6). The flow rates of buffer and ninhydrin were 100 ml/hr and 60 ml/hr, respectively. The column temperature was 55°C. Norleucine (0.10 mM) was used as an internal standard.

The sample was dissolved in sodium citrate buffer (pH 2.2). 1 ml of sample was injected on the column followed by the following elutants: sodium citrate buffer in about 3%N ethanol, pH 3.25, for 50 min; at pH 4.25 for 30 min and at pH 6.55 for 80 min. In addition to loss of amino acids in leakage studies, loss of RNA has been tested for by the presence of ribose in supernatant fluid since the orcinol method is a test for pentose (Haight and Morita, 1966). The supernatant was tested for sugars by the orcinol method (1.5g orcinol, 0.5g $FeCl_3$ in 100 ml of concentrated HCl). Ribose was used to establish a standard curve (0.1 ml sample to 4 ml of reagent). Samples were boiled for 10 min and absorbance determined at 660 nm.

Agar-plate studies

DPA solution was prepared by dissolving 3.34g and 8.85g of dipicolinic acid in 0.1N NaOH and adjusting the pH to 7 with HCl. This solution was used to make up nutrient agar having a DPA concentration of 20 mM and 53 mM. As a control, nutrient agar without any DPA was used. The plates were streaked and observed for growth after 6 days for the mesophilic *Bacillus* and after 48 hr for the thermophilic *Bacillus*.

The following mesophilic species of *Bacillus* were used: *B. megaterium*, ATCC 14581; *B. pantothenticus*, ATCC 14576; *B. cereus*, ATCC 14579; *B. pumilus*, ATCC 7061; *B sphaericus*, ATCC 14577; *B. firmis*, ATCC 14575; *B. lentus*, ATCC 10840; *B. circulans*, ATCC 4513; *B. subtilis*, ATCC 6051; *B. licheniformis*, ATCC 14580; *B. laterosporus*, ATCC 64; *B. brevis*, ATCC 8246; *B. badius*, ATCC 14574; *B. macerans*, ATCC 8274; and *B. coagulans*, ATCC 7050.

The strains of *B. stearothermophilus* used in this research are listed in articles of Fields and Harris (1972) and Harris and Fields (1972); 47 strains were used.

RESULTS & DISCUSSION

Respiration studies

The inhibitory effect of DPA on the respiration of vegetative cells of *B. stearothermophilus* strain M is illustrated in Table 1. Fields and Frank (1973) reported that germinated spores of strain M did not elongate and that DPA inhibited vegetative growth of strain M. Energy is needed for vegetative growth and this energy is obtained from carbohydrate metabolism. When the DPA, cells and substrates were mixed immediately, less inhibition occurred than if the DPA was left in contact with the cells for 1 hr before mixing with the substrates. These data are in agreement with those of Hachisuka et al. (1965) who found that the longer a cell-free extract was in contact with DPA the more inhibitory the effect on the anaerobic oxidation of glucose by vegetative cells of *B. subtilis*. The presence of Ca ions reduced the degree of inhibition for some of the substrates although the effect was minimal but for alpha-keto-glutarate and pyruvate. These data partly explain the reason the germinated spores of strains of *B. stearothermophilus* did not elongate and grow in the study of Fields and Frank (1972).

Leakage studies

The data in Table 2 indicate that vegetative cells held in contact with DPA for 72 hr at 55°C leaked more amino acids than cells held in distilled water for the same length of time. This increased loss of amino acids may be due to the removal of cations from cell membranes resulting in membrane damage. DPA caused more of some kinds of amino acids to leak than others. A fairly long incubation time was used to show these leakages.

Not only were amino acids lost from cells treated with DPA but also more sugars were lost from DPA-treated cells than from control cells. In the supernatant from the DPA-treated cells there was a mean of 255 μg/ml of sugar (expressed as ribose) and 84 μg/ml from the control cells. The loss of amino acids and sugars from treated cells as compared to control cells helps to explain in part the reasons for growth was inhibition. It appears that the damage to the cells was both physical and chemical.

Agar-plate studies

In the previous study of Fields and Frank (1973), it was shown that the growth of *B. stearothermophilus* strain M was inhibited by DPA at concentrations of 4 mM. When other strains of *B. stearothermophilus* were tested by the agar-plate method at 20 mM DPA (Table 3), none of the 47 strains grew. At 53 mM DPA, the same concentration used in the leakage investigation and near the concentration used in the respiration studies (50.6 mM), only two of the mesophilic species grew well, and four species showed slight growth after 6 days at room temperature.

B. coagulans, the other species besides *B. stearothermophilus* which some bacteriologists considered to be a thermophilic species, grew well on agar containing both levels of DPA. Although only one strain of *B. coagulans* was used in this study, it is the type strain for that species. Growth on nutrient agar might be used as a taxonomic characteristic to

Table 3—Effect of DPA in nutrient agar on the growth of species of Bacillus

Type	Species	Reaction[a] 20 mM DPA	53 mM DPA
Mesophiles			
	B. cereus ATCC 14519	†[c]	–
	B. pantothenticus ATCC 14576	†[c]	–
	B. pumilus ATCC 7061	+	–
	B. sphaericus ATCC 14577	+	–
	B. firmus ATCC 14575	+	–
	B. lentus ATCC 10840	+	sl[b]
	B. circulans ATCC 4513	+	sl[b]
	B. subtilis ATCC 6051	+	+
	B. licheniformis ATCC 14580	+	+
	B. laterosporus ATCC 64	+	–
	B. brevis ATCC 8246	+	–
	B. badius ATCC 14574	–	–
	B. macerans ATCC 8244	+	–
	B. megaterium ATCC 14581	†[c]	sl[b]
Thermophiles			
	B. coagulans ATCC 7050	+	+
	B. stearothermophilus[d]	–	†[c]

[a] Growth or no growth at 6 days at room temperature
[b] Slight growth
[c] Not tested
[d] Results of 47 strains at 48 hr at 54°C

separate *B. coagulans* from *B. stearothermophilus* if tests with more strains show the same results as the results in Table 3.

CONCLUSIONS

DPA depresses respiration and causes the cells of *B. stearothermophilus* to become leaky and lose amino acids and pentose sugars (ribose as part of RNA). The effect of DPA on growth varied with the species of *Bacillus*.

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RHEOLOGICAL PROPERTIES OF HYDROCOLLOIDS

INTRODUCTION

HYDROCOLLOIDS are used to generally improve or manipulate the texture of food products because of their ability to retard flow, modify gelling characteristics, and preserve emulsion and suspensions. Rheological properties are the most important factors governing these desired properties of hydrocolloids (Glicksman, 1969).

In this study, the rheological properties of natural and synthetic hydrocolloids from different sources, seaweeds, cellulose and plant seeds, were investigated. One sample from each of these three types of hydrocolloids, namely sodium alginate (Stein Hall & Co., N.Y.), sodium carboxymethylcellulose (Hercules, Inc., Wilmington, Del.), and guar gum (V-7-E series 7, Burtonite Co., Nutley, N.J.) were selected for the study. Of course each type of hydrocolloid contains a family of chemical compounds with a wide range of variation in molecular weight, number and position of the functional groups, as well as arrangement of the basic units. Thus, they have rheological properties of wide range and study of one sample from each type would by no means characterize the whole family. However, this would still provide some information on the respective rheological behavior of the hydrocolloids.

The deviations from Newtonian behavior, apparent viscosities, yield stresses and time dependencies of these hydrocolloids at different concentrations and temperatures were determined.

EXPERIMENTAL

A BROOKFIELD Synchro-Lectric viscometer (Model LVT, Brookfield Engineering Laboratories, Inc., Stoughton, Mass.) with cylindrical spindles and helipath stand was used to take viscometric measurements. The measurements were made in the controlled temperature rooms at 40, 50, 60 and 70°F with ± 1°F fluctuation.

Preparation of sample

Hydrocolloid suspensions in the concentration range of 0.5% to 2.0% (by weight) were made as follows:

1. 350 ml of stock distilled water (kept at 190–200°F) was poured into the blender cup and preweighed hydrocolloid was added.
2. The blender cup was covered and the Waring Blendor (Model 1249, Dynamic Corp. of America, New Hartford, Conn.) was turned on at "stir" position for 20 sec. A glass rod or spatula was used to break any existing lumps in the resulting mixture.
3. An additional 350 ml of hot distilled water was added and the blender turned on at "stir" for 40 sec.
4. The mixture was then poured into a 600 ml Griffin beaker and kept undisturbed for approximately 15 min.
5. Foamy layer at the top of the sample was skimmed off.
6. The samples were tightly covered with aluminum foil and then placed in the controlled temperature rooms.

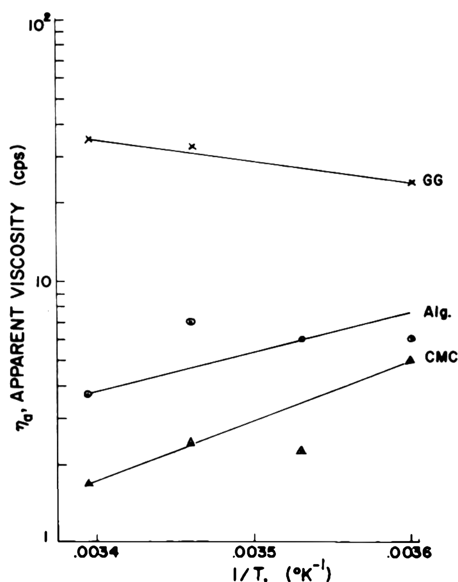

Fig. 1—Apparent viscosity as a function of temperature for all three samples at 4% concentration.

Viscometric measurements

1. The samples were kept at the constant temperature desired (40, 50, 60 or 70°F) at least 12 hr prior to the measurements in order to reach thermal equilibrium.
2. A suitable spindle was selected and attached to the viscometer which was mounted on the helipath stand.
3. The spindle was then lowered until the surface of the sample reached the marked level of the spindle.
4. The viscometer spindle was allowed to stand for 3 min to eliminate the effects of immediate time dependency.
5. Viscometer rotational speed was selected and driving motor was turned on.
6. After three revolutions, a reading was taken for every revolution until the readings approached a constant value.
7. Motor was turned off and readings were taken after 1, 2 and 3 min to obtain the equilibrium yield value.
8. Each set of readings was taken by placing the spindle into a fresh sample and following the above steps.

RESULTS

AN EXAMPLE of the experimental data obtained is presented in Table 1. The general power law equation with yield stress,

$$\tau = b\,\gamma^s + C \qquad (1)$$

where τ = shear stress; γ = shear rate; b and s = constants and C = yield stress, was assumed to express the flow behavior of binder suspensions under the experimental conditions and concentrations studied. This equation was employed to determine the values of the power law constants b and s using experimentally determined yield values, shear stress and shear rate.

Charm (1963a, b, c) analyzed the relationships between b, s, C, the rotational speed and torque exerted on the spindle in a cylindrical viscometer as follows:

$$2\pi N \left(\frac{b}{C}\right)^{1/s} = \int_{R_1}^{R_2} \left(\frac{A}{2\pi LCR^2} - 1\right)^{1/s} \frac{dR}{R} \qquad (2)$$

$$R_2 = \sqrt{\frac{A}{2\pi LC}} \qquad (3)$$

and

$$C = \frac{A_0}{2\pi R_1{}^2 L} \qquad (4)$$

where: A = torque exerted at surface of cylinder; N = speed of cylinder, RPS; L = length of cylinder in contact with fluid; R_1 = radius of cylinder; R_2 = distance from center of cylinder to the point where shear stress equals yield stress and streamline velocity = 0; and A_0 = torque exerted at surface of cylinder at zero shear rate.

Rewriting Charm's equation in another

form and defining K as a constant which is a multiple of $2\pi R_1$,

$$KN = \left(\frac{A}{2\pi LCR^2} - 1\right)^{1/s} \left(\frac{C}{b}\right)^{1/s} \quad (5)$$

shows that the logarithmic plot of N vs. $[(A/2\pi LCR_1) - 1]$ gives a straight line with slope of 1/s (Charm, 1963a, b).

An attempt was made to obtain C from the plot of $\sqrt{N}$ vs. $\sqrt{A}$ (Charm, 1963a). However, the extrapolation to N = 0 gave negligible values for A_o. Therefore, the measured values of A_o were used for computing C. A_o was obtained from the experimentally measured force exerted by the sample to the wall of the viscometer spindle after viscometer rotation was stopped (readings taken in 7).

The constant values of the shear stresses at each rotational speed were used in determining s (last reading taken in step 6). With the values of s and C, equation (2) was numerically integrated with the aid of Simpson's rule to give the value of b (Table 2).

The above analysis was repeated for the different sets of data at various temperatures and concentrations to obtain the values of power law constants. Using these values, plots of shear stress against shear rates were prepared.

Apparent viscosity, η_a at different shear rates was determined from the derivative of τ with respect to γ of equation (1):

$$\eta_a = \frac{d\tau}{d\gamma} = b\, s\, \gamma^{s-1} \quad (6)$$

Plots of this apparent viscosity vs. shear rates were prepared at various concentrations at each of the four temperatures. The variation of viscosity with temperature was shown by plotting η_a against 1/°K at a particular value of γ (=1.0 sec^{-1}) (Fig. 1).

To illustrate the time dependency, ratio of instantaneous apparent viscosity (η_{ia}), (instantaneous readings taken in step 6) to equilibrium apparent viscosity (η_∞) (constant readings in step 6) was plotted against time of shearing (Fig. 2).

The effect of concentration on yield stress was shown by graphs of yield stress vs. concentration at different temperatures for the three hydrocolloids.

DISCUSSION

AS NOTED ABOVE, the yield stress C was obtained from the shear stress readings after the viscometer spindle rotation was stopped. Therefore, the yield stress being considered here corresponds to the yield stress of a disturbed ("worked") sample rather than the yield stress prior to deformation or flow. It is the recovery yield stress rather than the yield stress required to initiate flow. Such yield stress of "worked" sample is more meaningful in food processing due to the fact that in practice the binder would have to be disturbed since it has to undergo mass movement in order to be mixed with or brought adjacent to other food ingredients which are to be bound. Furthermore, the yield stress of the binders at the time will dictate if the binder would remain at that preferential position for adhesion. In the foregoing discussion, sodium alginate will be designated as alginate and sodium carboxylmethylcellulose as carboxylmethylcellulose or CMC. The plots of yield stress vs. concentration for each sample are given in Figures 3–5.

In all three samples, the yield stress increases dramatically with an increase in concentration. This dramatic increase occurs between 1.0 and 1.5% for alginate; between 3.0% and 4.0% for CMC; and although not so distinct, between 1.0 and 2.0% for guar gum.

The graphs (Fig. 3–5) do not show a generally expected trend of decrease in yield stress with increase in temperature in a clear cut manner. In CMC, yield stress decreases as temperature increases from 50°F to 60°F to 70°F. This is reasonable considering the fact that the gel is physically more fluid at high temperatures. The yield stress at 40°F, however, is less than those at 50° and 60°F at

Table 1–A sample of each set of viscometer readings (Alginate 0.7%; T = 40°F)

Cylinder no.	Rotational speed (rpm)	Readings (after each revolution) A_i	Readings (after rotation is stopped) 1 min	2 min	3 min	A_c	A_o
1[a]	3.0	13.7, 13.8, 13.9, 14.0, 14.1, 14.2, 14.2, 14.2	5.4	5.3	5.3	14.2	5.3
1[a]	6.0	26.7, 27.0, 27.2, 27.4, 27.5, 27.6, 27.7, 27.8, 27.8, 27.8	5.2	5.0	5.0	27.8	5.0
1[a]	12.0	61.6, 61.9, 62.0, 62.1, 62.2, 62.3, 62.3, 62.3	5.4	5.3	5.3	62.3	5.3
2[b]	12.0	10.4, 10.7, 10.8, 11.0, 11.2, 11.3, 11.3, 11.4, 11.5, 11.6, 11.7, 11.8, 11.8, 11.8	5.7	5.7	5.7	11.8	5.7

[a] Dimensions of Spindle 1: L = 7.493 cm; R = 0.9421 cm
[b] Dimensions of Spindle 2: L = 6.121 cm; R = 0.5128 cm

Table 2–Power law constants (b and s) for various concentrations at different temperatures

Temp.	40°F		50°F		60°F		70°F	
% Concentration	b[a]	s	b[a]	s	b[a]	s	b[a]	s
				Alginate				
0.5			0.39	1.7			0.51	1.6
0.7	1.7	1.4	1.5	1.5	0.99	1.4	0.99	1.1
1.0	6.3	0.95	4.7	1.3	5.0	1.4	2.2	1.2
1.5	32	1.1	28	1.4	16	1.3	13	1.1
2.0	110	0.88	83	0.96	79	1.1	5.5	0.98
				Carboxymethylcellulose				
0.5	0.69	1.1						
1.0	3.9	1.3	2.1	1.1	2.2	1.1	1.4	1.2
2.0	51	0.82	33	0.87	27	1.1	17	1.3
3.0	130	0.89	190	0.85	150	0.81	130	0.84
4.0	310	0.72	780	0.68	660	0.62	600	0.65
				Guar Gum				
0.5	2.6	1.2			2.8	1.1		
0.7					9.2	0.98	14	1.3
1.0	26	0.91			39	0.82	48	0.74
1.5	100	0.83						
2.0	330	0.56					150	1.0

[a] dynes $(sec)^s/cm^2$

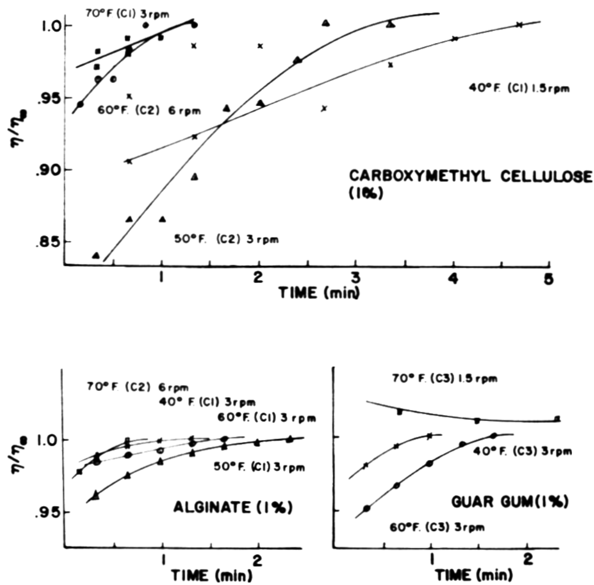

Fig. 2–Ratio of viscosities (η_i/η_{max}) vs. time of shearing. (Note: C = cylindrical spindle)

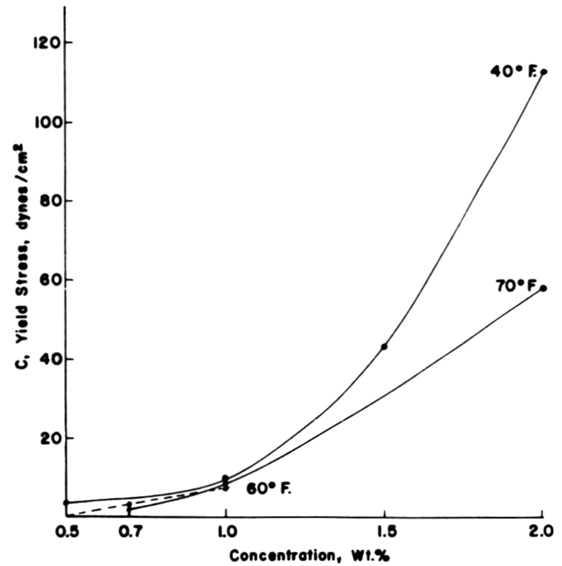

Fig. 5–Effect of concentration on yield stress for guar gum.

above 2.5% concentration. This may be explained by the occurrence of syneresis at this low temperature in the CMC gel sample which indicates the disruption of a portion of the relatively rigid gel structure, hence the lower bonding density per unit volume, thus low yield value. It should also be noted that the rigid gel requires a high shear force to disrupt its structure. However, elastic recovery is very limited in this type of rigid gel with higher hydrocolloid concentration and once it is disrupted, reorganization does not take place as readily as it does at the higher temperatures. Therefore, equilibrium is perferencially toward less bonding density per unit volume, thus lower yield stress.

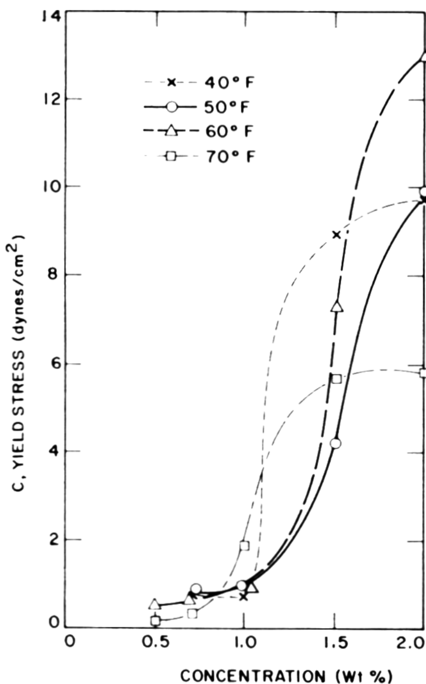

Fig. 3–Effect of concentration on yield stress for alginate.

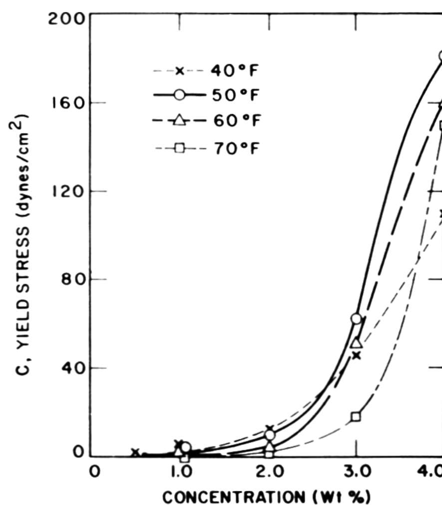

Fig. 4–Effect of concentration on yield stress for carboxymethylcellulose.

If this argument is applied to the behavior of yield stress for alginate, at concentration above 1.5%, equilibrium net bond formation appears to override the overall thinning effect of higher temperature such as increase in mobility, decrease in density, etc. Further increase of the temperature to 70°F causes the thinning effect to dominate resulting in lowest yield value. The limited data on guar gum suggests a simple trend of decrease in yield value with temperature up to 2.0% concentration. It is worthwhile to note that while there is competition between the two resulting mechanisms, thinning effect and increase in rate of bond formation, due to increase in temperature, the thinning effect seems to dominate at lower concentrations (up to approximately 1.4% concentration for alginate and 2.5% concentration for CMC) and yield stress has a simple trend to decrease with increase in temperature.

Yield stress of alginate and CMC is approximately the same order of magnitude while that of guar gum is about ten times. Therefore, among these three hydrocolloids, theoretically guar gum would be more functional or effective when used as a shape holding agent in the molded food products such as aspic, paté, pudding, custard and icings.

The power law constants (b and s) are presented in Table 2. In all cases, the viscosity constant, b, increases with an increase in concentration. There is no clear trend with respect to temperature except for alginate where b generally decreases with an increase in temperature. In all three samples the value of s is mostly

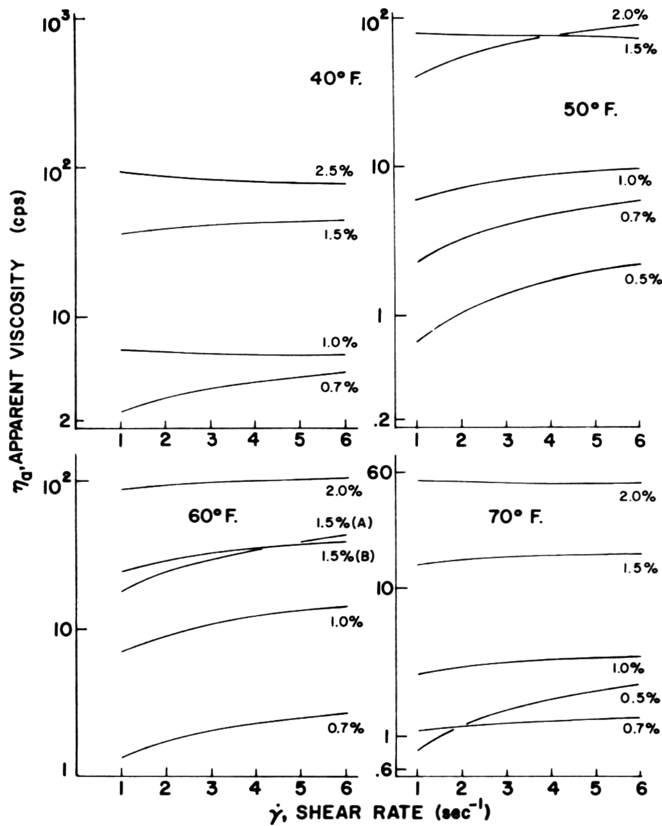

Fig. 6—Apparent viscosity vs. shear rate for alginate.

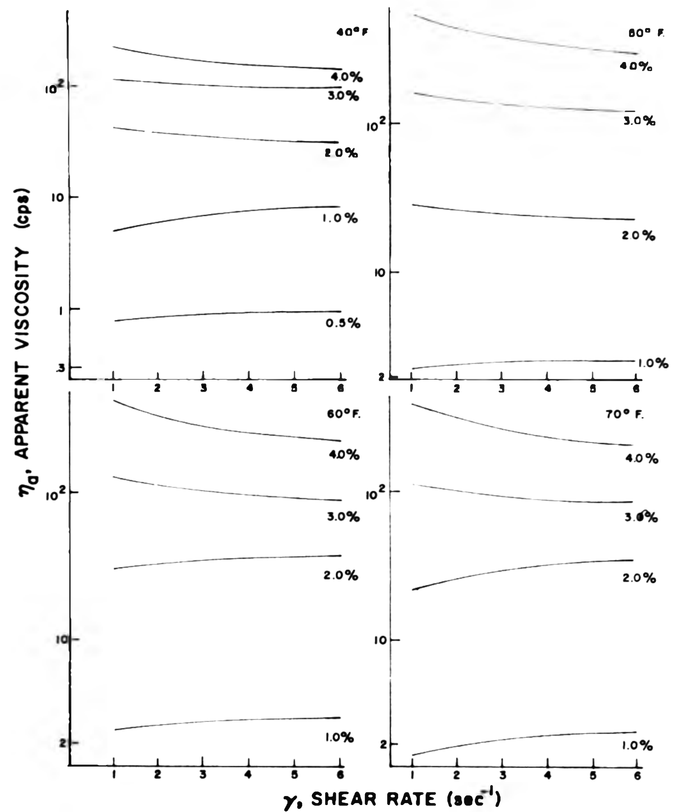

Fig. 7—Apparent viscosity vs. shear rate for carboxymethylcellulose.

greater than 1 at higher temperatures and lower concentrations, indicating some dilatant behavior. At lower temperatures and higher concentrations, s tends to be between 0 and 1, showing pseudo-plasticity.

Alginate shows a general increase in apparent viscosity with an increase in shear rate (Fig. 6). For CMC, at higher concentrations, the apparent viscosity decreases with increasing shear rate. At lower concentrations, the opposite effect was found. This switch from an increase in viscosity to a decrease in viscosity with increasing shear rate was found to occur at progressively higher concentrations as the temperature increases (see Fig. 7). A similar behavior was detected for guar gum (Fig. 8).

This phenomenon of increasing viscosity with shear rate in alginate and CMC at the concentrations studied or guar gum at lower concentrations may be effectively utilized in food processing in which attaining high viscosity at high shear is essential, such as in spinning and extrusion. In the processing of food which requires high viscosity of the end product and where low viscosity during processing which would ease handling and reduce cost of power and equipment, such as mixing, kneading and pumping, and thermal processing, CMC at a higher concentration would be desirable.

The apparent viscosity of alginate given in Figure 4 is in the same order of magnitude with the low viscosity alginate reported in the literature (Glicksman, 1969). The apparent viscosity of CMC given in Figure 6 also is within the same range with the low viscosity CMC reported in the literature (Glicksman, 1969). Considering that the viscosity of high quality guar gum has been reported as high as 3000–6000 cps (Glicksman, 1969), the guar gum used in this study

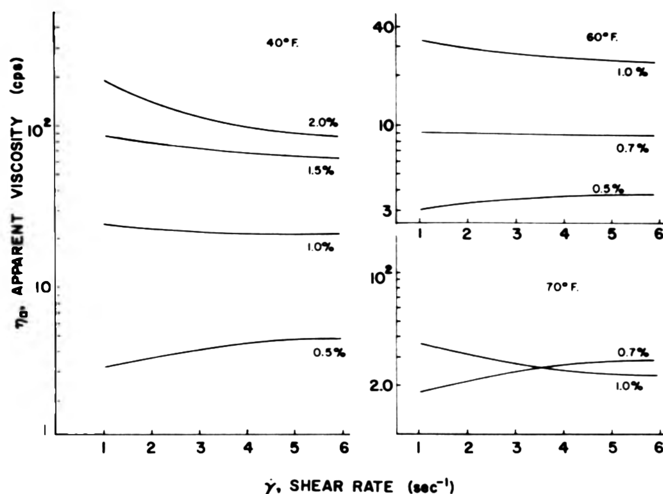

Fig. 8—Apparent viscosity vs. shear rate for guar gum.

had low apparent viscosity. The low apparent viscosities of all three hydrocolloids compared with reported values may be due to (1) the differences in the quality of the gums themselves; (2) the manner in which the hydrocolloids were suspended; (3) other conditions such as pH, absence of synergistic agents; or (4) condition of the measurements.

For alginate and CMC, the apparent viscosity, η_a, increases with a decrease in temperature (Fig. 1). This is what one would generally expect and in agreement with the trend reported in the literature (Glicksman, 1969). The increase seems to be linear with the reciprocal of the absolute temperature. CMC seems to be more temperature dependent than alginate. For guar gum, the apparent viscosity decreases with a decrease in temperature which could be explained by the pasty and lumpy consistency of the guar gum samples at lower temperatures.

CMC is therefore an effective hydrocolloid in imparting greater viscosity to the foods served at low temperatures and the foods processed at higher temperatures such as beverages, some canned foods, confectionary, dairy products, and jams and jellies. Unusual increase in viscosity behavior with increase in temperature of guar gum would be better utilized in foods served at high temperatures such as gravy, sauces, soups and stews.

Under the experimental conditions, all three hydrocolloid samples showed a time dependency which is rheopectic in nature (Fig. 2). The additional energy expended in stretching and aligning the gel structure before steady flow is attained might account for the rheopectic behavior.

CONCLUSIONS

THE FOLLOWING conclusions can be made from this study:

1. The general power law equation is an adequate model for describing the flow behavior of the hydrocolloids in the range of the experimental conditions used.
2. All three hydrocolloid suspensions showed a deviation from Newtonian behavior for the range of concentrations used and the viscosity was found to be dependent on shear rate and time of shearing.
3. Alginate showed a tendency for dilatancy while CMC and guar gum changed from dilatant to pseudo-plastic behavior with increasing concentration.
4. The viscosity of CMC was slightly more temperature dependent than that of alginate. The viscosity of guar gum did not decrease with increase in temperature.
5. All three hydrocolloids showed rheopectic properties in this experiment. Most of the time effects were within 3 min of shearing.
6. Yield stress increased with increase in concentration. At the same concentration, guar gum had higher yield values followed by alginate and CMC.

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MICROSCOPIC INVESTIGATIONS OF THE FREEZE DRYING OF VOLATILE-CONTAINING MODEL FOOD SOLUTIONS

INTRODUCTION

AQUEOUS SOLUTIONS of organic solutes are the basic fluids of biological systems, such as the extracellular and intracellular fluids of solid food materials. Extraction of these fluids from food solids results in organic-containing aqueous solutions of economic importance to the food industry. The behavior of these extracts during the freezing process is quite important in determining the final quality of freeze-dried products (Flink and Karel, 1970b; Flink and Labuza, 1972; Rulkens and Thijssen, 1972). This includes both the period of the lowering of temperature to a level at which the solvent, water, starts to crystallize as ice, as well as during the continuation of the freezing process when the increased conversion of water to ice results in the eventual solidification of the aqueous solution as a relatively complex system of ice crystals, concentrated solute phase or phases, and perhaps even pure solute phases.

The flavors of food materials are composed of numerous organic compounds present at low concentrations in aqueous solutions. Most studies on flavor quality retention have dealt with measuring the before, during or after retention of suitable volatile organic compounds from nonvolatile solute-containing aqueous solutions (Flink and Karel, 1970a,b; Thijssen and Rulkens, 1969; Rulkens and Thijssen, 1972; Chandrasekaran and King, 1972).

In a continuation of studies on the desorption of volatile organic constituents from aqueous carbohydrate systems during freeze drying (Flink and Karel, 1969; 1970a,b), microscopic analysis of the freeze-dried material showed changes which had taken place in the system during the freezing and freeze drying (Flink and Gejl-Hansen, 1972). Most notable were the partitioning of the carbohydrate nonvolatile solute (maltodextrin) into two fractions, and the appearance of the volatile organic constituent (hexanal) as liquid droplets. Flink and Gejl-Hansen concluded that the formation of the liquid droplets and their partitioning into the carbohydrate-rich phase were very important factors in accounting for the retention of the volatile organic compound during freeze drying.

To determine during which part of the freeze-drying process the partitioning occurs requires the development of a freeze-drying microscope stage on which the entire freeze-drying process could be observed. Various freezing and freeze-drying microscope stages have been reported in the literature (McCrone and O'Bradovic, 1956; MacKenzie, 1964; Chauffard, 1971; Freedman et al., 1972), though most studies using this equipment have dealt with investigations on conditions affecting the development and behavior of the ice phase (Luyet, 1968). Rey et al. (1966) have reported on microscopic observations of the solidification and freeze-drying behavior of organic mixtures and aqueous solutions of inorganic salts.

This paper reports on (1) the design of a new freeze-drying microscope stage capable of continuous observations at the high magnifications required for flavor retention studies and (2) the results of studies on the separation and retention of volatile organic compounds during freeze drying.

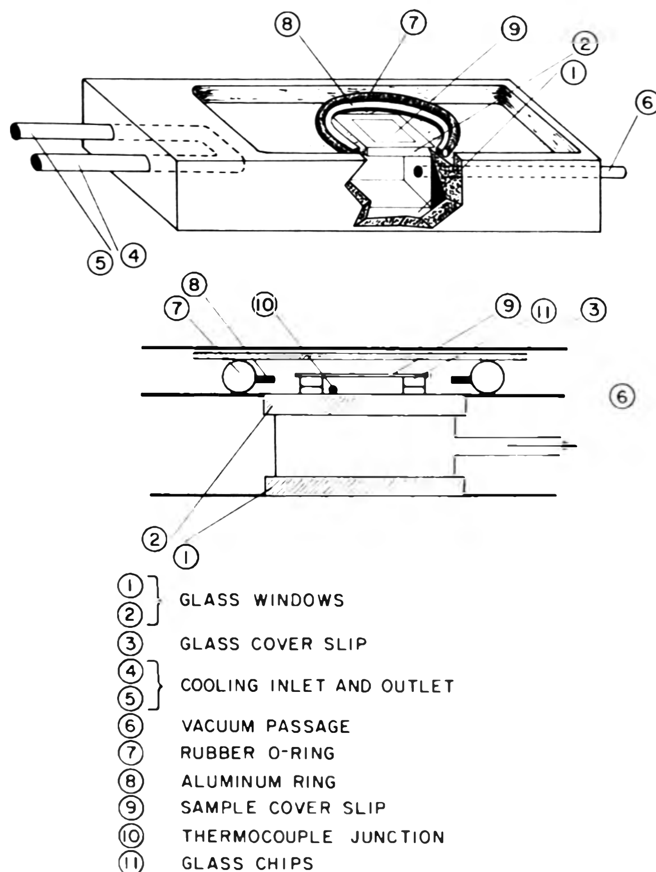

Fig. 1—Microscope stage freeze-drying system with detail of sample holder.

EXPERIMENTAL

The freeze-drying microscope

The freeze-drying microscope is capable of

being used for continuous observation of freeze-drying samples at magnification up to 600×. This high magnification is essential when investigating the formation and separation of droplets within the nonvolatile solute matrix.

The freeze-drying microscope consists of three systems which can be considered independently: the microscope system, the freeze-drying system and the data acquisition systems.

The microscope system. The freeze-drying microscope is based on an Olympus Model EH microscope body. As this microscope is modular, it is "custom designed" by being equipped with the following special options:

1. Trinocular head to allow for both visual and data acquisition;
2. Wide field and flat field photographic eyepieces of 15× which allow magnifications at the limit of resolution;
3. A polarizing filter set used to evaluate crystallinity of the sample;
4. A dry 60× objective which can be used for high magnification (900×) analysis of the freeze-dried material;
5. A long working distance 40× objective that enables the distance between the sample and front surface of the objective lens to be 1.3 mm. This distance is an important consideration when designing the freeze-drying chamber.

The freeze-drying system. The freeze-drying system consists of a freezing and freeze-drying chamber specially designed and constructed in this laboratory for use with the Olympus microscope and the associated support equipment.

The freeze-drying chamber is a vacuum-tight optical system which is capable of being refrigerated (Fig. 1). Three glass windows set into an aluminum block provide for sample holding (2) and vacuum seals (1, 3). The block also contains an internal path for the flow of chilled refrigerant (4, 5) as well as a vacuum passage to the lower vacuum chamber (6). The glass sample holder has a large contact area with the aluminum block at the refrigerated end, giving a large heat transfer surface. A gap is left between the sample holder and one wall of the block permitting air and water vapor to flow to the lower chamber and out the vacuum line. The lower window is semi-permanently sealed to the block with Apeizon putty. The upper window, a 0.17 mm thick cover glass (45 x 50 mm) rests on a rubber O-ring (7) that is held in circular shape by an aluminum ring (8). The total thickness of the O-ring and cover slip is less than 1.3 mm, allowing use of the long working distance objective. Chips of a cover slip are placed at the extremities of the sample holder to support a cover slip for the sample at an approximate distance of either 170 μm (1 chip) or 340 μm (2 chips). This enables control of the sample thickness which is optically important.

A dry ice-alcohol refrigeration system is used for freezing and temperature maintenance during freeze drying (Fig. 2). A bottom-emptying flask maintains a flooded suction head on the centrifugal pump. Fluid is pumped to the freeze drier through a copper coil immersed in a dry ice-alcohol bath before being returned to the suction line reservoir. Temperature can be regulated either by on-off cycles of the pump, or by control of the pump speed.

A single stage rotary oil vacuum pump is used in conjunction with a $CaSO_4$ desiccant vapor trap to remove fixed gases and water vapor from the freeze-drying chamber.

Dried compressed air is gently blown across the upper cover slip window to prevent condensation of environmental water vapor. The lower

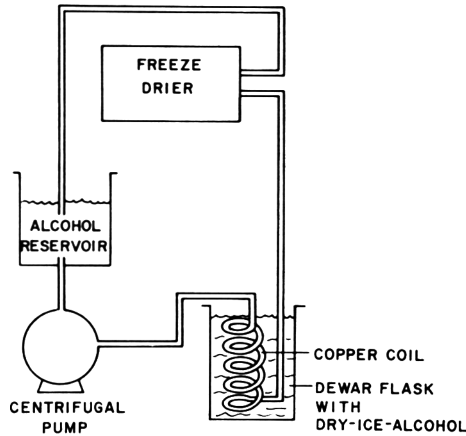

Fig. 2—Refrigeration system for freeze-drying microscope.

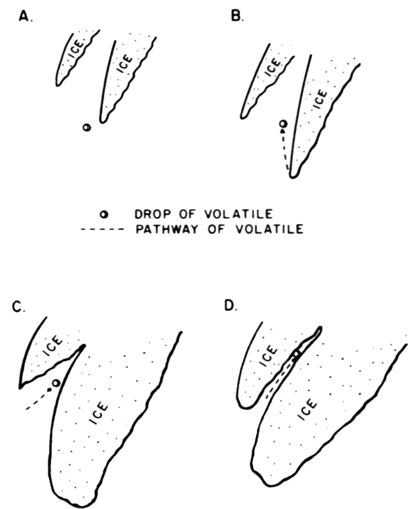

Fig. 3—Path taken by hexanol droplet during freezing of aqueous maltodextrin solution.

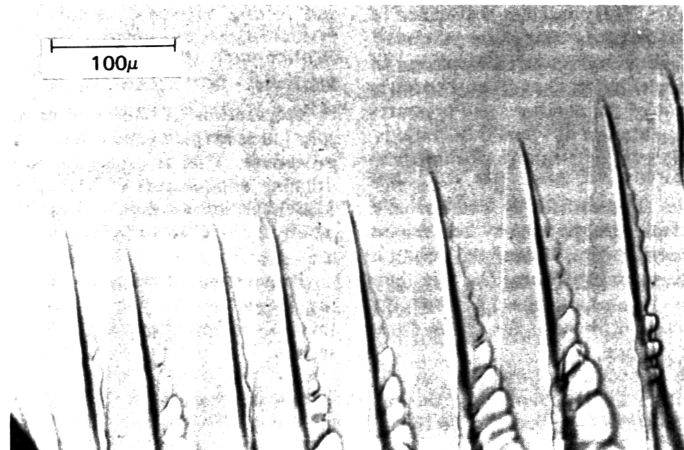

Fig. 4—Ice dendrites during freezing of 3.3% maltodextrin solution (150×).

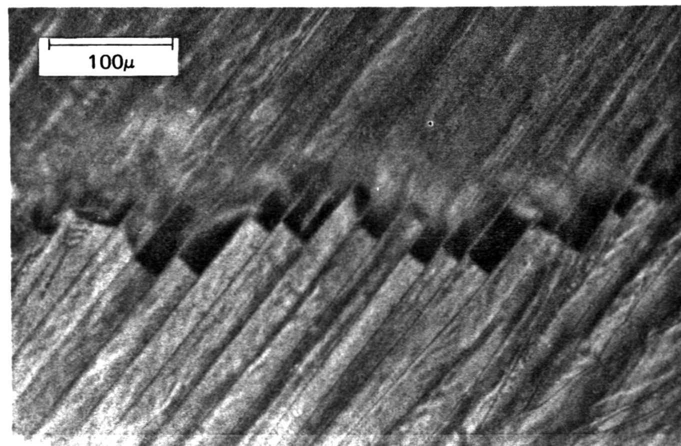

Fig. 5—Freeze-drying front in 3.3% maltodextrin solution (150×).

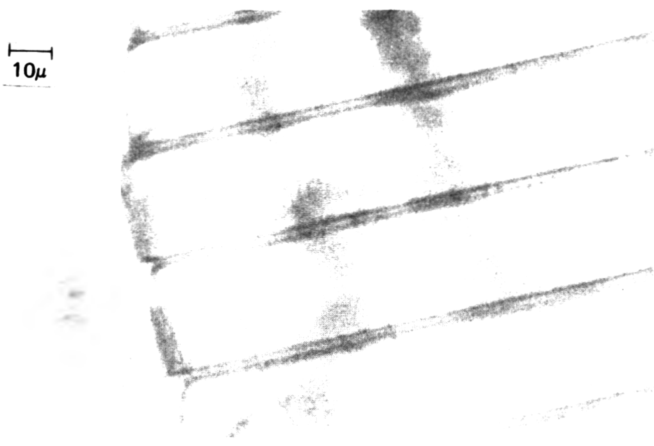

Fig. 6–Freeze-drying front in 3.3% maltodextrin solution (600×).

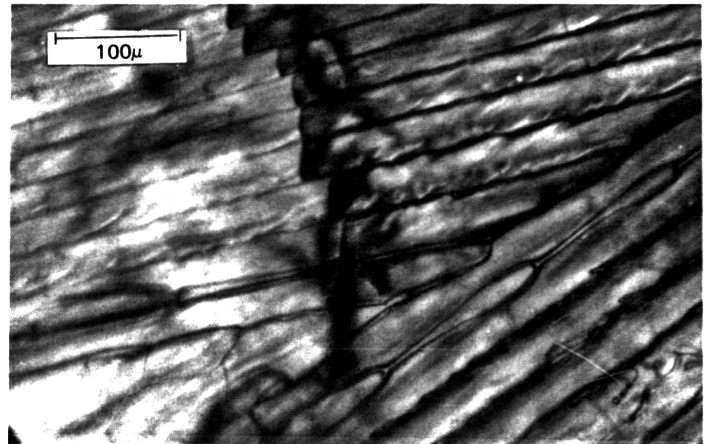

Fig. 7–Freeze-drying front in upper layer of 10% maltodextrin solution (shadow of lower region front is to left) (150×).

glass surface is within an insulation system and does not suffer condensation problems.

A carved balsa wood container is used for thermal insulation. This container is attached to the motion controls of the microscope stage.

Data acquisition systems. Evaluation of freezing and freeze-drying experiments requires the measurement of temperatures and pressures as well as documentation of visual observations. The following instruments supply this information.

Temperatures are measured by insertion of a microthermocouple junction into the samples (Omega Engineering Co., Stamford, Conn.). The junction diameter is 125 μm. The thin thermocouple wires (50 μm) are passed between the O-ring and aluminum block of the freeze dryer without loss of vacuum.

The system pressure is measured on the vacuum line with a thermocouple-type vacuum gauge having a range of 0-20 torr (Veeco Instruments).

Photographic records of typical visual observations are made with either a Polaroid ED-10 microscope camera or a 35 mm camera.

Visual observation is made via a closed circuit television system. This allows long visual observation periods without serious eyestrain and further, groups of people can observe and evaluate each experiment.

Methods

Preparation of model system. An aqueous solution is prepared according to a standardized procedure. This is especially important when utilizing components of limited solubility. The model system is either held at preparation temperature or chilled to 0°C by holding in crushed ice.

Preparation of microscope equipment. The dry ice-alcohol cooling system is prepared and the microscope stage connected to the cooling system. The freezing stage may be precooled to 0°C at this time, if desired. Dehumidified air sweeps the stage to prevent condensation of water vapor.

Sample freezing. The cooling system flow is adjusted to give the desired freezing conditions at the microscope stage. Freezing progress is followed either visually or photographically.

Subsequent steps prior to freeze drying. Upon the completion of the first freezing analysis, the sample can either be freeze dried or thawed and subjected to further freezing analysis. The latter is of interest when studying the resolubilization of the organic constituents or the influence of freezing history on subsequent freezing and freeze-drying behavior. Thawing is accomplished by stopping the coolant flow or additionally removing the heat absorbent from the lamp of the microscope optical system.

Sample freeze drying. Upon completion of the final freezing analysis, the chamber is evacuated and the frozen sample is freeze dried. Due to the small sample dimension, relatively rapid sublimation of the ice occurs. Following drying, the material can further be analyzed by more standard procedures as described by Flink and Gejl-Hansen (1972).

RESULTS & DISCUSSION

EXPERIMENTAL RESULTS can be divided into characterization of freezing and freeze-drying behavior in the micro-

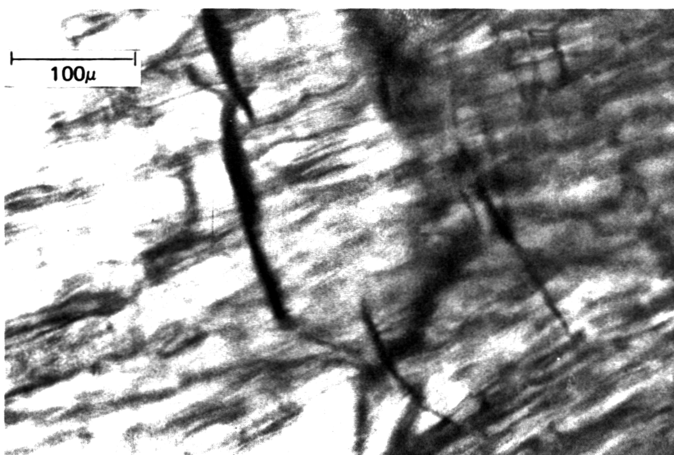

Fig. 8–Freeze-drying front in lower layer of 10% maltodextrin solution (shadow of upper region front is to right) (150×).

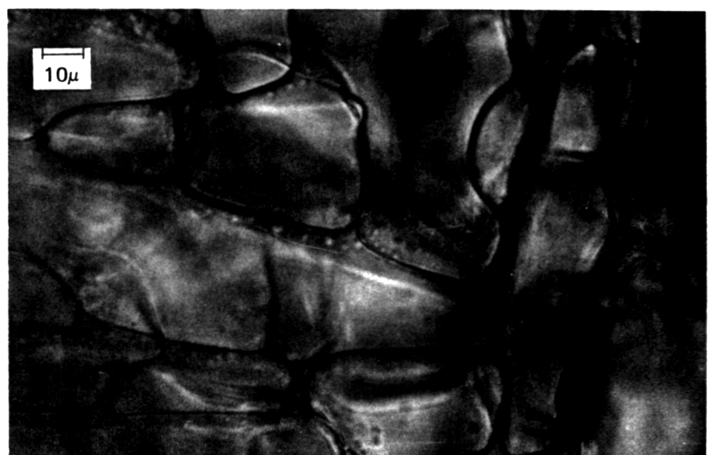

Fig. 9–Hexanol droplets at ice crystal grain boundries (600×).

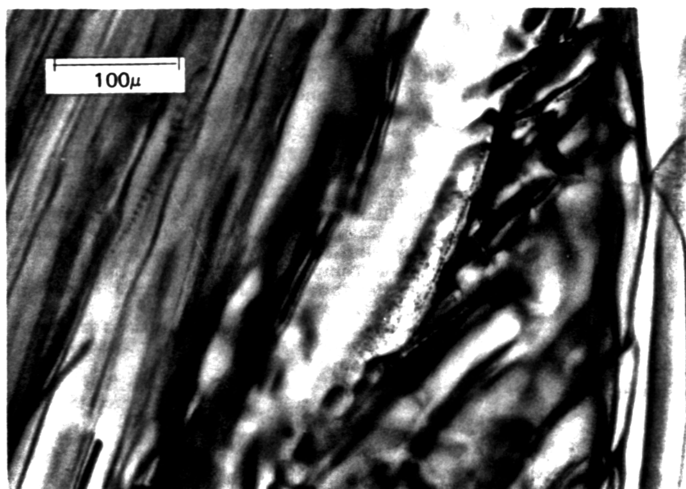

Fig. 10–Hexanol droplets in freeze-dried matrix, 100 microns into sample (150×).

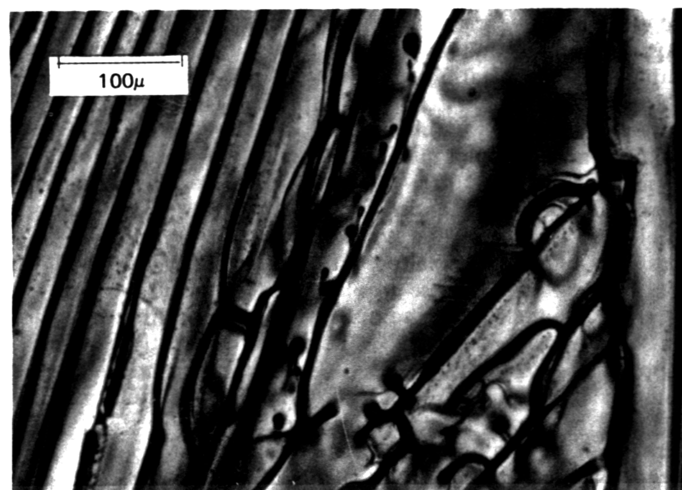

Fig. 11–Hexanol droplets in freeze-dried matrix; same view as 10, but at sample surface (150×).

scope, and studies on the freezing and freeze drying of aqueous solutions containing volatile organic compounds.

Freezing behavior

Freezing of water and aqueous maltodextrin solutions (10%) from room temperature generally occurs in 2–10 min. Temperature measurements indicated that the maltodextrin solution commences freezing at approximately −5°C and is completed at −7°C (Fig. 13).

Fast freezing is characterized by solidification of the sample in less than 20 sec (initial appearance of ice to complete solidification). The ice structure appears as plates or sheets without fine structure (Fig. 9).

Slow freezing is characterized by a fast freezing of a small part of the sample closest to the chilled surface followed by dendritic growth of ice crystals over a period of 0.5–10 min (Fig. 4). Some samples which have been slow frozen in thin slabs undergo dendritic crystallization in two layers; the bottom of the sample crystallizes first as disordered dendrites (Fig. 8), the upper region crystallizes later as ordered dendrites (Fig. 7).

Freeze-drying behavior

Freeze-drying fronts (the moving interface between frozen and dried regions) recede into the sample from all four sides. Samples approximately 1 cm × 1 cm, and 0.155–0.3 mm thick under a cover slip require from 50–80 min to freeze dry.

Separate freeze-drying fronts are observed in each of the different ice crystal orientations (and thus solute matrix orientations). The fronts are not completely planar, with small variations occurring among crystals having the same orientation (Fig 5, 6), and larger variations for crystals of different orientation (at different vertical locations) (Fig. 7, 8).

Freezing and freeze drying of aqueous solutions containing volatile organic compounds

Aqueous solutions of maltodextrin (10% w/v) and hexanol (0.3% w/v) are used for studying the phenomena associated with freezing and freeze drying which are responsible for retention of the volatile organic compounds in the dried material. The initial solution contains some liquid droplets prior to cooling. During the freezing process, the hexanol solubility limit is exceeded and many droplets of hexanol liquid appear. Often these droplets of hexanol move relative to their initial location due to bulk liquid fluid flow associated with liquid density differences resulting from temperature gradients and fluid flow associated with

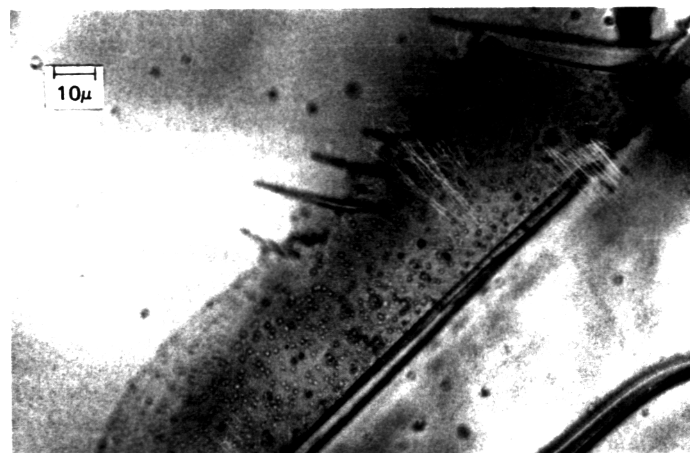

Fig. 12–Hexanol droplets in freeze-dried matrix; same field as 11 (600×).

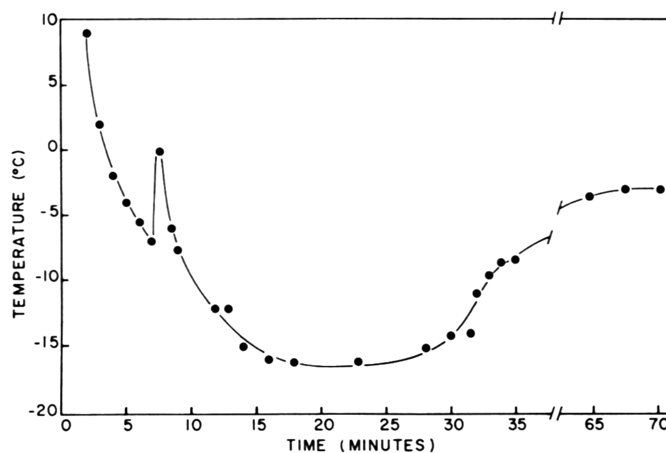

Fig. 13–Sample temperature during freezing and freeze-drying of droplet samples.

the growth of ice crystals. Figure 3 portrays the path taken by a hexanol droplet during the concentration steps associated with freezing. The extent of droplet movement is quite variable; in some cases it can be many droplet diameters, though in many cases it is little or none. This process results in the entrapment of the droplet in the interstitial solute matrix consisting of eutectic maltodextrin solution. Figure 9 shows these droplets at the ice grain boundaries of a completely frozen maltodextrin sample. These entrapped droplets of volatile (average diameter 2 microns) remain stationary during freeze drying and are found throughout the sample thickness in the dry amorphous matrix (Fig. 10 to 12). Similar behavior was observed in experiments at hexanol concentrations above (0.8% w/v) and below (0.1% w/v) that noted above.

The appearance of liquid droplets of alcohols in freeze-dried aqueous maltodextrin solutions has been related to the solubility of the volatile alcohols (Flink and Gejl-Hansen, 1972). Furthermore, the influence of molecular size, solubility and concentration on retention of the volatile following freeze drying has been demonstrated by Flink and Karel (1969, 1970a). A 0.5% (w/v) solution of a more soluble alcohol, n-butanol, behaved similarly, though the droplets formed during freezing are much smaller, making them more difficult to observe.

While experimental work is continuing with the freeze-drying microscope, it appears obvious that for some typical volatile organic compounds of limited aqueous solubility, retention after freeze drying is in the form of liquid droplets which primarily develop during cooling and freezing and are entrapped in the interstitial matrix after freezing. These droplets of volatile compounds are locked into the dry material following the freeze drying step.

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STRUCTURAL FUNCTIONS OF TASTE IN THE SUGAR SERIES: CYCLOHEXANE POLYOLS AS SWEET ANALOGUES OF THE SUGARS

INTRODUCTION

SHALLENBERGER'S sweetness hypothesis (Shallenberger et al., 1969; Shallenberger, 1966; Shallenberger and Acree, 1967) relates the sweetness of polyhydroxy ring structures to their AH,B systems where AH is a proton donor moiety and B is a proton acceptor. In the sugars, for example, the axial-equatorial and diequatorial α-glycol groupings fulfil the geometrical requirements for eliciting the sweet response, whereas the cis or trans diaxial arrangements are sterically disposed to prevent the effect.

Shallenberger's evidence has been criticized (Birch et al., 1970; 1971) because it has been accrued with the acid of reducing sugars, which, due to their free anomeric centers, equilibrate rapidly to mixtures of isomers. Nevertheless the quantitative examination of glycosidic structures has recently (Birch et al., 1971) supported the truth of the hypothesis.

If diequatorial or gauche arrangements of α-glycol groupings can cause sweet effects in ring structures, cyclohexane polyols present an obvious choice for experimental study. These molecules, being devoid of ring oxygen atoms, are simpler structures than the sugars and are not subject to mutarotational isomerisation when dissolved in the mouth. On the other hand, cyclohexane polyols resemble the sugars in being conformationally less rigid than analogous chair conformations of cyclohexane or a hypothetical pyranoid ring devoid of hydroxyl substituents (Stoddart, 1971).

This paper reports the sensory properties of some cyclohexane polyols containing from one to six hydroxyl substituents in relation to their configuration, conformation and analogy with the sugars.

EXPERIMENTAL

THE FOLLOWING cyclohexane polyols were obtained as gifts from Professor S.J. Angyal, New South Wales; Professor G.E. McCasland, San Francisco; and Professor L. Anderson, Madison, Wisc.:

Cyclohexane 1,2/4,5 tetrol	− Chiroinositol
(±) Viboquercitol	+ Chiroinositol
Alloinositol	D-(−)-Bornesitol
Mucoinositol	D-(+)-Pinitol
	Quebrachitol

Table 1—Sensory properties of cyclohexane polyols

Compound	Sweetness	Bitterness
Cyclohexane-1-ol	0	B
Cyclohexane cis 1,2 diol	0	B
Cyclohexane trans 1,2 diol	0	B
Cyclohexane cis/trans 1,3 diol	0	B
Cyclohexane cis 1,4 diol	0	B
Cyclohexane trans 1,4 diol	0	B
Cyclohexane 1,2/4,5 tetrol	0	0
(+) Cyclohexane 1,3,4/2,5 pentol [(+)-proto Quercitol]	tr	0
(−) Cyclohexane 1,2,4/3,5 pentol [(−)-vibo Quercitol]	tr	0
(±) Cyclohexane 1,2,4/3,5 pentol [(±)-vibo Quercitol]	S	0
Cyclohexane 1,2,3,4,5/6 hexol [epi Inositol]	tr	0
Cyclohexane 1,2,3,4/5,6 hexol [allo Inositol]	S	0
Cyclohexane 1,2,3/4,5,6 hexol [neo Inositol]	tr	0
Cyclohexane 1,2,3,5/4,6 hexol [myo Inositol]	tr	0
Cyclohexane 1,2,4,5/3,6 hexol [muco Inositol]	tr	0
(+) Cyclohexane 1,2,5/3,4,6 hexol [(+) chiro Inositol]	S	0
(−) Cyclohexane 1,2,5/3,4,6 hexol [(−) chiro Inositol]	S	0
3-0-methyl-myoinositol [D-(−)-Bornesitol]	S	0
3-0-methyl-(+) chiroinositol [D-(+)-Pinitol]	S	0
2-0-methyl-(−) chiroinositol [Quebrachitol]	S	0

D-Protoquercitol was extracted from common oak acorns (*Quercus robur L*) obtained locally. L-viboquercitol was prepared from D-protoquercitol (Angyal et al., 1962). Epiinositol was prepared from epi-myo-inosose (Reymond, 1957) which was itself prepared from myoinositol (Posternak, 1936). Neoinositol was prepared from epiinositol (Angyal et al., 1962). Myoinositol was obtained from British Drug House Chemicals, Poole, Dorset; all diols were obtained by fractional crystallization of commercial samples of cyclohexane 1,2- 1,3- and 1,4-diols obtained from Robertson Bros. Ltd., West Bromwich, Staffs.

Panellists were selected and trained according to a previous publication (Birch et al., 1972), and were asked to place a few mg of each substance on the tongue and to comment whether they were trace sweet (Tr.), sweet (S), intensely sweet (SS), trace bitter (tr.), bitter (B), or intensely bitter (BB). The decisions listed in Table 1 are those obtained in at least 70% of total judgments, each panellist carrying out duplicate tasting sessions. The total number

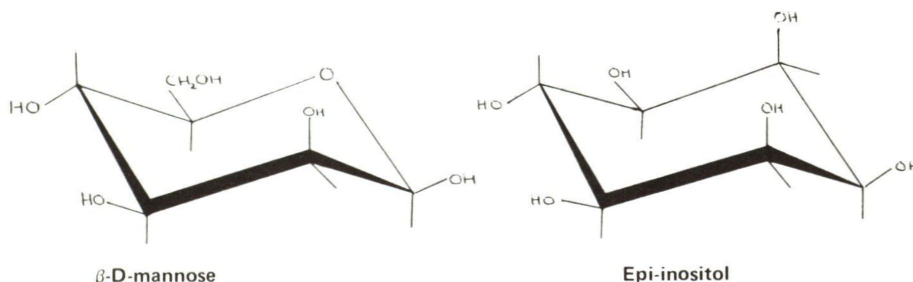

Fig. 1—Analogy of epi-inositol and β-D-mannose.

of panellists was ten. Each panellist tasted all 20 substances listed in the Table once each, at one session, rinsing with distilled water between substances, and pausing 1 min before passing on to the next substance.

RESULTS & DISCUSSION

THE RESULTS show that many of the inositols and other substances listed in the Table are sweet but their sweetness varies enormously. Epiinositol, for example, is only trace sweet even though, like many other structures presented in Table 1, it contains several possible AH,B systems meeting Shallenberger's (1969) gauche or diaxial requirements for α-glycol groups. This does not, however, invalidate Shallenberger's hypothesis since particular combinations of hydroxyl configurations around the ring may sterically prevent binding of AH,B systems to the taste bud protein. On the other hand, lack of sweetness in epiinositol may be explained by intramolecular hydrogen bonding in accordance with Shallenberger's own observations (Shallenberger et al., 1969; Shallenberger, 1966; Shallenberger and Acree, 1967).

Epiinositol is, as stated, trace sweet, and is an analogue of β-D-mannose (Fig. 1). Like 1-deoxy mannose, however, epiinositol is not bitter and this is strong supporting evidence of our previous deduction (Birch and Lindley, 1973) that bitterness in β-D-mannose is due to interaction of the β-anomeric hydroxyl group with the ring oxygen atom.

Substitution of a methyl group at position 3 of (+)-chiroinositol gives rise to (+) pinitol without loss of sweetness. Similarly substitution of a methyl group at position 2 of (−)-chiroinositol gives quebrachitol without loss of sweetness. These results suggest that since no change of sweetness or bitterness occurs after these substitutions, one of the nonmethylated hydroxyl groups may be AH in the AH,B system. Myoinositol, which is slightly sweet, is an analogue of β-D-mannose, β-D-galactose and α-D-glucose (Fig. 2). On the other hand 1,2/4,5 cyclohexane tetrol, which is also an analogue of α-D-galactose, is without taste (Fig. 3). Since the equatorial hydroxyl group at position 5 of the tetrol is smaller than the primary alcohol group in the analogous sweet α-D-galactose it would presumably offer no steric hindrance to binding. Also the methylene group which, in the tetrol, replaces the ring oxygen atom of α-D-galactose, does not prevent binding because D-viboquercitol (see below) is very sweet. Therefore we conclude that the third hydroxyl group of α-D-galactose is essential for sweetness in accordance with Hodge's recent observation (Hodge et al., 1972).

Among the cyclohexane pentols (quercitols or oak sugars) we have examined D-protoquercitol, L-viboquercitol and the crystalline racemic mixture D,L-viboquercitol. Only the last of these three is significantly sweet and so must be due to the D-viboquercitol which we have not, as yet, obtained as a pure enantiomorph. D-viboquercitol, as we have previously reported (Birch and Lee, 1971), is an analogue of α-D-glucose (Fig. 4) and therefore is predictably sweet, whereas L-viboquercitol has an axial hydroxyl group below the plane of the ring (Fig. 4) at position 5 which may be responsible for its lack of binding to the taste bud protein. D-viboquercitol has a strong sweet-

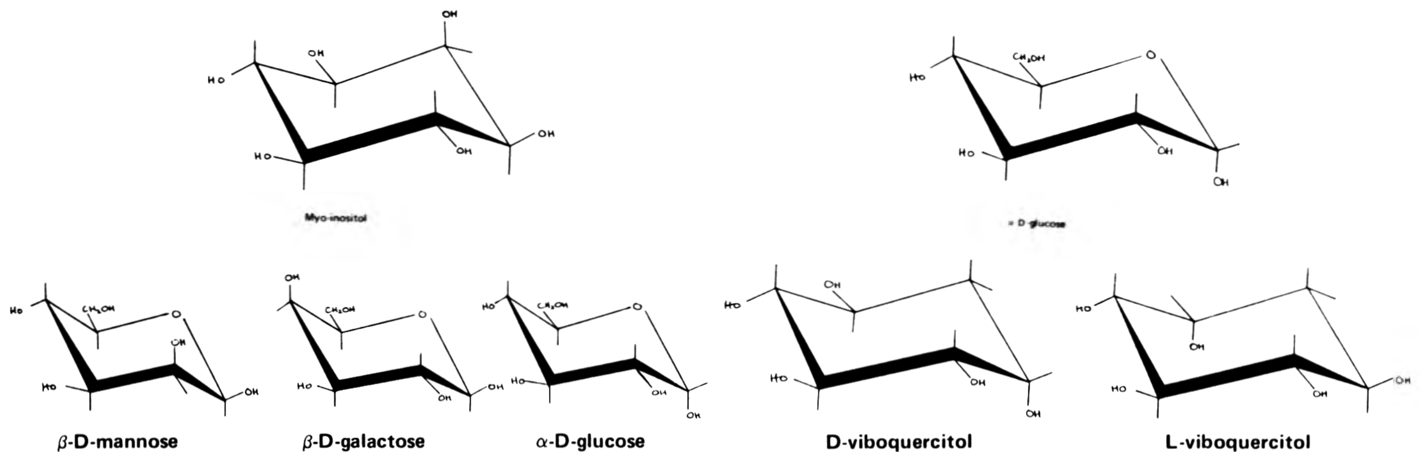

Fig. 2–Analogy of myoinositol, β-D-mannose, β-D-galactose and α-D-glucose

Fig. 4–Analogy of D-viboquercitol and α-D-glucose.

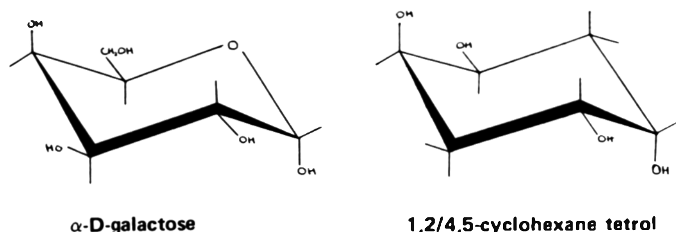

Fig. 3–Analogy of 1,2/4,5-cyclohexane tetrol and α-D-galactose

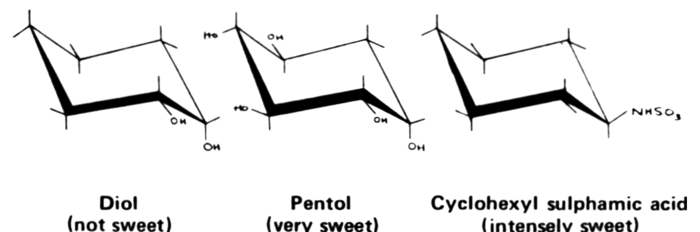

Fig. 5–Organoleptic properties of three related cyclohexane structures.

ness (judged between that of sucrose and fructose by the intensity of the D,L-mixture) but one less hydroxyl group than the inositols which have been tested. On the other hand all the cyclohexane derivatives with fewer hydroxyls (2–4) show no significant sweetness despite all possessing suitable AH,B systems. We can only explain these results by assuming that these molecules align themselves differently on the protein surface due to their greater hydrophobicity; hence some or all of the binding sites in such types are lipophilic in character. Kier (1972) has recently emphasised the significance of this third steric requirement of a lipophilic or "dispersion" site in any molecule capable of eliciting the sweet response and the tripartite functioning of an AH,B system and lipophilic site has been referred to by Birch and Shallenberger (1973) as "multiple group stereogeometry." This is a new concept and implies that combinations of many substituents at different points in the sugar ring may concertedly affect the total sensory properties of the molecule. The greater frequency of sweetness among the methoxy inositols reported here, and D-viboquercitol can presumably be explained on this basis.

If these substances had been tasted as solutions rather than crystals we would anticipate (as in previous studies) no qualitative differences, due to the intrinsic stability of the cyclitol structures. Some differences in intensity might occur due to absence of the hydrogen bonding which exists in the crystal lattice.

In studying many different carbohydrate structures we have never encountered a molecule with more than twice the sweetness of sucrose. Hence, although a polar moiety is likely needed to elicit sweetness, the preferable structural feature of saporific molecules may be an intact cyclohexane ring with a polar substituent, including an AH,B system, outside the ring (Fig. 5). No bitterness was observed in any substances containing more than two hydroxyl groups. Cyclohexanol and the diols were all bitter and devoid of sweetness, a feature which may again be possibly ascribed to the lipophilicity of these substances. The artificial sweeteners saccharin and cyclamic acid both possess bitterness as well as sweetness, as an intrinsic property of the molecule, which is in each case more lipophilic than either the polyols or the sugars.

CONCLUSIONS

CHANGES in configuration in polyhydroxy cyclohexanes cause alteration in their sweetness values from those of the sweetest known sugars down to nothing. These changes cannot be explained simply on the basis of hydrogen bonding and imply changes in binding mode due to alterations in the lipophilic character of the molecules.

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EFFECTS OF SUPERSATURATION AND TEMPERATURE ON THE GROWTH OF LACTOSE CRYSTALS

INTRODUCTION

INDUSTRIAL CRYSTALLIZATION of lactose from cheese whey is a relatively slow process based on gradual cooling of the concentrated whey from which the whey proteins may or may not have been removed (Nickerson, 1970). On the contrary, evaporative crystallization of sucrose from substantially purified sucrose syrups is a rapid process carried out at or above 80°C. It was shown by many workers that growth rate of sucrose crystals in solutions of comparable supersaturation is greatly accelerated by increase in temperature (the topic was reviewed e.g., by Van Hook, 1959).

The effect of temperature on crystallization velocity of lactose has received limited attention in the past, with studies at or below 30°C only. Whittier and Gould (1931) concluded that lactose crystallization was faster at 30°C than at any lower experimental temperature. Twieg and Nickerson (1968) showed that the temperature effect is dependent upon the supersaturation, the solutions of lower supersaturations crystallizing faster at lower temperatures. No information was found in the literature concerning the effects of temperatures above 30°C. This information is deemed essential for possible evaporative crystallization of lactose from deproteinated whey.

The first objective of this work was to evaluate the effect of temperatures above 30°C on growth rate of lactose crystals in pure lactose solutions of comparable supersaturations. The second objective was to determine the exact relationship between crystal growth rate and supersaturation; the general assumption that lactose crystallization rate increases linearly with supersaturation was recently questioned by Van Kreveld and Michaels (1965) and by Twieg and Nickerson (1968). These authors suggested that the rate may be proportional to a power of supersaturation greater than unity. The evaluation of the composite effect of temperature and supersaturation on growth rate of lactose crystals became the third objective of this work.

MATERIALS & METHODS

Experimental procedure

The investigation of the desired crystal growth rate relationships was carried out with single lactose crystals grown in pure lactose solutions of known concentrations. In essence, our method was based on the procedure used by Kucharenko (1928) for sucrose crystallization studies, modified by Van Kreveld and Michaels (1965). Large, well developed replicate lactose crystals of similar shape and weight were grown in supersaturated lactose solutions containing 10, 15, 20 and 25g anhydrous lactose/100g water above the solubility limits at 30, 50, 60 and 70°C. The overall growth rates of the crystals were measured as their weight increase per hour. Average growth rates for each supersaturation and temperature were calculated from the individual data deemed comparable because of the similarity of the experimental crystals.

Crystal growing technique

The experimental crystals were selected from a crop of crystals, spontaneously formed in a solution of analytical grade lactose held at room temperature for about a week. The selected crystals were perfected by growing them individually in further batches of lactose solutions for a considerable period of time. The crystals finally used for the experimental work were of a typical tomahawk shape and of about 0.25g in weight (Fig. 1).

Each crystal was placed into a separate 50-ml erlenmeyer flask with an indented ledge to keep the crystals tumbling while the tightly stoppered flasks with the various lactose solutions were rotated slowly (about 10 rpm) in a

Table 1—Solubility of lactose in water

Temp °C	Grams anhydrous lactose/100g water
30	24.0
50	44.0
60	59.2
70	77.8

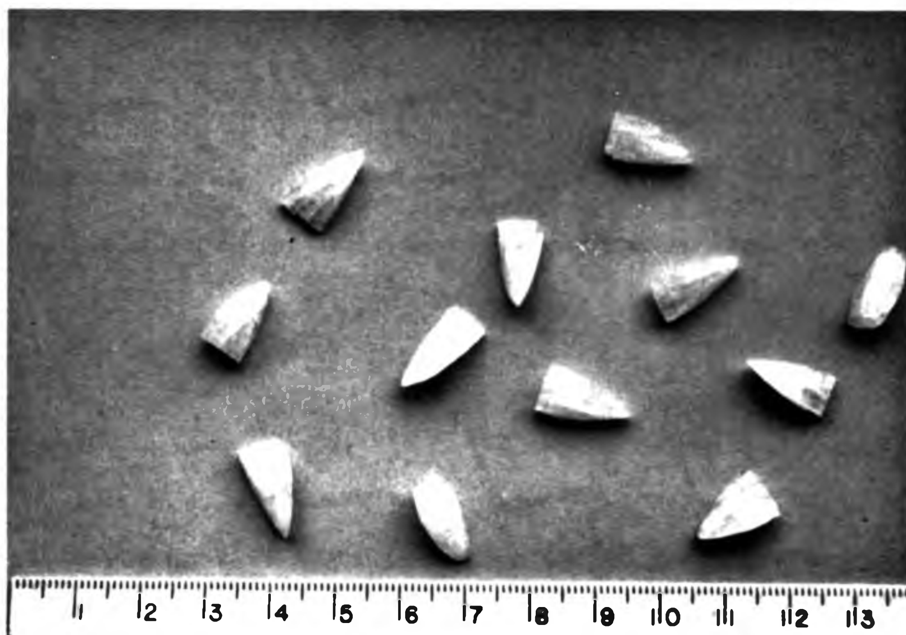

Fig. 1—Crystals of lactose used as seeds in the single crystal growth studies. (The scale is in cm).

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water bath held at constant temperature with about ± 0.2°C accuracy. The crystals were grown for 2 hr. At each temperature, an experimental run consisted of growing sets of four replicate crystals at each of the four different supersaturations; thus, 16 crystals were grown simultaneously in a run. The runs were repeated several times at each temperature with the sets of the initially randomly selected replicate crystals being rotated in order, so that the crystals were never grown in the same conditions more than once.

At the end of each run, crystals were taken out of the flasks with forceps, dipped momentarily into distilled water to get rid of the excess syrup, and then dipped into 95% alcohol and air dried. Before and after each run the crystals were weighed on an analytical balance with estimated precision of ± 0.2 mg.

Before the first run of each temperature block, the starting weights of all crystals were adjusted to about 0.25g by partially dissolving the crystals in water. However, such partially eroded crystals grew initially at much higher rates, and the first "conditioning" runs were thus excluded from the experimental results. The observation itself is consistent with the report of Van Kreveld and Michaels (1965) on rapid "healing" of broken lactose crystals. Similar reports on sucrose crystals were reviewed by Van Hook (1959).

At each experimental temperature, all the runs were replicated with the same sets of crystals without any intermittent weight adjustments. Thus, the starting weights in each run were slightly higher than those in a previous run. It follows that the crystal surfaces available for growth at each replicate run were not strictly identical. The error introduced by this procedure was entirely negligible as no systematic increase in weight gains in later runs of the same set could be detected.

Preparation of the lactose solutions

Mallinckrodt analytical grade lactose powder (lot 500 249) was used in preparing the experimental solutions. For each specific temperature, solutions of desired supersaturations were prepared by mixing appropriate amounts of the lactose hydrate powder and glass-distilled water in tightly glass-stoppered flasks. The weights were adjusted for the 5% water of crystallization. The solubility values given in Table 1 were used as a basis for preparing the solutions. These solubility values were obtained by averaging the literature data (Whittier, 1944, Rozanov, 1952, Foremost Foods, 1970) which showed certain discrepancies. The flasks were held in a boiling water bath until all lactose dissolved. The solutions were then evenly distributed into the appropriate crystal-growing flasks and brought to the experimental temperature before the crystals were placed into the flasks. The solutions were unbuffered, their natural pH being in the vicinity of pH 4.

Incidental false grain that had developed during or after a run was redissolved before the next run by placing the stoppered flasks into boiling water for the minimum time need to accomplish the dissolution. The false grain formation, sporadically observed in some of the replicate solutions at all experimental temperatures, did not appear to increase the variability of the results and thus all the data from such flasks were included.

Determination of the crystal surface area

An attempt was made to express the growth rates in units independent of the crystal size, such as a weight increase per unit crystal surface area. Following the procedure of Kucharenko (1928), the formula $A^3 = K\ W^2$ was used to estimate the average area A in cm^2 of the experimental crystals from their average weight W in grams. The correlation coefficient K (in units of cm^6/g^2) was assigned a value of 115, obtained by measuring the surface area of five well developed lactose crystals weighing 0.41–0.43g. Their surface areas were evaluated by tracing the boundaries of the five apparent crystal faces with a sharp pencil on paper and measuring the areas planimetrically. Using this method, the experimental crystal growth data were transformed into the crystallization velocity units of $mg/min/m^2$ to make comparison with other works easier. It is recognized that the inadequacies of the transformation may have introduced certain error, and that the large, well developed lactose crystals grow predominantly in one direction only—downwards from the apex of the crystal (Van Kreveld and Michaels, 1965).

Computer evaluation of the results

The relationships between the crystal growth rate and supersaturation at each experimental temperature were determined on an IBM 360/75 computer with the University of Illinois package of statistical programs SOUPAC. The heterogeneity of variances within the set of data was eliminated by using a fourth root transformation in the computations. The composite effect of temperature and supersaturation on the lactose crystal growth rate was evaluated by subjecting the entire experimental set to response surface analysis. Computer programs CENRS and PLTRS, from the computer library of the Pillsbury Company (Minneapolis, Minn.) were used to obtain a polynomial equation and a corresponding response surface plot, respectively, relating the three variables.

RESULTS & DISCUSSION

Analysis of experimental data

The general effects of supersaturation and temperature on crystal growth rate can be evaluated directly from the measured weight increases of the seed crystals. This is shown in Figures 2 and 3, where the mean weight increase of the experimental crystals at various temperatures is given as a function of supersaturation expressed as the excess ($C\text{-}C_S$) above solubility (absolute supersaturation) and also as a ratio C/C_S (relative supersaturation) used often by other workers.

The values used in the diagrams represent means of 12 (30°C and 60°C curves), or 16 (50°C and 70°C curves) measurements, collected in all the replicate runs. The complete record of the experimental data is available (Jelen, 1972). The average coefficient of variation for the whole experimental set was 13%, being somewhat higher (about 19%) for the lowest supersaturations. The 95% confidence intervals about the means are shown in Figure 3. The variability can be ascribed in part to differences in growth characteristics of the individual crystals. Some of them grew inherently faster at all supersaturations while others were noticeably slow.

The relationship shown in Figure 3, resembles a similar plot for sucrose (Van Hook, 1959, p. 155), demonstrating the sharp increase in the growth velocity with increasing supersaturation, especially at higher temperatures. The promoting effect of increased temperature on crystal growth velocity is also well demonstrated. The fact that the same growth rate is ob-

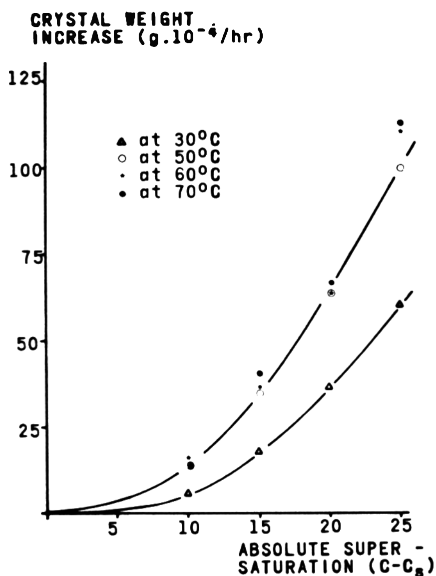

Fig. 2—Mean weight increase of experimental lactose crystals as a function of excess supersaturation at various temperatures.

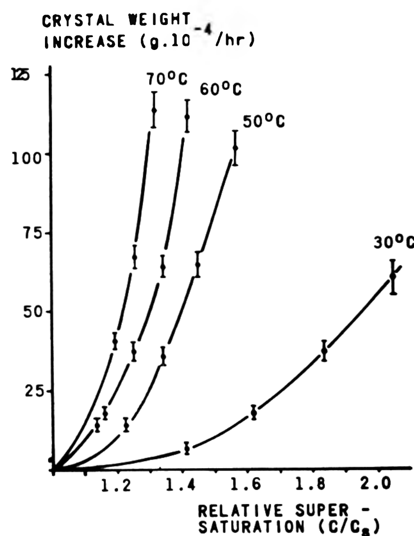

Fig. 3—Mean weight increase of experimental lactose crystals as a function of supersaturation ratio at various temperatures.

Table 2–Comparison of crystal growth velocities of the final (a) and preliminary (b) sets

Absolute super-saturation	Crystal growth velocity ($mg/m^2/min$) at							
	30°C		50°C		60°C		70°C	
(g/100g HOH)	a	b	a	b	a	b	a	b
10	47	82	112	128	138	–	112	119
15	143	140	280	270	297	–	328	282
20	295	306	520	435	512	–	535	–
25	482	485	810	700	895	–	905	–

Table 4–ANOVA table for the 50–60–70°C composite model

	df	MS
Model	2	25.47744
Heterogeneity of B & D	4	0.05210
Heterogeneity of A	2	0.04232
Deviations	3	0.03214
Error	204	0.08775

Table 3–Mathematical relationships between crystallization velocity of lactose and supersaturation at various temperatures

Temp °C	Functional[a] relationship	Parameters in the 4th root transformation: Constants	Standard error	Index of fit %
30	$Y = 0.148(C\text{-}C_s)^{2.52}$	B = 0.439	± 0.068	99.5
		D = 0.630	± 0.052	
50	$Y = 0.862(C\text{-}C_s)^{2.15}$	–	–	99.9
60	$Y = 1.371(C\text{-}C_s)^{2.05}$	–	–	98.8
70	$Y = 0.839(C\text{-}C_s)^{2.17}$	–	–	99.7
50–70	$Y = 0.914(C\text{-}C_s)^{2.13}$	B = 0.690	± 0.047	–
		D = 0.532	± 0.023	

[a] Y = crystal growth rate ($mg/m^2/min$); C, C_s = experimental concentration and concentration at saturation, resp. (g lactose/100g water)

Table 5–Response surface analysis of the lactose crystallization velocity data

Coefficient	95% Confidence limits
B0= –68.4800	–346.4428 to 209.4828
B1= 12.6260	4.5228 to 20.7291
B2= –43.8490	–64.7932 to –22.9048
B11= –0.1730	–0.2493 to –0.0967
B22= 1.6350	1.0937 to 2.1763
B12= 0.5822	0.4185 to 0.7459

Source	DF	SS	MS	F ratio
Adjusted total	15	1.1663×10^6	7.775×10^4	
Model	5	1.1603×10^6	2.3207×10^5	392.5
Residual	10	5.913×10^3	5.913×10^2	

tained with much lower relative supersaturation at higher temperatures may be significant for preventing induced nucleation; for sucrose, the supersaturation C/C_s = 1.2 is considered a limit above which induced nucleation occurs (Bretschneider, 1969).

When the same experimental data are plotted against excess supersaturation (Fig. 2) the result is similar as to the nonlinearity of the relationship. The promoting effect of temperature on crystal growth rate is again well demonstrated in the range from 30–50°C; the rate was about twice as high at 50°C as it was at 30°C at all supersaturations studied.

However, between 50 and 70°C the picture is quite unexpected in that there seems to be no significant increase in crystal growth rate with increasing temperature. This observation was first made in a preliminary experiment with a set of smaller crystals at 50 and 70°C; consequently, runs at 60°C were included in the main experimental set to find out whether the observation would be consistent for the whole 50–70°C range. The results of the 60°C runs concurred with those of 50 and 70°C runs, suggesting that there was indeed no significant increase in crystal growth velocity at temperatures above 50°C under these conditions. No explanation of this finding can be offered at this time, if a possibility of a gross error in available lactose solubility values is discounted. Considering the differences in the reported solubility values (Whittier, 1944, Rozanov, 1952, Foremost Foods, 1970) this possibility cannot be dismissed, although the discrepancies themselves do not offer an explanation at the present experimental temperatures. The data for growth of sucrose crystals at the same temperatures and supersaturations (listed by Hirschmuller, 1953, p. 24) show that the situation is not observed with sucrose.

Determination of the functional relationships

The average surface area A of the seed crystals was calculated to be 2.08 cm^2 using average crystal weight of 0.28g and the above mentioned value of K = 115. The underlying assumption that the shapes of all experimental crystals were similar and comparable to that of the crystals used for determining the K, was deemed justified.

The recalculated mean crystal growth velocities in $mg/m^2/min$ for all the experimental treatments are given in Table 2. The values, computed by the same procedure from the preliminary set of data where again K = 115 and average weight = 0.11g are also given. The agreement is satisfactory, suggesting that the crystal growth velocity was the same for these two crystal sizes. The error introduced by using average weights and area values rather than the individual experimental data constitutes about 7% deviations from the mean, as compared to the average experimental variability of 15%.

The recalculated individual experimental data (rather than the means) were used for the evaluation of the functional relationships between crystallization velocity and supersaturation at the four experimental temperatures. The power function $Y = B\,(C\text{-}C_s)^D$ was found to be the most appropriate description of the relationship between the crystal growth velocity Y and the supersaturation ($C\text{-}C_s$). The exact numerical forms of all the models obtained are summarized in Table 3. It can be seen that the crystallization velocity increased with approximately the square of supersaturation at or above 50°C, and with the 2.5 power at 30°C. Models with an additive constant ($Y = A + BX^D$) did not improve the degree of fit and the particular numerical values of A obtained were not significantly different from 0. The models for 50, 60 and 70°C were not significantly different from each other as evidenced by fitting a single curve to all the 50–60–70 data.

Table 4 is the ANOVA table for the composite model; the model itself is included in Table 3 and its parameters in the 4th root transformation compared to

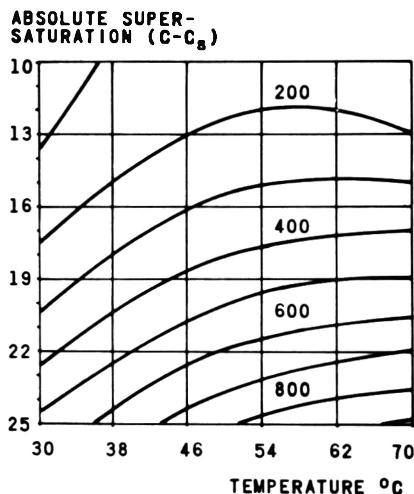

Fig. 4—Response surface plot showing a relationship of crystallization velocity (mg/m²/min), temperature (°C) and supersaturation ($C\text{-}C_S$, g lactose/100g water).

those of the 30°C model. Evidently, the two curves are significantly different at the $P < 0.05$ level. The degree of fit of the individual models to the mean experimental values is given in the last column of Table 3.

These results confirm speculations of Van Kreveld and Michaels (1965) and Twieg and Nickerson (1968) about the nonlinearity of the crystallization velocity vs. supersaturation relationships for lactose. The nonlinearity is much more pronounced than in the case of sucrose. All the results at each experimental temperature were obtained in a strictly comparable manner. Thus, extraneous effects such as strain, interruption, thermal shock, etc., known to affect the crystal growth velocity, can be dismissed as a possible explanation, and the results seem justified.

The composite effects of temperature T(°C) and supersaturation ($C\text{-}C_S$) (g lactose/100g H_2O) on the crystal growth rate are shown in the response surface plot in Figure 4. This plot is based on the following second power polynomial, describing the effects of temperature, supersaturation and of their interaction, on the crystal growth rate Y expressed in $mg/m^2/min$: $Y = -68.480 + 12.627\ T - 43.845\ (C\text{-}C_S) - 0.173\ T^2 + 1.635\ (C\text{-}C_S)^2 + 0.582\ T(C\text{-}C_S)$. The crystallization velocities, calculated from this model are sufficiently close to the mean experimental values within the whole experimental range, and the value of the F-ratio (392.6) proves that the second power polynomial fit is highly significant. Closer inspection of the 95% confidence limits of the numerical constants given in Table 5, reveals that all the linear, quadratic and interaction terms are statistically significant. This confirms the canonical equations derived above for the velocity-supersaturation relationships. The significance of temperature-supersaturation interaction again confirms that the effect of supersaturation is different at low and at high temperatures.

Despite the good fit of the model within the whole experimental range, caution must be exercised in its possible future use. As given above, the model is somewhat inaccurate outside the experimental range, especially at the very low supersaturation levels. The use of the second power, rather than of the slightly higher ones given in Table 3, leads to further minor inaccuracies. In addition, the growth rates of lactose crystals may vary with the crystal size; according to Van Kreveld and Michaels (1965), certain faces of small lactose crystals may eventually disappear in the process of crystal development, resulting in a slower overall rate.

CONCLUSIONS

INCREASING temperature from 30°C to 50°C doubled the growth rate of large lactose crystals in solutions of comparable excess supersaturation ($C\text{-}C_S$). Above 50°C, no significant rate increase was observed when solutions of equal excess supersaturation were compared. In solutions of comparable relative supersaturation (C/C_S) increasing temperature increased the crystal growth rate within the whole experimental range.

The crystal growth rate increased with approximately the second power of the absolute supersaturation at and above 50°C, and with the 2.5 power at 30°C.

The dependence of crystallization velocity on supersaturation and temperature can be described by a second degree polynomial formula, utilizing statistically significant linear, quadratic and interaction terms of both temperature and absolute supersaturation.

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EFFECTS OF CERTAIN SALTS AND OTHER WHEY SUBSTANCES ON THE GROWTH OF LACTOSE CRYSTALS

INTRODUCTION

CRYSTALLIZATION of lactose from impure solutions such as cheese whey is likely to be influenced by the amount and kinds of nonlactose substances present. Crystallization processes are usually retarded by the presence of impurities. For example, crystallization velocity of sucrose in natural sucrose syrups of 95% purity was shown to be about 1/2 of that in pure sucrose solution, and at 80% purity about 1/9 (Van Hook, 1959). In a recent review Smythe (1971) showed that most of the individual organic substances occurring in natural sucrose solutions retarded the crystallization velocity of sucrose both in natural and in model systems.

Several notable exceptions appearing in the literature (summarized by Van Hook, 1959, and Smythe, 1971) are associated with strong electrolytes such as salts which increased the sucrose crystallization velocity at low salt concentrations under certain conditions. In many other cases, however, the salts decreased the sucrose crystallization velocity just as any other impurity.

Studies of lactose crystallization in the presence of impurities are scarce. Michaels and Van Kreveld (1966) found the effects of several trace impurities on growth of small lactose crystals to be retarding; however, methyl and ethyl alcohol, sorbic acid, and formaldehyde to a certain point, showed an accelerating effect. Herrington (1934) observed a retarding effect of very high salt concentrations on lactose crystal formation. In addition, his work indicates the likelihood of a salt effect on lactose solubility, well known in the case of sucrose (Hirschmuller, 1953).

In general, the effect of salts on crystallization velocity of lactose is not well understood. This investigation was designed to evaluate the lactose crystal growth rates in the presence of certain salts and other substances found in cheese whey. The objectives were (1) to evaluate the effects of KCl, $CaCl_2$, NaH_2PO_4 and lactic acid, added at various impurity levels to a moderately supersaturated lactose solution at 50°C; (2) to compare the crystal growth rate in deproteinated clarified whey and in a pure lactose solution of similar lactose/water composition; and (3) to evaluate the effect of salt addition in the clarified whey.

Table 1—Proximate composition of the deproteinated clarified whey

Total solids (%)	40.0
Protein (N × 6.38, % total solids)	3.7
Ash (% total solids)	6.2
Lactose (g/100g of H_2O)	59.0

MATERIALS & METHODS

IN PRINCIPLE, the single crystal procedure described in our preceding paper (Jelen and Coulter, 1973) was used. The modifications and details pertaining to the present work are specified below.

Experimental procedure

Large replicate single lactose crystals were grown in lactose solutions containing 57g anhydrous lactose/100g water. At the experimental temperature of 50°C this corresponds to a supersaturation of 13g lactose/100g water above the solubility limit. Relative crystallization velocities were calculated as a ratio $C.V._{lactose+salt}/C.V._{lactose}$ from the mean growth rates, expressed as the crystal weight increase per hour. This procedure was used separately for each experimental run in which all the crystals were of similar shape and weight; no correction for the crystal surface area was then necessary.

Source of experimental crystals

The same crystals were used as in our previous work (Jelen and Coulter, 1973).

Crystal growing technique

The crystals were grown for 5–6 hr or for 10–12 hr depending on circumstances, as no significant variation in the crystal growth rates was observed due to varying time intervals. The effects of each salt were evaluated in a series of experimental runs with solutions of gradually increasing impurity. Four or more replicate crystals were grown at each impurity level, and two or more different impurity levels were run simultaneously. Thus, usually a total of 16 crystals were grown in a run, including control crystals. The runs were replicated three times so that data for 12 or more crystals were secured for each particular impurity level.

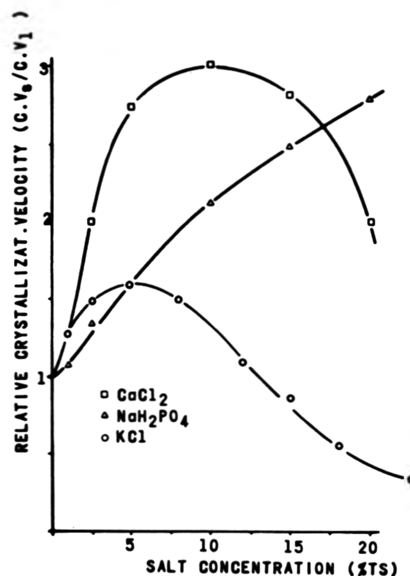

Fig. 1—Effects of salts at various concentrations on crystallization velocity of lactose at 50°C.

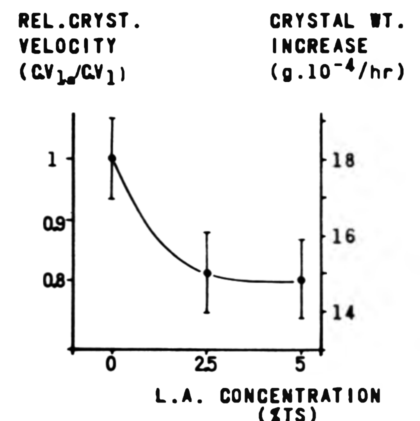

Fig. 2—Effect of lactic acid on crystallization velocity of lactose at 50°C.

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Table 2—Comparison of lactose crystallization velocities in pure and impure lactose solutions of 59g lactose/100g water concentration

	No. of observations	Mean crystal wt increase (g 10^{-4}/hr)	Variance	"t" value
Clarified whey	12	26.30	8.66	1.12
Pure lactose sol'n	12	24.99	6.30	
Clarified whey	15	26.41	5.93	7.4***
Clarified whey + NaH_2PO_4	15	33.99	10.02	

*** Significant at P X 0.001

The studies with the whey samples were carried out in a similar manner, except for the supersaturations which were kept at about 15g lactose/100g water above solubility limits with all samples.

Preparation of the experimental solutions

The "base" lactose solution was prepared for each set of runs in a glass-stoppered flask by mixing appropriate amounts of lactose monohydrate (Fisher certified lactose No. L-5 75212) and glass distilled water, and holding in a boiling water bath until all of the lactose dissolved. The solution was divided into smaller glass-stoppered flasks and appropriate amounts of a particular impurity were dissolved. All the reagents were of analytical grade. The solutions were distributed to the individual crystal growing flasks, tempered to 50°C, and the crystals introduced.

Preparation of the clarified whey samples

Rennet cheese whey from the pilot plant cheese production at the Dept. of Food Science & Nutrition, University of Minnesota, was heated to 95°C or more, acidified to pH 4.5 by HCl, separated, the supernatant concentrated to approx. 50% total solids (TS), further heated, and filtered, as described previously (Jelen et al., 1973). After determination of TS and lactose, the lactose-in-water concentration was adjusted by adding distilled water. Proximate composition of the final sample is given in the Table 1.

For investigation of the effect of NaH_2PO_4 in the clarified whey, the salt was added to 1/2 of the sample prepared for the investigation. Both samples were held in the same boiling water bath until the salt dissolved.

Analytical procedures

Total solids and ash contents of the clarified whey samples were determined according to AOAC (1970) for dairy products. Lactose content was determined by the phenol-sulphuric acid method of Dubois et al. (1956). The micro-Kjeldahl method was used for determining the residual nitrogen content of the clarified samples. The possibility of Ca presence in the lactose crystals was evaluated by the colorimetric method of Nickerson et al. (1964).

RESULTS

MEAN CRYSTAL growth rates in units of crystal weight increase/hr were calculated for each impurity level using all the replicate data secured. The median variability within the entire experimental design was 10% (range 5–15%). The mean values were used in calculating the relative crystallization velocities plotted in Figure 1 where the salt content is expressed as the weight percentage of the total solids content (including the salt), i.e., as % impurity. It can be seen that each of the three salts accelerated the growth of the lactose crystals at all or at certain impurity levels. The magnitude of the velocity increase varied with each of the salts and with their respective concentrations. The most dramatic effects were exhibited by $CaCl_2$, which substantially increased the C.V. at practically all the levels studied. A maximum effect is observed at 10% impurity level, accelerating the C.V. three times. A similar pattern, although in much lesser magnitude, was exhibited by the KCl, which increased the C.V. by a factor 1.5 at approximately 5% impurity level. However, at higher salt concentrations, the addition of KCl had an increasingly inhibitory effect on the growth of the experimental crystals.

The NaH_2PO_4 plot shows a different pattern as the growth promoting effect of this additive steadily increased with increasing salt concentration. In presence of NaH_2PO_4 the lactose crystals grew fastest at the 20% impurity level, the velocity being about 2.6 times higher than with pure lactose solutions. It may be that had the experiment continued, a point of maximum acceleration would have been reached at still higher salt concentrations. At low impurity levels, the crystallization velocity increase was less than with either $CaCl_2$ or KCl.

A possibility that an added salt could conceivably participate in growth of the experimental crystals, (as Herrington, 1934, reported with highly concentrated, boiled solutions) was tested with the $CaCl_2$-lactose solution. After the crystallization experiments, the $CaCl_2$-lactose solution of 20% impurity level was allowed to spontaneously nucleate and the nuclei were left to grow at room temperature for several days. The washed crystals were inspected under a microscope and analyzed for Ca content. No measurable Ca was detected and the crystals were found to be of the triangular shape characteristic for α-hydrate crystals. The entire weight increase of the experimental crystals was thus assumed to be attributable only to lactose deposition.

Minor pH changes caused by the salts were noticed in the unbuffered lactose solutions (initial pH about 4). Additions of KCl and NaH_2PO_4 raised the pH slightly to approximately 4.3–4.5, while the $CaCl_2$ lowered the pH to 3.3. This fact, as well as the subsequent crystallization experiments with lactic acid, show

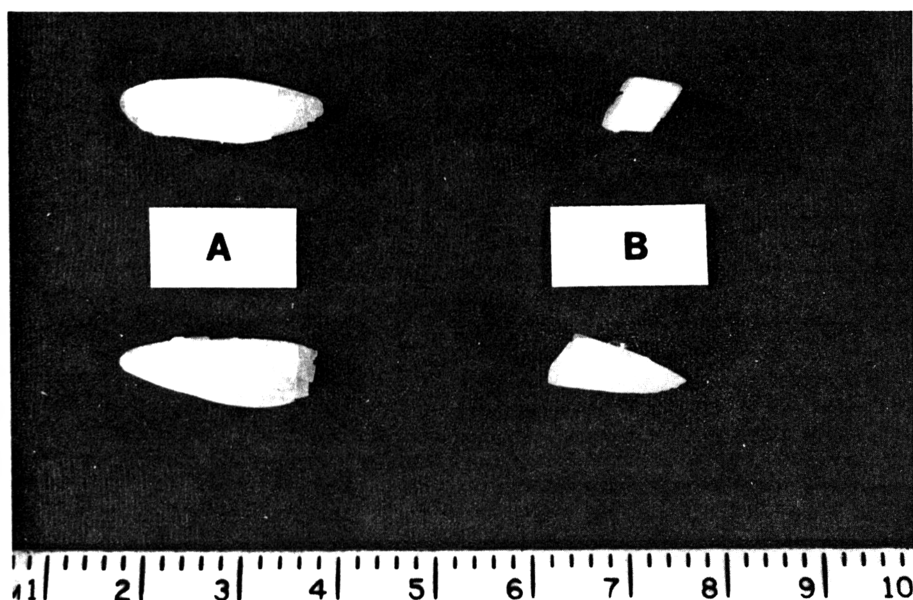

Fig. 3—Experimental lactose crystals grown for 12 days in a lactose solution with (A) and without (B) $CaCl_2$ addition at 50°C.

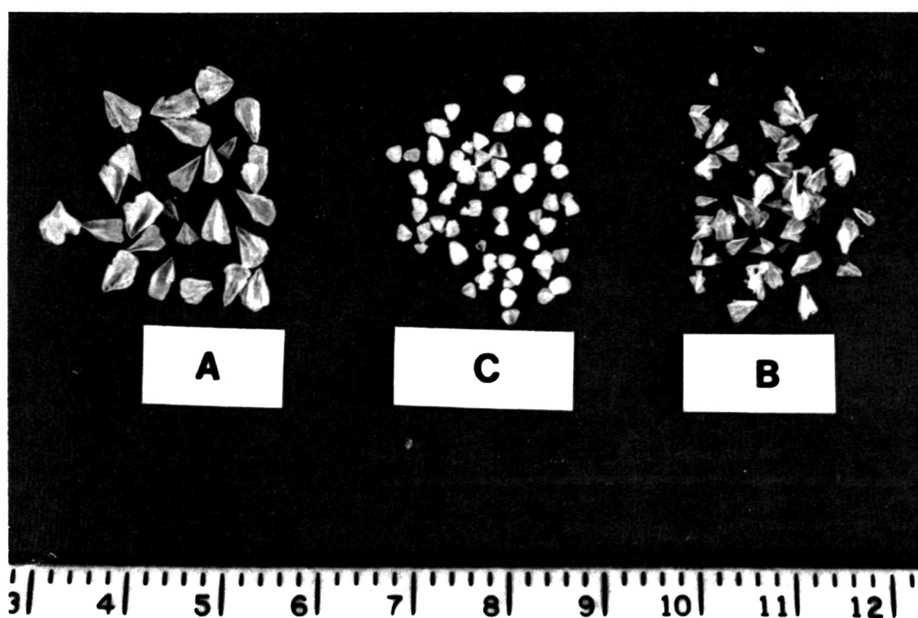

Fig. 4—Lactose seed crystals (20–40 mesh) grown for 10 days at 50°C in a lactose solution with $CaCl_2$ (A) and NaH_2PO_4 (B) addition and in the control solution with no additive (C).

that the pH per se did not affect the crystal growth rate.

Lactic acid was included in the model investigations as a representative of a weak electrolyte and of a major organic impurity occurring in clarified whey. Substances of both of these types seem to retard crystallization processes with no anomalies reported.

The results are summarized in Figure 2. It can be seen that addition of lactic acid caused a decrease of the crystallization velocity at both the 2.5% and 5% impurity levels. The pH of the solutions with lactic acid was 3.2, about the same as in the case of the lactose-$CaCl_2$ solutions. The 95% confidence intervals in Figure 2, to be read with the right hand scale, are typical of all the crystallization experiments reported here.

To evaluate the effect of the total impurity level in the deproteinated cheese whey on the lactose crystallization velocity, replicate single crystals were simultaneously grown in deproteinated whey and in a pure lactose solution of the same lactose/water composition. The mean values and other pertinent data are given in Table 2. The "t" test showed no significant difference between the crystal growth rates in these two systems. This is in contrast to the demonstrated negative effect of decreasing purity in natural sucrose solutions on the C.V. of sucrose. It may be that the electrolytic effect of some of the naturally occurring whey salts is evident here.

When NaH_2PO_4 was added to the deproteinated whey in an amount representing 10% of the lactose content, the purity was lowered to about 80%. Yet the crystallization velocity was significantly increased as shown in the lower part of Table 2.

Two different experiments are summarized in this table; thus, the agreement in the C.V. values for the clarified sample is surprisingly good. The pH of the clarified samples was about 4.3–4.5; the phosphate addition did not have any major effect on pH.

The increase by about 30% is much smaller than exhibited in the model system at this salt concentration level; nevertheless, the difference between the two means is still highly statistically significant.

It is likely that the impurities in the clarified whey counteracted to some extent the growth-promoting effect of the phosphate. On the other hand, it is obvious that the increasing impurity level does not necessarily decrease crystallization velocity of lactose in whey.

Addition of $CaCl_2$, which exhibited the largest accelerating effect in model studies, was also attempted in this experimental series. However, the viscosity of the samples with $CaCl_2$ rapidly increased up to the point of gelation, and no growth experiments were thus initiated.

DISCUSSION

THE PROMOTING effect of $CaCl_2$ on the crystallization velocity of sucrose was reported several times (Van Hook, 1959). Its effect in lactose crystallization, and the promoting effects of KCl and NaH_2PO_4 in either sucrose or lactose crystallization have not been reported insofar as we know. In fact, KCl has been considered a retarding agent in sucrose crystallization (Van Hook, 1959).

The results presented here seem to be consistent with the activity theory of Van Hook (1944), who proposed that crystallization velocity should be considered a function of thermodynamic activity rather than of supersaturation. Using the activity theory, the salt effects can be explained on the basis of the Debye-Huckel theory of strong electrolytes (Van Hook and Shields, 1944). The activity coefficient of a crystallizing sugar may be expected to change with increasing ionic strength of the salt solution, exhibiting a maximum at a certain low salt concentration, and a steadily decreasing behavior thereafter. Correspondingly, the crystallization velocity of lactose should be fastest at a certain low salt concentration if the theory is indeed applicable to lactose crystallization.

It is likely that the salt accelerates the surface deposition step of the crystallization process, perhaps in addition to lowering the lactose solubility and accelerating the mutarotation reaction (Haase and Nickerson, 1966). However, in the context of the presently used single crystal technique any possible effect of mutarotation could be meaningfully considered only in the layer immediately surrounding the crystal surface.

The acceleration of the crystal growth rate results in an altered shape of the crystal, as can be seen in the Figures 3 and 4. Four similar representative experimental crystals were grown in the experimental lactose solution at 50°C for 12 days. The presence of 6g $CaCl_2$/100g water caused a considerable flattening of the crystal base (Fig. 3, A); without the $CaCl_2$ the crystals continued to grow in the pyramid-like shape (Fig. 3, B).

The flattening effect was also observed with a mass of small seed lactose crystals (20–40 mesh) grown in similar conditions for 10 days (Fig. 4). The seeds formed flat, triangular flaky crystals in the presence of $CaCl_2$ (A) or NaH_2PO_4 (B) at the 10% impurity level, while in the control solution, the crystals again assumed the pyramid-like form (C).

The salt effects observed here might offer an explanation for the several forms of α-lactose crystals found in various dairy products (Van Kreveld and Michaels, 1965). The effect of an impurity on crystal habit is not unusual; e.g., similar effects in sucrose crystallization were recently reviewed by Smythe (1971).

CONCLUSIONS

THE EFFECT of salts on crystallization velocity of lactose varied with the species

and concentration of a salt. Acceleration, no change and retardation of the crystal growth rate were all observed at various salt concentrations.

The maximum growth-promoting effect observed here was exhibited by $CaCl_2$. At the 10% impurity level, the crystal growth rate was accelerated three times.

The composite effect of the naturally present whey salts is difficult to predict, as some of the more abundant whey salts like KCl may retard the crystallization velocity at higher salt concentrations.

Crystal growth rate in deproteinated whey of about 90% purity was the same as in comparable pure lactose solution. The addition of NaH_2PO_4 to the deproteinated whey accelerated the growth rate by 30%, while lowering the purity to about 80%.

The relationship of crystal growth rate and purity of the lactose solution cannot be predicted without specifying the nature of the impurities present.

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APPLIED SCIENCE and ENGINEERING

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TECHNIQUES FOR STUNNING CHANNEL CATFISH AND THEIR EFFECTS ON PRODUCT QUALITY

INTRODUCTION

THE ENACTMENT of the Humane Slaughter Law of 1958 focused attention on animal slaughtering procedures used in meat processing plants. Five methods for slaughter of livestock were approved as humane by the USDA. These methods were electrical stunning, carbon dioxide immobilization, mechanical bolt stunning, gunshot and ritual slaughter.

Kotula and Helbacka (1966) found that poultry slaughtered by the standard knife method of stunning and cutting lost more blood than birds slaughtered by electric shock. However, Anthony (1935) previously stated that birds bled well when stunned by an electrical shock. Mountney et al. (1956) found that birds receiving electric shock bled slower than those not shocked. When sufficient time was allowed for bleeding to stop, no significant differences were observed between those shocked and those not shocked. Goodwin et al. (1961) noted that humane slaughter treatments resulted in an increased shear value for the thigh muscle, but not the breast muscle. May and Hamdy (1966) calculated the economic losses to a poultry processing plant resulting from downgrading of eviscerated carcasses due to handling procedures. Wilson and Brunson (1968) found that electrical stunning produced the most severe hemorrhaging regardless of method of handling. Immobilization of broilers with carbon dioxide following gentle handling produced the lowest amount of hemorrhaging.

Tretsven et al. (1972), working with trout and salmon, found that stunning by electrical narcosis caused tiny hemorrhages in the flesh of 45% of the fish and resulted in blood discoloration in fillets. Blood retained in the unbled control samples also caused discoloration in the fillets and poor product quality.

It has been observed that catfish struggle during slaughter unless immobilized. Immobilization also enables more efficient and economic handling of the fish while in the initial phases of processing. Stunning catfish with an electric shock not only causes immobilization, but when a-c current is used, it has been reported to break the spine (Spencer, 1967). Investigations of the problem have been scarce.

In this study five methods of catfish slaughtering were compared with regard to their effects on the quality of frozen and stored product.

MATERIALS & METHODS

CHANNEL CATFISH (*Ictalurus punctatus* Rafinisque) were grown in tank culture (Andrews et al., 1971) to about 1½ lb live weight, under controlled conditions at Skidaway Institute of Oceanography, Savannah, Ga. They

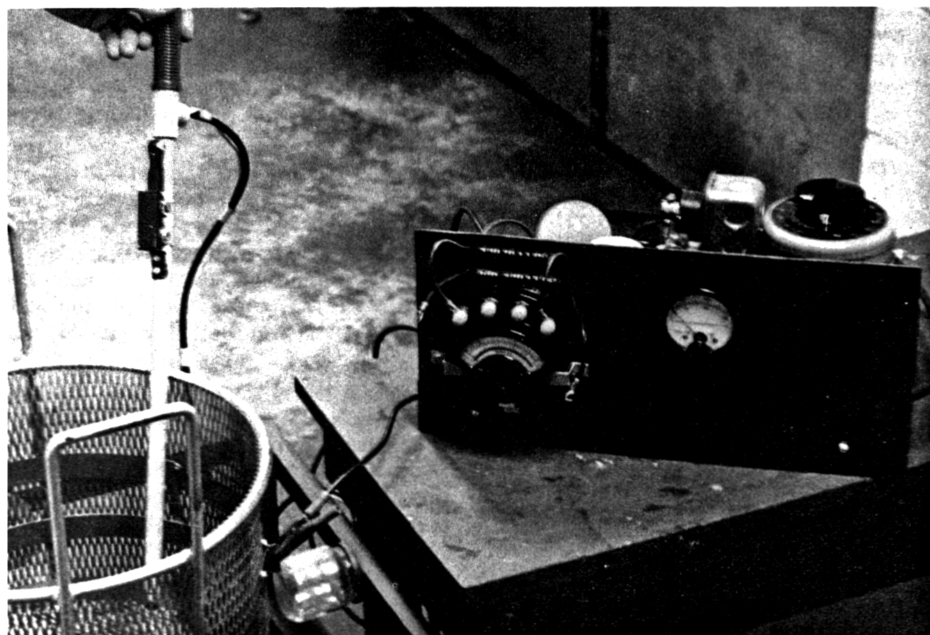

Fig. 1—Electric stunning device with basket and stunning shaft with micro-switch on the left and electronic gear on the right.

Table 1—Effect of storage time on weight loss and firmness of raw and cooked channel catfish[a]

	Wt. loss		Firmness	
Storage time	Thawed (%)	Cooked (%)	Raw (kg/g)	Cooked (kg/g)
Initial	1.45	20.52[a]	4.39[a]	2.69[a]
2 months	1.37	17.60[b]	6.30[b]	3.21[b]

[a] Values within a column followed by the same letter or letters do not differ significantly at the 5% level. Means not tested had insignificant F-value in ANOV.

Table 2—Effect of method of killing and storage on the Gardner "L" value of the raw fillets of channel catfish[a]

	Method of slaughter				
Storage time	Bleeding "L"	CO_2 "L"	a-c Shock "L"	d-c Shock "L"	Ice pack "L"
Initial	43.33[a]	39.47[a]	39.15[a]	40.72[a]	41.88[a]
2 months	43.90[a]	48.33[b]	41.88[a]	43.00[a]	41.15[a]

[a] Values followed by the same letter do not differ significantly at the 5% level.

were transferred live in aerated tanks to the Dept. of Food Science Lab. at Experiment, Ga., where the fish were slaughtered, dressed, frozen, stored and evaluated.

The following methods of slaughtering were included: (1) a-c electrical shock; (2) d-c electrical shock; (3) CO_2 immobilization; (4) gill and tail bleeding; and (5) ice immobilization.

Stunning procedures

Electric stunning was accomplished by a special device illustrated in Figure 1. The device was necessary for the comparisons included in the study. Because of its unique character and possible application in other investigations, a diagram of its construction is given in Figure 2. The laboratory stunner included a powerstat to control the amount of voltage. The current was controlled through a ballast resistor of 300 watts. A television transformer at 400 volts, rectified with a diode and a filter condenser was used for d-c current. The input voltage was controlled by a powerstat. The choice of a-c or d-c current was accomplished with three jacks, one being the ground and the other two being either a-c or d-c.

CO_2 immobilization was accomplished by immersing the fish in CO_2-saturated water for 5 min. The fish appeared to be immobilized in less than 3 min.

Bleeding

In the bleeding, a gill and tail cut served to immobilize the fish.

Ice immobilization was accomplished by covering the fish with crushed ice and holding for 3 hr at 1.7°C. After immobilization, the fish were hand-skinned, eviscerated, decapitated at the collar bone, washed and drained. Six fish were used for each stunning method. Each lot of fish was then packaged under atmospheric conditions in separate plastic bags and frozen at −23.3°C. They were stored at −20.6°C and evaluated after 4 days and after 2 months.

Quality evaluations

Evaluations included per cent weight loss during thawing and cooking, color difference measurements, shear press firmness, and subjective panel ratings on the raw and cooked fish (Boggess et al., 1971). For the initial evaluations, three fish were selected at random from each slaughtering treatment after 4 days of freezer storage. The remaining fish were held at −18°C for 2 months and evaluated in a similar manner.

Thawing. Upon completion of the designated storage period, each fish was weighed in the frozen condition. The fish were then thawed in the plastic bags in running water. After thawing, they were removed from the plastic bags, the excess free water blotted from the fish surface, and reweighed. Weight losses during thawing were recorded.

Color difference. A Gardner color difference meter model C-4(L) was used for measuring the color differences of the raw and cooked samples. A 1-in. square section was taken from each side similar to the 1/4-in. plug used for texture evaluations. This 1-in. fillet was sliced horizontally and the color differences between internal surfaces were read. Readings from each side were used as duplicates for each sample. The meter was set against a standard tile with the following values: L 76.6; A −1.1; b +24.2.

Organoleptic evaluation. The thawed fish was placed in white trays and a panel of five judges, all experienced in differentiating parameters of fish quality, rated each lot of the raw fish for appearance, color and aroma, using a hedonic scale of 1 (below fair) to 9 (very good).

The samples were cooked in heat-resistant plastic cooking bags at 177°C for 25 min or until fish would flake with a fork.

The cooked fish were placed in white trays and the same five judges rated each fish on appearance, color, aroma, texture and flavor of the baked product. Baking was selected since it had less effect on masking the quality differences than deep fat frying. The same 9-point hedonic scale was employed as for raw fish.

Shear press firmness. A 1/4-in. cylindrical plug was removed from behind the collar bone on the left side of the raw carcass and a similar plug from the right side of the cooked carcass for shear press firmness measurement. A Food Technology Corp. Texture Test System Model TP-1 with a continuous chart recorder was used throughout. The instrument was equipped with a 300-lb transducer ring and operated at a downstroke time of 30 sec and a range setting

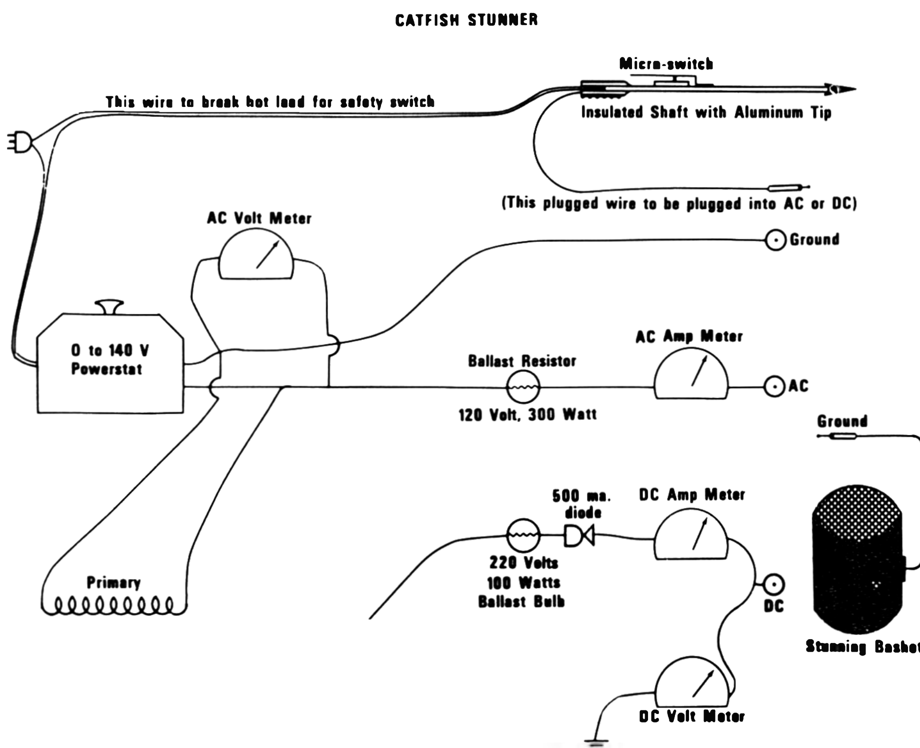

Fig. 2—Diagram of electric stunning device developed for catfish slaughtering investigations.

of 20. The shear values were calculated as g of force per g of fish tissue.

Statistical analyses. Statistical technique was analysis of variance (ANOV) with Duncan's multiple range test (DMRT) applied to determine differences in appropriate means at the 5% level. Sources of variability analyzed were method of killing and storage time. The method of killing × storage time interaction was also included as a source of variability to determine if the two factors were acting independently of each other.

RESULTS & DISCUSSION

THE FOLLOWING observations were made on the fish from the five methods of stunning. The bled unstunned fish appeared to skin the easiest, and also had less blood in the tissue after dressing. The fish stunned with CO_2 were rated slightly under the bled fish for appearance and ease of skinning. When d-c current was applied the fish developed tonic spasms and did not bleed as well as fish slaughtered by the other two methods of stunning. The a-c current made the fish have convulsions, confirming the possibility of spinal fracture (Spencer, 1967). These fish also bled poorly when compared with the first two methods of stunning. Ice-packing was the poorest method of stunning in that the fish were hard to skin, bled very poorly and their gall bladders were distended, making it almost impossible to remove the heads without rupturing this organ.

Statistical analyses of the data showed that in general, the interaction was insignificant at the 5% level for all variables studied, indicating that the method of killing and storage time acted independently of each other relative to the quality of channel catfish. Data for the thaw and cooking losses and firmness for raw and cooked channel catfish at each of the two storage times are presented in Table 1. None of these four measurements was significantly affected by method of killing. Also, thawed loss was not related to storage time. However, mean values for cooked loss, and for raw and cooked firmness were all significantly different for the two levels of storage time analyzed.

Table 2 summarizes the results for the Gardner "L" values (% total color reflectance). The interaction was not significant for four of the five methods tested, indicating that method of killing and storage time do not vary independently relative to the Gardner L value. As noted by Duncan's multiple range test of interaction means, the significant interaction was due to the failure of CO_2 to affect the Gardner L value over storage time in the same manner as the other four methods of killing.

Results for the Gardner *a* (redness), *b* (yellowness) and *a/b* ratio are summarized in Table 3. There was no significant interaction for any of these three measurements. Consequently, it was concluded that method of killing and storage time acted independently for these three variables. None of these three measurements was significantly related to method of killing. Also, the Gardner *b* value (yellowness) was not significantly affected by storage time. The Gardner *a* value (redness) was significantly related to storage time as indicated by a change from pink to gray color. Also differences in the *a/b* ratio for the initial and 2 months were highly significant, indicating a change in the dominant wavelength during storage. There were no significant differences in the Gardner values after cooking.

Mean organoleptic scores for appearance, color and aroma of the raw thawed catfish subjected to the five slaughter treatments are presented in Table 4. Again, the method of killing × storage time interaction term was not significant. Also, none of the variables was significantly related to storage time. Aroma ratings were not significantly affected by the method of killing. Ratings for raw appearance and color were lower for the a-c stunning treatment than for the other methods of slaughter.

In Tables 5 and 6, the mean organoleptic values are given for the baked channel catfish. The interactions were not signifi-

Table 3–Effect of storage time on the Gardner a, b and a/b ratio value of the raw fillet of channel catfish[a]

Storage time	Gardner values		
	a	b	a/b
Initial	2.29[a]	4.32	0.523[a]
2 months	1.33[b]	4.67	0.180[b]

[a] Values within a column followed by the same letter or letters do not differ significantly at the 5% level. Means not tested had insignificant F-value in ANOV.

Table 4–Effect of killing methods on the organoleptic ratings for appearance, color and aroma of whole raw fish evaluated by a panel of five experienced judges[a]

Method of killing	Appearance	Color	Aroma
Bleeding	7.17[a]	7.10[a]	7.17
CO_2	7.60[a]	7.40[a]	7.43
a-c Shock	6.57[b]	6.20[b]	6.93
d-c Shock	7.33[a]	7.00[a]	7.03
Ice	6.80[a]	6.63[a]	7.13

[a] Values within a column followed by the same letter or letters do not differ significantly at the 5% level. Means not tested had insignificant F-value in ANOV.

Table 5–Effect of killing methods on the organoleptic quality ratings of whole baked channel catfish evaluated by a panel of 5 experienced judges[a]

Killing methods	Appearance 9-1	Color 9-1	Aroma 9-1	Texture 9-1	Flavor 9-1
Bleeding	6.67[a]	6.90[ab]	6.83	7.93	7.23[a]
CO_2 Gas	7.07[a]	7.20[a]	7.33	7.93	7.17[a]
a-c Shock	6.00[b]	5.40[c]	6.60	7.67	6.37[ab]
d-c Shock	6.37[a]	6.17[bc]	6.87	7.30	6.07[b]
Ice	6.03[b]	5.87[c]	7.00	7.50	6.33[ab]

[a] Values within a column followed by the same letter or letters do not differ significantly at the 5% level. Means not tested had insignificant F-value in ANOV.

Table 6–Effect of storage time on the organoleptic qualities of whole baked channel catfish evaluated by a panel of five experienced judges[a]

Storage time	Appearance 9-1	Color 9-1	Aroma 9-1	Texture 9-1	Flavor 9-1
Initial	6.25	6.40	7.27[b]	7.64	6.95[b]
2 months	6.60	6.21	6.59[a]	7.69	6.32[a]

[a] Values within a column followed by the same letter or letters do not differ significantly at the 5% level. Means not tested had insignificant F-value in ANOV.

cant for any of the factors analyzed. Appearance and color were significantly related to method of killing but not storage time. Aroma was found to be related to storage time but not to method of killing. Texture was not significantly related to either storage time or method of killing. Flavor was found to be related to both method of killing and storage time. Fish stunned with d-c current were rated significantly lower than for the other slaughter methods. In general, immobilization by gas (CO_2) appears to cause less hemorrhaging and the blood was more completely removed in the dressing operation. Although appearance, color and texture were not affected by storage, aroma and flavor ratings were substantially lower after 2 months of storage. Poor bleeding of the fish appeared to be a factor in reducing the storage quality of frozen channel catfish.

Immobilization by gas and bleeding (unstunned) produced the best appearing product. The a-c shock produced the poorest and the d-c and ice-pack methods yielded an acceptable product. The completeness of bleeding appeared to affect appearance and color of the raw product. Ice-packed fish were harder to skin than those stunned by other methods.

After frozen-storage for 2 months, appearance, color and texture were not significantly affected by the stunning methods studied. But aroma and flavor were lowered, and these were associated with poor bleeding of the fish.

It is noteworthy that the two methods producing the highest quality product are not normally employed as immobilization techniques by catfish processors. This was not anticipated at the outset of the investigation when a unique laboratory stunning device was developed for the comparisons. However, the device enabled more complete control of the treatments and hence more valid comparisons. The improved product quality provided by bleeding or CO_2 gas immobilization over that of electrical stunning or icing suggests that more serious consideration be given to the economics and adoption of revised slaughtering techniques for catfish.

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QUALITY COMPARISONS OF ALBINO AND REGULAR (GRAY) CHANNEL CATFISH

INTRODUCTION

INTENSIVE CATFISH culture in the South expanded rapidly in the 1960's. Initially, catfish were produced exclusively in open ponds. Then, about 1970, interest in high-density production in raceways increased. Other methods, including cage and tank culture, are being investigated by researchers and producers (Madewell, 1971).

Catfish fingerlings in the 6 or 7-in. groups grown in any of these culture systems during the warm season growing period of 6 months will average 1 lb, but may range from 0.25–2.00 lb. The small fish may be sold or held for further growth. Since present processing methods require that fish be handled singularly, the processing cost is higher for the small fish. Processed catfish are generally sold as small, medium, or large with no special weight designation given for categories nor price differences charged for various sizes (Anon., 1972). Although the information available on the size preference of catfish by consumers is limited, it is generally recognized that some restaurants serve small fish and others medium or large ones. However, specific information is lacking.

Wide seasonal variations exist in production and processing of catfish with 78% of the processing occurring during the 6-month period of October through March. Since a regular and consistent supply of catfish should benefit both the growers and processors, there is a need for information on the comparative processing characteristics and qualities of various sizes of fish. Heaton et al. (1970) reported a processing evaluation of tank-cultured channel catfish. With the expansion of high-density culture and increased interest in albino fish, more information on comparative quality of regular and albino channel catfish is needed.

The purpose of this study was to evaluate the yield and quality of cage-cultured albino and regular channel catfish of varying sizes.

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Table 1—Effect of strain and weight of channel catfish on dressing percentage and waste

		Harvest dates					Restocked fish
		12-9-70	7-8-71	7-22-71	8-18-71	Mean	10-22-71
Live wt	albino	0.23	0.50	0.68	0.99	0.60	1.07
(lb)	regular	0.27	0.62	0.70	1.10	0.67	0.83
Dress-out	albino	57.3	59.7	58.9	59.8	58.93	60.0
(%)	regular	58.2	59.6	59.6	60.0	59.35	60.9
Head	albino	22.0	18.9	18.2	19.2	19.58	21.1
(%)	regular	21.7	19.3	18.4	18.7	19.53	20.6
Skin	albino	7.4	5.9	5.9	6.0	6.30	6.1
(%)	regular	7.6	6.0	5.8	6.3	6.43	6.0
Viscera	albino	9.0	8.3	7.9	6.5	7.93	5.8
(%)	regular	8.3	7.8	7.5	6.9	7.63	5.1
Visceral Fat	albino	2.8	5.5	7.5	7.5	5.83	6.5
(%)	regular	3.4	5.8	7.9	7.1	6.05	6.9
Misc	albino	1.5	1.7	1.6	1.0	1.45	0.5
(%)	regular	0.8	1.5	0.8	1.0	1.03	0.5

MATERIALS & METHODS

THE CATFISH utilized in this study were grown in cage culture experiments at the Coastal Plain Station at Tifton, Ga. Fish were harvested when they had attained mean live weights of approximately 0.25, 0.50, 0.75 and 1.00 lb. Those fish weighing less than 0.75 lb at the last harvest were restocked in cages and harvested after additional growth. The fish were transported live to the Food Science Laboratory at the Georgia Station and, after holding for 18 hr, were stunned by electric shock, skinned and eviscerated by hand. Eight fish of each weight were used for evaluations of dress-out factors. Four of these were selected at random for sensory evaluations, and the remaining four were used for chemical analyses.

Dress-out characteristics

Dressing percent values were determined by weighing each fish immediately after stunning and again after dressing. The waste from each fish was separated into head, viscera, visceral fat, skin and miscellaneous (blood, juices, etc.) categories, weighed, and reported as a percentage of the total fish weight.

Regression analyses were used to estimate the linear relationship between the observed dress-out factors and live weight. Regression equations calculated from the dress-out data were used to evaluate changes associated with fish size and date of harvest.

Chemical analyses

Moisture and fat were determined on raw and cooked flesh according to AOAC methods (1960). Four raw fish from each species at each harvest date were analyzed for fatty acid composition. Each fish was analyzed in duplicate essentially by procedures described previously by Worthington et al. (1972) with the exception that the samples consisted entirely of loin muscle tissues from the back area. The fatty acid data were subjected to an analysis of variance to test for significance of differences between strains of fish and differences related to time of harvest.

Texture measurement

One side of each cooked fish was used for texture measurements. Duplicate samples of approximately 10g were taken from the side of the fish beginning at the dorsal fin and extending back toward the tail. Texture measurements were made using a Food Technology Corp. recording shear Texture Press Model TP-1 according to a procedure described previously (Heaton et al., 1972).

Organoleptic evaluations

The dressed carcasses were held at 0°C for 24 hr. Each carcass was wrapped individually in aluminum foil and baked in an oven at 205°C until fully cooked, approximately 77°C internal. A six-member panel experienced in judging parameters of fish quality evaluated the car-

casses. Sensory ratings for appearance, color, aroma, texture and flavor were performed using a 9-point hedonic scale (9 = excellent to 1 = extremely poor). Four albino and four regular catfish were scored at each session.

RESULTS & DISCUSSION

THERE WERE pronounced differences in the appearance and color of the two types of dressed catfish. The dermal membranes and flesh of the skinned albino fish were almost white whereas the regular fish had typical gray membranes and light gray flesh.

Dress-out

Data on edible carcass, head, viscera, visceral fat pad, skin and miscellaneous for albino and regular catfish are presented in Table 1. Since differences between albino and regular catfish were statistically nonsignificant, the data were combined for the regression analyses.

Only minor differences in dressing percentages were recorded after the fish reached a weight of 0.50 lb. The low yield in the 0.25 lb fish was probably due to the disproportionately smaller edible carcass in the small fish. There was a significant decrease in head, viscera and skin relative to edible carcass as fish size increased.

The proportion of waste components varied with fish size. The percent head decreased until the fish weighed about 0.75 lb with the exception of the restocked fish which had percentage values almost as high as for the 0.25 lb fish. This characteristic was probably associated with delayed growth. The percent skin followed a pattern similar to that of the head. The percent viscera decreased with fish size and, apparently, with age. The relative weight of the visceral fat pad increased rapidly in fish up to 0.75 lb and then decreased gradually. This condition was probably associated with water temperature which was increasing until the fish weighed about 0.75 lb. Andrews and Stickney (1972) reported that total carcass lipids increased as water temperature increased between 18° and 34°C.

Table 2—Effect of size of albino and regular channel catfish on moisture, and total fat of raw and cooked fish

Quality factor		Live weight (lb)				Mean	Restocked fish
	albino	0.23	0.50	0.68	0.99	0.60	1.07
	regular	0.27	0.62	0.70	1.10	0.67	0.83
	average	0.25	0.56	0.69	1.05	0.635	0.95
Moisture[a] (Raw flesh)	albino	–	77.4abc	78.0ab	77.2abc	77.5	76.7bc
	regular	–	76.9abc	78.4a	78.2ab	77.8	76.1c
	average		77.15ab	78.2a	77.7ab	77.68	76.4b
Moisture (Cooked flesh)	albino	–	75.6a	76.2a	73.0b	74.9	72.9b
	regular	–	76.0a	76.3a	73.1b	75.1	74.1b
	average		75.8a	76.25a	73.05b	75.0	73.50b
Fat (Raw flesh)	albino	–	22.2ab	21.0ab	23.1a	22.1	20.6ab
	regular	–	20.0ab	21.4ab	20.4ab	20.6	18.0b
	average		21.1	21.2	21.75	21.35	19.3
Fat (Cooked flesh)	albino	–	12.8bc	16.7ab	17.8a	15.77	13.5bc
	regular	–	11.0c	16.2ab	19.1a	15.43	13.7bc
	average		11.9c	16.45ab	18.45a	15.60	13.6bc
Shear Force (Cooked flesh)	albino	0.846de	0.573e	0.702e	1.483ab	0.901	1.593a
	regular	0.719e	0.655e	0.625e	1.260bc	0.815	1.113cd
	average	0.7825b	0.614b	0.6635b	1.372a	0.858	1.353a

[a] Values within each quality factor having unlike letters are significant at the 5% level.

Moisture and fat

Data showing the effect of size of the catfish on moisture and on total fat of both raw and cooked fish are presented in Table 2. Analyses of the raw fish showed significantly more moisture in the 0.75 lb size than in the restocked fish. Moisture in the cooked flesh was significantly lower in the 1.00 lb and the restocked fish. It is uncertain whether these results were caused by fish size or some other factor.

Mean total fat in the raw fish was 20.8% (D.W.) and ranged from 18.0–23.1%. There was apparently no significant relationship between total fat of the raw fish and fish size. Total fat in the cooked fish increased significantly with size. It is uncertain whether this increase in fat was due entirely to size or whether it resulted from the increasing environmental temperature as in the study by Andrews and Stickney (1972).

Table 3—Effect of strain and size of channel catfish on sensory scores

Quality factor		Live weight (lb)				Mean	Restocked fish
	albino	0.23	0.50	0.68	0.99	0.60	1.07
	regular	0.27	0.62	0.70	1.10	0.67	0.83
	average	0.25	0.56	0.69	1.05	0.635	0.95
Appearance[a]	albino	7.8bc	8.5a	8.1ab	7.1de	7.88	7.3cd
	regular	6.5e	7.8bc	7.7bc	6.8de	7.20	7.3cd
	average	7.15c	8.15a	7.90ab	6.95c	7.54	7.3bc
Color	albino	7.9ab	8.2a	8.1a	7.1cdef	7.83	6.9ef
	regular	6.4f	7.6abcd	7.7abc	6.9def	7.15	7.3bcde
	average	7.15b	7.90a	7.90a	7.00b	7.49	7.10b
Aroma	albino	7.3ab	7.5ab	7.5ab	6.0d	7.08	6.9bc
	regular	7.0bc	8.1a	8.0a	6.9bc	7.50	6.5cd
	average	7.15ab	7.80a	7.75a	6.45b	7.29	6.70ab
Texture	albino	7.3cd	8.0a	7.0de	6.7e	7.25	7.5bc
	regular	7.6b	8.3a	7.3bcd	7.3bcd	7.63	7.6b
	average	7.45bc	8.15a	7.15cd	7.00d	7.44	7.55b
Flavor	albino	6.8b	7.2b	6.6bc	6.1c	6.68	6.6bc
	regular	6.7bc	8.1a	6.7b	6.7bc	7.05	6.7b
	average	6.75b	7.65a	6.65b	6.40b	6.87	6.65b

[a] Values in the various quality factors having unlike letters are significant at the 5% level.

Organoleptic

Mean values of sensory data for albino and regular channel catfish from the four size categories and the restocked fish are presented in Table 3. The score panel rated the 0.50–0.75 lb fish highest in both strains of fish. Albino fish were rated higher than regular fish for all samples except the restocked fish which were rated equal. This resulted from the whiter

Table 4–Percent fatty acid composition of muscle tissues from albino and regular channel catfish as affected by harvest date

	Albino Harvest date					Regular Harvest date				
Fatty acid	7/8	7/22	8/18	10/22	Mean	7/8	7/22	8/18	10/22	Mean
14:0[a]	1.45	1.52	1.34	1.21	1.38	1.34	1.46	1.27	1.24	1.33
15:0	1.13	1.17	0.94	1.23	1.12	1.24	1.11	1.23	1.32	1.22
16:0[a]	20.66	20.86	19.35	18.72	19.90	20.89	20.40	20.01	19.23	20.13
16:1ω7	3.83	3.68	3.26	3.05	3.45	3.31	3.65	3.22	3.05	3.31
17:1[b]	1.94	1.87	1.87	2.28	1.99	2.20	2.01	2.21	2.36	2.21
18:0	7.11	7.24	7.03	7.28	7.17	7.86	7.27	7.76	7.41	7.58
18:1ω9	33.76	34.15	34.67	29.83	33.11	31.82	34.90	33.54	31.23	32.87
18:2ω6	6.30	6.24	6.33	6.71	6.40	5.90	6.30	6.10	6.73	6.26
20:1ω9+ 18:3ω3[a]	1.32	1.26	1.24	1.48	1.32	1.22	1.29	1.21	1.29	1.25
20:4ω6	1.49	1.46	1.32	1.53	1.45	1.64	1.40	1.37	1.50	1.48
20:5ω3[a]	3.98	4.09	4.32	5.58	4.50	4.53	4.09	4.45	5.29	4.59
22:5ω3	1.68	1.72	1.79	2.13	1.83	1.76	1.67	1.75	1.92	1.78
22:6ω3[a]	11.76	11.11	11.92	14.66	12.36	12.33	10.87	11.95	13.41	12.14

[a] Effect of time of harvest significant at 5% level
[b] Tentative identification

and brighter flesh of the albino fish. The differences were statistically significant in the 0.25 and 0.50 lb fish, but in the other size categories the differences became smaller and were not significant.

Color ratings indicated a preference for the albino fish in every group except the restocked fish. The aroma scores were higher for the 0.50–0.75 lb fish with a slight preference for regular fish over albino. A strong odor described as "musty" or "muddy" was noted in the 1.00 lb fish harvested in August and also in the restocked fish which were harvested in October.

The pond in which the catfish were grown was not heavily fed, but was fertilized on a regular basis with superphosphate to enhance the largemouth bass-bluegill population. The organic wastes from the caged catfish plus the inorganic fertilization resulted in dense plankton blooms which may have been responsible for the off-flavor of the larger catfish. The flavor ratings for fish larger than 0.50 lb were lowered because of a musty flavor and those for the 0.25 lb fish were lowered because of weak flavor. Lovell (1971) reported that an objectionable earthy-musty flavor is frequently found in intensively cultured catfish.

Texture ratings showed significantly higher values for the 0.50 lb fish with very little difference between albino and regular catfish. Mean shear press values on cooked fish were significantly higher for both the larger and restocked fish. A sharp increase occurred between the 0.75 and 1.00 lb fish and also in the restocked fish but none of the other data reflected such marked changes. Moisture differences may have influenced shear press values.

The albino and regular channel catfish differed significantly in only one fatty acid, tentatively identified as heptadecenoic (17:1). This fatty acid was present in the diet at a level of approximately 1% of the total fatty acids. Significant differences related to time of harvest were observed for several of the fatty acids (Table 4); however, the magnitudes of the differences were small. The high levels of 20:5ω3 and 22:6ω3 found in the muscle tissues, 4.5% and 12.2% of total fatty acids, respectively, were a reflection of high dietary levels of these acids.

The preference shown for the albino catfish strongly suggests this variety be considered as a means of expanding the market for channel catfish. These results showed that fish of the 0.50–0.75 lb (live weight) size were easy to process and were preferred for serving as whole fish. Smaller fish were more tender and difficult to skin. Larger fish (1.00–1.50 lb live weight) were easier to process, and were considered desirable for baking or broiling rather than frying. Some restaurant operators cut larger fish into fillets, steaks, or pieces. As the industry expands and becomes more specialized, specific sizing will be necessary.

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EFFECT OF PACKAGING ON SHELF LIFE OF FROZEN SILVER SALMON STEAKS

INTRODUCTION

SHELF LIFE of frozen seafoods is dependent on several factors: the handling of fish prior to freezing, packaging, freezing rate and storage temperature (Slavin, 1963). Careless practices in any of these processes could induce undesirable physical and chemical changes and render the products unacceptable.

The effect of storage temperature on shelf life of frozen seafoods has been intensively studied by many researchers (Young, 1950; Dyer and Morton, 1956; Peters and McLane, 1959). Their results indicated that the product shelf life could be extended by lowering the storage temperature to −18°C or below. Dyer (1959) pointed out that storage temperature fluctuation to above −18°C caused quality deterioration and shortened the storage life of cod fillets. Palmateer et al. (1960) measured the increase in lipid oxidation caused by temperature fluctuation in frozen rockfish fillets. Temperature fluctuation to −10 to −4°C has been reported by Lentz and Rooke (1960) on frozen fish shipped by road in refrigerated trailers. A survey conducted by Lane (1966) indicated that some frozen seafoods in retailers' freezer cabinets reached −4°C mainly because of overloading of the products in the cabinets. These conditions are undoubtedly detrimental to the products.

Some types of deterioration, such as desiccation and oxidative rancidity, which occur during frozen storage, can be minimized by proper packaging of the products. Stansby (1955) and Yu et al. (1969) reported that vacuum packing frozen fish in tin cans preserved their fresh qualities better than other packaging methods. Moyer (1960) suggested vacuum packaging of fish in low oxygen permeable films to protect the products from desiccation and oxidative changes.

This experiment studies the quality changes in silver salmon steaks during frozen storage and the use of vacuum packaging to minimize these changes. The accelerated quality deterioration induced by storage temperature fluctuation was also demonstrated.

EXPERIMENTAL

20 MEDIUM-SIZED iced silver salmon, 1 day post-extraction, were obtained from a fish plant at Astoria, Oreg. The fish were immediately eviscerated, cleaned and frozen at −27°C overnight. Steaks (3/4 in. thick) were cut from the middle sections of the fish with an electric saw. Each steak was adjusted to weigh 200 ± 10g by trimming off the excess weight from the heavier steaks. The steaks, while still frozen, were packed individually by one of the packaging methods described below.

Sample No. 1, control

Each steak was placed in a 1 mil polyethylene bag and twist-closed with a wire.

Sample No. 2, vacuum packed

The steak was placed in a 4 mil Mylar-polyethylene laminated pouch. (Permeabilities per 100 sq in./24 hr: Water vapor, 0.1g at 37°C, 90% RH; O_2 from air, 1 cc at 1 atm, 25°C) The pouch was sealed under 29 in. vacuum.

Sample No. 3, vacuum packed with added antioxidants

The steaks were packed exactly the same way as Sample No. 2, except that 10 ml of dilute antioxidant solution was added to the pouch before vacuum sealing. The antioxidant solution was added to the fish in such a way that the entire surface of the fish was covered. The antioxidant solution was prepared as follows:

Concentrated antioxidant solution. 3.00g butylated hydroxyanisole (BHA) was dissolved in 54.00g propylene glycol, and 3.00g butylated hydroxytoluene (BHT) was dissolved in 40.00g of warm Tween 20. The two solutions were mixed. This antioxidant preparation is water miscible.

Dilute antioxidant solution. 1.67g concentrated antioxidant solution was diluted to 100 ml with water. 10 ml of this solution added to 200g of fish will add a concentration of 0.005% antioxidants to the fish.

The packaged samples were stored in a freezer-room maintained at −18°C. In order to evaluate the effect of fluctuating storage temperature on quality changes of the fish, half of the packages from each sample, after being stored at −18°C for 3 months, were transferred into a 15 cu ft food freezer. The temperature of the freezer was regulated by a timer which turned off the electricity for 2 hr each day. The freezer temperature rose slowly from −18°C to a maximum of −4.5°C and then returned to −18°C. It took approximately 4 hr to complete the temperature change cycle.

Four packages from each sample, while still frozen in the pouches, were heated in boiling water for 20 min. The fish, with skin and bones removed, were cut into small pieces, mixed and served warm to the taste panel. The taste panel consisted of 18 trained judges. Each sample was rated for texture, juiciness, flavor (specifically, the development of rancid flavor) and overall desirability on a preference ballot with a 9-point hedonic scale. Based on the hedonic scale, an arbitrary value of 5.0 was selected as the division between "acceptable" and "unacceptable." All sensory data were analyzed by analysis of variance at the 5% significance level. The samples were evaluated at 3-month intervals.

The level of oxidative rancidity that developed during frozen storage was estimated by determining the peroxide value (PV) (AOCS, 1946) of fat extracted by the method of Folch et al. (1957) from ground fish sample, including skin. Two packages were randomly selected from each treatment and the PV determined on each sample.

RESULTS & DISCUSSION

THE RESULTS of sensory evaluation of the fish samples stored at constant −18°C and at a fluctuating frozen temperature are shown in Figures 1 and 2 respectively. At −18°C storage temperature, the overall quality of the control sample deteriorated quite rapidly while the vacuum packed samples suffered only slightly.

The taste panels rated the texture of the three samples acceptable at the end of 12 months' storage. The mean scores of the vacuum-packed sample No. 2 were significantly ($P < 0.05$) higher than the control at 9 and 12 months (Fig. 1-A).

The mean juiciness scores of the samples were lowered only slightly during 12 months' storage. The three samples did not vary significantly ($P < 0.05$) in their scores (Fig. 1-B).

The flavor score of the control sample decreased steadily and approached the division line of acceptability at the end of 12 months (Fig. 1-C). The 1 mil polyethylene bag, being oxygen permeable, did not effectively protect the fish from autoxidation.

Samples 2 and 3, vacuum-packed in Mylar pouches, retained the desirable flavor of the fresh fish. High mean scores were given to these samples at the end of 12 months. The mean scores of the two samples did not vary significantly ($P < 0.05$), indicating that vacuum packaging efficiently prevented autoxidation of the fish, with or without the presence of antioxidants.

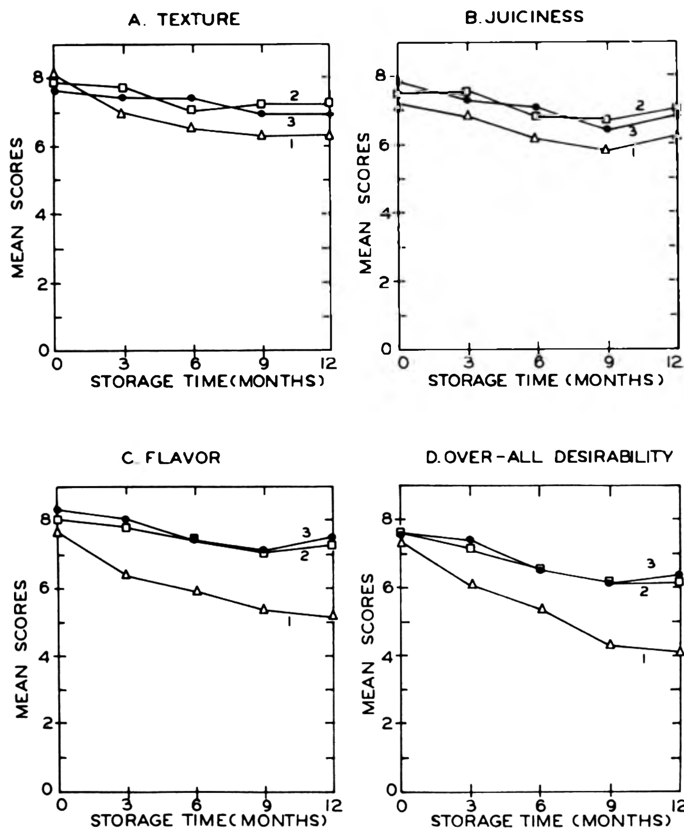

Fig. 1—Sensory evaluation of silver salmon steaks stored at −18°C: 1. Control; 2. Vacuum packed; 3. Vacuum packed with added antioxidants.

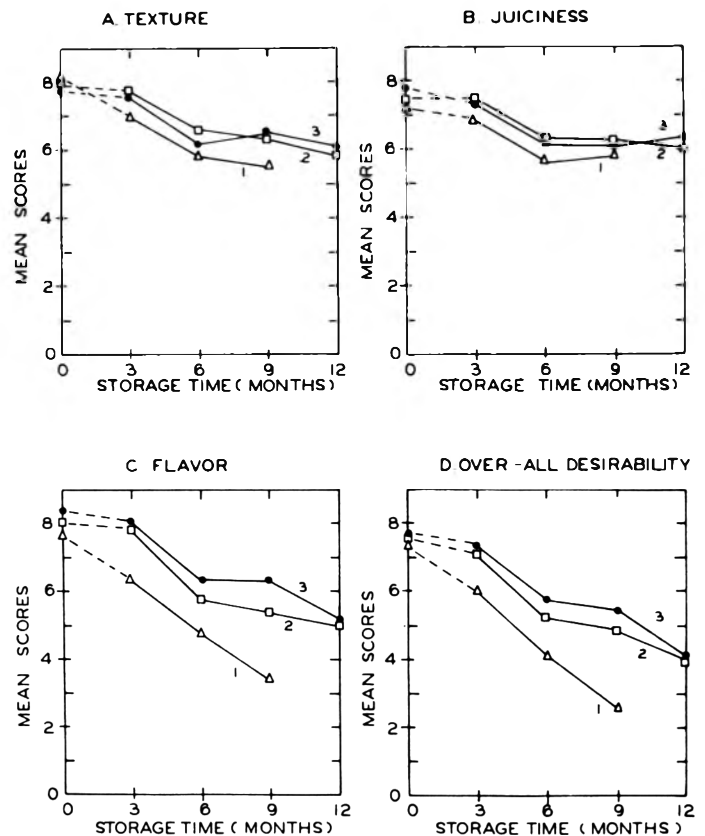

Fig. 2—Sensory evaluation of silver salmon steaks stored at fluctuated temperatures: 1. Control; 2. Vacuum packed; 3. Vacuum packed with added antioxidants.

The overall desirability curves (Fig. 1-D) resembled closely the flavor curves (Fig. 1-C). This suggested that the panels, in rating the overall acceptability, relied heavily on flavor of the fish. The panels had also taken into account the changes in texture and juiciness of the fish. Consequently, the mean overall acceptability scores were consistently lower than the mean flavor scores. The overall quality of the control sample was unacceptable at the end of 9 months' storage, while the two vacuum-packed samples (No. 2 and 3) were judged desirable and of good quality through 12 months' storage.

At constant −18°C storage temperature, the PV of the control sample increased steadily and reached 29 at the end of 12 months' storage (Fig. 3-A). This is in agreement with sensory results. The mean flavor score of this sample also decreased steadily (Fig. 1-C).

The PV of vacuum-packed fish (Sample No. 2) was significantly higher than that of the antioxidant treated fish (Sample No. 3) at 9 and 12 months. The taste panels did not differentiate these two samples in their flavor evaluation. The discrepancy between sensory test and chemical test can be explained by the fact that the fish samples used in the chemical test included skin, while samples served to the panels contained no skin. The skin and the fatty portions immediately next to the skin are generally more susceptible to autoxidation. Removal of the skin would therefore minimize the rancid flavor of the samples.

Sensory results (Fig. 2) showed that storage temperature fluctuation to above −18°C was very damaging to fish in every respect. The mean scores of juiciness and texture for samples stored at fluctuated temperature were lower than respective samples stored at constant −18°C. The most detrimental change induced by temperature fluctuation was the development of rancid off-flavor (Fig. 2-C). The flavor of the control sample became unacceptable after 6 months' storage (3 months' temperature fluctuation). The overall desirability of the control was also unacceptable at the end of 6 months' storage (Fig. 2-D). At the end of 9 months, the rancid flavor of the control became very strong. The storage test of this sample was therefore terminated.

Vacuum packaging was again shown to be effective in slowing down the rate of development of rancid flavor in the samples even under fluctuating storage conditions. The mean flavor score of Sample No. 3 (with antioxidants) was significantly ($p < 0.05$) higher than Sample No. 2 after 9 months' storage (Fig. 2-C). The overall desirability (Fig. 2-D) of Samples No. 2 and No. 3 became unacceptable at the end of 12 months' storage despite the fact that the mean flavor scores of these samples were about 5, which is the division line of desirability (Fig. 2-C). The PV of the control sample was significantly higher than that of the vacuum-packed samples (Fig. 3-B).

There were some chemical as well as physical changes not evaluated by the panels. A large quantity of ice crystals was formed in the packages of the control samples stored at fluctuating frozen temperatures. Only a small quantity of ice crystals was formed in control samples stored at constant −18°C. No ice crystal was found in any of the vacuum-packed samples, including those stored at fluctuating temperatures. The skin-tight vacuum packaging restricted the movement of water vapor.

The orange-red pigments of the control sample faded slightly after 6 months'

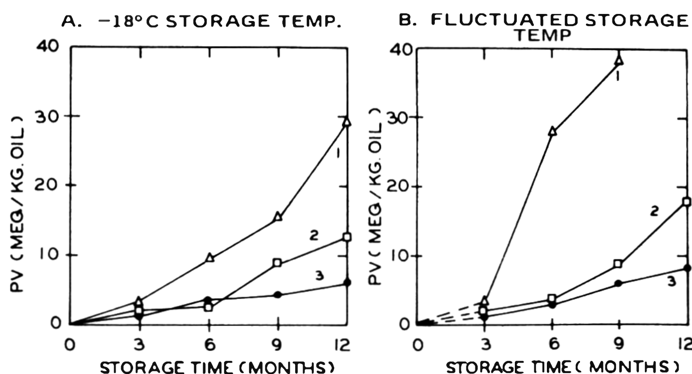

Fig. 3—Peroxide determination on silver salmon steaks stored at −18°C and fluctuating temperatures (Average of analysis of duplicate samples): 1. Control; 2. Vacuum packed; 3. Vacuum packed with added antioxidants.

storage at fluctuated temperature. At the end of 9 months, the color of the fish became yellowish. Vacuum packaging and antioxidant treatment reduced the rate of color fading but did not completely inhibit the color change. No color change was noticed in those samples stored at constant −18°C.

The weight loss of the control and vacuum-packed samples was insignificant through 12-months' storage, at either constant −18°C or at fluctuating temperatures. Despite the formation of ice crystals in the control packages, the polyethylene film appeared to be quite effective in preventing the ice and vapor from escaping.

The results of this experiment demonstrated that the shelf life of frozen silver salmon steaks could be extended substantially by vacuum packaging of the fish in low oxygen permeable film. The quality damage inflicted on the fish by storage temperature fluctuation could also be lessened.

Frequently sport fishermen and housewives store their fish in home freezers for extended periods of time. The two-door, top-freezer refrigerators appeared to be the most popular model used in homes. The air temperature of ten of these freezers was checked. Of the 10 freezers, three maintained a steady −23°C. Four fluctuated from −23° to −12°C; two from −23° to −15°C; and one freezer fluctuated to 7°C. The temperature fluctuation occurred from three to 12 times each 24-hr day, depending upon the brand of the freezer-refrigerator. Fish to be stored for several months in home freezers should be carefully packaged to minimize quality changes.

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PHOSPHOLIPID CHANGES AND LIPID OXIDATION DURING COOKING AND FROZEN STORAGE OF RAW GROUND BEEF

INTRODUCTION

THE PHOSPHOLIPID content of various meats and muscle tissues has been determined (Hornstein et al., 1961, 1967; Turkki and Campbell, 1967; O'Keefe et al., 1968; Terrell et al., 1968). Meat phospholipids are quite susceptible to oxidation (Lea, 1957; Younathan and Watts, 1959, 1960; Watts, 1962). Phospholipid oxidation and degradation causes objectionable changes in meat quality, i.e., off-flavors, off-odors and discoloration, (Lea, 1957, 1962; Watts, 1962; Younathan and Watts, 1959, 1960; Chipault and Hawkins, 1971). Phospholipids play an important role in governing the quality of hamburger meat. Campbell and Turkki (1967) studied the composition of phospholipids in hamburger but changes in the phospholipids of hamburger meat during cooking and frozen storage have not been determined.

Oxidation of meat lipids during cooking has been reported (Younathan and Watts, 1959, 1960; Chang et al., 1961; Keskinel et al., 1964; Greene, 1969; Jakobsson and Bergtsson, 1972), and oxidative changes in the phospholipids of heated bovine muscle were determined by Nakanishi and Suzama (1967). The extent of degradation of phospholipids in ground beef during cooking has not been investigated.

Various workers have shown that tissue lipids are stable during frozen storage (Caldwell et al., 1960; Keskinel et al., 1964; Evans et al., 1967; Witte et al., 1970). However, breakdown of triglycerides and phospholipids by lipases has been observed in frozen muscle tissues held at various temperatures and different storage intervals (Awad et al., 1968; Davidkova and Khan, 1968; Bosund and Ganrot, 1969). Hamburger patties are generally stored at −18°C in commercial and home freezers and the stability of lipids and hamburger quality at this temperature has not been established.

In the present investigation the phospholipid composition of three grades of hamburger meat was determined and the changes occurring in total lipids, phospholipids, TBA values and lipid soluble carbonyls during cooking and frozen storage were quantified. The results are discussed in relation to quality changes that may occur during domestic cooking and frozen storage of raw hamburger meats.

MATERIALS & METHODS

Materials

Fresh ground round, ground chuck and ground beef were obtained from the Dept. of Animal Science at Cornell University. Meat samples were freshly ground in a conventional manner to obtain fat contents representative of each commercial "hamburger" grade.

Patties were wrapped in commercial freezer paper and stored at −18°C. After thawing at 30°C patties were cooked on a metal frying pan, on a teflon-coated Sunbeam electric skillet and they were charcoal broiled on an aluminum foil-covered grill. All cooking progressed until patties were well done.

All solvents used were Mallinckrodt AR grade. Purified n-hexane was prepared according to Ellis et al., (1958). Water for phosphorus analysis was prepared by passing distilled water through an ion exchange column and redistilling in a Corning AG 1B Distillation Unit (Corning Glass Works, Parkersburg, W.Va.).

Phospholipid and fatty acid standards were obtained from Nutritional Biochemicals Corporation, Cleveland, Ohio; Applied Science Laboratories, Inc., State College, Pa.; The Hormel Institute, Austin, Minn.; and Nu-Chek-Prep, Inc., Elysian, Minn.

Analytical methods

The total lipids were extracted from the hamburgers by the method of Folch et al., 1957. The phospholipids were separated by two-dimensional thin-layer chromatography (TLC) (Parsons and Patton, 1967) using solvent systems of chloroform:methanol:water:28% aqueous ammonia (130:70:8:0.5, v/v) and chloroform:methanol:water:acetone:acetic acid (100:20:10:40:10, v/v). Silica Gel HR (E. Merck AG Darmstadt) coatings (375 thickness) were used. Phospholipid spots were located with iodine vapor and ninhydrin spray. The spots were identified by comparison with Rf

Table 1—The distribution of phospholipid classes in three grades of fresh hamburger meat

Phospholipid class	Ground round	Ground chuck	Ground beef
	(% Distribution)[a]		
Lysophosphatidylcholine (LysoPc)	0.60	–	–
Sphingomyelin (SP)	5.32	4.83	6.38
Phosphatidylcholine (Pc)	57.22	55.85	53.40
Phosphatidylinositol (PI)	5.41	6.25	6.60
Phosphatidylserine (Ps)	1.92	3.18	3.67
Phosphatidylethanolamine (Pe)	23.86	24.51	24.92
Cardiolipin (CAR)	5.72	5.38	5.04

[a] Means are values of four determinations.

Table 2—Drip loss, lipid and phospholipid content of three grades of ground beef patties cooked on a teflon skillet

Data	Hamburger grade		
	Ground round	Ground chuck	Ground beef
Initial patty Weight (g)	86.29	58.92	70.27
Total weight Loss (g)[a]	24.84	23.11	23.44
Weight of lipid in collected drip (mg)	0.86	1.55	4.09
Weight of phospholipid in collected drip (μg)[b]	0.91	tr	2.8

[a] Total weight loss represents drip and volatiles
[b] Mean value from a triplicate phosphorous analysis of an aliquot of drip lipids

Table 3—Changes in the total lipid and phospholipids of three grades of hamburger meat during cooking[a]

	Total lipids			Phospholipids			Phosphatidylcholine			Phosphatidylethanolamine			LysoPc[c]			LysoPe[c]		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
Treatment	(g/10g meat)			(mg/g meat)			(mg/g meat)			(mg/g meat)			(10^2 mg/g meat)			(10^2 mg/g meat)		
Fresh[b]	1.038	1.852	2.647	5.46	4.65	3.35	3.12	2.60	1.78	1.30	1.14	0.85	3.0	–	–	–	–	–
Teflon skillet	1.013	1.042	1.491	6.69	4.41	5.45	3.46	2.50	2.83	1.54	0.92	1.39	5.4	2.7	2.5	1.36	0.4	1.4
Metal frying pan	0.797	1.497	1.780	5.08	4.49	4.49	2.78	2.36	2.37	1.26	1.13	1.07	2.4	3.0	2.9	0.61	0.57	–
Charcoal broiled	1.108	1.485	2.029	5.18	2.52	4.45	2.88	1.26	2.34	1.20	0.76	1.08	4.1	1.0	3.4	0.51	0.52	0.72

[a] A = Ground round; B = Ground chuck; C = Ground beef
[b] Mean value of ten determinations
[c] LysoPc = Lysophosphatidylcholine; LysoPe = Lysophosphatidylethanolamine

values of known phospholipid standards. The phospholipids were quantified according to the method of Rouser et al., (1966).

For fatty acid analyses, approximately 6.4 and 3.0 mg of phosphatidylcholine (Pc) and phosphatidylethanolamine (Pe), were separated from total lipids by TLC (Parsons and Patton, 1967) and transferred to vials. The fatty acids of Pc and Pe were transesterified by the method of Glass, (1971).

Fatty acid methyl esters were analyzed using a Perkin-Elmer Mark II Gas Chromatograph (Model F11) equipped with a flame ionization detector and a coiled stainless steel column (1.8m long × 4 mm i.d.) packed with EGSP-Z on 100-120 mesh Gas Chrom Q (Applied Science Laboratories, Inc., State College, Pa.). Analyses were conducted isothermally at a column temperature of 180°C, injection temperature of 225°C and a detector temperature of 230°C. Nitrogen, hydrogen and air flow rates were maintained at 38.5, 38.5 and 300 ml/min, respectively. Chromatographic peaks were identified by comparison of their retention times to those of known standards. Peak areas were measured by triangulation.

TBA analyses were made according to the procedure of Tarladgis et al., (1960). Mean values are reported as mg malonic dialdehyde/kg meat.

The concentration of total carbonyl compounds in patties was measured by a modification of the procedure of Lawrence, (1965). 10g of meat were homogenized with 40 ml of water using a semi-micro jar Waring Blendor combination for 15 sec at low speed. The homogenate was transferred into a glass stoppered 250 ml flask by rinsing with 50 ml of 2,4-dinitrophenylhydrazine (DNPH) reagent (2g 2,4-dinitrophenylhydrazine in 1 liter 2N HCl). After vigouous shaking to give a uniform dispersion, the mixture was allowed to stand for 2 hr permitting the formation of total carbonyl DNP derivatives (2,4-dinitrophenylhydrazones). The DNP hydrazones formed were twice extracted from the mixture using 60 and 40 ml portions of purified N-hexane. The concentration of total carbonyl DNP derivatives was determined by reading the optical density of the hexane extract at 340 mμ in a Beckman DU spectrophotometer. The concentration of total carbonyl DNP derivatives was calculated from the absorbance using a molar extinction coefficient of 22,500 (Schwarz, et al., 1963).

Table 4—Changes in the fatty acids of phosphatidylcholine and phosphatidylethanolamine of ground round beef during cooking

		Phospholipid class			
		Phosphatidylcholine		Phosphatidylethanolamine	
Fatty acid	Carbon no.	Fresh	Teflon cooked	Fresh	Teflon cooked
		(moles %)		(moles %)	
Palmitic	(C16:0)	25.0	28.0	2.9	6.7
Stearic	(C18:0)	11.5	14.5	26.2	28.8
Oleic	(C18:1)	33.7	28.1	16.6	18.4
Linoleic	(C18:2)	22.4	21.8	15.3	17.6
Linolenic	(C18:3)	0.6	0.7	–	–
Arachidonic	(C20:4)	6.8	6.9	39.0	28.5

Table 5—Changes in mean TBA numbers and the concentration of total carbonyl compounds in three grades of hamburger meat upon cooking

Treatment	Hamburger grade	TBA no. Freshly cooked	TBA no. Cooked and stored 36 days at –18°C	Total carbonyls
		(mg malonaldehyde/kg meat)		(μMoles per g meat)
Fresh	Ground round	0.49	–	0.32
	Ground chuck	0.35	–	0.17
	Ground beef	0.53	–	0.45
Telfon skillet	Ground	3.97	5.50	4.98
	Ground chuck	4.41	6.08	0.44
	Ground beef	4.51	5.80	0.43
Metal frying pan	Ground round	4.05	6.17	4.18
	Gound chuck	4.91	7.02	4.30
	Ground beef	4.84	6.88	4.93
Charcoal broiling	Ground round	3.92	5.60	4.12
	Ground chuck	4.21	6.08	4.56
	Ground beef	4.51	5.59	4.24

RESULTS

Compositional studies

The lipid and phospholipid content of fresh hamburger patties varied from grade to grade i.e., ground round, ground chuck and ground beef contained 9.92, 13.87 and 25.60g lipid and 0.54, 0.46 and 0.33g phospholipid per 100g meat respectively. Neutral lipids comprised 94.73–98.72% of the total lipids and reflected the amount of fatty adipose tissue present in the respective samples. The phospho-

Table 6–Changes in total lipids and phospholipids of three grades of hamburger during storage at –18°C[a]

	Total Lipids			Phospholipids			Phosphatidylcholine			Phosphatidylethanolamine			LysoPc		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
	(g/10 gm meat)			(mg/g meat)			(mg/g meat)			(mg/g meat)			(μg/g meat)		
Fresh storage	1.04	1.85	2.65	5.46	4.65	3.35	3.12	2.60	1.78	1.30	1.14	0.85	30.3	–	–
2 wk	0.96	1.96	3.33	3.81	2.95	2.71	2.03	1.57	1.36	0.89	0.69	0.63	37.0	26.4	44.0
4 wk	1.03	1.83	3.16	3.76	3.87	3.49	1.96	2.02	1.85	0.96	0.93	0.84	26.4	27.0	38.0
8 wk	1.03	2.06	2.31	2.59	3.57	3.25	1.32	1.98	1.87	0.63	0.81	0.81	24.4	32.0	43.0
16 wk	1.17	2.63	2.46	3.40	3.78	3.14	2.13	1.98	1.70	0.94	0.88	0.74	37.7	28.8	34.9

[a] A = Ground round; B = Ground chuck; C = Ground beef

lipid content of the meat decreased from grade to grade as the total lipid content increased. Compositionally, the relative amounts of individual phospholipid classes varied slightly between hamburger of different grades (Table 1).

Changes in lipids during cooking

The cooking time required to reach a medium well-done state varied with method of cooking i.e., 7, 22 and 15 min at internal temperatures of 60, 70 and 65°C for teflon skillet, frying pan and charcoal broiling, respectively. The patties decreased in weight during cooking because of drip loss. The percentage diminution in weight upon cooking of ground round, ground chuck and ground beef was 29.2, 29.1 and 33.5 for teflon skillet; 36.6, 32.6 and 41.4 for frying pan, and 32.3, 32.1 and 39.8 for charcoal broiling respectively. Negligible amounts of phospholipids passed into the drip regardless of cooking method and representative data are presented in Table 2. This was consistent with the suggestion that the phospholipids of meat tissues exist in a bound state (Hornstein et al., 1961).

The effects of different cooking methods on the lipids of different hamburgers showed that the cooking loss (i.e., drip) of total lipids was proportional to the amount of fat initially present (Table 3). The ground round lost the least fat while ground beef showed the greatest decrease in fat. Relative increases in total phospholipids occurred in the ground round sample cooked on a teflon skillet and in all ground beef patties. Decreases in phospholipids occurred in the other samples.

Changes in individual phospholipid classes i.e., Pc and Pe were small but closely paralleled the respective increases and decreases in total phospholipids during cooking (Table 3). This is logical since Pc and Pe constituted the bulk of the total phospholipids. The data revealed no preferential degradation of individual phospholipids i.e., both Pc and Pe decreased comparably. Increases in lysophosphatidylcholine (LysoPc) and lysophosphatidylethanolamine (LysoPe) occurred in all hamburger grades upon cooking (Table 3).

Table 7–Changes in mean TBA numbers and the concentration of total carbonyl compounds in three grades of raw hamburger stored at –18°C

Treatment	Hamburger grade	TBA no. (mg malonaldehyde /kg meat)	Total carbonyls (μMoles/g meat)
	Ground round	0.49	0.32
Fresh	Ground chuck	0.35	0.17
	Ground beef	0.53	0.45
	Ground round	0.27	0.74
2 wk	Ground chuck	0.18	0.73
	Ground beef	1.55	1.25
	Ground round	0.38	1.05
4 wk	Ground chuck	0.65	1.38
	Ground beef	1.23	1.56
	Ground round	0.39	1.24
8 wk	Ground chuck	0.66	0.34
	Ground beef	1.50	1.17
	Ground round	0.35	0.64
16 wk	Ground chuck	0.88	–
	Ground beef	1.46	1.56

The relative proportion of unsaturated fatty acids in Pc and Pe decreased during all cooking treatments and representative data are shown in Table 4. Noteworthy was the high content of arachidonic acid in the Pe which decreased by 25% during cooking.

In order to determine if the changes in unsaturated acids were attributable to oxidation, appropriate indices of oxidative degradation were determined. The TBA numbers of all cooked samples markedly exceeded those of fresh meat (Table 5) and TBA values of cooked samples, that were subsequently stored in the frozen state, were greater than those of freshly cooked samples indicating continuing oxidation of lipids during storage.

The carbonyls increased markedly in all cooked samples. The magnitude of carbonyl increases during cooking closely paralleled the corresponding TBA increases in cooked hamburgers. Teflon cooked ground round and ground beef (duplicates) did not demonstrate corresponding increases in measurable carbonyl content.

Changes in lipids during frozen storage of raw patties

The total lipids of the hamburgers remained relatively constant, whereas the phospholipids, especially those of the ground round and ground chuck decreased during frozen storage (Table 6). Phospholid changes were most pronounced after the initial 2 wk of storage. Both Pc and Pe losses apparently accounted for most of the diminution of the PL in all samples except the ground beef where the changes were negligible. Minor increases in LysoPc occurred in all samples whereas LysoPe was not detected.

Analyses of the fatty acids during frozen storage revealed negligible changes in the composition of the Pc. The arachidonic acid content of the Pe decreased by approximately 25% and the other fatty acids fluctuated to a minor extent during storage.

Evidence of oxidation during storage was provided by analyses of TBA and total carbonyls in the stored samples (Table 7). The TBA values behaved differently with each grade of meat, i.e., a negligible change in ground round; a progressive increase in ground chuck after an initial loss, and a marked increase during the first 2 wk in the case of the ground beef. The carbonyl content of all samples increased during initial storage. Both TBA values and carbonyl content were much lower in stored than in cooked samples.

DISCUSSION

THE TOTAL LIPID content of hamburger meat was much higher than the total lipid content of beef muscle reported by Hornstein et al., 1961; O'Keefe et al., 1968; Turkki and Campbell, 1967. This is because beef muscle usually lacks the adipose tissue normally included in hamburger.

The phospholipid content of each fresh hamburger sample was within the range reported for various samples of beef muscle, i.e., 0.43 – 1.00 g/100g (Hornstein et al., 1961, 1967; O'Keefe et al., 1968; Turkki and Campbell, 1967). The phospholipid content of ground chuck agreed closely with the value reported in an earlier study (Campbell and Turkki, 1967). The variability of phospholipid content from grade to grade of hamburger meat paralleled the variation in muscle content indicating that the phospholipids of hamburger are mostly associated with muscle. Many studies have shown that tissue phospholipids are integral parts of cellular membranes and exist in tissue as phospholipoproteins (Hornstein et al., 1961; Watts, 1962; Kinsella, 1972).

The distribution of phospholipid classes in hamburger meat agrees with those reported in porcine, chicken and fish muscle (Davidkova and Khan, 1968; Kuchmak and Dugan, 1963; Peng and Dugan, 1965; Shuster et al., 1964), i.e., a high content of Pc is typical. Complete distributions of phospholipid classes in beef muscle are unreported.

The fatty acid composition of Pc and Pe from fresh ground round differed from the composition of fatty acids from total phospholipids reported in ground chuck (Campbell and Turkki, 1967). The latter study reported a higher content of palmitic acid and a lower amount of arachidonic acid in Pe compared to the present study. The distribution of fatty acids of Pc in this study compares favorably to that of total phospholipids from beef muscle (Hornstein et al., 1967; O'Keefe et al., 1968; Terrell et al., 1968), but the distribution of fatty acids in Pe from hamburger was different. Numerous studies have shown a high content of unsaturated fatty acids in phospholipids from various muscle tissues (Campbell and Turkki, 1967; El-Gharbawi and Dugan, 1965; Giam and Dugan, 1965; Hornstein et al., 1967; O'Keefe et al., 1968). Our present data corroborate these reports and show that the preponderance of arachidonic acid was associated with Pe.

The observed loss of Pc and Pe upon cooking of ground round and ground chuck was not a drip loss since negligible amounts of phospholipid phosphorus were observed in the drip. Hence the losses may be attributed to degradation via oxidation or thermal hydrolysis. The small increase in LysoPc and LysoPe upon cooking suggested a breakdown of Pc and Pe, respectively. Formation of these lysophosphatides may have been caused by phospholipases and/or selective thermal hydrolysis. Total degradation of Pc and Pe may have occurred to a marked extent and it has been postulated that negligible amounts of lysophosphatides occur in cooked meats because of rapid cleavage of both fatty acid moieties (Davidkova and Khan, 1968; Lea, 1962).

With the exception of arachidonic acid, the changes observed in the fatty acids of hamburger after cooking agreed with earlier studies of changes in fatty acids of egg, beef and pork upon cooking (Campbell and Turkki, 1967; Chang and Watts, 1952; Evans et al., 1967; Zipser and Watts, 1961). The decrease in arachidonic acid is consistent with its greater propensity to undergo autoxidation and this is accentuated when it is associated with Pe (Love and Pearson, 1971).

The high TBA values obtained from all samples of cooked hamburger meat was consistent with previous assays of cooked meats (Chang et al., 1961; Greene, 1969; Jakobsson and Bengtsson, 1972; Keskinel et al., 1964; Younathan and Watts, 1959). The marked increases in TBA values of cooked hamburgers were consistent with the observed decreases in phospholipids and particularly the arachidonic acid of Pe, reflecting lipid oxidation during cooking.

The marked increases in TBA numbers of frozen-stored cooked samples indicated that uncured cooked meats should not be stored for prolonged periods. Upon removal of cooked hamburger from frozen storage discoloration and off-odors were perceptible. Tims and Watts (1958) observed similar quality changes in cooked beef and pork stored under similar conditions.

Close agreement between TBA numbers and total carbonyl values confirmed the occurrence of some lipid oxidation during the cooking of hamburger meat. Carbonyls including those of cooked hamburger, contain both flavor and off-flavor compounds depending on their relative and absolute concentrations (Sink and Smith, 1972; Kinsella, 1969). Off-flavor carbonyls were quite apparent in frozen-stored cooked hamburgers which became rancid.

Negligible changes in the total lipids were expected during frozen storage of raw ground beef because the neutral lipids which compose most of the total lipids oxidize very slowly compared to phospholipids (El-Gharbawi and Dugan, 1963; Lea, 1957; Watts, 1962; Younathan and Watts, 1959, 1960). Tissue lipids in general have been shown to be quite stable in the frozen state (Caldwell et al., 1960; Keskinel et al., 1964; Witte et al., 1970).

Lipolysis of phospholipids during frozen storage has been observed in bovine, fish and chicken muscle (Awad et al., 1968; Bosund and Ganrot, 1969; Davidkova and Khan, 1968; Takama et al., 1967). Conceivably the phospholipid decreases noted in this study may have been partly caused by enzymatic hydrolysis. Phospholipases occur in tissues, and release fatty acids from phosphoglycerides. Mammalian tissues contain phospholipases which continuously release fatty acids from phosphoglycerides (McMurray and Magee, 1972).

The decrease in Pc and Pe to a constant level during frozen storage could be explained by the fact that grinding of fresh hamburger increased surface area and liberated bound phospholipids which were then easily oxidized (Castell, 1971; Lea, 1957). Phospholipid breakdown during the frozen storage of meat results in rancidity and browning (Caldwell et al., 1960; Greene, 1971; Lea, 1957; Love and Pearson, 1971).

Previous studies reported negligible changes in the fatty acids of egg and beef during frozen storage (Evans et al., 1967; Keskinel et al., 1964; Terrell et al., 1968) which disagrees with the observations made on the fatty acids of Pc and Pe in this study especially in case of arachidonic acid.

Increases in TBA values during frozen storage indicated that oxidation occurred. This also was in contrast to other studies which showed negligible lipid oxidation in beef at various frozen storage temperatures (Caldwell et al., 1960; Keskinel et al., 1964; Ledward and MacFarlane, 1971; Witte et al., 1970). The progressive increase in TBA values of raw hamburger during frozen storage may be associated with the increased adipose content of each hamburger type. Thus neutral lipids may have been oxidized in frozen stored hamburger.

Since carbonyls are the principal products of autoxidation (Sink and Smith, 1972), increases in total carbonyls indicated that oxidative changes occurred in hamburger patties during frozen storage. In view of the TBA and carbonyl increases in raw hamburger patties during frozen storage, at −18°C, this temperature may not be optimum for prevention of lipid oxidation.

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EFFECTS OF END POINT AND OVEN TEMPERATURES ON BEEF ROASTS COOKED IN OVEN FILM BAGS AND OPEN PANS

INTRODUCTION

OVEN FILM cooking bags are offered to homemakers as a new method of meat cookery. Film bags are marketed under several brand names, and one manufacturer suggests that lower cost, less tender cuts of meat ideally are suited to cooking in film. That agrees with the usual recommendation that moist heat (braising or pot roasting) be used to cook less tender cuts to soften collagenous connective tissue. Manufacturers sometimes imply in advertising that their product will produce a juicier, more flavorful roast in a shorter time than is otherwise possible.

In general, research data have indicated that tender cuts have less weight and nutritive loss, are more palatable and require longer cooking time when cooked by dry heat (roasting, broiling, deep fat frying) than when cooked by moist heat.

Schock et al. (1970) studied effects of two dry heat treatments (oven roasting, deep-fat frying) and two moist heat treatments (oven braising, pressure braising) on beef semimembranosus muscle from top round cooked to an internal temperature of 70°C. They found no significant differences attributable to heat treatment for flavor, tenderness, or overall acceptability. Total moisture and press fluid yield were higher ($P < 0.05$) for oven roasted pieces, whereas, the rate heat penetrated the muscle was slower ($P < 0.05$) and the cooking time was longer ($P < 0.05$) for oven roasting than for any other treatment.

Reid and Harrison (1971) reported that selected histological characteristics of the beef cooked by Schock et al. (1970) did not vary significantly among heat treatments. They suggested that variations among similar data appearing in the literature may be attributable to variations in methods of measurement used; age, maturity or grade of animals; or treatments other than heat treatments.

Ferger et al. (1972) studied effects of: (1) roasting and cooking in oven film on boned and rolled leg of lamb roasts cooked from the frozen state at 163°C to an internal temperature of 75°C, and (2) roasting and cooking in oven film on beef rib roasts cooked from the frozen state at 149°, 163° or 177°C to an internal temperature of 60°C. They concluded that (1) there is little difference between dry and moist heat in cooking time, percentage total cooking losses, flavor, juiciness or tenderness of meat cooked from the frozen state to the same internal temperature; (2) dry heat produces meat that appears less well-done than meat cooked by moist heat to the same internal temperature; and (3) time for cooking beef rib roasts from the frozen state by dry or moist heat is not shortened significantly by increasing the oven temperature above that usually recommended. Both cuts of meat used in the study by Ferger et al. (1972) were tender cuts.

No data were found for cooking a less tender cut of meat from the frozen state by roasting and cooking in oven film. Manufacturers of oven film recommend oven temperatures of 177–205°C. This study was designed to investigate effects on beef top round of roasting and cooking in oven film from the frozen state at two oven temperatures (177° and 205°C) and to three end point (internal) temperatures (60°, 70°, 80°C).

MATERIALS & METHODS

SIX PAIRS of USDA Good Grade whole rounds (30.2–42.3 kg) were purchased from a commercial packing company. Seven days postmortem, the top round was separated, the outside fat covering was removed, and the semimembranosus and adductor muscles were divided into three roasts (Fig. 1), ranging from 708–1666g. Dimensions of roasts, in cm, were: length, 17–26; width, 5–11; and depth, 6–10. At the time of cutting, all roasts were wrapped in oven film bags (nylon 66 with a heat stabilizer), frozen in a blast freezer at −26°C, then stored 3–10 wk in an upright household freezer at an average temperature of −22°C.

At each of 12 evaluation periods, three roasts were cooked from the frozen state, according to the experimental design, by dry or moist heat in a rotary hearth gas oven maintained at 177° or 205°C to end-point temperatures of 60°, 70° or 80°C. The experimental design was a split plot with three replications, with the two oven temperatures the main plots and the type of heat and end-point temperatures (six treatment combinations) the sub plots. For each replication, 12 roasts from two

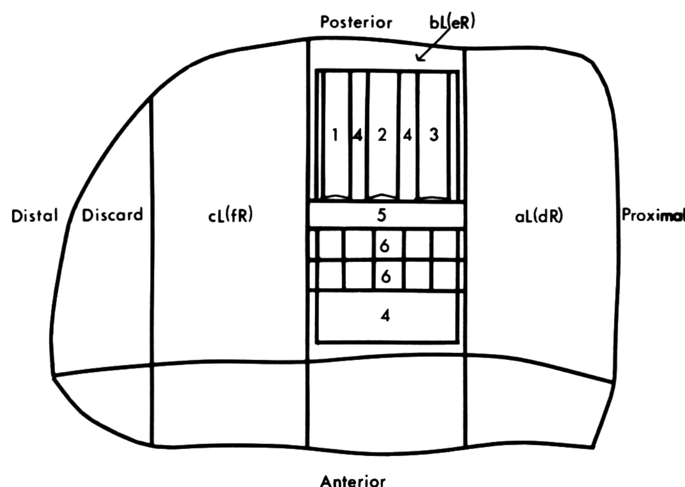

Fig. 1—Plan for division of top round into three roasts and sampling plan for individual roasts. (1) Distal shear core (1.3-cm) and water-holding capacity (WHC); (2) Central shear core (1.3-cm) and water-holding capacity (WHC); (3) Proximal shear core (1.3-cm) and water-holding capacity (WHC); (4) Total moisture and Gardner color-difference; (5) Apparent degree of doneness (Slice, 1.3-cm thick); (6) Palatability samples (1.3-cm cubes); aL, bL, cL—Sections a, b, c of top round, left side of carcass; dR, eR, fR—Sections d, e, f of top round, right side of carcass

pairs of rounds were assigned randomly to the six types of heat and end-point temperature treatment combinations for each oven temperature.

A hole was bored into the center of each roast for a centigrade thermometer. Roasts cooked by dry heat were removed from the oven film bags and placed on a low rack in a shallow roasting pan. Roasts cooked by moist heat remained in the bags and six slits (approximately 3.5 cm long) were made in the top of each bag to allow steam to escape and to prevent the bag from breaking.

Rate of heat penetration, cooking time and losses

Rates heat penetrated the muscle were observed by recording the time, in minutes, required for the internal temperature of each roast to reach 0°C, to increase from 0° to 10°C, and for each 5°C increase between 10°C and the end-point temperature. Total cooking time, in minutes, was recorded and cooking time in min/kg was calculated.

Percentages of total, volatile and dripping losses, based on weight of the frozen roast, were calculated for roasts cooked by dry heat. Percentages of total and dripping losses were calculated for roasts cooked by moist heat. Drippings from all roasts were collected in 250-ml graduated cylinders and allowed to stand 45 min. After the fat had stabilized at the top of the drippings, total volume of drippings and volume of fat were read, and the percentage fat in the drippings was calculated.

Post-oven temperature rise and objective measurements on cooked muscle

The maximum post-oven internal temperature, °C, of each roast, and the time required to reach that temperature was recorded. Roasts cooked by moist heat remained in the oven film bags during that period. Samples for measurements on cooked muscle were taken from roasts at the locations indicated in Figure 1.

Tenderness was measured by shearing three 1.3-cm cores (proximal, central and distal positions) on a Warner-Bratzler shearing apparatus with a 11.4-kg dynamometer. Triplicate measurements were made on each core, and the overall shear value was the average of the three shear cores.

Triplicate measurements for water-holding capacity (WHC) were made by the method of Miller and Harrison (1965) on 0.3-g samples from the center of the cores used for Warner-Bratzler shear values. The ratio of the area of pressed muscle to the area of expressed liquid marked on filter paper on which the sample was pressed was designated as the expressible-liquid index. Values for WHC were obtained by subtracting the expressible-liquid index from 1.0,

Table 1–Means and F-values for effects of type of heat on top round beef roasts cooked from the frozen state[a]

	Type of heat			
Measurement	Dry	Moist	Difference	F-value
Initial weight, kg	1.08	1.16	0.08	2.24ns
Rate of heat penetration, min				
Initial temp to 10°C	58.44	68.39	9.95	11.65*
Min/5°C, 10°C to end point	4.90	3.28	1.62	–
Cooking time				
Total, min	112.39	107.83	4.56	1.79ns
Min/kg	104.88	94.83	10.05	14.39**
Cooking losses, %				
Total	27.69	34.86	7.17	132.85***
Dripping	2.16	19.06	16.90	372.94***
Volume of drippings, ml	7.78	197.78	190.00	123.06***
Lipids in drippings, % of volume	18.14	4.05	14.09	12.43**
Total moisture, %	67.03	63.63	3.40	49.06***
Water-holding capacity	0.80	0.76	0.04	11.70**
Shear value, kg/1.3-cm core	3.4	3.7	0.3	0.51ns
Color-difference, Gardner				
Rd (reflectance)	20.15	20.54	0.39	0.60ns
a+ (redness)	6.05	2.59	3.46	46.06***
b+ (yellowness)	10.50	10.49	0.01	0.00ns
Sensory scores				
Flavor[c]	5.4	5.7	0.3	1.80ns
Juiciness[c]	5.3	4.7	0.6	2.30ns
Tenderness[c]	5.7	5.3	0.4	4.44*
Over-all acceptability[c]	5.3	5.1	0.2	1.75ns
Apparent degree of doneness[d]	2.0	2.6	0.6	16.87***

[a] End point and oven temperature data combined
[b] 1.0–(expressible liquid index); the larger the value, the greater the amount of liquid expressed
[c] 7–(extremely desirable, juicy or tender); 1–(extremely undesirable, dry or tough)
[d] 3–well done; 2–medium done; 1–rare
* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

Table 2–Means, F-values, and LSD's for effects of end-point temperatures on top round beef roasts cooked from the frozen state[a]

	End point temp, °C				
Measurement	60	70	80	F-value	LSD*
Initial weight, kg	1.10	1.12	1.15	0.40ns	–
Rate of heat penetration, min					
Initial temp to 10°C	63.33	62.00	64.92	0.33ns	–
Min/5°C, 10°C to end point	3.62	3.72	3.91	–	–
Cooking time					
Total, min	99.42	107.25	123.67	11.69***	10.68
Min/kg	91.34	98.29	109.94	16.79***	6.76
Cooking losses, %					
Total	27.91	31.59	34.32	35.67***	1.59
Dripping	10.83	12.24	8.76	5.33*	2.24
Volatile	21.04	24.98	30.15	40.53***	2.34
Volume of drippings, ml	101.67	125.83	80.83	2.31ns	–
Lipids in drippings, % of volume	8.85	6.59	17.85	2.96ns	–
Total moisture, %	66.95	65.32	63.72	14.97**	1.23
Water-holding capacity[b]	0.79	0.78	0.77	1.37ns	–
Shear value, kg/1.3-cm core	3.13	3.85	3.71	1.45ns	–
Color-difference, Gardner					
Rd (reflectance)	19.73	19.90	21.41	4.40*	1.30
a+ (redness)	6.30	4.19	2.47	18.78***	1.30
b+ (yellowness)	10.63	10.65	10.19	0.82ns	–
Sensory scores					
Flavor[c]	5.4	5.6	5.6	0.30ns	–
Juiciness[c]	5.2	5.5	4.4	2.85ns	–
Tenderness[c]	5.6	5.4	5.4	0.29ns	–
Over-all acceptability[c]	5.2	5.3	5.0	1.98ns	–
Apparent degree of doneness[d]	2.1	2.1	2.4	2.11ns	–

[a] Dry and moist heat and oven temperature data combined
[b] 1.0–(expressible liquid index); the larger the value, the greater the amount of liquid expressed
[c] 7–(extremely desirable, juicy or tender); 1–(extremely undesirable, dry or tough)
[d] 3–well done; 2–medium done; 1–rare
* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

arbitrarily chosen as the maximum expressible-liquid index. Because the expressible-liquid index is inversely related to the amount of liquid expressed from the sample, the larger the value for WHC, the greater the amount of liquid expressed.

Percentage total moisture was measured by drying 10-g samples of ground muscle in a C.W. Brabender Semi-Automatic Rapid Moisture Tester for 60 min at 121°C.

Color-difference was measured with a Gardner Color Difference Meter standardized using a satin finish ceramic tile with calculated values of: Rd (reflectance), 38.0; a+ (redness), 6.6; and b+ (yellowness), 14.7. Approximately 25g of cooked, ground muscle was packed in a plexiglas cell so that light could not filter through the sample. Duplicate measurements were made for each color-difference factor. After the first measurement, the cell was rotated 90° for the second measurement.

Sensory evaluation

Six experienced panel members evaluated each roast for desirability of flavor, overall acceptability and intensity of juiciness and tenderness using a 7-point scale with 7 most desirable or intense. A slice of muscle covered with a transparent household plastic wrap was placed under a MacBeth Skylight and rated as rare, 1; medium-done, 2; or well-done, 3. Instructions for evaluation were given to panel members during preliminary work. Panel members selected at random 1.3-cm cubes of muscle that were placed in the top of 1-pint enamel double boilers and set over hot water. The entire system was held at a constant low heat (35°C ± 1°C) on an electric hot tray (not more than 30 min) until the samples were evaluated.

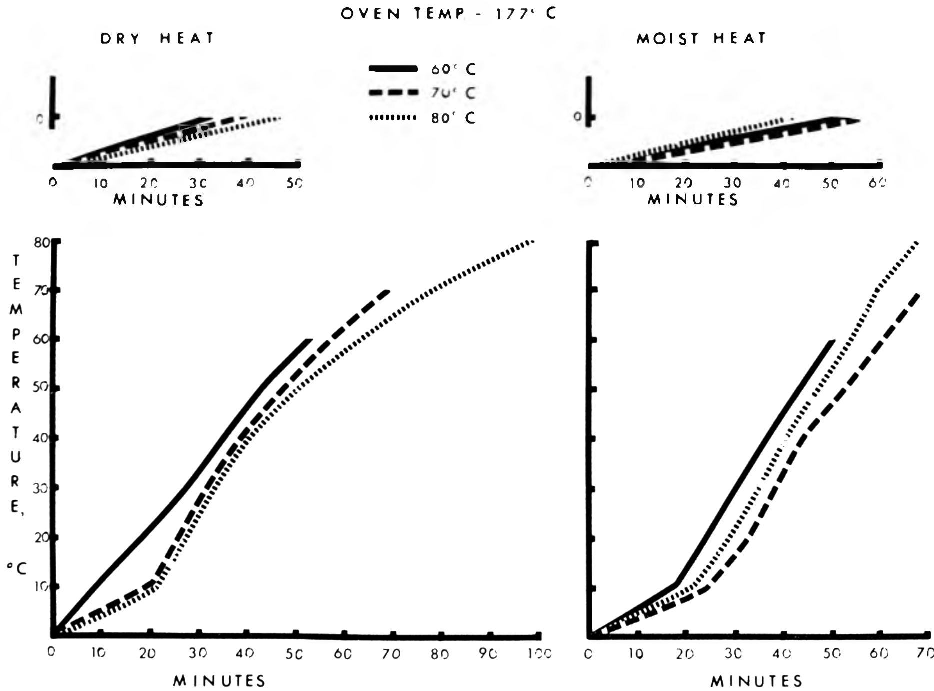

Fig. 2—Rate of heat penetration from initial temperature to 0°C and from 0° to 60°, 70° or 80°C for top round roasts cooked by moist and dry heat at 177°C.

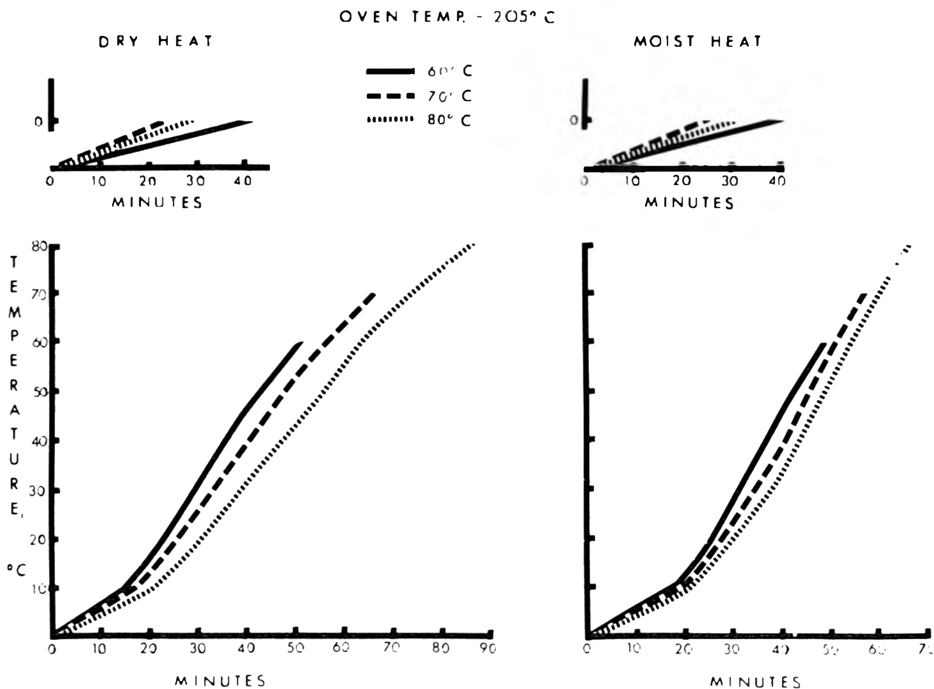

Fig. 3—Rate of heat penetration from initial temperature to 0°C and from 0° to 60°, 70° or 80°C for top round roasts cooked by moist and dry heat at 205°C.

RESULTS & DISCUSSION

Initial weight of roasts, rate of heat penetration

Analysis of variance indicated no significant differences among weights of the roasts assigned to the two types of heat, the three end point temperatures, or the two oven temperatures (Tables 1, 2 and 3).

The time required to raise the internal temperature from the initial temperature to 10°C was greater ($P < 0.05$) for roasts cooked by moist heat than by dry heat (Table 1), but that period of cooking was not affected significantly by end point or oven temperature (Tables 2 and 3).

Details of mean data for rate of heat penetration in Tables 1, 2 and 3 can be observed in Figures 2 and 3. With both moist and dry heat at both oven temperatures, heat penetrated the muscle at a fairly constant rate. At both oven temperatures, the internal temperature of roasts cooked by moist heat increased more rapidly than the internal temperature of those cooked by dry heat. There were no significant differences between dry and moist heat for the time required for each 5°C increase from 15° through 35°C.

Less ($P < 0.05$ to $P < 0.001$) time was required to increase the internal temperature from 35° to 40°C and from 45° through 80°C when cooking by moist heat than by dry heat. The magnitude of difference increased from 0.78 min/5°C at 45°C to 4.83 min/5°C at 80°C. With both dry and moist heat at the two oven temperatures, the average time for a 5°C increase in internal temperature was similar for all three end-point temperatures.

Heat penetrated muscle more rapidly

Table 3–Means and F-values for effects of oven temperatures on top round beef roasts cooked from the frozen state[a]

Measurement	Oven temp, °C		Difference	F-value
	177	205		
Initial weight, kg	1.08	1.16	0.08	0.15ns
Rate of heat penetration, min				
Initial temp to 10°C	67.39	59.44	7.95	0.77ns
Min/5°C, 10°C to end point	4.38	3.77	0.61	–
Cooking time				
Total, min	115.83	104.39	1.44	0.71ns
Min/kg	107.64	92.07	15.57	4.51ns
Cooking losses, %				
Total	31.13	31.39	0.23	0.05ns
Dripping	11.90	9.32	2.58	2.29ns
Volatile	24.10	26.68	2.58	2.09ns
Volume of drippings, ml	111.28	94.28	17.00	0.99ns
Lipids in drippings, % of volume	13.18	9.02	4.16	1.08ns
Total moisture, %	65.26	65.40	0.14	0.09ns
Water-holding capacity[b]	0.77	0.79	0.02	0.87ns
Shear value, kg/1.3-cm core	3.3	3.8	0.5	1.51ns
Color-difference, Gardner				
Rd (reflectance)	21.08	19.61	1.47	1.43ns
a+ (redness)	4.26	4.37	0.11	0.04ns
b+ (yellowness)	10.34	10.65	0.31	0.90ns
Sensory scores				
Flavor[c]	5.5	5.6	0.1	0.51ns
Juiciness[c]	5.1	4.9	0.2	0.45ns
Tenderness[c]	5.5	5.4	0.1	0.32ns
Over-all acceptability[c]	5.2	5.2	0.1	0.15ns
Apparent degree of doneness[d]	2.2	2.3	0.1	0.01ns

[a] Dry and moist heat and end-point temperature data combined
[b] 1.0–(expressible liquid index); the larger the value, the greater the amount of liquid expressed
[c] 7–(extremely desirable, juicy or tender); 1–(extremely undesirable, dry or tough)
[d] 3–well-done; 2–medium-done; 1–rare
* P < 0.05; **P < 0.01; ***P < 0.001

(P < 0.05) between 65° and 70°C and between 75° and 80°C when roasts were cooked at 205°C than when cooked at 177°C. There were no other differences attributable to oven temperature.

Type of heat

Percentages of total and dripping cooking losses and the volume of drippings were less (P < 0.001) for roasts cooked by dry heat than for those cooked by moist heat, whereas percentage total moisture was greater (P < 0.001) in roasts cooked by dry heat. Both Gardner a+ (redness) values and apparent degree-of-doneness scores indicated that roasts cooked by dry heat were redder (P < 0.001; appeared less well-done) than roasts cooked by moist heat (Table 1).

Although, on the basis of min/kg, cooking time was longer (P < 0.01) for dry than for moist heat, the total cooking time (min) was not affected significantly by type of heat (Table 1). Other measurements which type of heat did not affect significantly were: Gardner Rd (reflectance) and b+ (yellowness) values, flavor, juiciness and overall acceptability scores.

Roasts cooked by dry heat were scored more tender (P < 0.05) and juicier than those cooked by moist heat. Although the difference between dry and moist heat was greater for juiciness than for tenderness, the difference between the two types of heat was statistically significant for tenderness, but not for juiciness (Table 1). That may be explained by the mean square error term being four times greater for juiciness than for tenderness. Ferger et al. (1972) also found that at oven temperatures of 149° and 177°C beef rib roasts cooked by dry heat were slightly more tender and juicier than those cooked by moist heat in oven service film.

Total cooking losses were less (P < 0.001) with dry than with moist heat, despite the longer cooking time required with dry heat (Table 1). Similarly, Schock et al. (1970) found that although the cooking time was longer for oven roasted pieces of beef semimembranosus muscle than for oven braised, deep-fat fried or pressure braised pieces, losses from oven roasted pieces were lower (P < 0.05) than losses from any of the other pieces. They also reported that percentage total moisture was higher in oven roasted beef than in oven braised beef. Moreover, Hood (1960) reported that beef triceps brachii roasts cooked by dry heat required longer cooking time, but lost less weight during cooking than those wrapped in heavy aluminum foil during cooking. Dripping losses from roasts cooked by moist heat were greater than those from roasts cooked by dry heat (Hood, 1960).

End-point temperature

Cooking time, both total and min/kg, increased with each 10°C increase in end-point temperature. Differences in cooking time between any two end-point temperatures were significant (P < 0.05) except for the difference in total minutes between 60° and 70°C. Also, percentages of total and volatile cooking losses increased significantly (P < 0.05) with increasing end-point temperature from 60° to 80°C, whereas dripping losses increased slightly between 60° and 70°C, then decreased (P < 0.05) between 70° and 80°C (Table 2).

As expected, percentage total moisture was inversely related to volatile losses. Moisture was greatest in roasts cooked to 60°C, and decreased (P < 0.05) with each succeeding increase in end-point temperature; whereas volatile losses were least for roasts cooked to 60°C and increased (P < 0.05) with each 10°C increase in end-point temperature (Table 2).

Gardner Rd and a+ values indicate that the meat became lighter (Rd values) and lost redness (a+ values) with increase in end-point temperature between 60° and 80°C. Rd (reflectance) values increased (P < 0.05) between 60° and 80°C and between 70°and 80°C, whereas Gardner a+ (redness) values decreased (P < 0.05) between each increasing end-point temperature. Differences in Gardner b+ (yellowness) values attributable to end-point temperature were not significant (Table 2).

The end-point temperature did not affect significantly volume of drippings, percentage lipids in drippings, WHC, or

Table 4–Means, F-values, and LSD's for significant type of heat × end-point temperature interactions

Measurement	Type of heat	End-point temp, °C 60	70	80	F-value	LSD*
Cooking time						
Total, min	Dry	96.17	107.00	134.00	3.89*	15.10
	Moist	102.67	107.50	113.33		
Min/kg	Dry	91.14	104.69	118.80	4.17*	9.57
	Moist	91.54	91.89	101.07		
Cooking losses, %						
Total	Dry	22.98	27.92	32.19	6.79**	2.24
	Moist	32,85	35.27	36.45		
Dripping	Dry	1.84	2.60	2.04	4.08*	3.16
	Moist	19.81	21.88	15.48		
Total moisture, %	Dry	69.25	67.33	64.51	3.68*	1.74
	Moist	64.65	63.32	62.93		
Color-difference, Gardner						
a+ (redness)	Dry	9.69	5.68	2.78	12.32***	1.84
	Moist	2.90	2.70	2.15		
Post-oven temperature rise, °C	Dry	0.3	0.0	0.0	–	–
	Moist	7.8	5.6	0.8		
Sensory scores						
Flavor[a]	Dry	5.0	5.3	5.9	6.15*	0.64
	Moist	5.8	5.8	5.3		
Apparent degree of doneness[b]	Dry	1.6	1.8	2.5	6.07**	0.53
	Moist	2.6	2.7	2.4		

[a] 7–(extremely desirable, juicy or tender); 1–(extremely undesirable, dry or tough)
[b] 3–well-done; 2–medium-done; 1–rare
* P < 0.05; **P < 0.01; ***P < 0.001

Warner-Bratzler shear values. Likewise, none of the sensory measurements was affected significantly by end-point temperatures (Table 2).

Hood et al. (1955) broiled beef biceps femoris steaks to 71.1° and 80°C, and found those cooked to 71.1°C juicier than steaks cooked to 80°C, but there was no significant difference in tenderness or flavor of steaks cooked to those end points.

Similar to the results of this study, Visser et al. (1960) reported that as the internal temperature of beef roasts from seven muscles increased, the average cooking time and percentage total cooking losses also increased. In contrast to the findings of this study, they found that average tenderness scores and shear values did not differ significantly among end-point temperatures, but juiciness and press fluid yields generally decreased as end-point temperature increased.

Oven temperature

None of the measurements was affected significantly by oven temperature (Table 3). Although cooking time was slightly shorter when roasts were cooked at the higher oven temperature, total cooking losses averaged nearly the same for the two oven temperatures.

Similarly, Ferger et al. (1972) compared three oven temperatures for cooking beef roasts from the frozen state by dry or moist heat (in oven film) and found that the time required for cooking was not shortened significantly by raising the oven temperature above that generally recommended.

Type of heat × end-point temperature interactions

Data in Table 4 for type of heat × end-point temperature interactions help explain some of the data in Tables 1, 2 and 3.

Significant differences in total cooking time between 70° and 80°C (Table 2) are attributable to the effects of dry heat (Table 4). The only significant ($P < 0.05$) difference in total cooking time between dry and moist heat was for roasts cooked to 80°C. However, the difference was not large enough for a difference to be detected when only type of heat was considered (Table 1). When cooking time was expressed in min/kg, differences ($P < 0.05$) occurred between each end-point temperature for dry heat, and between dry and moist heat at internal temperatures of 70° and 80°C (Table 4). Those differences between dry and moist heat indicate that using cooking bags decreased the time required for cooking roasts to 70° and 80°C by approximately 13–18 min/kg. In contrast, data from this laboratory for both leg of lamb (end point, 75°C) and boneless beef rib roasts (end point, 60°C) indicated that using oven service film for cooking by moist heat did not reduce cooking time (Ferger et al., 1972). With moist heat, there were no significant differences in min/kg attributable to end-point temperature (Table 4).

Percentages of total cooking losses were greater ($P < 0.05$) for moist heat than for dry heat at all three end-point temperatures, but the difference between the two types of heat decreased at each 10°C increment in end-point temperature (Table 4). Percentages of dripping cooking losses were greater ($P < 0.05$) for moist heat than for dry heat at each end point. Values for both treatments increased between 60° and 70°C, then decreased between 70° and 80°C, with the decrease significant ($P < 0.05$) for moist heat. That accounts for the decrease ($P < 0.05$) in dripping losses between 70° and 80°C when data were summarized on the basis of end-point temperatures (Table 2).

Percentage total moisture decreased ($P < 0.05$) at each end-point temperature when roasts were cooked by dry heat. Values for roasts cooked by moist heat followed the same trend, although differences between end points were not significant. Therefore, it may be concluded that significant differences in total moisture among end-point temperatures (Table 2) are attributable to dry heat cooking. Roasts cooked to 60° or 70°C by dry heat contained more ($P < 0.05$) moisture than those cooked by moist heat. The difference at 80°C was not significant, which indicates that the difference ($P < 0.01$) in moisture between dry and moist heat seen in Table 1 is attributable to differences at 60° and 70°C.

Gardner a+ (redness) values were greatest for roasts cooked by dry heat to 60°C and decreased ($P < 0.05$) as the internal temperature was increased to 70° and 80°C. There were no significant differences among a+ values for roasts cooked by moist heat, which indicates that the differences among end-point temperatures (Table 2) are attributable principally to the effects of the dry heat treatment. The values for moist heat were less ($P < 0.05$) than those for dry heat at 60° and 70°C, which contributed to the differences ($P < 0.001$) between a+ values for dry and moist heat (Table 1).

There were significant interactions between type of heat and end-point temperature for two sensory characteristics, flavor and apparent degree of doneness. The panel preferred the flavor of meat cooked by moist heat to 60° and 70°C, with the difference between dry and moist heat being significant ($P < 0.05$) at 60°C. However, they preferred meat cooked by dry heat to 80°C, although the difference between the two types of heat

was not significant. Roasts cooked by moist heat appeared medium- to well-done at all three end-point temperatures. Apparent degree-of-doneness scores for meat cooked by dry heat were less ($P < 0.05$) than those for roasts cooked by moist heat to internal temperatures of 60° and 70°C. This suggests that the flavor of the meat was more desirable to the taste panel when it appeared more well-done.

Apparent degree of doneness, post-oven temperature rise

Apparent degree-of-doneness scores were higher ($P < 0.001$) for roasts cooked by moist heat than for those cooked by dry heat, and were not affected by oven temperature or end-point temperature. Roasts cooked by dry heat to an internal temperature of 60°C appeared bright red in the center (5–7 cm diam) with the red color fading to grey-brown around the edges, and exuding red juice upon standing. Roasts cooked by dry heat to 70°C were pink in the center (3–4 cm diam), and faded rapidly to grey-brown about halfway through the roast. Those cooked to 80°C by dry heat generally were slightly pink in the center (2–3 cm diam), but faded quickly to grey-brown throughout the remainder of the roast. The surface of roasts cooked by dry heat was a rich, dark brown, and their surface texture was fine-grained.

Roasts cooked by moist heat to an internal temperature of 60°C were slightly pink in the center (2–3 cm diam), fading quickly to light grey-brown through the remainder of the roast. Those cooked by moist heat to 70° and 80°C were uniformly grey-brown throughout. The surface of roasts cooked by moist heat was grey-brown, rather than the rich, dark brown of oven roasted meat, and the surface texture of the muscle appeared coarse.

Post-oven temperature rise was observed most frequently when roasts were cooked by moist heat. Those roasts remained in oven film bags during observation of post-oven temperature rise. The greatest increase in temperature (mean = 7.8°C) occurred in roasts cooked to 60°C by moist heat. Roasts cooked to 70°C by moist heat had a 5.6°C mean post-oven temperature rise. Roasts cooked by moist heat to 80°C and by dry heat to 60°C both averaged less than 1°C increase, and those cooked by dry heat to 70° and 80°C exhibited no post-oven temperature rise. Time required to reach the final post-oven temperature ranged from 8.5–28 min. The post-oven temperature rise observed for roasts cooked to 60° and 70°C by moist heat may account, partially, for the lack of pinkness usually expected, but not observed, at those end-point temperatures. Greater post-oven temperature rise at lower end point temperatures also was reported by Rogers et al. (1967), who found that post-oven temperature rise in turkey breast and thigh-leg pieces was greatest at 25°C internal temperature, and generally decreased with each 10°C increment in end-point temperature from 25° to 55°C.

CONCLUSIONS

UNDER the conditions of this study, it was concluded that:

1. Cooking in an oven film bag (moist heat) or roasting in an open pan (dry heat) is satisfactory for cooking beef top round from the frozen state. (a) In general, the palatability of the meat was similar for roasts cooked by either method at 177° or 205°C. (b) Using a cooking bag required significantly less total time to cook meat to an end-point temperature of 80°C at 177° or 205°C. (c) Cooking in an open pan produced significantly less weight loss from roasts cooked to end-point temperatures of 60°, 70° or 80°C at oven temperatures of 177° or 205°C.
2. Cooking in an oven film bag produces beef that appears more well done than beef cooked in an open pan to the same end-point temperature.

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EFFECT OF VARYING THE RATIO OF BEEF AND TEXTURED VEGETABLE PROTEIN NITROGEN ON PROTEIN NUTRITIVE VALUE FOR HUMANS

INTRODUCTION

VEGETABLE PROTEIN products processed to resemble meat are being used with greatly increased frequency by American populations as foodstuffs for humans. Although these products are finding many uses, one popular employment is as a partial or total replacement for ground beef.

Questions have arisen concerning the advisability of making such replacements on the basis of good nutrition even though soy is recognized as being a good protein source. Earlier studies from this laboratory were designed to evaluate protein quality of several plant protein products in comparison to ground beef. Doraiswamy (1972) found that three plant protein products processed to resemble ground beef gave distinctly poorer nitrogen retention than did either ground beef or whole egg when all were fed at a 4.0g N intake level. This would indicate a poorer protein value for these products. During 5-day experimental periods nitrogen balance values obtained by the eight adult human subjects for egg, beef, extruded defatted soybean product, spun concentrated soybean protein product, and wheat protein produce were −0.34, −0.42, −1.16, −1.31 and −0.99g N per day, respectively.

In another study (Kies and Fox, 1971), an extruded defatted soybean product referred to as TVP (textured vegetable protein product), a 1% DL-methionine enriched TVP product, and ground beef were compared using adult human bioassay techniques at both a 4.0 and a 8.0g N intake level. At the 4.0g N intake level, mean nitrogen balances of the 10 adult human subjects were −0.30, −0.70 and −0.45g N per day, respectively, for beef, TVP and methionine enriched TVP. This again suggests a lower protein nutritive value for TVP which can be overcome at least in part by methionine supplementation. It also indicates methionine as being the first limiting amino acid in the product.

At the 8.0g N intake level, feeding of the three products resulted in almost identical nitrogen retentions. This should not be interpreted as meaning the products were equal in protein value but rather that if sufficient quantities are fed, the TVP can meet the protein nutritional needs of humans.

Reports issued by the USDA in connection with rulings concerning school feeding programs have indicated that a food composed of 30% soy analog and 70% beef gave a PER value equal to that of 100% beef.

There are several possible explanations for the apparent disagreement in these results. It is possible that the PER methodology was not sufficiently sensitive to detect differences in protein value. Failure to detect a difference does not necessarily mean that no difference exists. Since it is unknown how the protein contents of the rations were calculated, it might be that different nitrogen conversion factors were used for the soy analog material and the beef. To feed at equal protein intakes is not necessarily the same as feeding at equal intakes of nitrogen. A third possibility might be that the mixture of soy analog and beef resulted in a total amino acid pattern having improved protein value and thus promoting better growth or better nitrogen retention than predicted on the basis of performance of the two products individually. Methionine (or total S-containing amino acids) is assumed to be the first limiting amino acid in soybeans and in beef. Mutual supplementation would be not likely to take place by mixtures of these two materials. However, protein quality is not merely a matter of supply of the first limiting amino acid but is also related to interplays among all amino acid constituents.

In the current project, comparative protein value of ground beef and a defatted extruded soy product processed to resemble ground beef (referred to as TVP, textured vegetable protein, in this project) was further investigated. The specific objective was to determine the effect of changing the beef to TVP nitrogen ratio on nitrogen balances of adult humans.

Table 1—Experimental plan

Period[a]	No. of days	N intake (g N/day) Beef	TVP[b]	Total[c]	Beef N/day TVP N Ratio
Depl	2	0	0	0.8	—
Adj	5	4.0	0	4.8	4/0
Expt 1	5	4.0	0	4.8	4/0
Expt 2	5	3.0	1.0	4.8	3/1
Expt 3	5	2.0	2.0	4.8	2/2
Expt 4	5	1.0	3.0	4.8	1/3
Expt 5	5	0	4.0	4.8	0/4

[a] Experimental periods randomly arranged for each of the eight subjects
[b] TVP (soy textured vegetable protein), an extruded soy product processed to resemble beef
[c] Includes 0.8g N provided by basal diet

PROCEDURE

THE 32-DAY study consisted of a 2-day nitrogen depletion period, a 5-day nitrogen adjustment period, and five experimental periods of 5 days each. The experimental plan is shown on Table 1. Order of the experimental periods was randomly arranged for each subject.

Nitrogen intake during the depletion period was maintained at 0.8g per subject per day as provided by the basal diet. This procedure has been found in our laboratory to hasten the adjustment of subjects to low protein diets. The basal diet used throughout the study is described in Table 2.

During the adjustment period and during all experimental periods, nitrogen intake was maintained at 4.8g nitrogen per subject per day. In previous studies from this laboratory this level of nitrogen intake has been established as being a rather ideal level for evaluation of protein adequacy in that it is slightly inadequate to maintain protein nutriture in adult humans

even by almost ideally balanced proteins. It is impossible to do comparative evaluations among resources when they are fed at levels which will meet subjects' needs in that all will give similar results.

During the adjustment, ground beef (ground round with all visible fat removed) was fed to provide 4.0g N per subject per day. The basal diet as previously described provided 0.8g N for a total of 4.8g N. The beef N to TVP N ratio was thus 4/0.

During the five experimental periods beef provided 4.0, 3.0, 2.0, 1.0 or 0.0g N while in concurrent periods, TVP provided 0.0, 1.0, 2.0, 3.0 or 4.0g N. Thus, nitrogen intake from the test sources was maintained constant at 4.0g N per say but the beef N to TVP N ratio was altered from 4/0 to 3/1 to 2/2 to 1/3 to 0/4. As in previous periods, the other items in the basal diet provided to 0.8g N per day.

Vitamin and mineral supplements were given each day to provide adequate nutrient intake. Caloric intake was adjusted to meet each subject's individual needs for weight maintenance. Details pertaining to the diets used were given in an earlier paper (Kies and Fox, 1971).

Details regarding the men and women who were subjects for the study are given in Table 3. All were students or spouses of students of the University of Nebraska who maintained their usual life schedules while participating in this project except for reporting to the Dept. of Food & Nutrition metabolism laboratory for consumption of meals. Signed subject consent forms were obtained from all subjects for participation. Subjects were all in good health as determined by physicians of the University of Nebraska Student Health Division.

The nitrogen balance technique was used as the primary method of evaluation. Urine, stools and food samples were collected and prepared for analysis as previously described (Linkswiler et al., 1958) and were analyzed for nitrogen content by the boric acid modification of the Kjeldahl method (Scales and Harrison, 1920). Urine samples were preserved under toluene and analyzed daily for 24-hr excretion of nitrogen and creatinine (Folin, 1914). Daily fecal nitrogen values were based on 5-day stool composites.

Fasting venous blood samples were drawn from each subject on day 1 of the study and on the morning following the completion of each experimental period. These were analyzed by a hospital laboratory via routine procedures as previously described (Kies et al., 1967), as a screening device for possible gross abnormalities in protein nutriture in particular and in normal health in general. Amino acid composition of the beef and TVP product as shown in Table 4 were done by the Dept. of Agronomy, University of Nebraska, via auto analyzer procedures.

Statistical analysis of data, including analysis of variance and Duncan's Multiple Range Test, were by the Statistical Laboratory of the Nebraska Agricultural Experiment Station, University of Nebraska.

RESULTS & DISCUSSION

MEAN NITROGEN balances of subjects while receiving the five beef to TVP nitrogen ratios are shown in Figure 1. For reference purposes data from an early comparison of beef and TVP protein are also given. Mean nitrogen balances of the eight subjects while receiving the beef to TVP nitrogen in the ratios of 4/0, 3/1, 2/2, 1/3 and 0/4 were −0.44, −0.56, −0.75, −0.90 and −1.11g N per day, respectively. Mean nitrogen balance figures were significantly different from

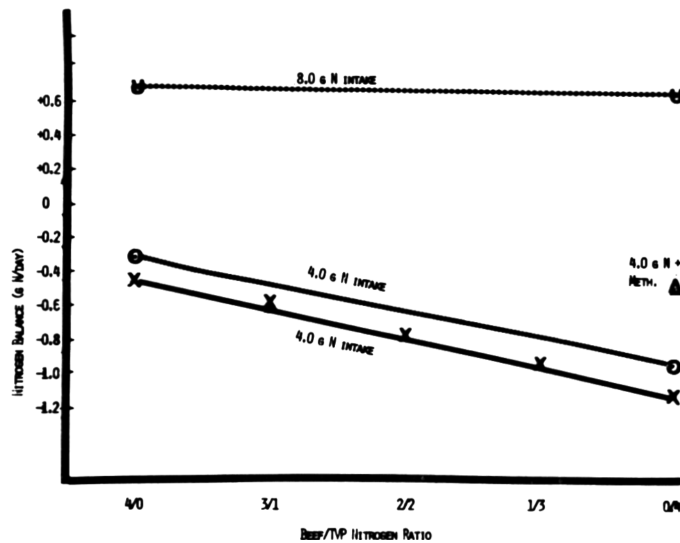

Fig. 1—Effect of change in beef/TVP nitrogen ratio on nitrogen balances of human adults. "X" symbol indicates mean values for each change in beef/TVP N ratio at 4.0g N intake. Solid line shows theoretical values. "o" and "U" symbols represent mean data collected in an earlier study (Kies and Fox, 1971). Dotted lines are theoretical values from that study at a low (4.0g N) and adequate (8.0g N) intake levels of total protein.

Table 2—Basal diet

	Amount
Breakfast	
Applesauce	100g
Starch bread	1/3 recipe
Jelly	Varied
Butter oil	Varied
Tea or coffee	Varied
Lunch	
Casserole	
Green beans	50g
Stewed tomatoes	50g
Test product	Varied
Peaches	100g
Starch bread	1/3 recipe
Jelly	Varied
Soft drinks	Varied
Butter oil	Varied
Vitamin supplement	
Mineral supplement	
Supper	
Casserole	
Green beans	50g
Stewed tomatoes	50g
Test product	Varied
Pears	100g
Starch bread	1/3 recipe
Jelly	Varied
Hard candy	Varied
Butter oil	Varied

Table 3—Description of subjects

No.	Sex	Age (yr)	Height (cm)	Weight (kg)	Nationality/ ethnic classification
570	F	21	166	58.6	Am/W
572	M	22	175	84.0	Am/W
573	M	24	188	85.8	Am/W
574	F	21	162	72.6	Am/W
576	F	27	168	64.0	Am/W
577	F	22	162	53.1	Am/W
578	M	18	165	58.1	Am/W
579	M	26	175	74.9	Am/W

Table 4–Selected amino acid contents of beef and TVP

Amino acid	Amount (g AA/4g N) Beef	TVP
Lysine	2.2	1.6
Methionine	0.7	0.3
Cystine	–	0.2
Tryptophan	0.3	0.3
Phenylalanine	1.1	1.3
Leucine	2.0	2.0
Isoleucine	1.2	1.2
Valine	1.3	1.3
Threonine	1.2	1.1

Table 5–Effect of beef/TVP N ratio on several blood constituents

Blood constituent[a]	Level of blood constituent while receiving following diet having beef/TVP N ratio diet[b] 4/0	3/1	2/2	1/3	0/4	Normal diet
Glucose, mg/100 ml	93	93	90	90	93	87
Phosphorus (inorganic), mg/100 ml	3.9	3.8	3.9	4.0	4.1	4.3
Urea N, mg/100 ml	7.1	7.2	5.8	6.4	6.8	14.8
Phosphatase (alkaline) units	49	47	46	51	48	36
Total protein, g/100 ml	7.0	6.9	7.0	7.0	7.0	7.0
Lactic dehydrogenase, units	148	148	150	150	144	162
Albumin, g/100 ml	4.9	4.8	4.8	5.0	5.0	5.1
Bilirubin, mg/100 ml	0.71	0.91	0.78	0.78	0.84	0.89
Globulin, g/100 ml	2.4	2.4	2.4	2.1	2.2	2.0
Uric acid, mg/100 ml	6.2	6.2	6.1	5.8	5.8	5.7
Albumin/globulin ratio	2.6	2.6	2.7	2.9	2.9	2.7
Calcium, mg/100 ml	9.0	9.1	9.0	9.0	9.0	9.1
SGPT, Babson units	19	23	21	20	18	15
Hematocrit, ml/100 ml	41	42	41	42	44	44
Hemoglobin, g/100 ml	14.5	14.4	14.4	14.4	14.9	14.6
Cholesterol, mg/100 ml	177	175	170	195	180	203
Triglycerides, mg/100 ml	89	91	104	85	110	76

[a] Determinations were by standard laboratory procedures of the St. Elizabeth's Hospital Lab., Lincoln, Neb. Information regarding methodology and quality control (within 1% for method used) is available from this Laboratory.

[b] Venous blood samples were drawn in the morning before consumption of the first meal of the day. Mean value for eight adult human subjects are shown.

one another at the 5% level with each change in ratio.

Seemingly a straight line relationship exists as a result of changing the beef/TVP nitrogen ratio. This is what would be expected if the difference in nitrogen retention as a result of feeding 4.0g nitrogen from either source were the result of either a lower total protein content or lower methionine content in the case of the TVP. The improvement in nitrogen retention in moving from a 0/4 to 4/0 beef/TVP nitrogen ratio is a simple mathematic relationship predictable from the simple rules of ratio relationships since intermediate points where mixtures were made fall on the straight line. If mixing the two resources resulted in diminishing weakness of both as in the case of the mutual supplementation effect, the intermediate points should fall above the straight line resulting in a positively curved line. The opposite, a negatively curved line, would be the result of mixing the two sources resulted in an intensifying of unfavorable tendencies. This was clearly not the case. Examination of the amino acid proportionality patterns of the beef and of the TVP product shown on Table 4 indicate these results to be expected.

Methionine (total sulfur containing amino acids) has been clearly demonstrated as being the first limiting amino acid in the TVP product (Kies and Fox, 1971). The methionine content of beef is only slightly higher than that of TVP which suggests that methionine is the first limiting amino acid in that product as well.

Lysine content is the most dramatic difference between the amino acid patterns of beef and TVP. Even though much lower in lysine content, the TVP product fed at the 4.0g N intake level supplies sufficient amounts of lysine to meet the recommended intake of lysine for young men (Rose, 1957).

When the mixing of two or more sources of dietary protein are mixed together resulting in a better total quality protein than any of the sources individually, this is referred to as the mutual supplementation effect. This is usually achieved by the mixing of protein resources having different first limiting amino acids. That methionine is probably the first limiting amino acid in both products examined here, it should not be surprising that a mutual supplementation effect was not demonstrated.

No significant differences were found in the following fasting blood analyses as a result of changing the beef/TVP nitrogen ratio: calcium, inorganic phosphorus, glucose, urea nitrogen, albumin, cholesterol globulin, total protein, albumin, globulin ratio, uric acid, phosphatase, lactic dehydrogenase, SGPT, hematocrit and hemoglobin. Mean values are shown in Table 5.

In conclusion, a straight line relationship exists as a result of changing the beef/TVP nitrogen ratio on the nitrogen retention of human adults. No mutual supplementation effect was demonstrated. demonstrated.

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RELATIONSHIP OF TENDERNESS MEASUREMENTS MADE BY THE ARMOUR TENDEROMETER TO CERTAIN OBJECTIVE, SUBJECTIVE AND ORGANOLEPTIC PROPERTIES OF BOVINE MUSCLE

INTRODUCTION

MANY OBJECTIVE methods used to evaluate meat tenderness have been developed and tested (Bouton and Harris, 1972; Pearson, 1963; Szczesniak and Torgeson, 1965); however, most of these methods require removal of the test sample from the carcass, cooking it and then objectively evaluating it. Armour and Co. (Chicago, Ill.) have developed an instrument called the Armour Tenderometer (AT) (U.S. Patent Application No. 776,234), which is designed to make a nondestructive tenderness measurement on the longissimus muscle while it is still in the carcass and that predicts the tenderness of meat after cooking. The design and original work leading to development of the AT have been reported by Hanson (1972).

The objectives of our studies were to determine the relationship of measurements made by the AT to certain organoleptic, objective and subjective properties of beef muscle. Three separate studies were done:

(1) AT values of wholesale ribs and chemical, Warner-Bratzler (W-B) shear and organoleptic properties of steaks from wholesale ribs were measured at three postmortem times.
(2) AT values were measured on 211 bovine longissimus muscles between 12th and 13th thoracic vertebrae, and these measurements were compared with organoleptic and W-B shear evaluation of top round steaks from the same carcasses.
(3) Selection of two groups of bovine carcasses that differed widely in tenderness, as measured by AT values of the longissimus between 12th and 13th thoracic vertebrae, and the organoleptic and W-B shear evaluation done on rib roasts and steaks from these two groups.

MATERIALS & METHODS

Study I

Wholesale ribs from typical A-maturity, U.S. Choice grade carcasses having small to modest amounts of intramuscular fat were obtained 24 hr postmortem from a central Iowa packing plant and were transported to the Iowa State University Meat Laboratory. AT measurements were taken on the longissimus of the wholesale ribs in an upright position on a meat cutting table at the junction of the 12–13th thoracic vertebrae, and steaks were then removed for organoleptic evaluation (2.54 cm thick), for chemical analyses (1.27 cm thick) and for W-B shear (2.54 cm thick) at the Meat Laboratory. After 3 days postmortem storage at 2°C, AT

Table 1—Mean values ± s.e. for certain chemical, physical and organoleptic properties of bovine longissimus at different postmortem times

Properties	Postmortem time (days) 1	3	7	Combined
% water	70.5 ± 0.2 (41)[a]	70.0 ± 0.3 (33)[a]	70.0 ± 0.2 (47)[a]	70.2 ±0.1 (121)[a]
% water-cooked	65.0 ± 0.2 (20)		64.4 ± 0.2 (20)	64.7 ±0.2 (40)
% fat (WWB)[b]	7.10± 0.3 (41)	7.93± 0.3 (41)	8.03± 0.2 (47)	7.66±0.2 (121)
% fat-cooked (WWB)[b]	8.83± 0.4 (20)		8.87± 0.4 (20)	8.85±0.2 (40)
% protein	21.2 ± 0.2 (12)	21.0 ± 0.2 (12)	20.4 ± 0.2 (12)	20.9 ±0.1 (36)
NPN (mg/ml)	3.64± 0.07(36)	3.11± 0.08(24)	3.78± 0.07(40)	3.56±0.04(100)
Salt insoluble N (mg/ml)	5.57± 0.59(17)	4.02± 0.57(18)	4.93± 0.67(13)	4.84±0.35(48)
Sarcoplasmic proteins (mg/g DFF)[c]	349.4 ± 4.0 (39)	335.9 ± 4.9 (27)	337.5 ± 3.9 (41)	342.8 ±2.4 (107)
Sarcoplasmic proteins-cooked (mg/g DFF)[c]	115.5 ± 4.8 (20)		105.1 ± 4.8 (20)	110.3 ±3.4 (40)
Myofibrillar proteins (mg/g DFF)[c]	509.6 ±11.9 (36)	540.0 ±13.8 (27)	568.3 ±11.3 (40)	540.4 ±7.1 (103)
Myofibrillar proteins-cooked (mg/g DFF)[c]	96.7 ± 5.5 (20)		96.0 ± 5.5 (20)	96.3 ±3.9 (40)
Collagen (mg/g DFF)[c]	17.4 ± 0.81(39)	17.3 ± 1.0 (27)	15.8 ± 0.8 (41)	16.8 ±0.6 (107)
Collagen-cooked (mg/g DFF)[c]	18.0 ± 0.81(20)		18.3 ± 0.8 (20)	18.1 ±0.5 (40)
Tenderness[d]	4.9 ± 0.1 (59)[e]	5.7 ± 0.1 (33)[f]	6.3 ± 0.1 (59)[g]	5.6 ±0.1 (151)
Flavor[d]	6.0 ± 0.0 (59)	6.3 ± 0.1 (33)	6.2 ± 0.0 (59)	6.2 ±0.0 (151)
Juiciness[d]	6.3 ± 0.1 (59)	6.6 ± 0.1 (33)	6.2 ± 0.1 (59)	6.3 ±0.1 (151)
% cooking loss	16.0 ± 0.3 (59)	15.6 ± 0.4 (33)	16.2 ± 0.3 (59)	16.0 ±0.2 (151)
W-B shear (kg/cm^2)	3.27± 0.07(59)[e]	2.86± 0.10(33)[f]	2.55± 0.07(59)[f]	2.91±0.05(150)
Armour Tenderometer (kg)	7.61± 0.11(59)[e]	7.47± 0.21(33)[e]	7.28± 0.14(59)[e]	7.45±0.09(151)

[a] Number in parenthesis represents number of steaks.
[b] WWB—wet weight basis
[c] DFF—dry, fat-free basis
[d] 8-point hedonic scale with 8 being the most desirable
[e,f,g] Means with different superscripts are significantly different ($P < 0.05$).

Table 2–Correlation coefficients between AT values and percentages of water and fat of bovine longissimus at different postmortem times

Chemical measurement	Postmortem time (days)		
	1	3	7
% water, 1 day	−0.25(41)[a]	−0.43*(27)[a]	−0.17 (41)[a]
% water, 3 days	−0.19(33)	−0.36*(33)	−0.50**(33)
% water, 7 days	−0.22(47)	−0.24 (33)	−0.32* (41)
% fat, 1 day	0.11(41)	0.38*(27)	0.22 (41)
% fat, 3 days	0.12(33)	0.34*(27)	0.42* (33)
% fat, 7 days	0.17(47)	0.32 (33)	0.26 (47)

[a] Numbers in parentheses represent number of steaks tested.
* Significantly different from zero at $P < 0.05$
** Significantly different from zero at $P < 0.01$

Table 4–Correlation coefficients between organoleptic tenderness scores and W-B shear values of bovine longissimus at different postmortem times

Organoleptic tenderness scores	Postmortem time (days)		
	1	3	7
1 day	−0.51**(59)[a]	−0.43**(33)[a]	−0.22 (58)[a]
3 days	−0.28 (33)	−0.60**(33)	−0.48**(32)
7 days	−0.19 (59)	−0.50**(33)	−0.57**(58)

[a] Numbers in parentheses represent number of steaks tested.
* Significantly different from zero at $P < 0.05$
** Significantly different from zero at $P < 0.01$

Table 3–Correlation coefficients between AT values and W-B shear values and organoleptic tenderness scores of bovine longissimus at different postmortem times

Tenderness measurement	Postmortem time (days)		
	1	3	7
W-B shear at 1 day	0.19 (59)[a]	0.26 (33)[a]	−0.03 (59)[a]
W-B shear at 3 days	0.52**(33)	0.47**(33)	0.25 (33)
W-B shear at 7 days	0.26* (58)	0.16 (32)	−0.12 (58)
Organoleptic score, 1 day	−0.40**(59)	−0.28 (33)	−0.34**(59)
Organoleptic score, 3 days	−0.49**(33)	−0.28 (33)	−0.03 (33)
Organoleptic score, 7 days	−0.34**(59)	−0.37* (33)	−0.09 (59)

[a] Numbers in parentheses represent number of steaks tested.
* Significantly different from zero at $P < 0.05$
** Significantly different from zero at $P < 0.01$

measurements were made on the remaining part of the wholesale rib at the junction of the 10–11th ribs, and steaks were again removed for organoleptic, chemical and W-B shear determinations. This same sampling procedure was repeated after 7 days postmortem with the AT measurements being made at the junction of the 8th and 9th ribs. Steaks for organoleptic and W-B shear were broiled to an internal temperature of 65°C in a 232°C broiler. Tenderness, flavor and juiciness of steaks were scored on an eight-point hedonic scale by a ten-member panel, consisting of five men and five women with an average age of 26 yr. W-B shear was determined by shearing across the fibers of three, 1.27-cm diam cores obtained from the lateral, central and medial positions of the rib steak after cooling to approximately 25°C. Each core was sheared twice for a total of six values on each steak, and the average of these six values represented the W-B shear value for that particular steak. Percentage moisture, fat and protein were determined by the procedures of AOAC (1965). Sarcoplasmic and myofibrillar protein solubility and nonprotein nitrogen of raw and cooked samples were determined according to the procedures outlined by Chaudhry et al. (1969). Salt-insoluble nitrogen and collagen were determined on the fraction remaining after sarcoplasmic and myofibrillar protein extraction. Salt-insoluble nitrogen was determined by micro-Kjeldahl, and collagen by the hydroxyproline method of Goll et al. (1963). Cooking loss was determined by recording steak weights before and after cooking.

Study II

This study used 211 A-maturity carcasses from animals from an Iowa State University cross-breeding project. These animals were slaughtered at a northwestern Iowa packing plant at an approximate age of 1 yr; 116 of the carcasses graded U.S. Good, 84 graded U.S. Choice, 7 graded U.S. Prime and 4 graded U.S. Standard. After 24 hr postmortem, AT measurements were made on the cross-section of the longissimus muscle of the left side at the level of the 12–13th rib, and two experienced evaluators subjectively scored marbling, color, firmness and texture of lean in the 12–13th rib section of the longissimus. These same evaluators also scored percentage kidney knob, conformation, and gave an overall quality score to each carcass. Color, firmness and texture of lean were evaluated on a three-point scale with three being dark, firm or coarse. Palatability evaluation and W-B shear measurements were made on 2.54 cm thick top round steaks removed after 7–8 days postmortem and frozen. These steaks were subsequently thawed at 2°C and broiled to 65°C internally in a 232°C broiler. Three, 1.27-cm diam cores were removed for W-B shear from top round steaks after they had cooled to 25°C, and each core was sheared twice. Organoleptic evaluation was carried out as previously described in Study I.

Study III

In the third study, two groups (12 each) of wholesale ribs from carcasses differing widely in AT values were obtained from a north-central Iowa packing plant. Carcasses graded U.S. Choice, were A-maturity and exhibited small-to-modest amounts of marbling in the longissimus between the 12–13th thoracic vertebrae. AT readings on the longissimus of left sides were taken between the 12–13th rib at 1 day postmortem. Two, 2.54-cm thick steaks were removed at 7 days postmortem from the posterior end of the wholesale rib, and a 7.62-cm thick roast was removed from the anterior end of the rib for organoleptic evaluation and W-B shear. Firmness of lean-cut surface was subjectively evaluated on a three-point scale with three being most firm. Roasts and steaks were wrapped and frozen at −7°C for subsequent organoleptic evaluation and W-B shear. Steaks were thawed for 24 hr at 2°C, broiled to 65°C internally in a 232°C broiler and served to an organoleptic panel as previously described in Study I. Roasts were thawed for 48 hr at 2°C and roasted in a 163–177°C oven to an internal temperature of 65°C. One, 2.54-cm slice was taken for organoleptic evaluation and another for W-B shear cores.

Temperatures of the longissimus muscle were always 2–4°C when AT measurements were taken in Studies I, II and III.

Correlations and analyses of variance were carried out according to Snedecor and Cochran (1967).

RESULTS

Study I

A number of chemical analyses were done on both raw and cooked muscle in the anticipation of determining how AT readings were influenced by chemical compositions of the muscle, and how the content and changes of chemical components were related to other measures of tenderness. Data of the various chemical, physical and organoleptic measures of beef longissimus at 1, 3 and 7 days postmortem and combined data for all three postmortem sampling times are given in Table 1. Of the three measures of tenderness (organoleptic panel, W-B shear and AT) tested for significant difference among days postmortem, it was found that the AT did not significantly detect differences in tenderness during the post-

Table 5–Means and standard deviations for certain bovine carcass and palatability characteristics

Characteristic	Mean	Std deviation
REA (cm^2)[a]	73.94	6.45
FT (cm)[b]	4.01	1.64
CWT (kg)[c]	292.88	42.63
Conformation (Ch = 13)[d]	13.1	2.00
Marbling (Sm = 14)[e]	13.7	3.77
Grade (Gd^+ = 11)[f]	11.4	1.37
Color (3 = dark)[g]	1.8	0.67
Firmness (3 = firm)[h]	2.3	0.82
Texture (3 = coarse)[i]	1.6	0.61
AT (kg)	8.31	1.23
W-B shear (Kg/cm^2)	3.01	0.61
Tenderness[j]	5.3	0.69
Flavor[j]	5.7	0.38
Juiciness[j]	5.1	0.94
% cooking loss	22.5	3.46

[a] Rib eye area
[b] Fat thickness at the junction of 12–13th rib
[c] Carcass weight
[d] Subjective score of 1–17 with 13 being mid-point Choice carcass conformation
[e] Subjective score of 10–30 with 14 being small amount of rib eye marbling
[f] Subjective score of 1–17 with 11 being high Good carcass grade
[g] Subjective score of 1–3 with 3 being a dark colored rib eye muscle
[h] Subjective score of 1–3 with 3 being a firm rib eye muscle
[i] Subjective score of 1–3 with 3 being a coarse textured rib eye muscle
[j] 8-point hedonic scale with 8 being the most desirable. Sensory evaluation of semimembranosus

mortem aging times of 1, 3 and 7 days. The organoleptic panel, however, detected significant changes among the three storage times and the W-B shear detected significant changes between 1 and 3 days storage.

Study I was further designed to test the relationship of the AT to certain chemical, physical and organoleptic measures of tenderness. Few of the measures of chemical composition, however, were consistently related to AT readings. Some of the more significant correlations were observed between percentage water or percentage fat with AT values (Table 2). Higher correlation coefficients, not shown herein, were found between percentage protein at day 1 and AT values at day 1 (–0.74) and between sarcoplasmic protein (dry-fat-free) at day 7 and AT values at day 1 (0.43). Few other significant correlations were found between chemical parameters and W-B shear or organoleptic measures of tenderness, and no significant ($P < 0.05$) relationships were found between chemical parameters of cooked steaks and AT, W-B shear or organoleptic values. These results would indicate that measures of chemical composition in this study are not very valuable in assessing tenderness or relating them to either sensory or mechanical means of measuring tenderness.

Although AT measurements were not highly related to measurements of chemical composition of muscle, some significant relationships did exist between AT measurements and W-B shear or organoleptic measures of tenderness (Table 3). In general, correlation coefficients between AT values and W-B shear values or organoleptic tenderness scores seemed higher after 1 day of postmortem storage than after 3 or 7 days postmortem (Table 3); this suggests that the AT may be most useful for predicting tenderness if it is used before carcass aging. Even those AT measurements made at 1 day, however, were not highly related to W-B shear and organoleptic tenderness scores at 7 day postmortem. On the other hand, the relationships between W-B shear values and organoleptic tenderness scores were higher than those between AT values and W-B shear or organoleptic tenderness scores (Table 4) and these show that the W-B shear accounts for about 25–40% of the variation in tenderness assessed by the panel. Although there is a range in correlation coefficients between W-B shear values and organoleptic tenderness scores it should be noted that the strongest relationships were between the simultaneous day of W-B and organoleptic measurement and the lower and statistically nonsignificant correlation coefficients were

Table 6–Correlation coefficients between organoleptic, objective and subjective properties of beef

Properties	Flavor[a]	Tenderness[a]	Juiciness[a]	W-B shear[b]	% Total cooking loss	Armour Tenderometer[c]	Carcass wt	% kidney knob	Rib eye area	Fat thickness
Flavor[a]	1.00									
Tenderness[a]	0.50**	1.00								
Juiciness[a]	0.64**	0.52**	1.00							
W-B shear[b]	–0.22**	–0.30**	–0.09	1.00						
% total cooking loss	–0.14*	–0.08	–0.06	0.13	1.00					
Armour Tenderometer[c]	0.06	–0.04	0.05	0.04	–0.17*	1.00				
Carcass weight	0.16*	0.17*	0.14*	–0.03	0.11	–0.21**	1.00			
% kidney knob	0.24**	0.24**	0.25**	–0.15*	–0.06	–0.08	0.52**	1.00		
Rib eye area	–0.04	0.08	–0.02	0.02	0.15*	–0.24**	0.61**	0.31**	1.00	
Fat thickness	0.11	0.14*	0.11	–0.24**	0.03	–0.27**	0.19**	0.20**	0.05	1.00
Conformation 1[d]	0.05	0.14*	0.10	–0.14*	0.16*	–0.26**	0.22**	0.19**	0.24**	0.53**
Conformation 2[d]	0.04	0.15*	0.07	–0.21**	0.11	–0.26**	0.07	0.12	0.15*	0.67**
Marbling 1	0.22**	0.32**	0.28**	–0.35**	–0.14*	0.14*	0.22**	0.35**	0.09	0.28**
Marbling 2	0.22**	0.29**	0.27**	–0.38**	–0.11	0.09	0.24**	0.34**	0.09	0.35**
Grade 1	0.21**	0.31**	0.28**	–0.32**	–0.11	0.10	0.29**	0.41**	0.16*	0.33**
Grade 2	0.18*	0.28**	0.22**	–0.32**	–0.05	0.03	0.25**	0.31**	0.16*	0.44**
Color 1	–0.10	–0.12	–0.04	0.13	–0.04	0.22**	–0.02	–0.04	–0.03	–0.20**
Color 2	–0.10	–0.16*	–0.02	0.20**	0.01	0.23**	0.04	–0.03	–0.07	–0.30**
Firmness 1	0.10	0.05	0.19**	–0.06	–0.09	0.23**	–0.05	0.06	–0.16*	0.00
Firmness 2	–0.18**	–0.02	–0.15*	0.15*	0.14*	–0.20**	0.00	–0.18**	0.05	0.21**
Texture 1	0.03	0.03	–0.01	–0.02	0.06	–0.14*	0.13	0.00	0.11	0.06
Texture 2	0.11	0.00	0.10	–0.05	–0.02	0.21**	0.12	0.21**	0.08	–0.13

[a] Sensory evaluation of semimembranosus broiled to 65°C internally was carried out by a 10-member panel using an 8-point hedonic scale, 8 being the most desirable.
[b] Three 1.27 cm cores were sheared twice each from semimembranosus cooked to 65°C internally.
[c] Armour Tenderometer measurements were made on the left rib eye muscle.
[d] 1 and 2 represent evaluator 1 and evaluator 2.
* Significantly different from zero at $P < 0.05$
** Significantly different from zero at $P < 0.01$

for measurements made on separate days, i.e., 1 vs. 7 days, 3 vs. 1 day and 7 days vs. 1 day.

Study II

Our second study involved measuring the longissimus of the left side of 211 beef carcasses with the AT at the 12–13th rib after 1 day postmortem and the data from these measurements, which includes means and standard deviations of carcass and palatability characteristics, are presented in Table 5.

One of the potential uses of the AT is as a nondestructive measure of tenderness of the longissimus at the level of the 12–13th rib in the anticipation that this measure of tenderness would accurately reflect the tenderness throughout the musculature of the carcass. This study, at least in part, tested the validity of this potential use by taking AT measurements of the longissimus and then determining the relationship between these measurements and organoleptic and W-B measurements of top round steak. Correlation coefficients presented in Table 6, however, show that organoleptic flavor, juiciness, W-B shear, percentage kidney knob, degree of marbling and grade are better measures of top round steak tenderness than were AT values measured on the longissimus. AT values were more significantly ($P < 0.01$) related to rib-eye area and color, firmness and texture of rib-eye lean and carcass conformation, although direction of correlation was sometimes different for the measurements made by the two different evaluators. Consequently, it is highly questionable whether these significant correlation coefficients have any biological or practical significance.

The correlation coefficients between AT values of longissimus and organoleptic tenderness of top round steaks were 0.03 (84 sides) for U.S. Choice carcasses and

Table 7—Means of left and right sides and side difference, standard deviation, and the "t" test of significance for the Armour Tenderometer

Left side (kg)	8.21
Right side (kg)	7.57
Side difference (kg)	0.64
Side difference (kg) standard deviation	0.22
"t" (with 31 degrees of freedom)	2.92**

** P < 0.01

Table 8—Means of organoleptic, objective and subjective properties of two groups of beef steaks and roasts differing in Armour Tenderometer values

	Steaks			Roasts		
Properties	Low[c]	High[d]	s.e.[e]	Low[c]	High[d]	s.e.[e]
Flavor[a]	6.2*	5.9	± 0.07	5.7	5.9	± 0.10
Tenderness[a]	6.1*	5.1	± 0.20	6.0	5.7	± 0.15
Juiciness[a]	6.3	6.2	± 0.13	5.4*	5.9	± 0.10
Overall acceptability[a]	6.2*	5.6	± 0.11	5.6	5.8	± 0.11
% cooking loss	17.6	19.0	± 0.52	21.6	20.3	± 0.36
W-B shear (Kg/cm²)	2.77	3.21	± 0.11	2.39	2.61	± 0.13
Tenderometer (kg)	6.13**	10.17	± 0.10	6.13**	10.17	± 0.10
Cooking time (min/lb)	16.4	18.4	± 0.53	40.5	41.3	± 0.55
Firmness score[b]	1.0**	3.0	± 0.00	1.0**	3.0	± 0.00

[a] Based on an 8 point hedonic scale with 8 being the most desirable
[b] Based on a 3-point scale with 3 being the most firm
[c] AT mean value of longissimus at 12–13th rib of 6.13 kg
[d] AT mean value of longissimus at 12–13th rib of 10.17 kg
[e] Pooled standard error (s.e.)
* P < 0.05
** P < 0.01

Table 9—Correlation coefficients between organoleptic, objective and subjective properties of two groups of beef steaks and roasts differing in Armour Tenderometer values

Properties	Flavor[a]	Tenderness[a]	Juiciness[a]	Overall acceptability[a]	% total cooking loss	W-B shear	Tenderometer	Cooking time
Steaks								
Tenderness[a]	0.81**							
Juiciness[a]	0.74**	0.73**						
Overall acceptability[a]	0.90**	0.92**	0.77**					
% evaporation	−0.36	−0.42*	−0.59**	−0.53**				
% drip	−0.57**	−0.35	−0.48*	−0.47*				
% total cooking loss	−0.55**	−0.49*	−0.67**	−0.62**				
W-B shear	−0.75**	−0.83**	−0.69**	−0.80**	0.54**			
Tenderometer	−0.57**	−0.50*	−0.17	−0.55**	0.30	0.45*		
Cooking time	−0.45*	−0.28	−0.41*	−0.48*	0.82**	0.34	0.38	
Firmness	−0.48*	−0.46*	−0.10	−0.48*	0.29	0.40*	0.98**	0.37
Roasts								
Tenderness[a]	0.59**							
Juiciness[a]	0.78**	0.38						
Overall acceptability[a]	0.93**	0.77**	0.78**					
% evaporation	0.15	0.21	0.10	0.12				
% drip	0.19	0.24	0.12	0.21				
% total cooking loss	0.22	0.30	0.14	0.22				
W-B shear	−0.37	−0.72**	−0.20	−0.51**	−0.21			
Tenderometer	0.24	−0.26	0.40*	0.12	−0.38	0.18		
Cooking time	−0.13	0.07	0.04	−0.04	−0.21	−0.04	0.16	
Firmness	0.26	−0.21	0.45*	0.15	−0.36	0.18	0.98**	0.16

[a] 8 point hedonic scale with 8 being the most desirable
* Significantly different from zero at P < 0.05
** Significantly different from zero at P < 0.01

0.10 (116 sides) for U.S. Good carcasses; hence, quality grade seems to have no effect on the relationship of AT values to tenderness of the cooked meat. Similarly marbling score was not related to AT value. In this study, we also measured the paired left and right sides of 32 carcasses with the AT. If it is assumed that the two sides of the carcass have the same tenderness, measurements on paired sides should provide an estimate of the precision of the AT measurement. AT values from two sides of the same carcass differed significantly ($P < 0.01$, Table 7), and the correlation coefficient between AT measurements on right and left sides of the same carcass was only 0.08.

Study III

This study was designed to determine the ability of AT values to differentiate carcasses having tough meat from carcasses having tender meat. Thus, if the AT could accurately distinquish between these two categories of carcasses, it would be expected that two groups of carcasses differing widely in AT values should also differ widely in W-B shear force values and in organoleptic ratings for tenderness. Therefore, Study III involved an investigation of two groups of wholesale ribs from carcasses differing widely in AT values (12 ribs from carcasses with a mean AT value of 6.13 kg and 12 ribs from carcasses with a mean AT value of 10.17 kg), but being of A-maturity and having small-to-modest amounts of marbling in the cross-section of the longissimus at the 12–13th rib. Organoleptic ratings of flavor, tenderness and overall acceptability of rib steaks differed significantly between these two groups of carcasses selected to differ widely in AT values (Table 8); steaks from the groups of carcasses with low AT values were rated more desirable in these three characteristics. Although there was a significant difference in overall acceptability of steaks with low AT values compared with high AT values, steaks from both of these AT groups were scored as acceptable in palatability. Moreover, organoleptic ratings of tenderness on rib roasts from a group of ribs having low (6.13 kg) AT values did not differ significantly from organoleptic tenderness ratings on rib roasts from ribs having high (10.17 kg) AT values (Table 8). In addition, W-B shear values on either rib steaks or roasts were not significantly different between the two groups of carcasses. Subjective firmness scores for ribs in the carcasses having low AT values were lower (softer) than those for ribs in the high AT group (Table 8); this suggests that AT values may be more highly related to firmness of lean than to tenderness or palatability per se.

Correlation coefficients between organoleptic, objective and subjective measurements are given in Table 9. The very high correlation coefficient ($r = 0.98$) between AT value and subjective score for firmness of lean indicates that the AT is a measure of rib-eye firmness. Although AT values were significantly related to flavor, tenderness and overall acceptability of rib steaks and to juiciness of rib roasts, none of these relationships was very high, and it seems that AT values would be of only limited value as a predictor of tenderness of cooked meat. Conversely, W-B shear force values were more highly correlated with organoleptic evaluation of tenderness in rib steaks than were AT values. Furthermore, W-B shear values were also significantly related to tenderness of rib roasts, whereas AT values were not. Overall acceptability was highly related to flavor, tenderness and juiciness in both rib steaks and rib roasts; this indicates that all three of these factors are important in determining acceptability of beef.

DISCUSSION

THE RELATIONSHIP of the AT measurements on bovine longissimus muscle to certain organoleptic, objective and subjective measurements was tested by using three different experimental designs: (1) analyses of steaks from wholesale ribs at three different postmortem aging times to determine factors influencing AT values; (2) attainment of AT measurements on longissimus at the 12–13th rib of carcasses under in-plant conditions with subsequent evaluation of top round steaks; and (3) use of AT measurements to classify carcasses into two groups having widely different AT values followed by measurement of rib steaks and roasts to determine whether these two groups also differed widely in tenderness.

All three of these studies indicated that AT values were not highly related to organoleptic evaluation of tenderness. Indeed, W-B shear values were in practically every instance more highly correlated with organoleptic tenderness than were AT values. These results are in contrast to those of Hanson (1972) who reported correlation coefficients of 0.77 and 0.69 between AT and panel for U.S. Choice and U.S. Good Grade beef. However, results by both Hanson (1972) and Henrickson et al. (1972) confirm our results of a low relationship between the W-B shear and the AT. Study III showed that even when carcasses within the same grade and marbling score were grouped into two categories that differed widely in AT values, AT measurements were not highly related to organoleptic evaluation of tenderness. These relationships however, were some of the highest ones we obtained, but these had to be acquired under extreme differences in AT values. Even with the extreme difference in AT values the organoleptic panel still rated the steaks as acceptable from both groups. Furthermore, the inability of AT measurements to detect the increase in tenderness that occurs during postmortem storage (Study I) and the large differences in AT values taken on two sides of the same carcass (Study II) suggest that AT measurements are neither very precise nor very accurate indicators of tenderness in cooked beef. Furthermore, significant relationships were between AT values and subjective and objective carcass values rather than between AT and organoleptic values. Other investigators who have also found low relationships between AT values and other measures of tenderness are: (1) Carpenter et al. (1972) who reported that AT measurements might be useful to stratify carcasses into different tenderness groups, although this stratification was not very precise, and (2) Dikeman et al. (1972) who found some significant but low correlations between AT values and measures of tenderness on cooked meat.

From our studies, we must conclude that the AT is not a particularly useful nondestructive carcass level test to predict what the tenderness of meat will be after cooking. This is unfortunate because a nondestructive method of accurately assessing discrete differences in tenderness before marketing could be a potentially valuable tool in classifying meat into tenderness groups. Although it is possible that AT measurements could be used to group carcasses into "tough" and "tender" categories, our results indicate that even these extreme categories will not be very different with respect to tenderness (Study III).

A very potentially useful finding in Study III was the AT prediction of and the relationship to rib-eye muscle firmness score. Since AT measurements are a measure of the resistance of the longissimus muscle and the force required to thrust a set of probes a given distance into it, perhaps, it is not too surprising that AT values are highly related to firmness of muscle. The questions of what causes muscle firmness and of how it influences meat quality, however, remain largely unanswered. It would appear that the property of muscle firmness merits much more attention than it has presently received.

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^{15}N TRACER STUDIES OF NITRITE ADDED TO A COMMINUTED MEAT PRODUCT

INTRODUCTION

CONCERN has recently been expressed by scientists and consumers about the addition of nitrite to food. Nitrite can react with secondary amines and some other similar compounds to produce nitrosamines (Sen et al., 1969; Sen et al., 1970; Wolff and Wasserman, 1972; Tannenbaum, 1972), and some of the nitrosamines are potent carcinogens (Magee and Barnes, 1967).

Sodium nitrite, used as a curing agent in meat, produces the typical cured meat color and distinctive flavor; and it also acts to inhibit outgrowth of botulinum spores in the event of contamination and mishandling of cured meat products (Urbain, 1971; Greenberg, 1972). Nitrite (which can be legally added at a rate of 156 ppm) is converted rapidly to forms undetectable as nitrite during processing and continues to be converted with time after processing until a fairly constant, low level is reached (Greenberg, 1972). This conversion depends on such factors as pH and processing and storage temperatures (Nordin, 1969). Some of the added nitrite forms nitrosomyoglobin and/or nitrosylhemochrome (Woolford et al., 1972; Bard and Townsend, 1971). Gaseous products such as NO and N_2O are formed (Woolford et al., 1972). The nitric oxide (NO) is believed to be involved in the formation of nitrosomyoglobin and nitrosylhemochrome (Bard and Townsend, 1971). Some of the nitrite may also become bound to proteins (Mirna and Hoffman, 1969; Olsman and Krol, 1972).

A determination of the fate of the nitrite ion in cured meat is of importance in decisions concerning the addition of nitrite to meat products. We used the stable isotope of nitrogen (^{15}N) as a label for sodium nitrite in order to establish quantitatively the amount of nitrite nitrogen in various fractions of a typical cured meat as a function of processing temperature and storage time.

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MATERIALS & METHODS

A COMMINUTED luncheon meat was prepared under conditions that simulated commercial production. The meat used was 80% lean picnic and included 3% water, 3% salt (sodium chloride), 0.25% dextrose and 0.25% sucrose. Sodium nitrite labeled with ^{15}N (96.1% enrichment from Prochem., Lincoln Park, N.J.) was added at 156 ppm. Ascorbate was not used in the formulation because it accelerates breakdown of nitrite (Mirvish et al., 1972). Even though such accelerated breakdown may be desirable, ascorbate was purposely avoided in order to simplify our system.

The meat was ground and mixed in a Keebler paddle mixer for 8 min with the salt and sugar. The nitrite and water were then added, the mixer cover was immediately closed and mixing continued under vacuum for 8 more min; product temperature after mixing was 2°C. The vacuum line to the mixer included two liquid nitrogen traps, connected in series, to retain volatiles which might be produced during mixing.

After mixing, the meat was placed in a stainless steel stuffer and 300 × 308 cans were each filled with 11 oz of product. The cans were closed under vacuum within 15 min of filling. Some of the cans were frozen at −55°C immediately and the rest were held at 0°C for 24 hr. After 24 hr, half of the remaining cans were cooked for 60 min in 71°C water and half were retorted for 80 min at 107°C. Following heat processing, the cans were chilled in 15°C water. The frozen samples were stored in a freezer (−18°C). The 71°C samples were stored at 5°C and the 107°C samples were stored at room temperature (22°C).

Analysis for ^{15}N involved a conversion of sample nitrogen to $(NH_4)_2SO_4$ which was in turn converted to N_2 by hypobromite on a Consolidated Nier isotope ratio mass spectrometer (Burris and Wilson, 1957). The conversion to $(NH_4)_2SO_4$ may be accomplished by the Devarda reduction method for nitrate, nitrite and ammonia (Bremner and Keeney, 1965), the Kjeldahl method for organic nitrogen (AOAC, 1970) or a combination of these two (Davisson and Parsons, 1919). In our work, the samples were fractionated as illustrated in Figure 1; analysis for ^{15}N was conducted on each fraction. Sample analyses were conducted at 2, 23, 40, 49 and 65 days after processing.

The samples were powdered by first immersing them in liquid nitrogen followed by pulverization in a Waring Blendor. The sample was then extracted with water according to the AOAC procedure for colorimetric determination of nitrite. Nitrite assay was conducted on the water supernatant by the Griess reagent method (AOAC, 1970). Total nitrogen was determined on the water supernatant by a combination of the Devarda and Kjeldahl methods (Davisson and Parsons, 1919). The residue from the water extraction was analyzed by the total nitrogen method and then subjected to extraction with 1.1N KI and 0.1N KH_2PO_4 (Helander, 1957). The supernatant from the salt extraction was checked for residual nitrite and also subjected to the total nitrogen assay. The residue was also examined for total nitrogen.

The amount of label from nitrite that was associated with the pigment fraction was estimated by extraction of the pigment with a solvent of 40 parts acetone and 3 parts water (Hornsey, 1956), followed by a simple Devarda reduction (Bremner and Keeney, 1965) which did not include the Kjeldahl digestion step.

For all fractions the resulting NH_3 was quantitated by titration in boric acid and then redistilled and collected in dilute (0.08N) H_2SO_4. The sample was then concentrated to approximately 1 ml and spotted and dried on strips of filter paper for conversion to N_2 by hypobromite on the mass spectrometer.

Head space gases were analyzed by puncturing the can through an attached rubber septum with a Vacutainer test tube under vacuum. The collected gases were then injected directly into the mass spectrometer and analyzed for various nitrogen-containing gases.

Processing gas or volatiles generated during vacuum mixing were trapped as previously described. The traps and contents were maintained in liquid nitrogen until analysis. At the time of analysis, the traps were warmed at room temperature and the gases were sampled by withdrawal into a syringe through a rubber septum which had been previously incorporated into the trap. The sample was then immediately injected into the mass spectrometer and analyzed for various nitrogen-containing gases. The sampling of the traps was done periodically during the thawing period to relieve pressure and to collect the gases as they were volatilized.

RESULTS & DISCUSSION

THE FIRST ANALYSIS was conducted 2 days after processing and less than one-half of the added ^{15}N was identified as residual nitrite (Fig. 2). Residual nitrite continued to decrease with time as described by Greenberg (1972). The residual nitrite content and change during storage for both the immediately frozen (stored at −18°C) and 71°C processed samples

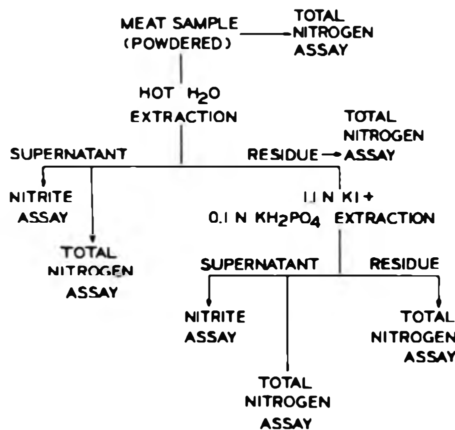

Fig. 1—Diagram for assay of meat samples.

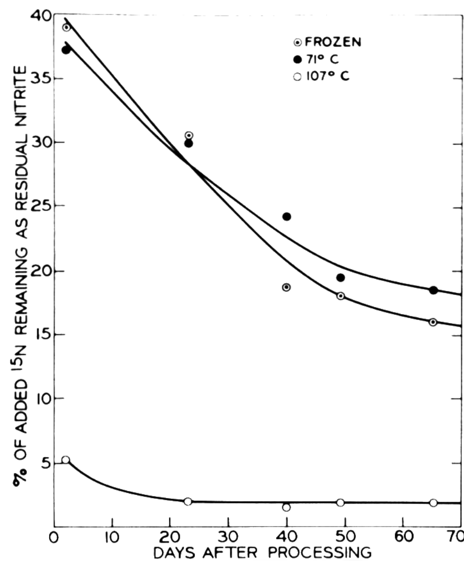

Fig. 2—Change with time of ^{15}N measured as residual nitrite.

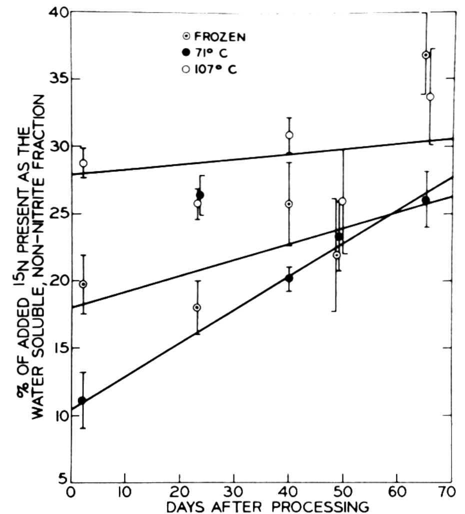

Fig. 3—Change with time of ^{15}N in nonnitrite water soluble compounds.

(stored at 5°C) paralleled each other closely and did not differ significantly The residual nitrite content of the sample retorted at 107°C and stored at 22°C, however, was already very low 2 days after processing. Nordin (1969) found nitrite conversion closely related to temperature. This was confirmed by comparison of the 71°C and 107°C samples. The frozen sample, in our experiment, however, showed an unexpected rapid conversion of nitrite; the cause for this is not known but an increased concentration of solutes due to freezing may have played a role.

The total nitrogen assay on the supernatant from the water extraction showed more ^{15}N than could be accounted for by the residual nitrite. This water soluble, nonnitrite fraction (calculated by subtracting nitrite ^{15}N from total ^{15}N of the extract) showed a significant increase over time for 71°C cooked samples and showed a slight although nonsignificant increase for the frozen and the 107°C sample (Fig. 3). The amount of label in this fraction accounted for a substantial portion of the total recovered ^{15}N.

The salt extraction failed to solubilize much protein. This low protein solubility was expected since the samples were subjected to rather severe heat treatment, both in processing and in the hot water extraction. The protein that was extracted, however, showed a small but significant increase with time in the amount of ^{15}N (Fig. 4). This established that some of the nitrite became involved with proteins since this extract after the water extraction was essentially all amino nitrogen. The colorimetric test for nitrite in this supernatant was negative.

The residue from the salt extraction consisted largely of heat denatured muscle proteins and connective tissue components. This residue contained a small amount of ^{15}N initially which increased markedly and very significantly during the first 23 days of storage after which it plateaued (Fig. 5). The ^{15}N in this fraction of the frozen and 71°C samples paralleled each other, while in the 107°C sample, it was significantly higher at 2 and 65 days of processing. The ^{15}N contained in this fraction, as well as that in the salt soluble extract, probably represented protein bound nitrogen. It has been suggested that nitrite binds to proteins through thionitroso bonding (Mirna and Hoffman, 1969; Olsman and Krol, 1972) as well as by other means.

The amount of ^{15}N associated with the pigment fraction appeared at first to be surprisingly large. However, it was necessary to perform this extraction on a freshly powdered sample and subsequent investigation showed that residual nitrite was carried along with the pigment in the small amount of water present. The amount of ^{15}N associated with the pigments was therefore corrected (for the 107°C sample) by subtracting the ^{15}N of residual nitrite from the total ^{15}N in the uncorrected acetone-water extract and crediting the difference to pigment bound ^{15}N (see Fig. 6 for the difference of the curves). This correction may over-compensate since nitrite may not be quantitatively recovered in acetone-water, but the error should be below 2% because less than 2% of the nitrite remained in the 107°C sample beyond the first 2 days. Figure 6 gives both the residual nitrite curves and the uncorrected acetone-water curve. This indirect method for pigment bound ^{15}N accounted for 8–9% of the ^{15}N in the 107°C sample. The frozen sample should have little or no cured pigment formed; therefore, all of the label in the uncorrected acetone water extract was presumed to be due to residual nitrite. Since residual nitrite levels were approximately the same for the frozen and 71°C samples, the difference in ^{15}N content of the uncorrected acetone water extract of these two samples should be attributable to the pigment bound ^{15}N contained in the 71°C sample. The difference was 9–12%, which gave an estimation of the amount of pigment bound ^{15}N in the 71°C sample.

Assuming that myoglobin comprises about 0.36% by wet weight of muscle (Lawrie, 1966) and the molecular weight of myoglobin is 17,000, calculation reveals that on a mole of ^{15}N-nitrite per mole of myoglobin basis about 10% of the added label should be bound to myoglobin. Our results ranged between 9 and 12%. Meat will, however, contain varying amounts of myoglobin as well as some other heme pigments such as hemoglobin, cytochromes and vitamin B_{12}. Also, if cooked pigment has the capacity to bind two moles of ^{15}N nitrite as has been suggested (Tarladgis, 1962), then a maximum of 20% of the ^{15}N may be bound to pigments. Thus, our assumptions in correcting the pigment fraction seem reasonable. The N_2 of head space gases was also examined for ^{15}N and only a small amount of N_2 could be detected, which accounted for about 1% of the ^{15}N in the heat processed samples and none for the frozen sample.

Analysis of gas trapped during mixing showed a very small amount of N_2 and a

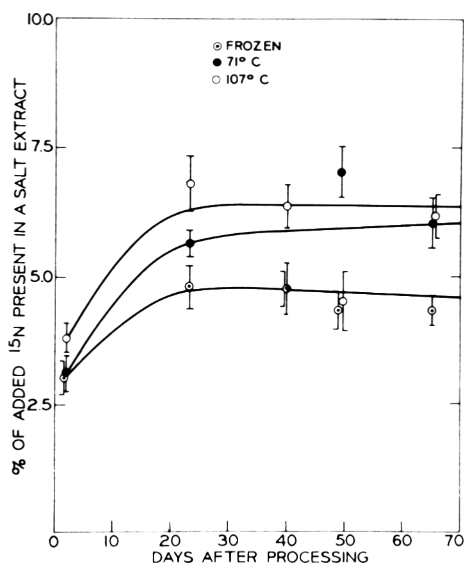

Fig. 4–Change with time of ^{15}N in compounds of a salt-soluble extract.

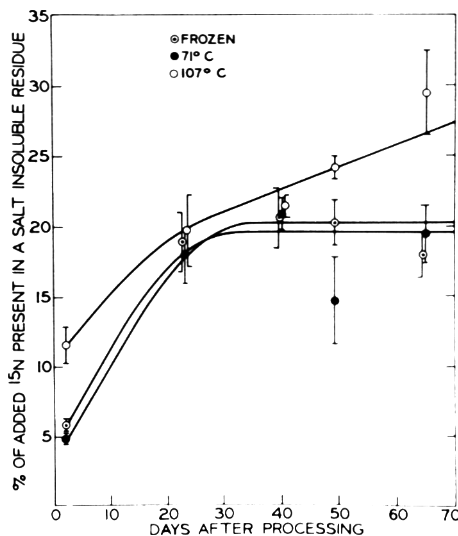

Fig. 5–Change with time of ^{15}N in compounds of a salt-insoluble residue.

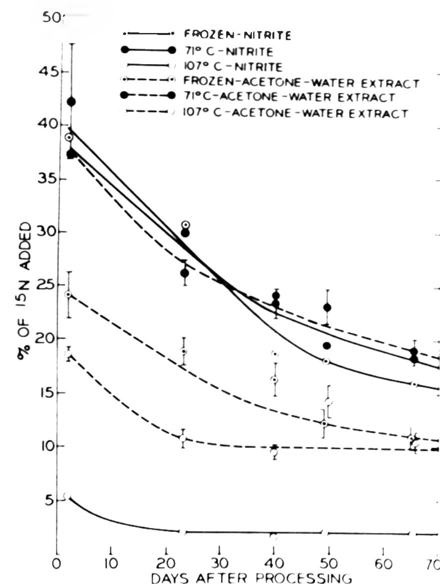

Fig. 6–Change with time of ^{15}N measured as residual nitrite and the change with time of ^{15}N in compounds in an uncorrected acetone-water extract.

somewhat larger amount of NO. Exact quantitation was not possible for the gas samples because of sampling limitations, but estimates were made by comparison of peak heights with a reference of known concentration. The ^{15}N content in these two gases together was approximately 5% of the total ^{15}N added.

The sample processed at 107°C also contained some exuded water and gelatin in the cans. This was examined by the total nitrogen assay and found to contain 2–3% of the total ^{15}N.

The total recovery of ^{15}N as determined by summing the individual fractions ranged from 72–86%.

CONCLUSIONS

IT WAS CONFIRMED that nitrite added to a meat product is rapidly changed to other compounds during and after processing and that the rate of change slows until a rather constant low level of residual nitrite is reached. As the residual nitrite as such was changed, we found an increase of ^{15}N occurred in two fractions: an apparent protein bound fraction and a nonnitrite, water-soluble fraction. The amount of ^{15}N in the pigment fraction was relatively constant during storage, did not vary greatly due to amount of heat processing and agreed with calculated predictions.

Despite considerable effort, total recoveries could not be improved above the range of 72–86%. However, because commercial conditions were being simulated, a low amount (156 ppm) of $NaNO_2$ was used. Use of larger amounts of nitrite might produce better recovery but result in artifacts and conclusions unrealistic for a commercial process.

We have not attempted to quantitate nitrate which might be formed (Möhler, 1970) in the product since nitrate would be included in our total nitrogen assay.

The implications which this work may have in regard to formation of nitrosamines or to the bacteriostatic "Perigo factor" (Perigo et al., 1967; Perigo and Roberts, 1968) are still unclear. The fact that nitrite is chemically reactive may indicate a good probability for nitrosamine formation. On the other hand, the conversion of nitrite to different forms or compounds drastically reduces the nitrite concentration. This may well reduce the likelihood of nitrite reacting with secondary amines when the two substances are brought together in the low acid environment of meat.

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MEASUREMENT OF SAUSAGE EMULSION STABILITY BY ELECTRICAL RESISTANCE

INTRODUCTION

A RAPID and simple method for the evaluation of the stability of sausage emulsions could be of substantial benefit to the industry. Cook stability (Townsend et al., 1968) and sensory rating of physical properties (Morrison et al., 1971) after cooking are the primary methods presently available for evaluating the stability of sausage emulsions. These tests require at least 30 min time and are not necessarily precise indicators of emulsion performance under actual production conditions.

Table 1–Composition of formulae used to prepare sausage emulsions for stability measurements

Formula	Ingredients	Percent
A	beef lean[a]	33.74
	soybean oil	48.52
	ice/water	16.26
	salt	1.48
B	pork lean[b]	37.00
	pork trim[c]	35.00
	ice/water	25.00
	salt	2.00
	cure mixture[d]	0.09
	sodium erythorbate	0.02
	dextrose	1.00
	spices	0.13

[a] Moisture = 76.01%, protein = 20.74%, fat = 2.10% and ash = 1.10%

[b] Moisture = 73.49%, protein = 21.65%, fat = 4.72% and ash = 1.22%

[c] Moisture = 29.57%, protein = 7.21%, fat = 63.56% and ash = 0.44%

[d] Composition: sodium nitrite (18%), sodium nitrate (18%), sodium chloride (64%)

Becher (1966) suggested electrical conductivity as a method for determining types of emulsions. Ivey (1969) suggested the use of electrical resistance as a rapid method for testing the stability of dilute emulsion systems. Webb et al. (1970) found electrical resistance to be a reliable objective method for determining the end-point for the emulsifying capacity measurement. Morrison et al. (1971) and Haq et al. (1973) reported that emulsions with relatively high total lipid content had lower a-c conductivities than those with low total lipid content; with conductivity decreasing sharply at the point of emulsion collapse.

A meat protein, under defined conditions of concentration, pH and ionic strength, exhibits a characteristic net charge (Wilson, 1960). In a meat emulsion, the protein is the emulsifying agent of the continuous phase and the dispersed particles are associated with the continuous phase by electrical charges (Saffle, 1968). When an electric field is applied to a dispersing system, such as emulsion, consisting of charged particles, the positively charged particles will migrate to the negative electrode and vice versa. This phenomenon has been designated as electrophoresis. When this migration occurs, the flow of current is impeded by the presence of the particles collected near the electrode(s). Also, polarization (electro-chemical reaction) may occur at the electrodes in an ionic system. Thus, the direct current (d-c) measurement of a dispersing system can be affected by these phenomena. However, with alternating current (a-c) measurement, the effect is reduced but depends upon the frequency of the alternating current.

The purpose of this investigation was to determine the validity of electrical a-c impedance and d-c resistance as methods for evaluating the stability of sausage emulsions.

EXPERIMENTAL

Sample preparation

Lean beef (semimembranosus) and pork muscle (longissimus dorsi) tissue samples were trimmed free of surface fat and connective tissue. Each was ground separately, twice, through a 3/8-in. plate, mixing thoroughly each time. Pork trim (60% fat), for the addition of the fat component, was ground twice through a 3/8-in. plate with thorough mixing each time. The samples were divided into lots of 400g each, placed in Whirl-Pak bags, frozen and stored at -27°C. The frozen samples were thawed at 2°C for 24 hr and used for analytical purposes and emulsion preparation.

Two formulae (designated as A and B) were used to evaluate the stability measurements. The ingredients and levels for each formula are shown in Table 1.

Emulsions with the composition of Formula A were prepared using beef samples prepared as previously described, soybean oil, water/ice and salt. The emulsions were prepared in three stages using the Mixer Omni Base 2 System reported by Haq et al. (1972). In the first stage lean beef, ice and salt were chopped (2°C) with a Brookfield counter-rotating mixer. In the second stage chilled soybean oil (2°C) was added stepwise in 12–15 ml portions, stirred with a rod and chopped (8.0 ± 0.5°C) with the Brookfield mixer. High speed comminution was accomplished in the third stage to simulate an emulsitator by chopping with a Sorvall Omni mixer until the final temperature attained 15.0 ± 0.5°C.

Emulsions with the composition of Formula B (commercial type emulsion) were prepared in two stages using the Cutter Mixer Base 2 System reported by Haq et al. (1972). Pork lean, salt, ice, cure and sodium erythorbate were chopped (1.5 ± 1.0°C) for 40 sec in the first stage. In the second stage, pork trim, dextrose and spices were added to the above lean mixture and chopped for various times. Each time of chopping was accomplished on a separate emulsion using Formula B. The chopping times for the second stage were: 5 sec (designated as B_1), 15 sec (designated as B_2), 40 sec (designated as B_3), 90 sec (designated as B_4) and 143 sec (designated as B_5). Emulsions were prepared and evaluated in triplicate.

Table 2–Terminal chop temperatures, physical properties scores and cook stability losses on sausage emulsions chopped for various times

Formula code designation	Chopping time, sec		Terminal chop temp °C	Physical properties score	Cook stability, % loss	
	Stage 1	Stage 2			Water phase	Oil phase
B_1	40	5	8.5±2.0	16±0	3.50±1.97	4.33±2.30
B_2	40	15	10.0±0.5	17±0	2.23±2.03	2.13±2.37
B_3	40	40	16.5±0.5	23±0	–	–
B_4	40	90	25.0±1.0	23±0	2.70±3.20	–
B_5	40	143	28.5±1.5	11±0	19.67±0.57	9.80±1.60

Evaluation procedures

The d-c resistance of the emulsion prepared with Formula A was recorded continuously on a Bausch and Lomb VOM-5 strip chart recorder ($\times 10^5$ ohm scale) during the third stage of chopping with the Mixer Omni Base 2 System.

Terminal point d-c resistance measurements were made using a Triplet VOM meter on the emulsions designated B_1, B_2, B_3, B_4 and B_5 immediately after completion of chopping. The values were recorded immediately after the connections were made.

The a-c impedance measurement system was developed as illustrated in Figure 1. A Hewlett Packard test oscillator (#651 B) was used to apply an a-c signal across electrodes fitted in the chopping bowl. The oscillator was set to give an output of 1 volt at 1 KHz. The a-c signal across the electrodes was converted to d-c through a specially designed bridge circuit (Edgerton et al., 1968). This converted d-c signal was applied to a Clevite Brush recorder (Mark 250). The recorder was calibrated with standard resistors. Before starting each experiment, connections were made as shown in Figure 1. The a-c impedance of the emulsions B_1, B_2, B_3, B_4 and B_5 was recorded continuously during the second and third stages of chopping with the Cutter Mixer Base 2 System. If a test unit having a different configuration from the unit developed for this experiment is used, it would be necessary to calibrate the particular system for impedance, thereby establishing the correlation between conductance and impedance.

Also, after the chopping was terminated, the emulsions (B_1, B_2, B_3, B_4 and B_5) were lightly tamped into the chopping bowl to remove voids and a terminal point a-c resistance measurement was made using a Y.S.I. (Model 31) conductivity bridge (Yellow Springs Instrument Co., Yellow Springs, Ohio). The resistivity was determined as instructed by the conductivity bridge manufacturer using the relationship:

$$\text{resistivity} = \frac{\text{measured resistance in ohm}}{\text{cell constant}} \text{ (ohm-cm)}$$

The cell constant for the electrodes fixed in the chopping bowl was determined using a standard 0.01 demal KCl solution.

A Leeds and Northrup Speedomax H & W Multipoint recorder was used to measure the temperature of emulsions with a thermocouple fixed in the chopping bowl as illustrated by Haq et al. (1972).

The method of Townsend et al. (1968), with modifications as described by Morrison et al. (1971), was used to measure the cook stability of all emulsions.

Sensory evaluation of physical properties was made on the cooked emulsions using the rating scale of Haq et al. (1973). This scale included the rating of the cooked emulsion, obtained from the cook stability test, for color, resilience, graininess, firmness and binding. A total score of 23 was considered to be an emulsion having excellent stability and physical properties.

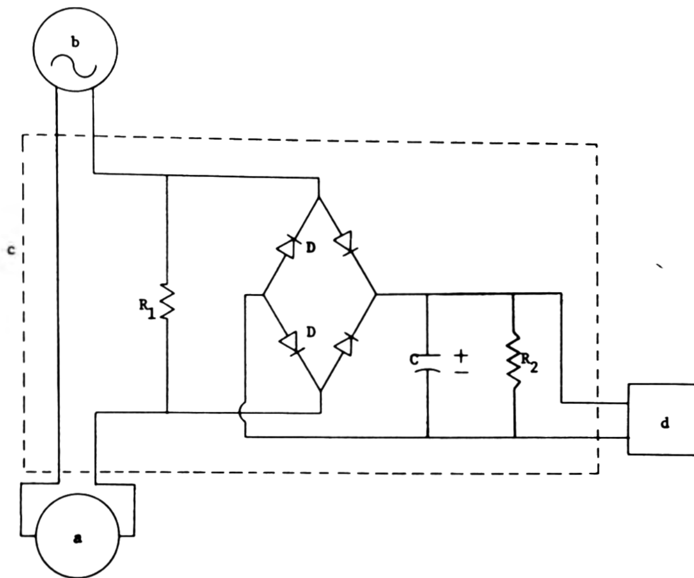

Fig. 1—a-c impedance measurement system. [(a) measuring cup with electrodes; (b) a-c source (oscillator); (c) bridge circuit; (d) recorder; (C) 1 μf; (D) type 1N663 silicon diodes; (R_1) 100 kilohms; (R_2) 1,000 ohms]

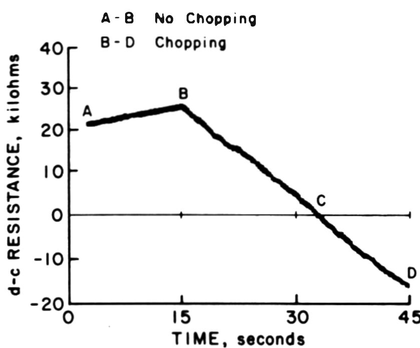

Fig. 2—Continuous d-c resistance measurement during emulsification of a sausage emulsion.

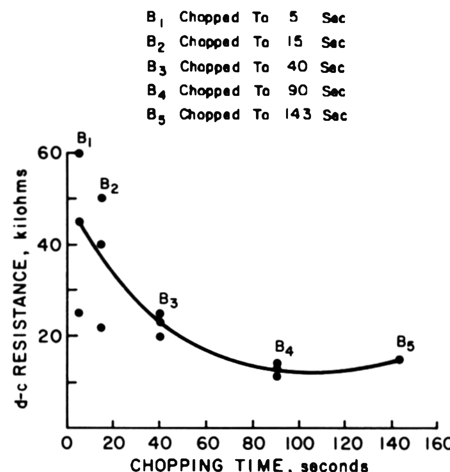

Fig. 3—Terminal point d-c resistance measurements during emulsification of sausage emulsions.

RESULTS & DISCUSSION

d-c Resistance

A typical continuously recorded d-c resistance of an emulsion prepared with Formula A (cook stability = 99.02 ± 0.39%; physical properties score = 23) is reproduced in Figure 2. When the emulsion was not being chopped, it was observed that the d-c resistance changed with time (segment A-B of the curve). It was also observed that the instrument recorded d-c resistance values virtually below zero (segment C-D of the curve). These were interpreted to be due to polarization (electrochemical reaction). When a d-c field is applied to an ionic system, an emf is produced at the electrodes and the electrodes act as a battery (Becher, 1966). This emf alters the original circuitry for the resistance measurements and, therefore, the initial calibration does not hold true during the measurement. Previous investigations in this laboratory indicated that the d-c resistance measurement was valid for highly dilute emulsion systems such as the measurement of emulsifying capacity (Webb et al., 1970). However, in highly viscous emulsion systems the d-c resistance measurement was not satisfactory. This difference was believed to be due to the ability of the system to become polarized under the conditions of this experiment. Therefore, additional methods of measurement were investigated.

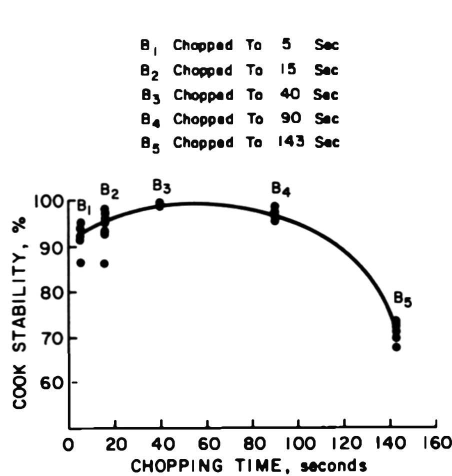

Fig. 4–Cook stability measurements during emulsification of sausage emulsions.

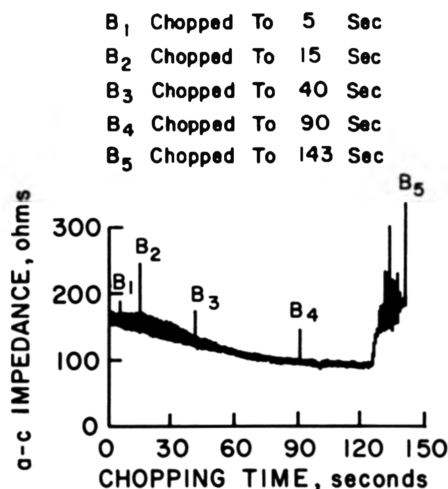

Fig. 5–Continuous a-c impedance measurement during emulsification of a sausage emulsion.

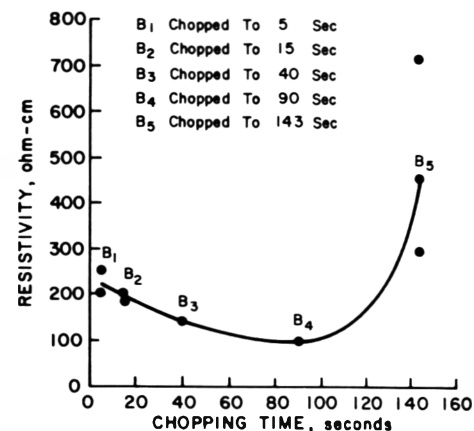

Fig. 6–Terminal point resistivity measurements of sausage emulsions.

The terminal point d-c resistance measurements made on the emulsions B_1, B_2, B_3, B_4 and B_5 are presented in Figure 3. While making measurements it was observed that the reading changed with time which verifies points A-B in Figure 2. Therefore, extreme care had to be taken to obtain a consistent time and d-c reading after preparation of these emulsions.

The cook stability data for the emulsions B_1, B_2, B_3, B_4 and B_5 are presented for the various chopping times in Figure 4. The terminal chop temperatures, water and oil losses from the cook stability tests and the physical properties scores by sensory evaluations are presented in Table 2. It is evident from the data presented in Figures 3 and 4 that the cook stability and the d-c resistance of the emulsions B_1 and B_2 were highly variable (B_1 : σ d-c = ± 17.56 Kilohms, σ cook stability = ± 2.85%; B_2 : σ d-c = 14.19 Kilohms, σ cook stability = ± 3.87%). Variability decreased as the emulsions became more completely emulsified with increased chopping time. The d-c resistance of emulsion B_5 was significantly higher ($P = 0.100$) than emulsion B_4, while the cook stability was significantly lower ($P = 0.001$). These emulsions had collapsed as evidenced by the oil and water separation during cooking (Table 2).

It was concluded that, under the conditions of this study, the d-c resistance measurements were not sufficiently reliable for evaluating the stability of sausage emulsions. The factors responsible for this lack of reliability were attributed to polarization, the large number of charges on particles due to the proteins and the high viscosity of an emulsion at a concentrated protein level. Webb et al. (1970) successfully used the d-c resistance measurement to determine the end-point of the emulsifying capacity measurement on dilute protein extracts while the system was being agitated. This difference in results was attributed to the very dilute system and the recording of the d-c resistance during continuous agitation for the emulsifying capacity measurement.

a-c Impedance

A typical continuous a-c impedance measurement on emulsion B_5 is shown in Figure 5. The continuous a-c impedance measurements were very reproducible. The recording patterns were similar for all trials on each emulsion (B_1, B_2, B_3, B_4 and B_5) and the patterns for all of the emulsions were reproducible from that of emulsion B_5, as illustrated in Figure 5.

The terminal point a-c resistivity data for the five emulsions are shown in Figure 6.

The cook stabilities and terminal a-c resistance measurements were highly variable during the early stages of chopping (B_1 : σ cook stability = ± 2.85%, σ a-c resistivity = ± 29.63 ohm-cm; B_2 : σ cook stability = ± 3.87%, σ a-c resistivity = ± 10.86 ohm-cm) which was attributed to the heterogeneity and coarseness in the structure of the emulsion system. These observations were confirmed by the physical properties scores in Table 2. The a-c resistivity of emulsion B_3 was less variable (σ = ± 3.55 ohm-cm) than that for emulsions B_1 and B_2. The cook stability and physical properties scores on emulsion B_3 were very desirable. An inverse relationship ($r = -0.880$) existed between the cook stability and the a-c resistivity of emulsions B_1, B_2 and B_3. When emulsion B_4 was chopped for 90 sec, the a-c resistivity increased and the cook stability was significantly ($\alpha = 0.001$) lower than that of emulsion B_3. Emulsion instability, as evidenced by lower cook stability and physical properties scores (Table 2) was reflected by a change in the slope of the impedance and resistivity curves (Fig. 5 and 6). This instability was attributed to an excessive chopping time (overchopping of 140 sec). Conversely, a resistivity of 130 ± 20 ohm-cm (18.0 ± 2.5°C) indicated a very stable emulsion on the basis of the cook stability and physical properties scores. Therefore, the results indicated that the a-c impedance measurement technique was of value as a test of emulsion stability. It was concluded that a-c impedance has potential as an objective technique for determining the stability of sausage emulsions. However, further research would be necessary to develop the system for practical application.

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CHANGES IN MEAT COMPONENTS DURING FERMENTATION, HEAT PROCESSING AND DRYING OF A SUMMER SAUSAGE

INTRODUCTION

APPROXIMATELY one-tenth of all meat produced in the United States is consumed in the form of sausage products. In 1971, 203 million pounds or 6% of the total sausage production in federally inspected meat plants were semidry and dry products (Filiatreau, 1972).

The manufacture of fermented sausage has been described as an "art" and has traditionally been practiced by only a few specialists (Kramlich, 1971). Early processes required approximately 3–5 days for fermentation and processing prior to drying. The fermentation was accomplished by the lactic acid bacteria present as a part of the natural meat flora, introduced from equipment, or introduced by adding back part of a freshly fermented meat batch (Deibel et al., 1961a, b; Everson, 1971; Niven, 1960). Production failures, such as the development of off-flavors and casing "explosions," were not uncommon when uncontrolled fermentations occurred (Jensen, 1954). Jensen and Paddock (1940) first introduced starter cultures for meat fermentation. The bacteria which have been proposed for starter cultures are *Micrococcus aurantiacus* (Niinivaara, 1955), *Pedicoccus cerevisiae* (Deibel and Niven, 1957) and a number of species of *Lactobacillus* (Jensen and Paddock, 1940). Stoychev et al. (1972a, b) have investigated the effects of pH and concentrations of NaCl, $NaNO_2$ and $NaNO_3$ on the growth and enzymatic activity of starter cultures of *Micrococcus* and *Lactobacillus* species. In addition, the growth characteristics of these bacteria as related to meat fermentation have been reported (Stoychev et al., 1972c).

The commercial introduction of lyophilized starter cultures in 1957 allowed processors to control fermentation and thus maintain desirable and uniform product characteristics from batch to batch. Starter cultures of *Lactobacillus plantarum* and *Pediococcus cerevisiae* are currently available as a frozen concentrate (Everson et al., 1970; Anonymous, 1972). Fermentation time requirements have been significantly reduced to 32–40 hr using the lyophilized culture form and to 18–24 hr using the frozen concentrate (Everson et al., 1970).

Acton et al. (1972) reported that fermentation of summer sausage at either 22, 30 or 37°C did not significantly affect product flavor although less lactic acid was produced at 22°C than at 30 or 37°C. A longer lag phase for growth initiation of the lactic acid bacteria occurred at the lower temperature. The water-holding capacity of the ground sausage mix gradually decreased during 72 hr, particularly at 30 and 37°C, which indicated progressive protein denaturation.

Bertling and Müller (1965a, b; 1966a, b, c) and Bertling (1967a, b) recorded the average composition for seven types of European dry sausage. These studies revealed that the composition of all types was within a narrow range for each of the major components. Approximate quantities summarized from these reports are: moisture, 31%; fat, 48%; and protein, 16%. Analyses for semidry products were not reported.

This study was conducted to determine where and at what rate compositional, microbiological and physical changes occur during the phases of summer sausage processing. While the term "summer sausage" can be used to describe a wide variety of product styles, the product utilized in this study would be classified as a fermented-cooked summer sausage.

EXPERIMENTAL

Sausage fermentation and processing

A summer sausage formula (Table 1) was used in this study. Fresh pork trimmings and boneless beef were coarsely ground through an 8-mm plate, reground through a 4-mm plate and mixed in a Hobart H-600 mixer. Cure and seasonings were thoroughly blended into the meat mixture before adding the starter culture, *Pediococcus cerevisiae*, to a level of 3.6×10^6 cells/g meat. Initial meat temperature was approximately 5°C and increased to approximately 12°C during 15 min of blending. Three replicate sausage batches were prepared utilizing pork and beef of the same source.

Each sausage batch was stuffed into 52 mm diameter Dry Sausage fibrous casing (Union Carbide). The sausage sticks, each weighing approximately 1.8 kg, were hung in a fermentation chamber maintained at 38°C and 95–98% RH. The sausages were held in this chamber for 36 hr.

Following fermentation, the sausage sticks were initially heated at 82°C for 60 min and then at 88°C until an internal temperature of 71°C was obtained. Most summer sausages are not heated more than 63°C internally before drying. The product in this study represents only a single product style of summer sausage. Following heat processing, the product was given a cold water spray rinse and placed in a 7.5 ± 2.0°C drying room having 10–15 air changes/hr. The air relative humidity ranged from 78 to 82%. The summer sausage was held in the drying room for 60 days. Samples of each batch were taken for analysis at the following processing intervals: (1) after blending; (2) after fermentation; (3) at 63° internal during heat processing; (4) at 71°C internal after heat processing; and (5) after 10, 30, and 60 days of drying.

Table 1—Summer sausage ingredients

Ingredient	Quantity
Meats:	
lean beef, boneless	4.54 kg
lean pork trimmings	4.54 kg
Cure:	
sodium nitrite	0.70 g
sodium erythorbate	4.24 g
sodium chloride	226.8 g
Seasonings:	
ground black pepper	17.0 g
ground white pepper	17.0 g
ground mustard	5.7 g
coriander	2.8 g
allspice	2.8 g
nutmeg	1.4 g
sage	5.7 g
sucrose	45.4 g
Starter culture:	
Pediococcus cerevisiae (Accel)[a]	7.1 g
Dextrose	90.7 g

[a] Trademark of starter culture produced by Merck & Company, Rahway, N.J.

Chemical analyses

Percentages of moisture, fat, protein, salt and lactic acid were determined for sausage samples of each replicate at the intervals indicated above. Moisture was determined by the AOAC (1970a) method. Ether extractables (Soxhlet) were used to calculate percent fat. The Kjeldahl nitrogen method following AOAC (1970a) was used for protein determinations. The salt content, expressed as percent NaCl, was measured with QUANTAB Chloride Titrators following the procedure of the AOAC (1970b) and Vander Werf and Free (1971).

Table 2–pH, percent lactic acid, percent salt, shear force values and preference panel scores of summer sausage during processing and drying[a]

Sample	NaCl %	Lactic acid %	pH	Shear force kg/g/cm²	Preference Score[b]
Sausage mix:					
initial	2.23[a]	0.00[a]	6.05[a]		
fermented	2.34[ab]	0.47[b]	4.85[b]		
63°C internal	2.40[ab]	0.47[b]	4.85[b]		
71°C internal (0 days drying)	2.60[bc]	0.46[b]	4.91[c]	0.22[a]	7.02[a]
Product (dried):					
10 days	2.92[c]	0.48[b]	5.05[d]	0.67[b]	7.28[a]
30 days	3.62[d]	0.50[b]	5.05[d]	1.31[c]	6.55[a]
60 days	4.19[e]	0.73[c]	5.19[e]	2.60[d]	6.95[a]

[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] Preference scale: 9 = like extremely; 1 = dislike extremely.

Table 3–Nitrogen fractions in meat during processing and drying of summer sausage[a]

Sample	Nitrogen Fraction (mg N/g wet sample)				
	Myofibrillar	Sarcoplasmic	Nonprotein	Insoluble	Total
Sausage mix:					
initial	3.34[a]	5.73[a]	2.98[a]	16.21[a]	28.26[a]
fermented	1.75[b]	0.62[b]	4.45[b]	22.07[b]	28.89[a]
63°C internal	0.68[c]	0.21[b]	5.16[bc]	24.26[b]	30.31[a]
71°C internal	0.18[c]	0.79[b]	4.51[b]	24.03[b]	29.51[a]
Product (dried)					
10 days	0.11[c]	0.08[b]	5.85[c]	33.48[c]	39.52[b]
30 days	0.00[c]	0.00[b]	7.91[d]	40.21[d]	48.12[c]
60 days	0.00[c]	0.00[b]	8.73[d]	42.18[e]	50.91[d]

[a] Any two means within a column having the same or one of the same letters are not significantly different at $P < 0.05$.

Lactic acid content was determined as previously described (Acton, et al., 1972) using meat homogenates. The pH of the sausage homogenates was recorded prior to titration for lactic acid.

The following nitrogen (N) fractions of the sausage samples were studied: myofibrillar protein N; sarcoplasmic protein N; nonprotein N (NPN); insoluble N; and total N. Total N was obtained by Kjeldahl N analysis (AOAC, 1970a) of the meat samples. Myofibrillar protein N, sarcoplasmic protein N and NPN fractions were obtained using the extraction and fractionation procedure of Khan and van den Berg (1964). 15g of finely ground meat from each processing interval previously listed were added to 300 ml of phosphate buffer (pH 7.0, ionic strength 1.0) and blended on a magnetic stirrer at 465 rpm for 60 min at 4°C. The slurry was centrifuged at 4°C and 30,900 × G for 30 min and the supernatant filtered through Whatman #1 paper. NPN in the supernatant was determined by using 5% trichloroacetic acid (TCA) filtrates. Myofibrillar proteins in the supernatant were fractionated at an ionic strength of 0.04 by diluting with 24 vol of distilled water at 4°C and centrifuging for 30 min at 12,000 × G. Proteins remaining soluble at 0.04 ionic strength were designated the sarcoplasmic fraction. Fraction N concentrations were expressed as mg N per g wet sample.

Microbiological analyses

Counts of total viable bacteria and lactic acid bacteria were made at each sampling interval. 10-g samples of the meats were blended with a Waring Blendor for 1 min with 90 ml quantities of 0.9% saline and subsequent decimal dilutions prepared with the same diluent. Duplicate 1-ml samples of the appropriate dilutions were mixed with standard plate count agar (APHA, 1968) or lactic agar (Frazier et al., 1968). Plates were incubated at 30°C for 48–72 hr before counting.

Shear and panel preference analysis

Summer sausage samples at 0, 10, 30 and 60 days of drying were analyzed for shearing force and preference scores. Meat slices 2 mm thick and of variable diameter (52–36 mm), due to stage of sausage dryness, were cut for both determinations.

For shear measurements, an Allo-Kramer Shear Press equipped with a 3000-lb ring was used with a 30 sec downstroke at range 300. Shearing force for each slice of meat was calculated as kg/g/cm² of surface area exposed to the shear blades. Shear values were recorded from groups of three slices randomly selected from each of the three sausage batches.

Sausage slices were served at room temperature (22°C) to 20 untrained panelists for preference rating. Panelists scored the samples on a nine-point hedonic scale (1 = dislike extremely; 9 = like extremely). Three rating sessions were conducted using the same panelists.

RESULTS & DISCUSSION

Chemical analyses

The initial sausage mixture contained 60.1% moisture, 15.8% protein, 19.9% fat (Fig. 1). No significant ($P < 0.05$) change occurred in the quantity of these components during fermentation. There was a slight increase in fat content during heat processing due to a 6.7% loss of moisture. During the 60-day drying period, the increase of protein and fat content of the summer sausage was significantly ($P < 0.01$) correlated with the decrease in moisture level. The amount of moisture removed over the drying period followed previously estimated shrinkage schedules (Wilson, 1960). At 10 days of drying the summer sausage was at the semidry stage (20% shrink). The medium dry stage (30% shrink) was attained at approximately 18 days and the dry stage (35–40% shrink) at 24–30 days. The summer sausage would be classified as "fully dried" at approximately 40 days, having reached a 40–50% moisture loss.

The NaCl content (Table 2) did not change during fermentation but was significantly ($P < 0.05$) higher after heat processing when compared to the initial level in the sausage mixture. Salt content significantly increased at each drying interval, being dependent on the rate of moisture removal during drying ($r = -0.95$). At 60 days, the salt content had increased by 87% over the initial level in the mixture.

The lactic acid content produced during fermentation (Table 2) remained relatively constant (0.47–0.50%) from the end of fermentation through 30 days of drying. The lactic acid quantity of 0.50–0.73% at 30–60 days is within the concentration range generally reported for summer sausage (Acton et al., 1972; Merck, 1969).

The sausage pH significantly decreased from an initial value of 6.05 to 4.85 during fermentation (Table 2), reflecting the production of lactic acid by the starter culture. Product pH significantly increased by 0.1–0.2 pH units during the 60-day drying period. It may be possible that the pH increase was due to an increase of basic NPN compounds accumulated during heat processing and drying (Table 3).

The quantities of soluble myofibrillar and sarcoplasmic protein N significantly ($P < 0.05$) decreased during the meat fermentation as shown in Table 3. These changes were reflected in the concurrent

increase of the insolubleN fraction. The sarcoplasmic protein fraction was more rapidly denatured during the 38°C fermentation than the myofibrillar protein fraction. Bendall and Wismer-Pederson (1962) reported that within pork muscle tissue a combination of low pH and high temperature precipitates sarcoplasmic proteins onto the myofibril. The low temperature heating (38°C) over the 36-hr fermentation apparently provided a continued thermal energy input which resulted in the substantial denaturation of muscle protein fractions prior to heat processing (Table 3). It should be noted that changes in the fraction solubilities on extraction are the result of the summation of partial denaturations occuring through the time gradient used for fermentation. In addition, the pH decrease accompanying fermentation may have contributed to the decreased solubility of both types of protein.

No significant change occurred in the total N quantity during fermentation and heat processing of the sausage. On drying, the quantities of NPN, insoluble N and total N increased, all changes being highly correlated with the rate of moisture removal.

Microbiological analyses

The lactic acid bacteria counts of the inoculated sausage increased by 2 log cycles during the 36-hr fermentation (Fig. 2) which is in agreement with the report of Acton et al. (1972). Heat processing of the fermented sausage reduced counts of the total viable lactic acid bacteria by 4.5 log cycles. During the 60-day drying period, one additional log reduction was found. Total plate counts from standard plate count agar were similar to, and in most cases, the same as those shown for the lactic acid bacteria.

Shear and panel preference analyses

Shear forces of slices of summer sausage samples from 0–60 days of drying are given in Table 2. At each analysis interval, there was a significant ($P < 0.05$) increase in the shear force values. Shear force and moisture content were highly correlated ($r = -0.90$).

In addition to sausage classification by moisture content, or shrinkage, it would be possible to rapidly assign dryness classification by degree of hardness as determined by the shear press. In this study, the 60-day "dry" summer sausage was approximately 3.9 times as tough as the "semidry" sausage of 10 days. There are no quantitative classification characteristics for fermented (or nonfermented) and dried sausage products, other than moisture content, known to exist at the current time.

Panel preference scores (Table 2) showed no significant difference between samples due to stage of summer sausage dryness. Panelists noted that the "dry" sausage samples were tougher than the other samples. This may be responsible for the slightly lower score of the 30- and 60-day sausage samples. Although flavor analysis was not conducted, the sausage had a mild degree of "tanginess" characteristic of this type of product (Acton et al., 1972; Borgstrom, 1968).

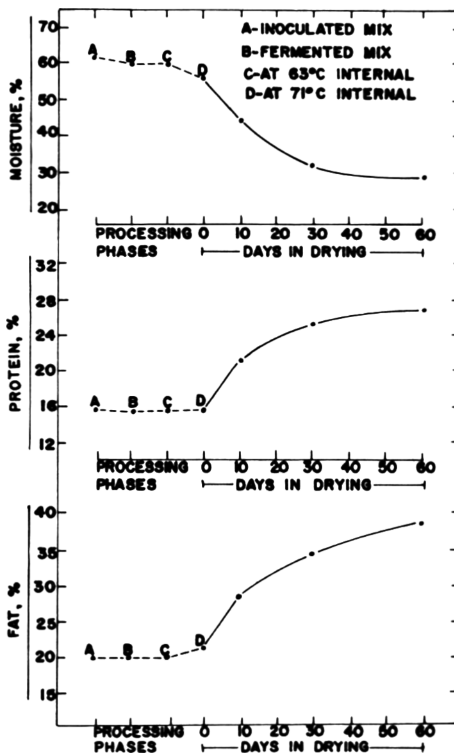

Fig. 1–Moisture, protein and fat contents during processing and drying of summer sausage.

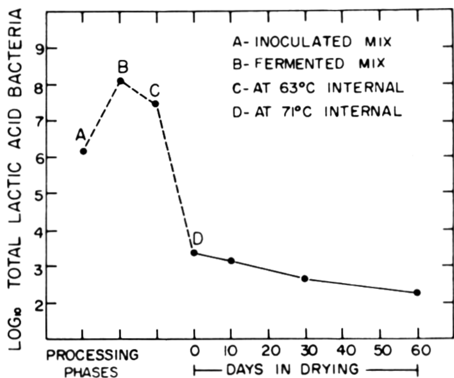

Fig. 2–Total lactic acid bacteria counts during processing and drying of summer sausage.

CONCLUSIONS

THE SEQUENCE of chemical, microbial, physical and sensory changes occurring during the primary processing phases (fermentation, heat processing and drying) of a fermented-cooked summer sausage were presented in this report. It was evident that (a) meat protein denaturation, (b) lactic acid bacteria growth and destruction, and (c) production of lactic acid by the starter culture were complete by the end of heat processing. Further chemical and physical changes in the product were dependent on the rate at which moisture was removed during the drying phase.

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CHICKEN LIPID CHANGES DURING COOKING IN FRESH AND REUSED COOKING OIL

INTRODUCTION

READY-TO-EAT chicken is now available throughout the U.S. from many sources, including franchised chicken specialty outlets, retail grocery stores and restaurants. Much of this chicken is breaded and cooked in heated vegetable oil, which deteriorates through continued use. Other pre-cooked chicken is merchandised in the frozen state. After frozen storage and reheating, fried chicken first loses its "freshly-cooked" flavor, then develops a "warmed-over" flavor and eventually a rancid flavor (Hanson et al., 1959). Fried chicken also darkens in color during holding or storage, may lose moisture and thus becomes less acceptable.

Oxidative rancidity is a major cause for flavor deterioration of meat during storage (Tims and Watts, 1958; Turner et al., 1954). Therefore, the lipids present in muscle tissues affect flavor quality, and are responsible in part for problems related to product stability. The susceptibility of natural fat to oxidative rancidity depends largely upon its degree of unsaturation and its fatty acid composition. Poultry fat has a total unsaturation of 60–70% (Chang and Watts, 1950), thus poultry meat tends to become rancid faster than beef or lamb. Hanson et al. (1959) found that flavor changes develop at a faster rate in muscle than skin. Katz et al. (1966) showed a higher phospholipid content in muscle than in skin, and that phospholipids contained more long-chain polyunsaturated fatty acids than did neutral lipids. Phospholipids have been considered an important component which may correlate with flavor deterioration.

Various chemical reactions such as hydrolysis, oxidation and polymerization occur in cooking oil during heating (Carlin et al., 1954). The free radicals formed in cooking oil are considered as the initiating agents for these chemical reactions, and the amounts formed may be related to the length of time cooking oils are heated or reheated. Absorption of cooking oil, or substitution for part of the moisture, can occur during cooking, thus changes in chicken fats during cooking and frozen storage may be influenced by reuse of cooking oil, and are therefore important in studies on the stability of precooked frozen chicken.

This study was conducted to evaluate changes in the fatty acid composition of the cooking oil and in chicken lipids during cooking in fresh and reused corn oil, and to evaluate changes during storage of raw and cooked chicken.

EXPERIMENTAL

COMPOSITIONAL CHANGES in the cooking oil (corn oil) were first evaluated. This included intermittent heating of water-saturated cotton balls in corn oil, and later by actual cooking of chicken pieces. Fatty acid changes in chicken muscle and skin were evaluated after the chicken pieces were cooked in fresh corn oil and in reheated corn oil.

Preparation of corn oil for analyses

600g of commercial corn oil (Miesel brand) were placed in a beaker, heated on an electric hot plate to 200°C and maintained at this temperature. Oil was heated for 6 hr, cooled overnight at room temperature, then reheated 6 hr; this process was repeated through 48 hr of heat.

Moist cotton balls, previously washed thoroughly, first with ethanol and then with redistilled hexane (Kawada et al., 1967), weighing 1g and containing 80% by weight of water, were fried in the oil every hour.

40g of oil were sampled at 1/2, 1 and 6 hr during the first period, then at the end of 24, 36 and 48 hr of heating. Samples were cooled to room temperature and kept in a vacuum desiccator at 0°C.

Preparation of chicken

7-wk old male broilers were obtained from a commercial farm and were processed in the University Poultry Laboratory. The birds were killed, defeathered and eviscerated in the usual manner, and chilled in water mixed with crushed ice for 3 hr. The birds were cut into portions identified as breasts, thighs, drumsticks and wings, packaged in "Cryovac®" bags and stored at 0°C.

Cooking procedure

Egg-milk dip. Eight large eggs were blended for 1 min in a Waring Blendor, two 415.8g (14.5 oz) cans evaporated milk and 1.9 liters (2 qt) cold water were added and mixed well immediately before use.

Breading. A basic mixture of 11.4 kg (25 lb) of breading, obtained by mixing all-purpose wheat flour and potato flour (5:1 ratio), 1.5 kg (3.25 lb) salt, and 738.4g (26 oz) commercial seasoning was used in these experiments.

Cooking. Chicken was cooked in a Mies commercial pressure fryer, Model C. 12.2 kg (27 lb) of commercial corn oil (Miesel brand) was placed in the cooker, and preheated to 205°C (400°F).

Four cut-up birds (as a group) were dipped in the egg-milk mixture for 10 sec to wet each piece evenly, drained and breaded. When the temperature of the oil reached 205°C, the pieces were added and cooked for about 1 min, depending upon the color. When the chicken was brown, the cooker was closed and the pressure regulated to 15 psi, and the chicken was cooked for 9.5 min. Immediately after cooking, the pressure was released and the pieces removed, placed on a wire rack and then transferred to a warming oven at 70°C to drain and darken in color. The pieces were held in the warming oven approximately 15 min and then packaged in a heat-sealed polyethylene bag.

Cut-up pieces were cooked in fresh corn oil and after 24 and 42 hr of heating. 40g of corn oil were sampled at the end of 6, 30 and 48 hr of heating. Moist cotton balls, previously described, were cooked every hour when chickens were not cooked. After 2 min of cooking, the cotton balls contained approximately 2.9% moisture and 91.4% of oil. 1.4 kg (3 lb) of fresh corn oil were added each day before cooking.

Three groups of chicken pieces were randomly selected for each of the following treatments: uncooked cut-up birds, cooked with fresh corn oil, and cooked with 24- and 42-hr heated corn oil. One group from each treatment was analyzed immediately after processing. The other two groups were packaged in Cryovac® bags and frozen at −37°C. Samples were stored at −18°C for 3 or 6 mo before analyses.

The samples of corn oil including fresh corn oil and corn oil heated for 6, 30 and 48 hr were kept in a vacuum desiccator and stored at 0°C until analyzed.

Lipids extraction and purification

Muscle and skin samples (without breading) were obtained from each group (four birds) and were ground prior to lipid extraction. Total lipids were extracted with chloroform-methanol-water 8:4:3 (v/v/v) according to the Folch method (Folch et al., 1957).

Methylation of lipids

Lipids were methylated by a rapid low temperature method introduced by McGinnis and Dugan (1965). This sample was kept at 0°C, in the dark, for not more than 12 hr prior to GLC analysis.

Gas-liquid chromatography

Fatty acid methyl esters were determined in an F & M (Model 810) dual column gas chroma-

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Table 1–Changes in fatty acid composition of corn oil during heating with cotton balls

Fatty acids[a]	Heating time (hr) 0	0.5	1	6	24	36	48
16:0	13.7	14.0	12.5	14.9	13.8	16.5	17.4
18:0	2.4	1.7	2.1	1.4	2.2	2.6	2.4
18:1	25.3	26.6	26.5	26.5	29.2	30.9	32.2
18:2	58.6	57.7	58.9	57.2	54.8	50.0	48.0
18:3	trace	trace	–	–	–	–	–
Total sat.	16.1	15.7	14.6	16.3	16.0	19.1	19.8
Total unsat.	83.9	84.3	85.4	83.7	84.0	80.9	80.2

[a] Number of carbons:number of double bonds; duplicate analyses

Table 2–Changes in fatty acid composition of corn oil during cooking with chicken

Fatty acids[a]	Heating times (hr) 0	6	30	48
	Percent (GLC peak area)			
14:0	–	0.4	0.3	0.7
15:0	–	trace	–	–
16:0	14.8	15.4	16.7	19.2
16:1	–	0.2	0.8	1.8
18:0	1.8	2.2	1.6	1.6
18:1	24.0	24.1	25.3	26.7
18:2	59.5	57.5	55.2	50.0
18:3	trace	0.2	–	–
Total sat.	16.6	18.0	18.6	21.5
Total unsat.	83.5	83.0	81.3	78.5

[a] Number of carbons:number of double bonds

Table 3–Changes in fatty acid composition of chicken muscle total lipids during cooking and frozen storage

	Storage time (mo) 0				3				6			
	Cooking treatments[b]											
Fatty acids[a]	Raw	A	B	C	Raw	A	B	C	Raw	A	B	C
	Percent (GLC peak area)											
14:0	1.1	1.0	1.2	0.9	1.3	0.7	0.9	1.1	2.2	0.8	1.2	1.7
15:0	1.1	1.3	0.8	0.5	1.2	0.6	0.8	0.7	3.3	0.7	1.1	1.0
16:0	20.6	21.4	20.3	21.4	25.3	20.6	20.0	23.3	26.5	19.8	21.9	23.8
16:1	5.0	3.9	3.7	3.4	5.3	3.0	3.9	4.3	6.7	4.8	4.8	4.8
18:0	7.0	4.7	5.1	5.1	7.6	4.8	5.6	4.6	6.7	3.9	5.0	4.9
18:1	33.8	31.3	30.7	29.8	37.9	31.2	33.2	30.5	36.8	31.5	33.7	33.6
18:2	24.9	33.8	37.2	37.4	20.1	38.5	34.8	34.1	16.2	37.3	31.6	29.5
18:3	1.5	1.6	1.2	1.4	0.7	0.6	0.9	1.4	0.4	1.2	0.8	0.9
20:3	2.1	1.0	–	–	–	–	–	–	–	–	–	–
20:4	2.8	–	–	–	0.6	–	–	–	1.2	–	–	–
Total sat.	29.8	28.4	27.4	27.9	35.4	26.7	27.3	29.7	38.7	25.2	29.2	31.4
Total unsat.	70.1	71.6	72.8	72.0	64.6	73.3	72.8	70.3	61.3	73.8	70.9	68.8

[a] Number of carbons:number of double bonds
[b] (A) Fresh corn oil; (B) Corn oil previously heated for 24 hr; (C) Corn oil previously heated for 42 hr

tograph, equipped with a flame ionization detector. A 72 × 1/4-in. copper column was packed with 15% diethylene glycol succinate and 3% phosphoric acid as liquid phase and chromosorb-W as solid support. Helium was used as carrier gas at a flow rate of 35 ml/min. The hydrogen flame was fed by using 60 ml/min stabilized at 200°C for 2 days. Column temperature was maintained at 190°C, with detector temperature at 260°C and injector at 250°C.

Separation of phospholipids from neutral lipids

The sample, dissolved in chloroform, was applied in several spots at a distance of 0.5 cm from each other along the bottom of a plate coated with silica gel G. The plate was allowed to stand until the chloroform evaporated from the spotting sample.

The solvent system was a combination of hexane, diethyl ether and acetic acid in a volume ratio of 80:20:1. Ascending chromatography was used in a vapor saturated chamber. This procedure allowed the solvent to rise to within 0.5 cm of the top of the absorbent. Average running time was 30 min.

After development, the solvent was evaporated from the plate and the spots were then made visible in iodine vapor. Phospholipids which remained at the origin, were scraped off the plate with the solvent containing chloroform and methanol at a ratio of 2 to 1 (v/v) through the filter paper. The remaining neutral lipids were scraped and washed with ethyl ether into small tubes. The purified phospholipids and neutral lipids were subjected to methylation for GLC analyses of the fatty acids.

RESULTS

Changes in corn oil composition

Composition of corn oil before heating and after heating with cotton balls for times up to 48 hr are reported in Table 1. Corn oil initially contained 59% linoleic acid, 25% oleic acid, 14% palmitic acid, 2% stearic acid and trace amounts of linolenic acid. During heating linoleic acid decreased, especially after 24 hr of heat, whereas oleic and palmitic acids increased.

The percentage of all fatty acids was calculated as 100%; therefore, when one of these fatty acids decreased, one or more of the other fatty acids increased. The change in fatty acid content of corn oil during heating, due mostly to the decrease in linoleic acid, was expected.

The cooking oil was sampled and analyzed frequently during intermittent cooking of chicken. Linoleic acid decreased from 59% in fresh corn oil to 50% in oil heated for 48 hr (Table 2). Linoleic acid in corn oil declined about the same during cooking of chicken and when the corn oil was cooked with moist cotton balls.

Table 4–Changes in fatty acid composition of chicken skin total lipids during cooking and frozen storage

	Storage time (mo)											
	0				3				6			
	Cooking treatments[b]											
Fatty acids[a]	Raw	A	B	C	Raw	A	B	C	Raw	A	B	C
	Percent (GLC peak area)											
14:0	1.9	0.8	0.9	0.9	2.0	1.6	1.2	1.0	1.6	1.2	1.1	1.4
15:0	0.9	0.2	0.2	–	0.5	–	–	–	0.7	0.5	0.6	0.7
16:0	26.3	20.6	20.5	20.9	24.3	20.9	22.0	20.8	26.1	20.5	20.5	21.7
16:1	6.1	3.3	4.1	4.1	6.3	3.5	3.6	2.9	6.3	4.1	4.5	4.5
18:0	6.3	3.6	3.6	4.1	7.5	3.9	4.7	4.4	6.4	3.8	4.5	4.9
18:1	38.4	30.6	33.1	32.1	40.2	31.3	33.4	30.8	40.9	30.6	33.3	32.6
18:2	18.3	39.9	37.1	37.5	18.9	38.4	34.8	39.9	16.7	38.4	34.3	33.4
18:3	1.9	1.1	0.5	0.6	0.5	0.4	0.2	0.3	1.3	1.1	1.1	0.7
Total sat.	35.4	25.2	25.2	25.9	34.5	26.4	27.9	26.2	34.8	25.7	26.7	28.7
Total unsat.	64.7	74.9	74.8	74.3	65.7	73.6	72.0	73.9	65.2	74.2	73.2	71.2

[a] Number of carbons:number of double bonds
[b] (A) Fresh corn oil; (B) Corn oil previously heated for 24 hr; (C) Corn oil previously heated for 42 hr

Table 5–Changes in fatty acid composition of chicken muscle phospholipids during cooking and frozen storage

	Storage time (mo)											
	0				3				6			
	Cooking treatments[b]											
Fatty acids[a]	Raw	A	B	C	Raw	A	B	C	Raw	A	B	C
	Percent (GLC peak area)											
12:0	0.5	0.2	0.4	0.4	0.3	0.6	0.1	0.8	0.5	–	0.3	0.3
13:0	0.3	0.6	0.3	0.3	trace	trace	–	–	3.5	3.1	2.8	1.7
14:0	0.4	0.3	0.3	0.5	0.2	0.2	trace	0.4	3.7	0.5	2.3	1.9
Unknown	–	0.4	0.4	0.3	–	–	–	–	–	–	–	–
15:0	7.7	8.0	4.9	6.2	13.9	11.6	4.6	7.4	17.2	14.4	15.1	14.8
16:0	13.8	14.4	14.8	18.0	19.4	14.9	16.7	16.7	17.1	16.2	15.2	15.7
16:1	0.4	0.8	1.2	0.7	3.1	–	–	–	–	–	–	–
17:0	3.3	3.9	2.3	2.6	1.7	2.2	1.1	2.3	4.5	5.3	5.7	6.0
18:0	17.9	10.1	9.9	7.6	12.1	6.4	6.5	7.8	13.4	11.1	12.3	13.8
18:1	12.8	18.7	18.7	18.6	23.4	20.5	22.5	16.8	14.0	13.5	15.3	15.8
18:2	22.3	30.6	37.0	38.9	19.4	35.4	42.7	40.9	15.8	26.2	27.7	26.9
18:3	3.0	0.6	–	–	trace	trace	0.1	–	–	–	–	–
20:3	2.4	0.6	–	–	–	–	–	–	–	–	–	–
20:4	15.3	10.9	9.7	6.0	6.7	8.3	5.8	6.9	11.4	9.7	3.2	3.0
Total sat.	43.9	37.5	33.1	35.6	47.6	35.9	29.0	35.4	59.9	50.6	53.7	54.2
Total unsat.	56.2	62.2	66.6	64.2	52.6	64.2	71.7	64.6	41.2	49.4	46.2	45.7

[a] Number of carbons:number of double bonds
[b] (A) Fresh corn oil; (B) Corn oil previously heated for 24 hr; (C) Corn oil previously heated for 42 hr

Changes in chicken lipids

Total muscle lipids. The major fatty acids from uncooked chicken muscle fat were oleic, linoleic and palmitic acids, accounting for 79% of the total. The remainder were minor fatty acids such as stearic, palmitoleic, linolenic and arachidonic acids. The total unsaturation was 70% (Table 3).

Losses of arachidonic, eicosatrienoic, oleic, stearic and palmitoleic acids and a large increase in linoleic acids were found in total muscle lipids after chicken was cooked in fresh corn oil. The total muscle lipids from chicken cooked in reheated corn oil were quite similar in both the 24 and 42 hr groups, although linoleic acid increased with an increase in oil heating time, and absolute values of several fatty acids decreased slightly but not significantly.

After 3 mo storage, samples showed normal flavor and appearance without any observable deterioration by casual observations. However, after 6 mo storage, a rancid odor was emitted from the cooked samples when the bag was opened, and a slight brown discoloration in the dark meat was found. Uncooked samples showed a yellowish color on the skin and a slightly brownish color in the dark meat. The percentage of polyunsaturated acids (arachidonic, eicosatrienoic, linoleic and linolenic acids) in muscles from chicken cooked in reheated oil decreased during the first 3 mo of storage (Table 3). After 6 mo, linoleic acid decreased further, with little change in other fatty acids. The total unsaturated fats of fresh chicken muscle, 70% decreased to 65 and 61% after 3 and 6 mo storage, respectively.

Total skin lipids. The major fatty acids in uncooked chicken skin fat were oleic,

Table 6—Changes in fatty acid composition of chicken skin phospholipids during cooking and frozen storage

	Storage time (mo)											
	0				3				6			
	Cooking treatments[b]											
Fatty acids[a]	Raw	A	B	C	Raw	A	B	C	Raw	A	B	C
	Percent (GLC peak area)											
12:0	–	1.2	–	–	–	–	–	–	–	–	–	–
13:0	2.8	0.9	1.0	3.6	0.9	0.6	0.3	0.3	1.2	0.9	1.2	1.3
14:0	–	0.7	0.4	1.6	0.7	0.7	0.4	0.8	0.7	1.0	1.9	2.0
15:0	3.4	1.6	0.6	0.6	2.3	0.6	0.3	0.4	3.4	0.9	1.2	1.3
16:0	18.1	17.9	15.5	15.1	21.0	18.1	18.8	19.9	22.1	18.5	19.2	20.1
16:1	2.6	2.5	0.6	1.6	3.5	1.2	0.9	1.4	3.6	1.5	1.2	1.2
17:0	1.4	–	0.5	0.5	4.4	1.1	0.4	0.8	5.1	1.7	0.9	1.0
18:0	4.2	4.0	2.8	2.1	7.4	2.8	2.9	3.9	7.5	2.9	3.1	3.5
18:1	36.0	22.0	22.4	22.4	35.7	21.9	23.1	22.7	35.0	22.0	22.8	22.5
18:2	18.6	43.8	52.1	49.5	20.3	49.1	51.2	49.8	19.8	50.0	48.5	47.1
18:3	1.5	1.1	0.9	trace	0.9	0.6	1.1	–	0.5	–	–	–
20:4	11.5	4.5	3.3	3.0	2.8	3.2	0.6	–	1.1	0.6	–	–
Total sat.	29.9	26.3	20.8	23.5	36.7	23.9	23.1	26.1	40.0	25.9	27.5	29.2
Total unsat.	70.2	73.9	79.3	76.5	63.2	76.0	76.9	73.9	60.0	74.1	72.5	70.8

[a] Number of carbons:number of double bonds
[b] (A) Fresh corn oil; (B) Corn oil previously heated for 24 hr; (C) Corn oil previously heated for 42 hr

Table 7—Changes in fatty acid composition of chicken muscle neutral lipids during cooking and frozen storage

	Storage time (mo)											
	0				3				6			
	Cooking treatments[b]											
Fatty acids[a]	Raw	A	B	C	Raw	A	B	C	Raw	A	B	C
	Percent (GLC peak area)											
12:0	0.3	–	–	–	–	–	–	–	–	–	–	–
13:0	0.1	–	–	–	–	–	–	–	–	–	–	–
14:0	0.9	1.1	1.3	1.2	1.3	0.8	0.7	1.0	1.7	0.8	1.6	1.0
15:0	1.8	0.4	trace	trace	0.6	–	–	–	0.7	0.7	1.6	0.4
16:0	20.9	21.3	22.5	20.2	25.1	21.0	20.8	20.2	27.5	19.6	18.8	23.9
16:1	4.9	4.6	4.8	3.9	6.1	2.9	4.2	3.9	7.1	4.1	4.2	4.2
18:0	6.5	4.2	4.0	4.1	6.0	4.7	5.3	4.1	6.1	3.7	5.5	4.6
18:1	35.9	32.0	30.0	34.9	40.7	32.2	35.2	34.9	39.3	33.8	34.4	34.8
18:2	23.2	33.9	36.1	34.9	19.1	37.7	33.0	34.9	16.3	36.2	32.5	29.7
18:3	1.4	0.7	1.3	0.9	1.1	0.7	0.9	0.9	1.4	1.0	1.3	1.2
20:3	2.8	1.4	–	–	–	–	–	–	–	–	–	–
20:4	0.5	0.3	–	–	–	–	–	–	–	–	–	–
Total sat.	31.4	27.0	27.8	25.5	33.0	26.5	26.8	25.3	36.0	24.8	27.5	29.9
Total unsat.	68.7	72.9	72.2	74.6	67.0	73.5	73.3	74.6	64.1	75.1	72.4	69.9

[a] Number of carbons:number of double bonds
[b] (A) Fresh corn oil; (B) Corn oil previously heated for 24 hr; (C) Corn oil previously heated for 42 hr

palmitic and linoleic acids, amounting to 83% of the total. The remainder were stearic, palmitoleic, myristic and linolenic acids. The total unsaturation was 65% (Table 4). Muscle fat contained a higher percentage of unsaturation and more highly unsaturated fatty acids than skin fat.

After chicken was cooked in fresh corn oil, linoleic acid in skin lipids increased from 18% to 40%, whereas most other fatty acids decreased. The total unsaturation increased from 65% to 75%. Additional changes in fatty acids in total skin lipids due to cooking in reused corn oil were minimal.

After 3 and 6 mo storage, the composition of skin lipids of raw chicken had changed only slightly; however, the changes following cooking were significant. Major changes were relative decreases in palmitic and oleic acids and an increase in linoleic acid.

Muscle phospholipids. The predominant fatty acids in phospholipids from uncooked chicken muscle were linoleic, stearic, palmitic, arachidonic and oleic acids, which totaled 82% (Table 5). The highly unsaturated fatty acid content and high levels of arachidonic acid and linoleic acid are characteristic of phospholipids.

Losses of arachidonic, eicosatrienoic, linolenic and stearic acids were found in the muscle phospholipids after the chicken was cooked in fresh corn oil. Linoleic acid and oleic acid increased, and an unidentified component was present.

The muscle phospholipids from the

Table 8–Changes in fatty acid composition of chicken skin neutral lipids during cooking and frozen storage

	Storage time (mo)											
	0				3				6			
	Cooking treatments[b]											
Fatty acids[a]	Raw	A	B	C	Raw	A	B	C	Raw	A	B	C
	Percent (GLC peak area)											
14:0	2.0	0.7	0.7	1.3	1.0	0.8	1.1	0.9	1.4	1.0	1.3	2.3
15:0	1.0	–	0.2	0.9	0.3	–	–	–	0.7	0.3	0.5	0.3
16:0	26.4	19.2	19.1	20.2	26.0	20.6	21.5	19.5	26.3	21.5	20.8	22.8
16:1	5.1	3.8	4.3	5.4	5.9	4.0	4.2	3.6	6.4	3.8	4.9	4.1
18:0	6.4	4.1	4.1	4.5	6.5	3.5	4.3	3.6	7.6	3.7	4.1	6.2
18:1	39.1	30.0	33.9	33.3	42.3	29.7	34.6	32.7	40.3	30.5	32.9	31.9
18:3	2.0	0.9	1.3	1.1	0.3	0.3	0.2	0.3	0.7	1.0	0.9	0.6
Total sat.	35.8	24.0	24.1	26.9	33.8	24.9	26.9	24.0	36.0	26.5	26.7	31.6
Total unsat.	64.2	76.2	75.8	73.0	66.3	75.2	73.2	76.0	64.1	73.5	73.3	68.4

[a] Number of carbons:number of double bonds
[b] (A) Fresh corn oil; (B) Corn oil previously heated for 24 hr; (C) Corn oil previously heated for 42 hr

chicken cooked in fresh corn oil contained 11% arachidonic and less than 1% eicosatrienoic and linolenic acids. When the chicken was cooked in corn oil previously heated for 24 or 48 hr, polyunsaturated acids decreased and linoleic acid increased.

Total unsaturated fatty acids in muscle phospholipids from uncooked birds decreased during storage, especially arachidonic, eicosatrienoic, linolenic, linoleic and stearic acids. Increased amounts of several short chain fatty acids were also found after storage.

The greatest loss of unsaturated fatty acids was found in the chicken cooked in corn oil previously heated for 42 hr, followed by that heated for 24 hr, and lowest in chicken cooked in fresh corn oil. In other words, the use of reheated corn oil accentuated the loss of unsaturated fatty acids in chicken during storage and contributed to the stability changes.

Skin phospholipids. The predominant fatty acids of phospholipids from uncooked chicken skin fats were oleic, linoleic, palmitic and arachidonic acids, amounting to 84% of the total (Table 6).

Primary changes in composition of skin phospholipids after the chicken was cooked in fresh corn oil were decreases in oleic and arachidonic acids, and increases in linoleic acid and some short chain acids. Fat changes were greater in skin phospholipids from the chicken cooked in reused corn oil.

The unsaturated fatty acid, arachidonic acid, in skin phospholipids from uncooked birds decreased during storage; however, cooking resulted in more severe changes than did frozen storage.

Muscle neutral lipids. The predominant fatty acids in neutral lipids from uncooked chicken muscle were palmitic, oleic and linoleic acids, amounting to 80%. About 28% of the muscle neutral lipids were polyunsaturated (Table 7).

The changes in muscle neutral lipids during cooking in fresh corn oil were mainly an increase in linoleic acid and slight decreases in several other acids. Storage of uncooked birds resulted in increases in palmitic and oleic acid and decreases in linoleic acid. Muscle neutral lipids from the chicken cooked in reused corn oil after frozen storage showed a decrease in polyunsaturated acids.

Skin neutral lipids. The major fatty acids in skin neutral lipids from uncooked chicken were oleic, palmitic and linoleic acids (Table 8). The relative amounts of fatty acids in neutral lipids had slightly higher percentages of oleic and palmitic acids but a lower percentage of linoleic acid.

DISCUSSION

THE FATTY ACID changes in poultry meat during cooking in heated corn oil can be the result of chemical factors such as oxidation, hydrolysis or polymerization, or physical changes associated with movement of cooking oil to the product or juices from the product to the cooking oil. Chemical reactions can also occur simultaneously in the heated oil and in the skin or muscle lipids during cooking.

Heat-induced free radicals and the unsaturated fatty acids present in corn oil are considered reactive components. Since the rate of autoxidation of linoleic acid is rapid, the decrease in linoleic acid in heated corn oil was expected. The losses of unsaturated fatty acids from chicken cooked in fresh corn oil, such as arachidonic and eicosatrienoic acids, indicate that specific chemical reactions occurred. The increase in linoleic acids after cooking may have resulted from adsorption or absorption of corn oil which contained 59% linoleic acid.

The occurrence of chemical reactions in heated corn oil is postulated to be initiated by the formation of heat-induced free radicals, with the rate of reactions depending upon the concentration of free radicals formed. In other words, the corn oil may contain more free radicals when heated for 42 than 24 hr, or than fresh corn oil. When the chicken was cooked in these reused corn oils, it was expected that the chemical reactions would occur at a faster rate. Thus, the increased losses of eicosatrienoic and oleic acids which occurred in chicken muscle fat when cooked in reused corn oil were expected.

Decreases in unsaturation of fatty acids in chicken muscle were found during storage. The absorption (or adsorption) of reused corn oil by chicken pieces undoubtedly affected product stability. The lower percentage of unsaturated fatty acids after storage represented oxidation of muscle fat.

The composition of phospholipids from chicken was similar to that reported by Katz et al. (1966), Peng (1965), Issacks et al. (1964) and Marion et al. (1967). Some fatty acids reported by Katz et al. (1966) such as 20:1, 20:2, 22:3 and 22:4 were not found in this study.

The fatty acid, 22:0 may be eluted at the same time as 20:3 with the GLC system used. However, in this study saturated fatty acid esters were separated from unsaturated fatty acid esters utilizing TLC prior to GLC analysis. The peak identified as 20:3 was found in the unsaturated fatty acid esters and is, therefore, assumed to be 20:3.

The corn oil used did not contain phospholipids, thus the absorption (or

adsorption) of oil by chicken pieces during cooking would not affect composition of phospholipids in cooked chicken. Such changes are presumed to be due to heat induced chemical reactions.

Losses of arachidonic. eicosatrienoic and linolenic acids in chicken muscle phospholipids are probably due to the oxidative deterioration which occurs during cooking. One double bond present in the unsaturated acids is first attached to form hydroperoxides as primary products. These undergo a variety of scission and dismutation reactions to form a wide spectrum of carbonyl compounds, hydroxy compounds and short chain fatty acids. The formation of carbonyl compounds may contribute to the aroma of cooked chicken (Bassette and Day, 1960). Hydrolysis may also occur in chicken fat when heat is applied. The increased amounts of linoleic and oleic acids may be due to the decreased amounts of higher polyunsaturated acids, since all fatty acids amounted to 100% or partially to the reconstitution of hydrolyzed phospholipids with the fatty acids from absorbed corn oil during cooking.

The lower unsaturated fatty acids in muscle phospholipids after storage indicated severe oxidation. This could have been catalyzed by heme protein (Younathan and Watts, 1960), since the ferric heme pigments are formed from oxidation of oxymyoglobin and myoglobin.

The greater losses of polyunsaturated acids in the phospholipids than in neutral lipids during cooking in reused corn oil shows that they are more readily susceptible to stress and are very important in determining meat quality. The oxidation of tissue lipids appears to occur in two stages, first in phospholipids, then in neutral lipids (El-Gharbawi and Dugan, 1965). The neutral lipids in skin also appear to be more stable than in the muscle.

Minimum chemical changes occurred in the fatty acids when fresh chicken was cooked in fresh corn oil. Frozen storage of chicken and use of reheated corn oil both contribute to increased oxidation and other chemical changes in the lipids.

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USE OF γ-IRRADIATION TO PREVENT AFLATOXIN PRODUCTION IN BREAD

INTRODUCTION

THE USE OF low level or pasteurizing doses of gamma irradiation to extend the shelf life of certain foods has been studied by numerous workers (Bellamy, 1959; Hannesson, 1972; Niven, 1958). These levels of irradiation do not sterilize food products but rather reduce the microbial population and thereby extend storage life. This application of irradiation was employed by Hartung et al. (1973) to extend the shelf life of fresh bread used in food systems for manned space flight missions.

Molds are a common and important cause of spoilage of bread (Frazier, 1967). Mycostatic agents, principally propionates, are added to bread to delay mold growth, but molding still occurs after extended storage. Mold spoilage of bread containing mold inhibitors was noted in early manned space missions (Hartung et al., 1973). By applying a 50 Krad dose of irradiation to flour and subsequently to bread, Hartung et al. (1973) showed a significant reduction in the amount of visible and total mold that developed on the bread during storage up to 20 wk. However, a small percentage of molds survived and were capable of outgrowth during storage.

A number of common spoilage molds are capable of producing secondary metabolites that are both toxic and carcinogenic to a wide range of animals (Detroy et al., 1971). One such group of these compounds are the aflatoxins, which are produced by certain strains of *Aspergillus flavus* and *A. parasiticus*. Miyaki et al. (1967) reported that aflatoxins were resistant to ionizing irradiation doses as high as 30 Mrad. Jemmali and Guilbot (1969; 1970a, b) observed that exposure of *A. flavus* spores to gamma irradiation doses of less than 200 Krad tended to induce or increase aflatoxin production in subsequent cultures.

Hartung et al. (1973) found that *Aspergillus* and *Penicillium* species predominated among molds isolated from unirradiated flour and bread. While irradiation treatment lowered the incidence of these organisms, surviving spores had the capability of outgrowth. The possibility that such survivors might be capable of toxin production or might have altered patterns of toxin production raises concerns about the use of irradiation as a method of food preservation, not only for space food systems, but food systems in general. This work was initiated to study the effects of selected low levels of gamma irradiation on the ability of strains of *A. parasiticus* to survive and produce aflatoxin in a fresh bread system.

EXPERIMENTAL

Organisms

Aspergillus parasiticus strains NRRL 2999 and NRRL 3000, obtained from the culture collection of the USDA Northern Regional Research Laboratory, ARS, Peoria, Ill., were used in this study. Both are known aflatoxin-producing cultures. Strain 2999 is generally a more potent aflatoxin producer than strain 3000. Stock cultures were maintained at 2–4°C on slants of Difco potato dextrose agar in screw cap test tubes.

Inoculum

Cultures of the toxic molds were grown on potato dextrose agar slants for 10 days at 25°C until well sporulated. The spores were washed from the slants with a sterile 0.01% solution of Tween 80. The harvested spores were suspended in 100 ml of sterile Tween 80 and aseptically filtered through sterile cheesecloth to remove mycelial debris. The filtered suspensions were quantitated using a Petroff-Hausser counting chamber. Portions of the suspenions were diluted to obtain two levels of inoculum, approximately 10^2 and 10^6 spores/bread slice. The dilutions were made so that the required number of spores were contained in 0.1 ml of spore suspension. One-half of this amount was applied to each side of each slice of bread.

Inoculation, packaging and irradiation of bread slices

The bread used in this study was baked without mold inhibitor by a local bakery and was obtained and used within 12 hr of baking. The slices of bread were aseptically removed from the packaged loaves and placed on sterile towels inside a bacteriological glove box. The interior surfaces of the glove box had previously been sanitized with a 50% solution of household bleach and exposure to germicidal UV light for 1 hr before use. The bread slices were exposed to germicidal UV light for 15 min per side prior to inoculation. The slices were inoculated and the inoculum was spread over the surface of the bread by brushing it with a flamed inoculating loop. The inoculated slices were individually packaged in polyethylene pouches (PL540, W.R. Grace Co.) and the pouches were sealed under an air atmosphere. The inoculated slices were irradiated at 0, 100 and 200 Krad at ambient (ca 25°C) temperature using a Cobalt 60 source similar to the one described by Teeny and Miyauchi (1970). Dosimetry for irradiating the bread was established using a Fricke Dosimeter, ASTM 01671-63. The bread was stored for 10 days at 25°C, and then analyzed for aflatoxins and total mold content.

Extended storage studies were also included wherein white bread slices were inoculated, packaged and irradiated in the same manner as described above. The bread slices were then stored at 25°C, and examined at 0, 1, 2, 4 and 6 wk. Samples were evaluated for aflatoxin content, total molds, yeasts and bacteria.

In all treatments duplicate samples were used and the studies were replicated three times.

Analyses of bread slices

The bread slices were observed for visible mold growth during the storage periods. After storage, duplicate samples were composited by blending in a sterile blender. Total bacteria, yeast and mold counts were made using standard methods (Sharf, 1966), except that instead of acidifying potato dextrose agar for yeast and mold counts, 30 ppm tetracycline was used to prevent bacterial growth.

The composited samples were also analyzed for aflatoxin content using a modification of the extraction method of Pons et al. (1966), in which 100% ethyl alcohol was substituted for acetone in the extraction solvent. This resulted in cleaner extracts that did not as readily form emulsions during the subsequent chloroform extraction step. The aflatoxin concentration in the extracts were estimated by visual comparison of the fluorescence of the samples to known standards on exposure to long wave UV light (Chromatovue Cabinet, Model C5) using thin-layer chromatography (TLC) plates (20 × 20 cm, 0.25 mm thick Silica Gel G-HR, Brinkmann Instruments, Inc.). The TLC plates were developed in toluene/ethyl acetate/90% formic acid (60/30/10) according to the method of Scott et al. (1970). Standard aflatoxins were obtained from the USDA Southern Utilization R & D Div., New Orleans, La.

RESULTS & DISCUSSION

Growth

The amount of growth of toxic strains of *A. parasiticus* in bread was reduced by low dose gamma irradiation of inoculated bread (Table 1). Both 100 and 200 Krad doses resulted in lower amounts of detectable toxic mold in all treated bread

Table 1–Effect of irradiation on total number of mold propagules per gram of white bread inoculated with Aspergillus parasiticus NRRL 2999 and NRRL 3000 and stored at 25°C for 10 days

Strain	Inoculum level/slice	Number of mold propagules/g of bread		
		Control	100 Krad	200 Krad
NRRL 2999	10^2	8.3×10^7	2.8×10^6	2.1×10^4
	10^6	1.7×10^7	1.5×10^6	4.1×10^4
NRRL 3000	10^2	3.3×10^8	4.4×10^4	9.5×10^5
	10^6	1.1×10^5	5.0×10^5	9.1×10^4

Table 2–Effect of gamma irradiation on the production of aflatoxins by Aspergillus parasiticus NRRL 2999 and NRRL 3000 on white bread stored at 25°C for 10 days

Strain	Level of spores/slice	Aflatoxin	µg Aflatoxin/g of bread[a]		
			Control	100 Krad	200 Krad
NRRL 2999	10^2	B_1	33	–	–
		G_1	218	–	–
	10^6	B_1	29	22	–
		G_1	164	77	–
NRRL 3000	10^2	B_1	49	< 0.1	–
		G_1	249	2	<0.01
	10^6	B_1	5	2	–
		G_1	50	15	–

[a] – None detected

Table 3–Effect of gamma irradiation on aflatoxin ($B_1 + G_1$) production by Aspergillus parasiticus NRRL 2999 and NRRL 3000 on white bread stored at 25°C for various periods up to 6 wk

Storage time (wk)	Strain 2999, 10^2 spores/slice, Control	100 Krad	200 Krad	Strain 2999, 10^6 spores/slice, Control	100 Krad	200 Krad	Strain 3000, 10^2 spores/slice, Control	100 Krad	200 Krad	Strain 3000, 10^6 spores/slice, Control	100 Krad	200 Krad
1	182	–	–	16	111	–	105	–	–	15	6	–
2	46	0.03	–	540	640	0.05	370	–	–	24	122	0.02
4	70	–	–	113	41	13	16	–	–	5	81	–
6	4	–	–	132	1061	–	34	–	–	264	227	–

µg Aflatoxins ($B_1 + G_1$)/g of bread[a]

[a] – None detected

samples except in one case. Visible mold was evident to varying degrees on all control samples after storage at 25°C for 10 days. The control cultures of both strains produced more apparent growth at the 10^2 spore inoculum than the 10^6 spore level. The effect was greatest with strain NRRL 3000. This may represent some type of competitive effect or auto-inhibition that is related to spore concentrations and limited growth conditions. Visible mold was not evident to any appreciable degree on the slices treated with a 100 Krad dose and was completely lacking on slices given a 200 Krad dose with strain NRRL 2999. There appeared to be little difference between a light and heavy inoculum in terms of survival and amount of subsequent visible growth after irradiation treatment. Strain NRRL 3000 was more variable than strain NRRL 2999 in amounts of growth noted within and between treatments. Strain NRRL 2999 appeared to be more resistant to the 100 Krad dose than was strain NRRL 3000.

Bread slices that were inoculated, irradiated and stored for up to 6 wk showed similar trends, as the bread stored for 10 days, toward lower amounts of toxic mold during the first 2 wk of storage. However, as the storage time increased, the molds tended to overcome the effects of irradiation and the amounts of mold growth in treated slices at 6 wk of storage approached the amounts found in the control samples. Peak amounts of growth were reached in less time in the control samples than in the treated slices. Peak amounts of mold occurred later in the 200 Krad treated samples than in 100 Krad treatment. There did not seem to be a noticeable difference in amount of toxic mold growth between a light and heavy inoculum within any of the treatments. Apparently, the long storage time allowed sufficient time for surviving spores to grow out and produce approximately the same amount of mold mass whether a few spores were present initially or whether the numbers were greater. Other factors such as nutrients, moisture and available oxygen within a given package also might have affected the amount of growth produced.

Examination of inoculated bread slices for yeasts and bacteria showed only a very low incidence of yeasts. Bacterial numbers were initially very low but tended to increase with storage times. The patterns of bacterial numbers did not reflect any particular trends as a result of the various treatments. The numbers of bacteria found in irradiated samples were of a similar order of magnitude as in control samples. Reduction in mold growth did not result in an increase in bacterial numbers.

Aflatoxins

Gamma irradiation of bread slices inoculated with spores of *A. parasiticus* NRRL 2999 and 3000 reduced the amount of aflatoxins produced on subsequent storage and incubation (Table 2). The 200 Krad dose essentially eliminated aflatoxin production by either strain at both levels of spore inoculation during 10 days of storage. These results agree with those of Jemmali and Guilbot (1970a) who reported that gamma irradiation doses of 200 Krad and above tended to reduce the ability of *A. flavus* to produce aflatoxins.

The 100 Krad treatment level also reduced the amount of aflatoxins detected in inoculated bread at the end of the 10 day storage period (Table 2). With strain NRRL 2999 no aflatoxins were detected at the 10^2 spore inoculum level but with

strain NRRL 3000 small amounts of toxins were found. Higher amounts of aflatoxins were noted in samples inoculated with 10^6 versus 10^2 spores at the 100 Krad dose treatment with both strains. The total mold counts did not differentiate between mold types and since complete sterilization of the substrate before inoculation was not attempted, it is possible that other mold types may have added to the load detected at the lower inoculum levels. With the larger inoculum it is likely that more spores survived the irradiation treatment and were more capable of competing with any other microbial growth that occurred.

Aflatoxin production in inoculated bread samples irradiated and stored for up to 6 wk followed the same general pattern as observed with the bread stored for 10 days (Table 3). With the 200 Krad dose, no aflatoxins were detected in any bread samples that were inoculated with 10^2 spores of either NRRL 2999 or 3000. With 10^6 spores at 200 Krads, trace amounts of aflatoxins were found with both organisms at 2 wk of storage. And with 10^6 spores of strain NRRL 2999 at the 200 Krad treatment level significant amounts of aflatoxins were detected at 4 wk of storage. Except for small amounts of aflatoxins found at 2 wk of storage with strain NRRL 2999, no toxins were detected in any of the bread inoculated with 10^2 spores and irradiated at 100 Krads. But with 10^6 spores both organisms produced very high amounts of aflatoxins after irradiation at 100 Krad and subsequent storage.

The data suggest that stimulation of aflatoxin production may have occurred at the 100 Krad treatment level in samples inoculated with 10^6 spores after 1, 2 and 6 wk of storage (Table 3). This would be supported by the findings of Jemmali and Guilbot (1970). However, it should be remembered that there is considerable natural variation in aflatoxin production by a given mold strain and between strains. Also, because of the substrate and the conditions of the experiment it was impossible to determine the amount of aflatoxins produced per unit mass of mold mycelia, which would be necessary to conclusively prove stimulation of toxin production. At this point, stimulation of toxin production at the 100 Krad level in a bread substrate certainly appears probable, but further studies are needed for more conclusive evidence. There seemed to be more stimulation of toxin production in strain NRRL 2999.

Production of aflatoxins by *A. parasiticus* strains on bread was effectively eliminated by treatment of the inoculated bread with 200 Krad doses of irradiation. The mold strain as well as the initial load of spores were factors in the organisms' ability to produce aflatoxins after irradiation treatment. Strain NRRL 2999 seemed more capable of toxin production after irradiation treatment than strain NRRL 3000. With the lower irradiation dose, cultures from either strain using 10^6 spores/slice were more capable of aflatoxin production after treatment than were cultures from 10^2 spores. The 100 Krad dose appeared to stimulate aflatoxin production at 1, 2 and 6 wk of storage when 10^6 spores/slice were used for inoculum. But this could not be shown when an inoculum of 10^2 spores/slice was used. It is apparent that the higher irradiation dose provided a greater margin of safety in preventing the development of aflatoxins in bread under the conditions of this study.

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MICROBIOLOGY OF A MODIFIED PROCEDURE FOR COOLING PASTEURIZED SALT YOLK

INTRODUCTION

COMMERCIAL salt yolk, used extensively in the manufacture of mayonnaise and salad dressing has a viscosity of about 500 cps at 32°C, 900 at 18.3°C and 1500 at 7.2°C (Lineweaver et al., 1969). Present pasteurization requirements for salt yolk (Fed. Reg., 1971) specify that the temperature must be reduced to 18.3°C or lower within 2 hr and to 7.2°C if held more than 24 hr. The high viscosity commonly causes excessive pressures when pumping pasteurized salt yolk through the cooling systems presently used in egg-pasteurizing equipment. Pressures of 80 to more than 160 lb/sq in. have been measured by the authors in commercial pasteurizers. This leads to damaged gaskets and plates when the press is tightened to the degree required to prevent leakage. Because of the pressure problem, the rate of salt yolk production is limited. This study was undertaken to seek a way to reduce the operating difficulty (and the cost) without reducing safety. The proposed procedure is to complete the cooling of salt yolk in 30-lb cans instead of pumping it through the cooling section of the pasteurizer. Theoretical justification for such a procedure is based on the fact that salt yolk is a very poor microbial growth medium because of the high salt content and consequently low water activity.

Salt yolk has a water activity (a_w) of about 0.90 for the usual formulation. This is the value for water containing 20.3g of salt per 100g of water (Scott, 1957), which is the composition of the water phase of salt yolk that consists of 10 parts of salt added to 90 parts of 43% solids yolk. At this a_w many microorganisms will not grow. Indeed salmonellae will not grow at a_w values below 0.945 (Christian and Scott, 1953), a value that is well above the a_w of salt yolk.

The micrococci are the most salt-tolerant microorganisms other than the halophiles, which require high salt concentrations for optimum growth. Many strains of micrococci grow, although slowly, in 25% sodium chloride (Larsen, 1962). Among the micrococci that will grow at the salt concentration of 10% salt yolk are various strains of salt tolerant staphylococci. Scott (1953) showed that 14 food-poisoning strains of staphylococci would grow at a_w values as low as 0.86. It is important, therefore, to establish that processing or handling methods for salt yolk provide a safe product. Although salt yolk might spoil due to growth of other micrococci, staphylococci are the main concern. Salt yolk must be handled so that staphylococci do not multiply and possibly produce enterotoxin. Staphylococci are more heat resistant than salmonellae (Angelotti et al., 1960) and would not be destroyed by pasteurization unless salt were to sensitize them to heat, which is not the case (see Results). Micrococci and staphylococci occur in pasteurized plain whole egg and yolk and thus evidently survive pasteurization (Shafi et al., 1970). These authors did not find coagulase positive staphylococci in the pasteurized egg but did find coagulase negative staphylococci.

In order to arrive at time-temperature conditions for safe and efficient handling of salt yolk we have determined (a) the heat sensitivity of coagulase-positive staphylococci in salt yolk, and (b) the microbial activity that occurs in yolk containing 10% salt and two higher salt levels at temperatures ranging from 7 to 29°C.

MATERIALS & METHODS

Egg yolk materials

Commercial salted yolks (10% salt) were obtained from a USDA inspected egg processing establishment about 80 miles from the laboratory. They were transported chilled and storage experiments were begun the same day that the samples were taken. For comparison of unpasteurized and pasteurized yolk, samples were taken from the same lot immediately before and after pasteurization at 65°C for 4½ min.

Laboratory prepared yolks were separated from aseptically broken eggs according to Garibaldi et al. (1969). Yolks with membrane intact were prepared as free of whites as possible and after blending, 13% whites were added to simulate a commercial yolk containing approximately 45% solids. 10% salt yolk was prepared by adding 10 parts by weight of oven sterilized NaCl to 90 parts of yolk. The 12.2% and 14.3% salted yolks were prepared by adding 2.5 and 5g of salt respectively to 100g of 10% salt yolk. Care was taken to assure that the salt was completely dissolved.

Cultures

The staphylococci cultures used consisted of four coagulase positive stocks carried in the laboratory culture collection (Nos. 413, S-30B, S-290, S-10) and two other isolates that are no longer carried. These organisms were grown in shaken cultures in Trypticase Soy (BBL) broth plus 2% yeast extract at 35°C for 3 days, at which time growth was well into the stationary phase. The cells harvested by centrifugation, were washed once in sterile deionized water and resuspended in sterile deionized water. Such cells were stable for several weeks at refrigerator temperature as determined by standard plate counts.

Microbiological determination

Total plate counts were determined by spread plating on Trypticase Soy (BBL) plus 2% yeast extract agar and incubation for 48 hr at 28°C.

Differential staphylococcal counts were obtained using Vogel Johnson Agar (BBL). Coliform counts were made on Violet Red Bile Agar (Difco).

Method of determining the cooling rate of warm salt yolk

Salt yolk (10%) was taken from a commercial plate and frame egg pasteurizer with the cooling water shut off. The product at 28–29°C was put into 30-lb cans and a full pallet load (54 cans) of uncooled material was collected. Two thermocouples, in each of two cans were placed so that one was centered and one was intermediate between the center and the wall of the can. Both were equidistant between top and bottom of the liquid. The thermocouples were connected to a multipoint recording potentiometer. One can was placed centrally in the pallet and the other was on the upper corner of the pallet load nearest the blower in the cold room. The temperature of the room was −20°C and air velocity at the pallet was 30–40 ft/min as measured by an Alnor anemometer.

Thermal resistance measurement

Decimal reduction times were determined for a mixture of two strains of staphylococci that were inoculated into yolk before the salt was added. The method used was essentially the same as that used for salmonellae by Garibaldi et al. (1969).

RESULTS & DISCUSSION

Rate of cooling of salt yolk in 30-lb cans

The temperature of pasteurized salt yolk was reduced to approximately 32°C in the regenerating section of the egg pasteurizer. At this temperature the yolk has a viscosity of about 500 cps and can be pumped readily. Even in the can located at the center of a 54-can pallet, salt yolk

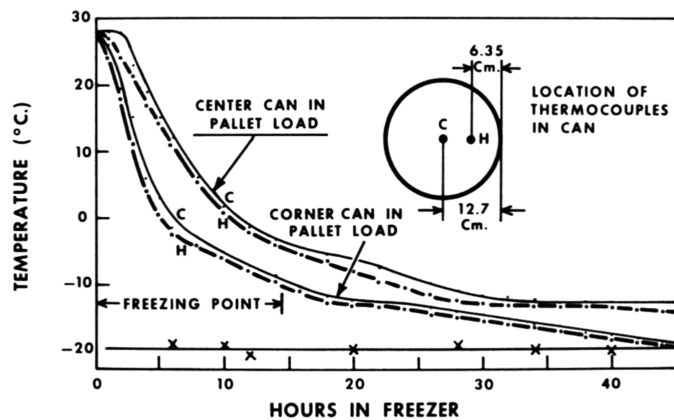

Fig. 1–Cooling curves for salt yolk in 30-lb tapered cans placed in a commercial –20°C room. Thermocouples C and H were both half way (12.7 cm) between top and bottom of liquid. Air movement was 30–40 ft/min at the pallet.

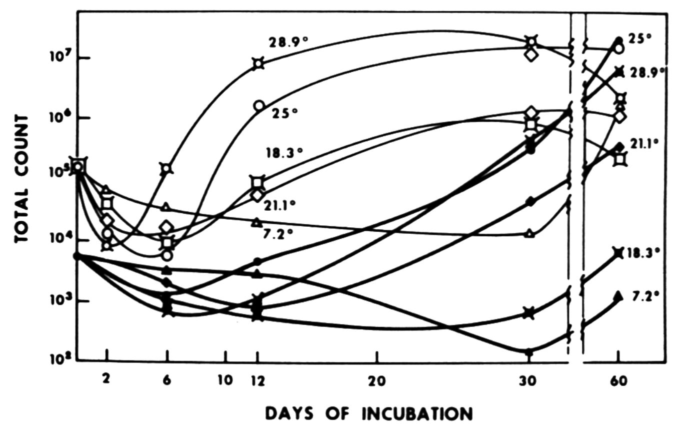

Fig. 2–Microbial activity in commercial pasteurized (closed points and heavy lines) and unpasteurized salt yolk at 7.2, 18.3, 21.1, 25 and 28.9°C.

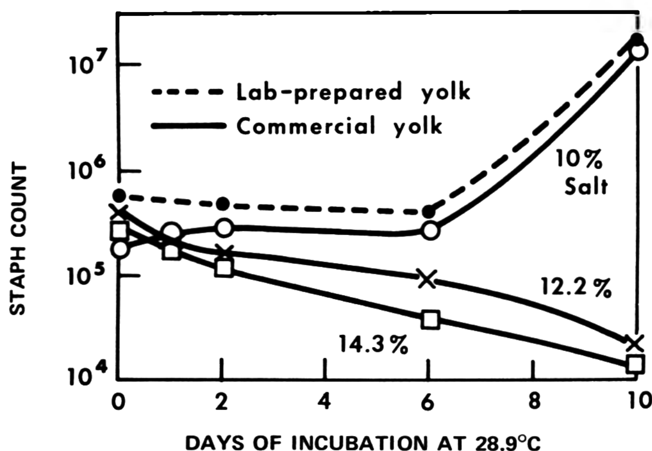

Fig. 3–Effect of salt content of yolk in the range 10–14.3% salt on growth staphylococci at 28.9°C. A mixture of five strains was added to the commercial yolk and a mixture of two strains (Nos. 413 and S-30B) was added to the laboratory prepared yolk.

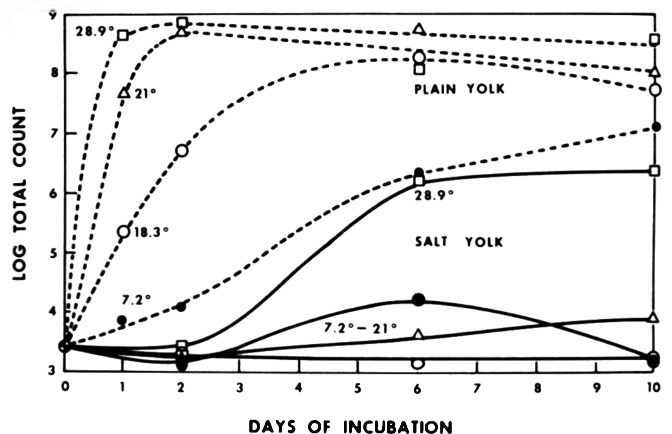

Fig. 4–Growth at 7.2–28.9°C of a mixture of two strains (Nos. 413 and S-30B) of staphylococci added to sterile, plain yolk at a level of 2.5 × 10^3 per g.

at 30°C, filled into 30-lb cans, cooled to less than 10°C in 7 hr when placed in a room at –20°C (Fig. 1). The air velocity in this room was 30–40 ft/min at the location of the pallet. The yolk in the most exposed corner cooled about twice as fast as that in the center can. The flatness of the curves at –13°C corresponds to the freezing point of salt yolk. Even in a room at 0°C, the temperature of the yolk in the center can would reach 10°C in about 12 hr. The room should, of course, have sufficient refrigeration capacity to handle the warm product load.

Microbial activity

At least in part because some microorganisms are killed in the salt environment, a net increase in total numbers was not evident in samples of unpasteurized salt yolk held at 29°C or below until the sixth day and was not evident until after 12 days in pasteurized yolk (Fig. 2). Therefore, for at least 10 days spoilage will not be a factor in pasteurized salt yolk even at 29°C. However, considerable growth will occur in 30–60 days at 21°C or above. Coliform counts on these samples decreased at all storage temperatures. The higher the temperature the faster the decrease. The coliform data are not shown because an increase was never observed even in 60 days.

Staphylococci introduced into 12.2 and 14.3% salt yolk at a level of approximately 3 × 10^5 per g tend to die off at 29°C but grow in 10% salt yolk after about a 6-day lag phase (Fig. 3). Total counts determined at the same time as the staphylococci counts for the commercial yolk samples were about twice the staphylococci counts throughout the 10-day test. Growth data were similar in laboratory-prepared yolk (broken line in Fig. 3). Growth tests, identical to those at 29°C were conducted at 21, 18 and 7°C for laboratory and commercial yolk at the three salt levels. In contrast to the results at 29°C counts did not increase at these temperatures nor did the counts decrease at these temperatures as was the case at 29°C in the 12.2 and 14.3% salt yolk.

Staphylococci grew rapidly without lag in plain yolk at 21°C and higher (Fig. 4). Total counts in Figure 4 reflect staphylococcal counts since the yolk was essen-

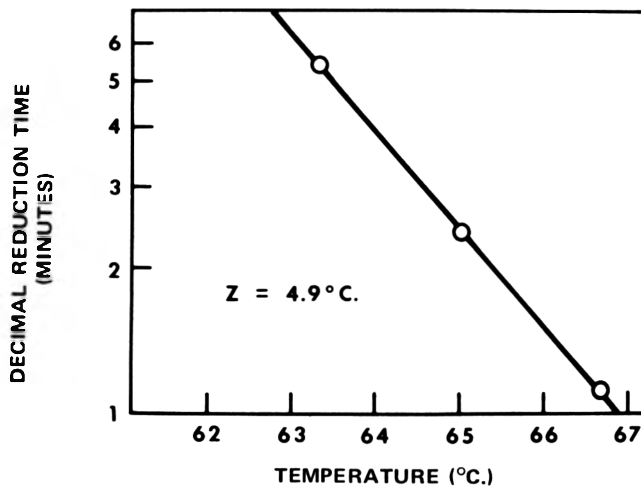

Fig. 5–Decimal reduction times for a mixture of two strains (Nos. 413 and S-30B) of staphylococci in laboratory prepared 10% salt yolk.

tially free of other microorganisms. In this case, a lag of at least 2 days occurred in salt yolk at 29°C (cf. Fig. 3 where the lag was 6 days). However, little or no growth occurred in salt yolk at 21°C and 7.2°C. Apparently the viability of staphylococci is not affected by introduction into the salt medium as is that of some of the adventitious microorganisms that occur in commercial yolk (cf. Fig. 2).

It was anticipated that staphylococci might not be killed in salt yolk by pasteurization at 63.3°C since Angelotti et al. (1960) found a decimal reduction time (D) of 2 min for staphylococci in custard at 63.3°C. A "D" value of 5.5 min at 63.3°C was found for a mixture of two strains of staphylococci in salt yolk (Fig. 5). A "z" value of 4.9°C was found for the short temperature range studied. (z is the number of degrees required for a thermal destruction curve to traverse one logarithmic cycle). Since less than 90% of the staphylococci are killed in 3.5 min at 63.3°C, the official minimum pasteurization conditions (Fed. Register, 1971), it is important to handle salt yolk in a way that will prevent growth.

It is unfortunate that staphylococci are not killed under the minimum required conditions for pasteurization of salt yolk but this in itself does not constitute a hazard. For example, use of unpasteurized salt yolk to manufacture salad dressing of specified degrees of acidity (Fed. Register, 1971) has been permitted because both staphylococci and salmonellae die off in such products (Wethington and Fabian, 1950, and others). The present results show how to handle salt yolk to avoid growth of staphylococci therein and hence how to avoid any accompanying hazard that might result from such growth.

CONCLUSION

BULK COOLING of pasteurized salt yolk in 30-lb cans placed in a room at −10 to −20°C provides a wide margin of safety. That is (a) at such temperatures salt yolk cooled from 30°C to less than 10°C in about 7 hr at the slowest cooling location in a 54-can pallet load and (b) observation for 10 days showed that neither spoilage nor staphylococci organisms grow in salt yolk (10% or higher salt) held below 25°C. Even at 29°C no growth occurred for several days.

It would be practical and would provide a wide margin of safety to fill 30-lb cans with pasteurized salt yolk at 30°C or lower if the cans, stacked in a way that permits free air movement, are placed in a room at −10°C or lower within 2 hr.

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METHOD FOR ESTIMATING LIMONIN CONTENT OF CITRUS JUICES

INTRODUCTION

LIMONIN contributes to the bitterness of grapefruit and orange juice (Maier and Dreyer, 1965; Higby, 1938). Since limonin has an effect on quality of citrus juice a study of the limonin content of grapefruit and orange juice products is of much interest to citrus processors. A quick, simple method for the determination of limonin concentration in citrus juice would be of great help in quality assurance programs.

Several methods are presently in use for determining limonin in citrus juices. Maier and Grant (1970) developed a thin-layer chromatographic (TLC) procedure that requires about 4 hr to complete four samples. Chandler (1971) developed a procedure based on TLC, which includes a quite lengthy and complex extraction procedure. Kruger and Colter (1972) developed a procedure based on GLC, which also is quite lengthy.

This paper presents a simplified TLC method for limonin estimation which requires no extraction or preparation when applied to commercial single strength or reconstituted juices, and is therefore less time consuming than any previous method. The limonin concentration can be determined on six juices in 1.5 hr. This method can also be applied to fresh juice, but considerable time is required in preparing the sample. The method was applied to a study of relationship between juice yield and limonin content of orange juice.

EXPERIMENTAL

Materials

TLC plates. Silica Gel G, 20 × 20 cm, 250μ (Analtech, Inc., Newark, Del.) thin-layer chromatographic plates were used. Plates were also prepared at our laboratory with Silica Gel G, (Warner-Chilcott, Richmond, Calif.; SMI, Emeryville, Calif.) where a Warner-Chilcott plate rack and a Research Specialities Co. adjustable spreader were used. These plates were useful but they were not as strong as the commercial plates, and more care had to be taken when spotting them with whole juice. The plates were prepared with 20 channels by using a TLC plate scriber.

Solvent systems and sprays. Sixteen solvent systems were developed for the separation of limonin from citrus juices (Table 1). Two spray reagents were used: (1) 10% sulfuric acid in ethanol (Chandler, 1966) and (2) 2% sulfuric acid, 1% p-dimethylaminobenzaldehyde in ethanol (Wasicky, 1962).

Standard. A limonin standard was prepared which contained 0.01 μg limonin/μl acetonitrile. A standard containing 0.02 μg limonin/μl was also used.

TLC procedure. On an area between 0.5 and 3.0 cm from the bottom of the plate 25 μl of untreated, well mixed (shaken 1 min) canned grapefruit or orange juice (or reconstituted concentrate) was applied with a 50-μl Hamilton #705 syringe. The plate was dried with a stream of nitrogen or air, and another 25μl of juice was applied. On the center five bands of the plate (between 0.5 and 3.0 cm) standards of 0.1 through 0.5 μg were spotted by using a 50-μl syringe. The most important step in the procedure was found to be the thorough drying of the plate before placing it in a solvent tank. After the plate was dried with a stream of air or nitrogen, it was further dried with a heat gun for 1 min. The heat gun was not used at the beginning of this work and it was not necessary with orange samples. The main problem encountered was obtaining consistent separation of the sample spots of grapefruit. Small amounts of residual water were probably causing trailing, spreading and inconsistent Rf's. After use of the heat gun for thorough plate/spot drying, no further problems were encountered.

The dried plate was placed in a solvent tank containing acetone. The acetone was allowed to travel to the 3.0 cm mark, and the plate was removed and air dried 2×. When the plate was properly dried the acetone would run up the channels evenly across the channel. When the plate was not dry the acetone would run faster on the edges of each channel and cause the sample to form a dense concentration in the center of each 1 cm channel and after development the limonin concentration would not be readable. When the plate was properly dried the limonin appeared as a rectangle on the 1 cm channel and the concentration was quite readable after the plate had been developed in one of the solvent systems. The prepared *dried* plate was placed in a paper-lined tank containing one of the solvent mixes (Table 1) and the solvent was allowed to travel to the top of the plate. The plate was removed from the tank, air dried and developed two more times when solvents 8 or 9 were used (Table 1 shows Rf's for these after three passes). The other solvent systems listed required only one pass of the solvent. The plate was sprayed with spray (1) or (2) until lightly wet in appearance and placed in an oven at 125°C for 6 min. After cooling, the concentration was estimated by observation of either visible color (gray to black) or of fluorescence. For determination by visible color, the plate was viewed from the back (i.e., glass side) with a white light on the support side, and the den-

Table 1–Solvent systems used in the separation of limonin

	Solvent system	ml	Rf
1.	Chloroform, acetic acid	98–2	.14
2.	Chloroform, acetic acid	98–1	.06
3.	Methylenechloride, acetic acid	98–2	.06
4.	Methylenechloride, diethylether, acetic acid	185–15–2	.18
5.	Carbontetrachloride, acetone	85–15	.06
6.	Benzene, diethylether, acetic acid, methanol	180–20–2–6	.12
7.	Benzene, methanol, acetic acid	190–5–5	.14
8.	Benzene, hexane, acetone, acetic acid[a]	65–22–10–3	.32
9.	Benzene, hexane, acetone, acetic acid	65–22–10–0.5	.18
10.	Benzene, chloroform, diethylether, acetic acid	85–85–30–10	.12
11.	Benzene, chloroform, diethylether, acetic acid	65–65–65–5	.14
12.	Chloroform, diethylether, acetic acid	130–65–2	.06
13.	Chloroform, diethylether, acetic acid	130–65–5	.20
14.	Chloroform, diethylether, acetic acid	150–50–5	.14
15.	Benzene, diethylether, acetic acid, methanol	180–20–4–2	.06
16.	Benzene, diethylether, acetic acid	160–40–9	.10
17.	Benzene, acetic acid, methanol	192–8–2	.05

[a] Solvent system was used in limonin determination by Chandler (1971). Solvent systems 1 through 7 were useful on orange juice, 13 through 17 on grapefruit juice and 8 through 12 on both orange and grapefruit juice.

sity of the unknown spot was compared with the limonin standards. For fluorescent estimation, the plate was placed over a UV light with light passing through the plate and coating. The limonin was red to brown fluorescent under UV light. The fluorescent intensity and area of the unknowns were compared with standards. Since 0.1 μg/100 μl = 1 ppm and 50 μl of whole juice was used, the estimated value of μg of limonin per 50 μl of juice, multiplied by 2 indicated the limonin concentration in ppm of the unknown.

Application to fresh juice. The following fresh fruit was checked: Duncan grapefruit: Valencia, Hamlin, Parson Brown and Navel oranges. The fruit was halved and juiced with a Sunbeam extractor with firm hand pressure. The juice was allowed to stand 10 min, then strained through cheesecloth. The sample was weighed and boiled for 5 min, cooled and the tare weight adjusted with distilled water. The sample was mixed for 5 min and then analyzed as above.

Preparation of low and high yield orange juice. Juice samples were prepared from different orange cultivars (Hamlin, Pineapple and Valencia) by using relatively low and high extraction pressures. Thirty boxes of each type fruit were randomized and 15 boxes each were used for a low yield and a high yield juice. Yield was controlled through changes made at the extractor. All juices were finished on a cylindrical screw finisher set with 12 lb (Hamlin) to 15 lb (Pineapple and Valencia) air pressure using a screen with 0.033 in. hole diameter. All juices were concentrated in a pilot plant falling film evaporator at 3–10 mm Hg. Yields (%) were calculated on the basis of unfinished juice/pound of fruit.

Recovery studies. A limonin standard, 0.5 μg/μl in acetonitrile was prepared. To 50-g samples of juice were added 0.3, 0.5 and 1.0 ml of the limonin standard to give an additional 3, 5 and 10 ppm limonin. These samples were stirred for 10 min with a counter rotating mixer and reweighed. The samples were then analyzed by the procedure outlined above and the limonin content was compared with the amount originally added. As shown in Table 2, samples A and B were separated by using solvent system 8. This solvent system required three developments of the plate. The samples were spotted in triplicate on the same plate. A panel of six judges compared the unknown samples to standards. Samples C and D were treated as follows. Four identical 50-μl aliquots were applied to four channels on a TLC plate with a blank channel between samples. On the blank channels the appropriate standards were applied. Each plate was prepared in triplicate or duplicate and run in a different solvent system to show reproducibility and reliability. The limonin concentrations were estimated and compared by using spray (1) and reading fluorescence intensity. To further determine whether a 50-μl sample is representative the following test was made. Two grapefruit juices found to contain 3 (sample I) and 7 (sample II) ppm were tested as follows: A 46-oz can of grapefruit juice was shaken by hand for 1 min. The can was opened and six 5-ml aliquots were removed at various depths and labeled 1 through 6. On a 20-channel plate these samples were spotted with experimental samples and standards as follows: Two spotting sequences were used and two plates each were prepared as shown below: "X" represents 50-μl samples of unknown taken from the 5-ml aliquots previously taken. Numbers represent μg of limonin from standard solutions:

Sequence I

.15 X .20 X .15 X .20 X .15 X .20 X

Sequence II

.30 X .35 X .40 X .30 X .35 X .40 X

Plates were judged independently and at different times. All sample I spots were judged equal to the 0.15 standard (i.e., 3 ppm) and all sample II spots were equal to the 0.35 standard (i.e., 7 ppm).

RESULTS & DISCUSSION

THESE STUDIES resulted in the development of a simple analysis for limonin, requiring no sample preparation since the whole juice was spotted directly on commercially available plates. If samples were well mixed, no problems were caused by juice pulp or clogging syringes. Duplicate determinations on 12 juices required about 2 hr, and on 24 juices, about 3 hr. This average time of around eight samples/hr should meet most plant quality control needs. Table 2 indicates the precision and accuracy of this method for limonin. As shown in Table 2, grapefruit C which had no limonin added was run in three different solvent systems. Values of 3.8, 3.7 and 4.3 ppm were found for the sample. The mean value was 3.9 ppm with a maximum deviation of 10%. Recoveries of limonin added to grapefruits A and C ranged from 94–123%. Six judges were used to evaluate the limonin content to show that most technically trained people could determine limonin by such a method and thus demonstrate its feasibility as a potential industrial quality control method. With grapefruit A and orange B, judge B showed high deviations and thus should not use this method. If this method is to be used for quality control, individuals should be tested by carrying out recovery experiments to assure they can read plate spot values correctly and reliably. Our results indicated most people can.

When performing analysis by this procedure, standard spots above 0.5 μg were not satisfactory and are not recommended since it is difficult to differentiate differences of 0.1 μg at these densities; spots of 0.1μg through 0.5 μg are easy to visually differentiate. Spot densities can easily be kept in a range by dilution, however. For example, as shown in Table 2 when the juice contained more than 10 ppm only 25 μl of juice was spotted and the reading was multiplied by 4 to derive the answer in ppm. When using standards of 0.10 through 0.50 μg, as with the lower levels shown in Table 2, a close approximation to the actual limonin value was obtained with a 50-μl sample. If greater accuracy were desired the approximate limonin content could be deter-

Table 2—Recovery of limonin added to commercially processed grapefruit and orange juices

Solvent system	Limonin added (ppm)	Limonin found by six judges (ppm) A	B	C	D	E	F	Mean value (ppm)	Expected value (ppm)	% recovery
					Grapefruit A					
8	0	3	3	3	4	3	3	3.2		
8	3	6	7	6	7	6	5	6.2	6.2	100
8	5	8	12	9	9	9	11	9.6	8.2	117
8	10[a]	14	20	16	14	16	18	16.3	13.2	123
					Orange B					
8	0	4	7	3	4	4	4	4.3		
8	3	7	10	8	8	9	8	8.3	7.3	114
8	5	10	12	10	9	10	10	10.2	9.3	110
8	10[a]	16	20	14	12	14	12	14.7	14.3	103
					Grapefruit C					
10	0	4.0	3.5	3.5	4.0	3.5	4.0	3.8		
10	5.0	9.0	7.0	8.0	9.0	8.0	9.0	8.3	8.8	94
12	0	4.0	4.0	4.0	3.0	3.0	4.0	3.7		
12	5.0	10.0	10.0	9.0	6.0	10.0	10.0	9.2	8.7	94
17	0	5.0	4.0	4.0	4.0	5.0	4.0	4.3		
17	5.0	10.0	10.0	9.0	8.0	10.0	10.0	9.5	9.3	98
					Orange D					
1	0	2.0	2.0	2.5	2.5	2.5	2.5	2.3		
1	5.0	9.0	8.0	9.0	8.0	8.0	8.0	8.3	7.3	114
12	0	2.0	2.0	2.0	2.5	2.5	2.5	2.2		
12	5.0	8.0	9.0	8.0	7.0	7.0	8.0	7.8	7.2	108

[a] Sample size 25 μl; all others 50 μl

Table 3–Relation of high and low yield extraction to the limonin content in orange juice

	Juice yield %		Limonin ppm single strength		% Oil on reconstituted basis	
	Low	High	Low	High	Low	High
Hamlin	54.1	66.8	1.5	5.5	0.005	0.01
Pineapple	58.8	74.2	<1.0	38	0.04	0.07
Early Valencia	55.5	69.4	<1.0	5.0	0.01	0.035
Late Valencia	57.6	79.8	<1.0	7.0	0.027	0.04

mined in a preliminary analysis and the sample rerun and bracketed closely with the standards. For example, if a 50-μl sample contained around 3 ppm, 0.10 μg, 50 μl, 0.15μg, 50 μl and 0.20 μg should be applied. This sequence would allow a very close approximation of the limonin content.

Two spray reagents were used in this work (see Experimental). Although spray (1) was easier to prepare and use, spray (2) was especially useful when it was important to see impurities in the limonin spot. In early stages of this work we were only developing the plate 2× with solvent system 8, and, on occasion, the limonin spot of the juice contained another color not seen in the standards. Spray (1) would not have shown this. Sixteen solvent systems were developed during this study for the separation of limonin from citrus juices. Solvent systems 8 and 9 required development of the plate 3×. These are not recommended since the others required only one development of the plate. In the general survey of orange juice, solvent system 1 was preferred, but if the limonin content were 6 ppm or less, solvent system 2 was recommended. For grapefruit, solvent systems 10 and 12 were preferred. All citrus fruits have not been examined, and each of these systems may not be satisfactory on all fruit varieties. Solvent systems 10 and 12 gave good separations of grapefruit juice that contained K. Early (a variety often added early in the citrus season to increase the Brix acid/ratio and color). When an unusual citrus variety is examined several different solvent systems should be tried and the best one selected for that application. The plate should be examined with ultraviolet light and the fluorescent areas marked before spray (1) or (2) are used. After the plate is sprayed and developed if there is overlap of spots it will be apparent from observing fluorescence of spots. If spray (2) is used overlap of spots may also be indicated by a color difference. It would be advisable to use spray (2) for spot indication until the method is worked out. Spray (1) is preferred after the correct separation system has been determined. In this procedure the actual origin is 3 cm above the bottom of the plate and the sugars (most usual interfering compounds) do not move above the 3 cm mark, three Rf's (Table 1) below 0.1 actually gave better separations than expected and visual determinations were still easily carried out.

The method was used in an experimental study conducted on a series of samples or orange juice concentrates prepared with high and low yields (see Experimental).

Juices were evaluated on a basis of limonin content, recoverable oil and flavor. Yields, limonin levels and oil levels are shown in Table 3. All taste tests were triangulation type, comparing low yield to high yield samples for detectable differences. The oil levels of the low yield juices were adjusted to the high levels and taste tests carried out. There were significant differences between low and high yield samples in all four runs. The low yield juices were preferred by our taste panel. As shown in Table 1 the limonin content and the oil level in the juice from Pineapple oranges was too high for objective taste testing. The Hamlin, early Valencia and late Valencia low yield juices were adjusted to the same oil levels and limonin contents as the high yield juices. In all three instances taste tests showed a significant difference still existed, and the low yield juices were still preferred by our panel.

A sample of the early Valencia low yield juice was divided, and half was used as a control. To the other half, limonin was added to 5.5 ppm. Taste tests showed a significant difference, with the control preferred. This procedure was repeated on several commercial concentrates and there was a significant difference in each case, with the control preferred. These taste test results indicate that limonin does affect the flavor of orange juice when its level is high enough, but it was not the entire problem with the high yield juices. Most tasters referred to the greenish, astringent, woody or immature flavors in high yield juice whereas limonin usually causes a pronounced bitterness.

This method was also applied to fresh juice of different varieties (see Experimental). Results indicated: Duncan grapefruit 2.0 ppm, Valencia orange 3.0, Hamlin 2.0, Parson Brown 1.5 and Navel 4.0 ppm. The method as outlined for fresh juice would not necessarily give the same limonin content if the juice were commercially extracted and processed. To test the general applicability of the method it was also carried out on canned single-strength orange juice. Five commercial samples of canned single-strength orange juice that were produced December 11, 12, 18, 18, 1972, and February 14, 1973, were found to contain 6.0, 2.0, 2.0, 2.0 and 2.0 ppm.

In most quality control applications the exact level of limonin is not required. Usually an indication that limonin is below some predetermined level is sufficient. For example, if a citrus producer decided that his product should contain 8 ppm limonin or less the following test could be run by quality control. Six juice samples could be checked on one plate. Duplicate 50-μl samples of juice would be spotted on two channels then 0.4 μg limonin and repeat across the plate. If all samples were less than or equal to the standards, his requirements for the product would be met and no further checks on limonin would be required.

This is a simplified reliable method for determining limonin which can be performed easily on a routine basis, and should provide a useful quality control test for grapefruit and orange juices. It was found applicable to fresh juice, commercial single-strength canned juice and orange juice concentrates, as well.

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Mention of brand names is for identification only and does not imply recommendation by the USDA.

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STORAGE QUALITY OF BANANAS PACKAGED IN SELECTED PERMEABILITY FILMS

INTRODUCTION

BANANAS are among the three most widely consumed fruits in the United States. The total annual import of this fruit is estimated to be above 3.5 billion pounds.

The losses during transport, wholesale and retail storage are much greater than with other fruits. Therefore, the extension of bananas shelf life represents an important problem.

Controlled atmosphere treatment at low temperature has been known for many years to create beneficial conditions for fresh fruits and vegetable storage. Atmosphere containing 1–10% of oxygen, 5–10% of carbon dioxide or a combination of both can retard ripening of ethylene untreated bananas for months (Young et al., 1962; Mapson and Robinson, 1966).

Complicated equipment is needed to maintain concentration of gases, humidity and temperature at the desired level, and under retail conditions it has not been a practical operation.

Many attempts have been made to control the atmosphere by packaging with both perforated and hermetically sealed films. Packages with perforated films were shown to reduce water losses and maintain higher CO_2 levels; however, oxygen concentration was too high to be effective in retardation of ripening. In a hermetic package a state of equilibrium is reached after some time when the rate of respiration is equal to the rate of permeation and as a consequence, steady concentrations of oxygen and carbon dioxide are maintained. In this case, the main problem to be solved is the selection of a film with proper permeability for a particular package system in order to get desirable steady concentrations of oxygen and carbon dioxide.

Marcellin (undated) studied packages with polyethylene film and silicone lastomer membranes. He concluded that these packages may be adapted to controlled atmosphere preservation of various fruits and vegetables. Tomkins (1960; 1961; 1962) determined oxygen and carbon dioxide concentrations in packages of produce using different parameters.

Beneficial conditions were reported to be obtained with hermetic packages for different fruits, especially for apples.

Polyethylene bags were used to eliminate premature ripening of ethylene untreated bananas (Karel and Go, 1964; Scott and Roberts, 1966; Badran, 1969). The graphical approach of Jurin and Karel (1963) for predictions of respiratory gases in packages was applied to bananas (Karel and Go, 1964) and found to give good correlation with actually observed concentrations.

A few mathematical solutions have been presented (Toll, 1962; Veeraju and Karel, 1966; Henig and Gilbert, 1973) to relate the variables affecting storage quality of packaged fruits.

Henig and Gilbert (1973) studied the dynamics of the permeation-respiration interaction in tomato and banana packages. They solved numerically the differential equations representing the change in O_2 and CO_2 concentrations within the packaging system using a computer. The computer-aided iteration technique enabled the determination of equilibrium concentration of these gases, and also the time in which they are established. Their theoretical solution was validated by experimental work.

Bananas exhibit a climacteric pattern of respiration with a very high peak of CO_2 evolution. This is probably why we have not found reports in the literature of packaging of ethylene-treated bananas in hermetic packages. The present commercial practice uses perforated films because of insufficient permeation through the commonly used low density polyethylene. Since a hermetic package represents many advantages, the objective of this work was to develop such a package for ethylene-treated bananas.

Table 1—Permeability[a] of the films used for packaging of bananas[b,c]

Film code	Film trade name[d]	Oxygen	CO_2
A	TPM–87	94	686
B	UE–630	1,828	9,411
D	VF–71	698	3,598
F	RMF–61	2,100	10,811

[a] cc/24 hr X 100 inch² X atm
[b] Coefficient of variation less that 5%
[c] Gilbert-Pegaz method (Gilbert and Pegaz, 1969)
[d] PVC films used were supplied by the Resinite Division, Borden Chemical.

MATERIALS & METHODS

BANANAS used in these experiments were Chiquita Valery brand from Honduras. The fruit had been harvested and shipped under commercial conditions and was about 7 days from harvest. The hands of bananas were treated with 1000 ± 50 ppm of ethylene for 24 hr. These were then cut into fingers and the cut end dipped into a 400 ppm fungicide solution (Merck W-7). For packaging, four films coded A, B, D and F were used. The identification and permeabilities of these films are shown in Table 1.

Three fingers weighing approximately 500g were put on a foamed polystyrene tray (#20 S). The films were used to tightly overwrap polystyrene trays with bananas. To provide a hermetic package, the film was sealed at the bottom with a hot plate. The type of package selected is similar to ones used in packaging of other food products. Packages were kept at (15°C ± 0.5) and at room temperature (22°C ± 2).

At predetermined storage times, the gas composition of the package headspace was determined by gas chromatography. The analysis conditions have been published previously (Daun et al., 1971). The color changes of the peel were recorded using color photography and compared with a banana's standard color index (United Fruit Company). Characterization of the pulp odor, taste and texture were made applying a sensory scoring method and descriptive terms. A five-point scale (1 bad, 2 poor, 3 fair, 4 good and 5 very good) was used. A panel containing five to seven graduate students conducted sensory evaluations.

RESULTS & DISCUSSION

THE MEAN VALUES for oxygen and carbon dioxide content in the headspace of the banana packages made with different films, during storage at 15°C and 22°C, are shown in Figures 1–3. The results from three experiments using the same conditions were combined for these figures.

Preliminary experiments with pro-

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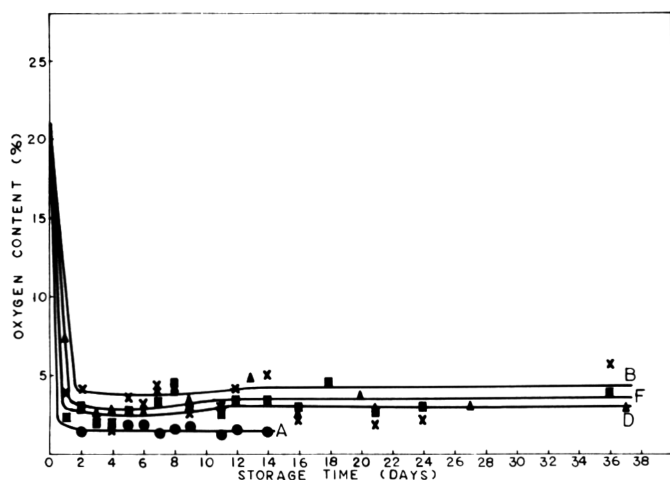

Fig. 1–Oxygen content of the headspace of banana packages using films of different permeability (stored at 15°C)

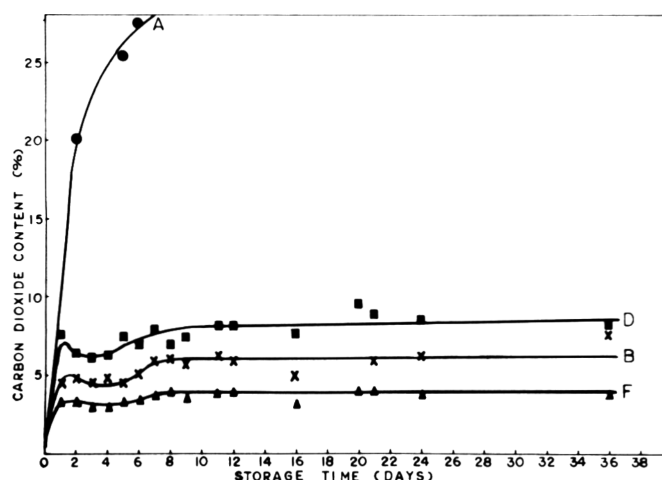

Fig. 2–Carbon dioxide content of the headspace of banana packages using films of different permeability (stored at 15°C)

longed storage periods (up to 30 days) showed an erratic delay of ripening of the bananas in high barrier film packages. The highest barrier films never ripened. The ethylene treatment was then introduced to obviate the variability in ripening rate of untreated fruit and provided a climacteric type of fruit whose respiration and physiological reactions were quite different than those discussed by Karel and Go (1964).

The bananas used in our experiments were treated with ethylene 24 hr before packaging and were physiologically very active at this time unlike preclimactric fruit of Karel and Go. Therefore, in all packages, the highest rates of oxygen consumption and carbon dioxide evolution were present at sealing. Since each package represented a closed system with restricted access of oxygen and outflow of carbon dioxide, a decrease in concentration of the oxygen and an increase of the CO_2 was observed in the headspace, particularly during the first days of storage. A state of dynamic equilibrium where the headspace concentration of oxygen and carbon dioxide remained relatively stable was reached between the first and second day for the packages kept at 22°C. At 15°C, however, this state appeared between the third and fourth day of storage. These stable concentrations are presented in Table 2.

As expected, the steady levels of oxygen and carbon dioxide are different in packages with various films and are temperature dependent. At 15°C in the packages with film A, the oxygen concentration was below a critical limit (2%), and the carbon dioxide concentration was 20.2%, i.e., far above the permissible 5% and was increasing continuously up to the sixth day of storage when it reached 40.2%. The bananas remained green throughout the experiment. They failed to ripen when the packages were opened after 18 days and they developed black spots within a few hours after exposure to the air. The increase in the CO_2 content during storage time indicated that aerobic respiration was shunted to fermentation, with undesirable quality effects. Film A was, therefore, eliminated from further experiments.

In the packages with B, D and F films, the oxygen concentration was within an acceptable range i.e., above 2%. CO_2 concentration, however, varied in these packages. In the packages with film B, oxygen and carbon dioxide concentrations were maintained at about 4% through the entire experiment. This was about 1% higher than in the packages with film F. These conditions were not injurous for bananas but ripening retardation was much less. In the packages with film D, relatively high CO_2 levels were observed during the first day of storage, reaching 7.7% and thus probably affecting physiological processes. In the next 2 days, CO_2 concentration decreased slightly and then started to increase again. In the packages with film F, CO_2 concentration reached only a level of 2.9% after 4 days of storage. The oxygen and CO_2 concentrations in the packages with film F remained constant during storage time. The packages with film F were found to be the most effective in retarding the ripening process and in extending shelf life of bananas to as much as 30 days. This film has gas permeabilities over twice that of the highest levels of the polyethylene films used in previously recorded work, including patents.

Packages with film A were not exam-

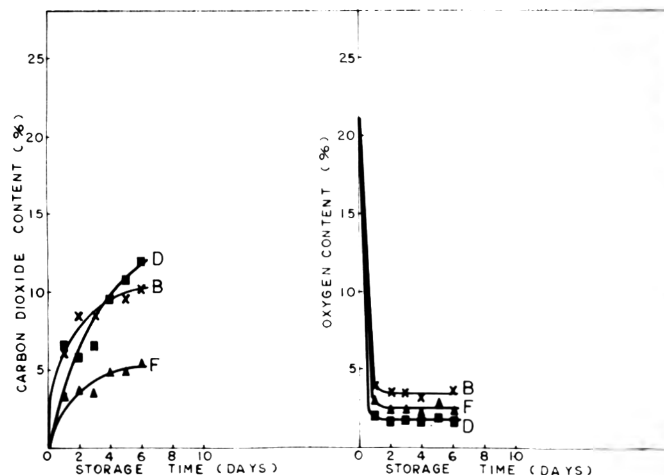

Fig. 3–Carbon dioxide and oxygen content of the headspace of banana packages using films of different permeability (stored at 22°C)

Table 2—Oxygen and carbon dioxide concentrations in the head-space of the packages at equilibrium state (%)

Film	Temperature 22°C		Temperature 15°C	
	O_2	CO_2	O_2	CO_2
A	Not used	Not used	1.4[a]	20.2[a]
D	1.7[a]	5.9[a]	2.0[b]	6.3[b]
F	2.5[a]	3.7[a]	2.8[b]	2.9[b]
B	3.4[a]	8.4[a]	4.1[a]	4.8[a]

[a] Second day
[b] Fourth day of storage

ined at 22°C because this film was already eliminated after the first experiment at 15°C. After 2 days of storage at 22°C, the oxygen and CO_2 levels in the packages with B, D and F films were close to the values obtained in 15°C with only the packages with film B having a higher CO_2 level of 8.4%. However, under the higher temperature condition, the rate of oxygen supply and CO_2 loss were inadequate to support biochemical processes determining proper quality.

Peel color changes of the bananas unpackaged and in packages made with films A, D and F stored at 15°C were recorded on color photographs which are available in our files. Control samples were rated 3 after 6 days, 5 after 10 days, and 7 after 13 days using a standard color index. Grade 7 was considered as indicating overripened fruit. Bananas in packages with film A did not change their color during the experiment and were rated 2. After 18 days of storage when the packages were opened, black spots developed within a few hours after exposure to the air. This was considered as an evidence of CO_2 injury. The peel color of the bananas stored in packages with film D yellowed very slowly. After 9 days, the color index was 3 and did not change up to the 20th day. After 28 days, peel color was rated 4 and when packages were opened, peel color reached grade 5 in 2 days. The conditions within the packages with film D supported aerobic color changes on a minimal level and when exposed to the air, the fruit was able to develop typical yellow color.

In packages with film F, peel color development was slower than in control but followed the same color change sequence. Peel color was rated 3 after 9 days, 5 after 16 days and 6.5 after 30 days. Traces of a marble type of mosaic pattern were detected in some packages.

Color changes in packages with film B were very similar to those obtained with film F but occurred sooner.

Peel color development was abnormal in all packages at 22°C as compared to the control which reached full ripeness (stage 6) after 6 days of storage. All packaged bananas formed a characteristic marble-type mosaic with slightly yellow and green areas, and this was unchanged up to the end of the experiment.

A summary of the sensory evaluation of samples stored at 15°C is presented in Table 3.

The optimal quality of the unwrapped control bananas was found between 10 and 12 days of storage at 15°C. The odor of the pulp was typical and full-bodied; however, the intensity was lower than the control ripened at room temperature. The taste was balanced, not too sweet and without "raw" note. The texture was firm, rather jelly-like and without "flour" note.

The bananas stored in packages with film A were of an unsatisfactory quality on the basis of external appearance which was confirmed during the preliminary sensory evaluation.

The bananas stored in packages with film B behaved very similarly to the control. Optimal quality was found after 13 to 15 days of storage i.e., only 3 days after control.

The odor and taste of the banana pulp of the samples stored in packages with film D was rated as fair after 30 days of storage. However, an untypical off odor and off taste were detected. Since color of the peel was fully acceptable, it appears that gas composition in these packages was sufficient for chlorophyll degradation, whereas biochemical changes in the pulp were not able to follow typcial aerobic patterns. An investigation of biochemical processes taking part under conditions created inside the packages was not conducted in these experiments.

Table 3—Sensory properties of the bananas stored at 15°C in packages with films of different permeability

Film	Optimal storage time (days)	Odor[a]	Taste[a]	Texture[a]
Control	10–12	5	5	5
A	None	1	1	1
B	13–15	4	5	5
D	27–33	3	3	4
F	27–33	5	5	4

[a] Five point scale: 1 Bad; 2 Poor; 3 Fair; 4 Good; 5 Very good

The optimal storage time for bananas in packages with film F was approximately the same as with film D i.e., 27 to 33 days, but the quality of the pulp was significantly better. The odor and taste were rated very good. The texture was good. In general, the sensory properties were found to be similar to the unwrapped control at the optimal stage, therefore, film F was considered as the best one among available films for the particular package system at 15°C.

The odor and taste development was abnormal in all packages at 22°C as compared to the control which reached optimal quality after 6 days of storage. All packaged bananas exhibited fermentation note with increasing intensity up to the 12th day of storage, i.e., the end of the experiment. The PVC films are of hydrophobic type with consequent good moisture vapor barrier properties. The effectiveness of these films was established in preliminary experiments. The anti-fogging agent present in the film formulation prevented undue water vapor condensation on internal surfaces of the packages. The initial fungicide treatment was effective in eliminating the problem of a fungal growth during the experiments.

CONCLUSIONS

A RETAIL PACKAGE was developed in which ethylene-treated bananas were maintained with excellent color, odor, taste and texture after 30 days of storage at 15°C. The beneficial conditions were obtained by the use of a film with proper gas permeability for the particular weight of the fruit and package dimensions. The desired oxygen, carbon dioxide and water vapor concentrations were generated by the system itself as a result of balance between the respiration of the bananas and the diffusion from surrounding atmosphere through the film.

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RESEARCH NOTES

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A Research Note

INFLUENCE OF FRESHNESS AND COLOR ON POTATO CHIP SENSORY PREFERENCES

INTRODUCTION

PRODUCING a uniform and desirable potato chip color throughout the year is an important problem of numerous potato chip producers. Most manufacturers attempt to remove dark-colored chips from their processing line since they feel that consumers object to the dark color. Thus, manufacturers may be forced to absorb considerable economic losses when whole batches of chips are judged to be too dark for marketing. Actually there may be a consumer market for these darker chips due to their unique and characteristic flavor.

Thus, this sensory study was designed to determine whether the difference between regular and dark-colored chips could be detected and which was preferred when the visual variable was eliminated. A secondary objective was to follow chip sensory preferences for both regular and dark-colored chips as influenced by storage.

EXPERIMENTAL

Samples and storage conditions

A batch of fresh chips was obtained from a local manufacturer. The chips were hand-sorted into a group representing regular colored chips (average Hunter Lab Color and Color Difference Meter L value of 40) and dark colored chips (L value of 25). Chips of each color were sealed in commercial potato chip bags and stored in the dark at anbient room temperature for up to 4 wk. Sensory evaluations were performed initially and at the end of each week's storage. Also, after each week of room temperature storage, samples were stored at −18°C for composite evaluation at the end of the storage study. Preliminary studies indicated that this frozen storage treatment did not adversely affect textural properties.

Panel and methods

The sensory panel consisted of 20 college-age students (15 female and 5 male), most of whom had experience with sensory evaluation techniques. Sampling was done by evaluating the requested sensory properties of 4–5 chips in a quiet and odor-free room in mid-afternoon on the same day for a 5-wk period. Rinse water was supplied and utilized by the panel between samples requiring tasting.

Each week a triangle test, composed of regular and dark chips, was performed by the blindfolded panel. The panel was simply asked to identify the odd sample.

This was followed by a second presentation of two samples to the blindfolded panel, one regular and one dark, and the panel was asked to select first the chip with the preferred odor and then that with the preferred flavor.

In a third presentation the panel was not blindfolded and was presented with one new sample each of regular and dark chips. This time they were to first select the chip with the preferred color then preferred odor and then flavor.

During the fourth week of the study the blindfolded panel was presented with samples of regular and dark chips representing each of

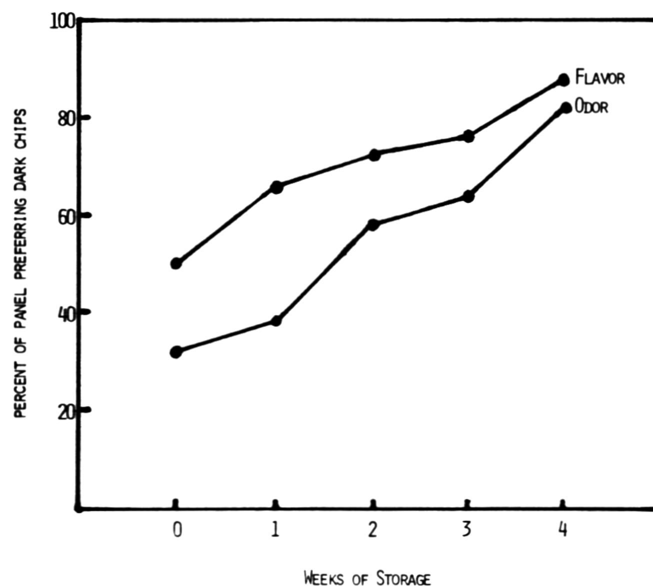

Fig. 1—Effect of storage on odor and taste preferences of dark- over regular-colored chips (blindfolded panel).

the storage times and they were asked to preferentially rank these samples.

RESULTS & DISCUSSION

INTERESTINGLY, no significant differences in sensory properties ($\alpha = 0.05$) between regular and dark chips were noted for relatively fresh chips (up to 2-wk storage) while the panel was blindfolded. Thus, it would appear that the flavor of freshly prepared dark chips would not be a detrimental factor on their marketibility.

When given only a visual choice between regular and dark chips, regular chips were preferred most of the time although not to the degree one might expect since regular chips were preferred only 55% of the time.

As can be seen from Figure 1, as storage time increased the blindfolded panel has a clear preference for the odor and flavor of the dark chips. Those who preferred the dark chips usually said that the dark chips had more flavor or a more characteristic potato chip flavor. A more prevalent rancid odor and/or flavor was given as the most prevalent reason for not preferring the regular color chips over the dark chips.

Table 1—Panel preference rankings of regular and dark chips as influenced by storage (blindfolded panel)

Rank	Color	Wk of storage
1	Dark	1
2	Dark	0
3	Regular	1
4	Dark	2
5	Dark	3
6	Dark	4
7	Regular	2
8	Regular	0
9	Regular	3
10	Regular	4

A composite of the blindfolded panel preference rankings of samples frozen after each storage period is summarized in Table 1. The dark chips stored for 1 wk were ranked the most preferable chips while the least desirable were the regular color chips stored for 4 wk. As can be seen, five out of the top six ranked chips were of the dark color type. Thus, it is quite apparent that overall dark chips were preferred to regular color chips even though the dark chips were held at room temperature storage longer.

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A Research Note
THE POSSIBILITY OF RECOGNIZING IRRADIATED AND NONIRRADIATED POTATOES BY THEIR WEIGHT LOSS

INTRODUCTION

IRRADIATION of potatoes with gamma rays to inhibit sprouting will probably be among the first commercial applications of nuclear technology to the preservation of agricultural products. Several factors contribute to make irradiation of potatoes a promising application in the food technology field: (a) The low radiation exposure required (10 Krads) and, consequently, the moderate cost of the irradiation equipment and process (Rondenay, 1971); (b) The ever-increasing demand from industry for a year-round supply of potatoes in good condition for processing (mashed potatoes, chips, crisps, etc.); and (c) The limited or only temporary efficiency of chemical antisprouting products.

The above factors account for the concerted efforts made by the European Community over the past three years to demonstrate in an unequivocally scientific manner the effectiveness of such a technology (Eurisotop, 1969). Moreover, since several countries (France, Germany, Israel, Holland, Canada, USA, USSR and Japan) have reached such an advanced stage of experimentation as to achieve commercial feasibility, coupled with the fact that some have authorized the sale of irradiated potatoes–even if only on a temporary basis and in limited quantities–it becomes rather urgent to establish whether a potato does not sprout because it has been chemically treated or, more simply, because it is in a physiologically inactive period.

In order to grant authorization for the irradiation of certain foodstuffs, many legislations request, in addition to documentation proving the wholesomeness of the product, a procedure for detecting the irradiation treatment and the absorbed dose. In this connection, mention should be made of the papers on an electric conductivity method for differentiating between irradiated and nonirradiated potatoes (Schertz, 1970) and on the identification of irradiated potatoes through modifications of its microstructure (Penner, 1970).

cases, this enabled actual irradiation to be established. The present experiment was carried out to determine whether potatoes reacted in the same manner. In this paper, an attempt is made to compare the loss in weight of nonirradiated control potatoes with that of irradiated potatoes as well as with potatoes treated with an antisprouting chemical (isopropyl-phenyl-carbamate) IPC.

Preliminary experiments (Magaudda, 1970) showed that there was a different weight loss in irradiated fruit as compared with nonirradiated controls and, in some

MATERIALS & METHODS

Potatoes

130 kg of potatoes (Tonda di Berlino variety) grown in the Avezzano area were used in this experiment. A high percentage of the tubers, as soon as delivered to the Laboratory, started sprouting buds approximately 14.5 mm long. Each experimental lot consisted of 10 kg set out in two one-layer boxes: 5 kg of potatoes with buds and 5 kg of potatoes whose buds had been removed.

Table 1—Angular b coefficient of the weight loss in IPC-treated potatoes stored for 150 days at 10°C, 70% RH. S.E. of b < 0.00000 in all the cases. (Values in brackets refer to IPC-untreated potatoes.)

Dose (krads)	Angular coefficient b: Potatoes with sprouts	Angular coefficient b: Potatoes with sprouts removed
0	−0.04205	−0.03425
	(−0.07146)	(−0.06828)
5	−0.03012	−0.02624
7.5	−0.03000	−0.03387
10	−0.03423	−0.02927
	(−0.04946)	(−0.04840)
12.5	− 0.03976	−0.02962
	(−0.04797)	(−0.04530)
15	−0.03229	−0.03144
	(−0.05443)	(−0.04576)
20	−0.03187	−0.03239
40	−0.03438	−0.03349
80	−0.03633	−0.03116

IPC at 20 mg/kg of potatoes (Sparenberg, 1970), was used for the purpose of reducing to a minimum the expected difference in weight-loss between nonsprouting irradiated samples and sprouting control samples. Before treatment, all samples were washed throughly with water.

After drying, the tubers were irradiated with radiation exposure of 0 (control)–5-7, 5-10-12, 5-15-20-40-80 krads in the industrial irradiator facility (^{60}Co source) of the Casaccia Nuclear Studies Centre, at a dose rate of 2,700 rads/min. The boxes, placed at random, were stored in a controlled cell at 10°C and 70% RH. During the 150-day storage period the boxes were weighed every 4–5 days. Results of these weighings were used to plot the regression line and its angular coefficient b, indicating whether the weight loss was rapid or slow and thus enabling comparisons to be made between the various treatments.

Potato "cores"

Experiments were also carried out using "core" tubers. For this purpose, potatoes were cut into small cylinders ("cores") 11 mm diam × 4 cm long. Every sample was represented by 60 "cores" collected from potatoes irradiated at 0-5-7, 5-10-12, 5-15-20-40-80 krads and from the control after 150-days storage. The "cores" were then analyzed over a 24-day period by weighing every 2 days. The potato "cores" were kept at room temperature (24°C) in Petri dishes. The weight loss during the storage period was calculated by applying the same method as that used for the boxes.

RESULTS

Potatoes

Values of the angular coefficient b of the weight loss in irradiated potatoes and in the controls (Table 1) show the differences between potatoes whose buds had or had not been removed. In agreement with many reports dealing with this subject (Nazir et al., 1971; Nys, 1968; Parks, 1960), we noted that, regardless of the chemical treatment or bud removal, weight losses were higher in the unirradiated controls than in the irradiated samples (Table 1). However, it should be noted that there is no connection between the loss in weight and the absorbed radiation dose, at least in the range of exposures applied.

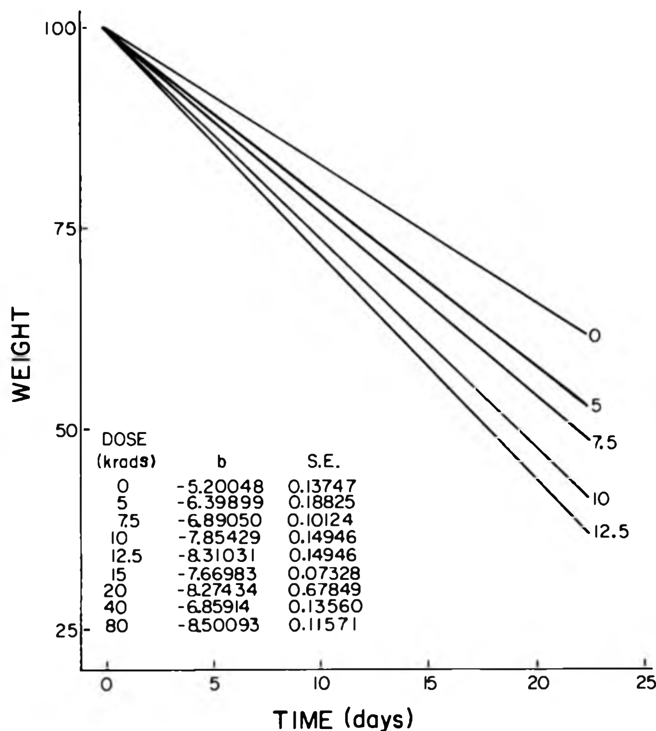

Fig. 1—Angular b coefficient of the weight loss in potato "cores" obtained from potatoes stored for 150 days at 10°C, 70% RH. "Cores" were stored at room temperature (18–23°C) for 24 days.

Potato "cores"

Values of the angular coefficient b of the weight loss in "cores" obtained from the debudded potato samples used in the above-mentioned experiment are shown in Figure 1. This figure shows that the nonirradiated control lost less weight than the irradiated samples. In Figure 1 the same phenomenon is also represented graphically but only for the irradiation dose range normally used for antisprouting purposes. The weight loss in potato "cores" (b value) is clearly correlated with the absorbed gamma rays.

DISCUSSION

FROM RESULTS obtained it appears that it is possible to perform experiments in potatoes, [as previously done on other vegetable products (Magaudda, 1970)] revealing "a posteriori" whether or not the naterial has been irradiated, and eventually also the absorbed dose.

However, a considerable obstacle in the case of potatoes is presented by the fact that, unlike citrus fruit and tomatoes, potato tubers have a great metabolic activity, so that the nonirradiated controls lose more weight than the irradiated samples, due to sprouting. Also in the nonirradiated samples, as shown in Table 1, there are differences in "b value" closely connected with sprouting. In fact, among the potatoes treated with IPC, those with buds lose more weight than those with buds removed. Similar behavior has been observed in samples not treated chemically. However, their weight loss is always higher than in the corresponding IPC-treated samples. In similar conditions it is rather difficult to establish how much weight has been lost through sprouting and how much as a consequence of irradiation.

One solution would be to perform analyses on the potato core which remains, as such, uninfluenced by the sprouting phenomenon. The experiment carried out on potatoe "cores" proves that there is a good relation between gamma ray doses and weight loss. In fact, the phenomenon is evident (Fig. 1) mainly when the applied irradiation doses are in the range used for sprouting inhibition. By adopting this method, it may be possible to overcome the difficulties involved in analyses made "a posteriori" (in the absence of unirradiated potatoes). In fact, it does not appear difficult to standardize the time and drying temperature for several varieties of potatoes, in order to find out a practical method for the commercial control of foodstuffs.

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A Research Note
MONOSODIUM GLUTAMATE INGESTION AND THIRST PRODUCTION

INTRODUCTION

THE DESCRIPTION of the Chinese Restaurant Syndrome (Kwok, 1968) resulting from ingestion of large amounts of monosodium glutamate (MSG) has focused attention on this compound. Interest was further increased when Olney (1969) reported brain lesions caused by injection of MSG. The MSG controversy has also resulted in various indications to our laboratory that some people experience a great thirst after meals containing MSG. It has therefore been suggested that MSG may have a direct thirst-producing effect on the central nervous system.

EXPERIMENTAL

RATS were maintained on lab chow ad lib. but were deprived of water and permitted to drink for only 1 hr each day. No experimental tests were attempted until the rats were capable of maintaining body weight on this regimen.

Four tests of the thirst-producing effects of MSG were performed. The test solutions (0.02 ml per gram of body weight) were injected orally by intubation one-half hour before the rats were placed in the drinking cages. Each solution was presented in a random order to each rat. The first was between 0.15, 0.6 and 1.2 molar solutions of NaCl or MSG. In the second test the preload was one molar solutions of the sodium, potassium and ammonium salts of glutamic acid. To elimate the possibility of a synergistic effect between various salts, the third protocol involved measuring drinking response after a preload of a combination of salts. The solutions used were 1M NaCl, and equal mixtures of molar solutions of NaCl, KCl, MSG, K-glutamate, NH_4-glutamate and NH_4Cl. To overcome the objection that forced loading of the solutions prevented the taste mechanism from functioning, the final experimental procedure consisted in dividing the 1-hr drinking period into two halves. During the first half hour 0.17M solutions of the various salts were available for the animals to drink. After the first half hour there was a 15 min delay and tap water was presented for the second half hour.

Due to the great variability in individual amounts which the rats drank, all values are for percent of control when the preload was distilled water. During the fourth experiment tap water drinking during the first and second half hour periods was used as the control.

Table 1—Thirst after salt preload

Preload solution	Water consumption (% control[a])
Experiment 1	
0.15M NaCl	152 ± 19[b]
0.15M MSG	118 ± 14
0.6M NaCl	211 ± 24
0.6M MSG	172 ± 19
1.2M NaCl	248 ± 30
1.2M MSG	223 ± 27
Experiment 2	
1M MSG	133 ± 22
1M NH_4-Glu	142 ± 21
1M K-Glu	143 ± 13
Experiment 3	
1M NaCl	241 ± 19
½M:½M NaCl:MSG	210 ± 16
½M:½M NaCl:NH_4-Glu	191 ± 19
½M:½M NaCl:K-Glu	207 ± 19
½M:½M NaCl:NH_4Cl	188 ± 19
½M:½M NaCl:KCl	214 ± 19

[a] Each rat was its own control and received a distilled water preload. Water consumed ad lib. after the preload = 100.
[b] Mean ± standard error

Table 2—Ad libitum salt solution consumption and thirst

Solution (0.17M)	Consumption (½ hr) % control[a]	Water (½ hr) % control[a]
NaCl	94 ± 2[b]	180 ± 35
MSG	104 ± 6	170 ± 23
NH_4-Glu	96 ± 7	124 ± 31
K-Glu	114 ± 3	89 ± 18
NH_4Cl	78 ± 6	196 ± 47
KCl	86 ± 4	236 ± 39

[a] Each animal was its own control; control consumption = 100.
[b] Mean ± standard error

RESULTS

TABLE 1 contains the data from the first series of experiments. No difference could be found between NaCl and MSG solutions at the concentrations tested in experiment one. Moreover, experiments two and three show that no combination of either salts of glutamic acid or of chlorine could produce a greater enhancement of the thirst sensation than NaCl alone.

The data in Table 2 are those obtained from the ad lib. situation which resembled the more usual method of MSG ingestion. It can be seen that only the NH_4Cl and KCl solutions proved to be less acceptable than the tap water control. The amount imbibed in the second half hour increased thirst resulting from the salt solution drunk in the first half hour. Except for the K-glutamate, the thirst produced by the various salt solutions are not demonstrably different from that produced by NaCl.

CONCLUSIONS

THE PRESENCE or absence of a thirst-producing effect of MSG has no relationship to the controversy surrounding the use of this compound in food preparation. It must be noted, however, that the lack of concern for the fact that MSG is as thirst evoking as NaCl has resulted in the addition of MSG to some foods in quantities that produce unpleasant effects. The failure of glutamate salts to have any special thirst-inducing effects is evident since K-glutamate did not produce an increase in drinking in the ad lib. experiment. This result can be explained by the reported low ability of some K salts to stimulate thirst (Fitzsimons, 1972).

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HYDROCYANIC ACID IN CANNED SWEET CHERRIES

INTRODUCTION

AMYGDALIN, first isolated from the seeds of the bitter almond and later from stone fruit kernels, is a cyanogenetic glycoside. Upon hydrolysis it yields gentiobiose, benzaldehyde and hydrocyanic acid (Liener, 1966). The hydrolysis, one of the earliest reactions studied in the history of enzyme chemistry (White et al., 1959), is catalyzed in the seed or fruit kernel by the enzyme, β-glucosidase.

Dickinson (1957) has presented evidence that β-glucosidase activity in the kernels of cherries and plums is not destroyed by heating at 100°C for less than 6 min. Sweet cherries are usually processed and canned with their kernels intact. The purpose of this study was to observe if underprocessed sweet cherries might produce levels of hydrocyanic acid of potential toxicologic significance due to the hydrolytic activity on amygdalin by active β-glucosidase.

EXPERIMENTAL

SWEET CHERRIES (*Prunus avium* var. Emperor Francis) were picked from 4 trees in the New York State Agricultural Experiment Station orchards. Lots were mixed, sorted and washed. The kernels were removed from a portion of the harvested fruit. The cherries were put into number 303 cans containing 20% sugar syrup and exhausted for 3-½ min at 82°C. Cans were retorted either for 2 min or 12 min at 100°C. All cans were stored in a 38°C room. Thus, there were four treatments: cherries canned with or without kernels and retorted for either 2 or 12 min.

Duplicate treated cans of cherries were taken from the storage room at 1, 3, 6, 12, 23, 32 and 50 wk and analyzed for hydrocyanic acid, after steam-generated distillation, by silver nitrate titrimetry (Pesticide Analytical Manual, 1967). At 50 wk of storage, the analysis of cyanide was also accomplished by the fluorometric method of Jeffrey and Wiebe (1971). Kernels were removed from the canned cherries prior to distillation. Both fruit and juice were used for the analysis.

RESULTS & DISCUSSION

TABLE 1 shows the results of hydrocyanic acid residues in canned sweet cherries. The control group was considered to be the fruit processed without kernels and retorted for 12 min. The amount of silver nitrate needed to titrate the distillate from the control group of cherries, considered the "blank," was subtracted from the amount of silver nitrate needed to titrate the other three groups.

Table 1—Hydrocyanic acid residue in canned sweet cherries after retorting 2 or 12 min at 100°C and stored at 38°C for 50 wk

Processed with kernels	Retort time (min)	HCN content (ppm) during storage (weeks)						
		1	3	6	12	23	32	50
+	2	2.1	1.6	1.8	1.4	1.1	1.9	2.0
0	2	0.1	0.4	0.4	0.3	0.1	0.4	0.2
+	12	0	0.2	0.3	0.1	0	0.1	0.1
0	12	Control or "blank treatment"						

The underprocessed cherries, i.e., 2-min retort, containing their intact kernels, had the highest cyanide level from the first week through the 50 wk of storage. The range of cyanide was 1.1–2.1 ppm during the entire period and did not appear to increase during the storage time. The HCN apparently was produced from the β-glucosidase activity of the kernels, since removal of the kernals or heat-destruction of the enzyme negated this effect. The levels of cyanide are lower than those reported by Dickinson (1957). However, we are in agreement with his observation that cyanide residues are not increased in stored canned fruit.

Recovery studies with 1–2 ppm of cyanide as sodium cyanide added to the cherries indicated an 89–120% recovery. Recovery of cyanide below 0.5 ppm was difficult and considered below the sensitivity of either the titration or the fluorometric analytical methods. The cyanide in cherries processed without kernels, or retorted for 12 min with kernels, was present in only trace amounts at or below 0.4 ppm.

Cyanide intake from consumption of wild black cherries (*Prunus serotina*) has been associated with malformed offspring of swine (Selby et al., 1971). Certain plant foods, especially cassava and beans, can contain in excess of 100 ppm of cyanide (Montgomery, 1964). The oral intake of cyanide salts have a minimum lethal dose of 2–3 mg/kg body weight in animals (Clark and Clark, 1967) and man (Rieders, 1965). The 1–2 ppm of hydrocyanic acid found in our study of under processed sweet cherries with intact kernels is assumed to be toxicologically insignificant.

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A Research Note
SIMPLE DETERMINATION OF PHOSPHORUS IN PET FOODS

INTRODUCTION

VARIOUS colorimetric methods are presently employed for the determination of phosphorus in pet foods (AOAC, 1970). However, all methods involve considerable reaction time, critical timing and/or preparation of unstable solutions (Kuhn, 1962). Therefore, it was of interest to attempt to adapt the method of Kennedy and Weetman (1971) for the determination of phosphorus in various pet foods.

EXPERIMENTAL

A BECKMAN ACTA III Ultraviolet-Visible Recording Spectrophotometer and a Bausch & Lomb Spectronic 100 Spectrometer were used to determine the spectra of the various solutions.

The pet foods were dry ashed at 600°C and taken up in an acid solution as outlined by Kuhn (1962). Following this, an aliquot of sample solution containing 0.05–0.35 mg of phosphorus was introduced into a 50 ml volumetric flask. Water was added until the volume was approximately 35–40 ml. Then 5 ml of 2.5M nitric acid was added followed by the addition of a 5 ml aliquot of 10% (w/v) ammonium molybdate solution (A.R. grade: $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$). Water was added to mark and solution was mixed thoroughly and allowed to stand 1 hr at approximately 20°C and then transferred to optical cells for absorbance measurements at 362 nm.

The preparation of the blank and standard solutions followed the same procedure, except only 10 min were needed for the color-producing reaction to go to completion. The standard solutions were prepared using dibasic sodium phosphate heptahydrate.

Table 1—Phosphorus content of meat and meat product samples by conventional and new methods

Samples	Percent phosphorus[a]	
	New method	Old method
Biskit #1	1.51 ± 0.05	1.46 ± 0.07
Puss 'n Boots Liver #1	0.28 ± 0.03	0.26 ± 0.03
Burger #1	0.75 ± 0.05	0.78 ± 0.04
Biskit #2	1.43 ± 0.05	1.41 ± 0.06
Puss 'n Boots Liver #2	0.29 ± 0.02	0.29 ± 0.03
Burger #2	0.90 ± 0.04	0.88 ± 0.05
Horsemeat	0.13 ± 0.02	0.15 ± 0.02
Bone	3.07 ± 0.15	3.17 ± 0.2

[a] Mean % P (six determinations) ± standard deviations

RESULTS & DISCUSSION

VERY GOOD agreement was obtained between this method and conventional methods (Table 1). This procedure is both reproducible and accurate and offers the advantage of using solutions whose concentrations are not critical and are stable over a period of time.

This is not the case for Kuhn's method. A number of solutions have to be prepared every day due to decomposition. The colorimetric measurements in the old method of analysis are extremely time dependent, meaning the time elasped between mixing the reagents and making the absorbance measurement is critical. In the new method, once the color producing reaction has gone to completion, the absorbance of the solution is stable for up to 5 hr, which enables the technician to run many samples at once without regard to time.

Kennedy and Weetman's work concentrated on simple salt solutions. Our method has been applied to the more complex case of pet foods in acidic media. The major change in procedure involved the length of time necessary for the absorbance to stabilize. For reasons which have not been determined, the color-producing reaction takes longer for solutions of product than for simple salt solutions.

Time studies illustrate that it took up to 50 min for absorbance to stabilize. No change in absorbance was observed up to 5 hr. However, past this time absorbance decreased as a yellow precipitate, probably phosphomolybdate, was observed to form. Although Kennedy and Weetman mentioned some concentrations of interfering species may be too high for applied work, no other interferences were observed. The reaction appears to be catalyzed by visible light, which was not evident from the earlier article. Solutions kept in the dark required 3 hr to equilibrate, thus all our solutions were allowed to stand in room light.

In its present form, this method is as accurate as other molybdate methods but has the advantage of being much simpler and less time consuming for doing many analyses simultaneously.

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A Research Note
RELATION BETWEEN SHEAR FORCE AND TENDERNESS OF BEEF

INTRODUCTION

ALTHOUGH shear force as an indication of tenderness is widely used in research and product control tests, the relation between tenderness as experienced by chewing and that indicated by shear force is still a controversial matter. Many investigations on the relation between shear force on one hand as measured by a variety of mechnical devices, and taste panel ratings on the other, have yielded varying but statistically significant correlations (Szczesniak and Torgenson, 1965). Results of many other studies, however, have given conflicting or poor correlations and consequently the validity of shear force measurements as an indication of meat tenderness has been questioned (Deatherage and Garnatz, 1952; Hurwiez and Tischer, 1954; Wells et al., 1962).

A major problem involved in correlating shear force with taste panel scores would appear to be the lack of homogeneity in muscle. Within a given muscle shear force varies from end-to-end (Ginger and Wier, 1958; Paul and Bratzler, 1955) and from location-to-location (Alsmeyer et al., 1965; Hedrick et al., 1968). Consequently, even by using core samples and taking other customary precautions (Kastner and Henrickson, 1969; Khan and Voisey, 1973; Szczesniak and Torgenson, 1965), samples for shear force measurement do not necessarily have the same tenderness as those submitted to the taste panel, nor are the samples submitted to the taste panel necessarily uniform in tenderness. Inherent variability in taste panel scoring further aggrevates the problem.

Studies of factors influencing tenderness often require information on the minimum difference in shear force that would constitute a difference in tenderness detectable by a taste panel (Khan and Lentz, 1973). Since this information cannot readily be deduced from the published correlations even where they are significant, experiments were designed to obtain a more definite and direct answer. Samples of cooked beef of similar shear force (within ± 0.2 kg of average) were selected from one or more muscles and compared with samples similarly selected for a different shear force level. This procedure permitted shear force and taste panel measurements on the same sample, minimized between-sample variability and enabled accurate determination of the minimum shear force differences detected by the taste panel. Additional factors considered were the level of tenderness in similar and different muscles and the method of cooking.

Table 1—Effect of shear force difference between samples on percentage of judgements correctly identifying the most tender sample[a]

Differences in shear force between samples (kg)	Judgements correctly identifying most tender sample (%)
3.2 ± 0.2	100
2.6 ± 0.2	100
2.0 ± 0.2	95[b]
1.4 ± 0.2	80[b]
0.9 ± 0.2	80[b]
0.5 ± 0.1	70[c]
Less than 0.4	50

[a] Shear force of samples varied from 1.7 to 7.3 kg. Results for all samples and for both cooking methods are included because level of tenderness and method of cooking had no noticeable effect on tenderness assessment.
[b] Significant at 1% level.
[c] Significant at 5% level.

EXPERIMENTAL

SAMPLES were obtained from the short loin and the round of choice steers (Canada Grade A-1) aged under commercial conditions for 7 days. Tests were made using biceps femoris, longissimus dorsi, obliquus abdominis internus, psoas major, semimembranosus, semitendinosus, vastus lateralis and vastus medialis. Meat for shear force and taste panel assessment was cooked either by boiling in water or by broiling. For cooking by boiling, four slices, each at least 1.5 cm in thickness, were cut parallel to the fibers from different locations in each muscle. Each slice was then cut longitudinally into two or three pieces of equal thickness (40–50g each), depending on the size of the muscle, and clamped inside metal-plate molds as described earlier (Khan and Voisey, 1973). The clamped slices were cooked to an internal temperature of 80°C in a boiling water bath. For cooking by broiling, slices of at least 2 cm in thickness were cut as described above and cooked under a broiler in an electric oven to an internal temperature of 70°C. Cooked samples were cooled to room temperature and strips 1 cm square in cross-section were cut along the fibers.

For taste panel tests, samples (about 1 cm long) were cut from each strip using a shear press (Food Technology Corp., Reston, Va.) equipped with a meat shear cell. Only samples having shear force differences between the two ends less than 0.2 kg were used in the taste panel tests. Samples having shear force differences of no more than ± 0.2 kg from the mean and in some cases no more than ± 0.1 kg, were placed in groups. Samples having shear force differences of no more than ± 0.1 kg, were placed in groups. Samples from two groups differing in average shear force between 0.4 and 3.4 kg were given to the taste panel for assessment. The paired comparison method was employed for detecting tenderness differences using an untrained panel of six members having discriminating judgement for tenderness. The panel was asked to identify the most tender sample. Each sample was supplied in duplicate and the panel was given two comparisons at each session. For each level of shear force difference, 24–36 judgements were obtained by

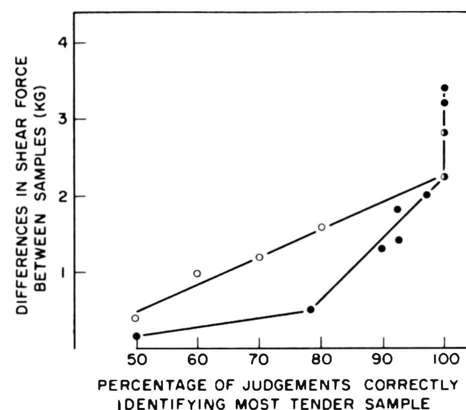

Fig. 1—Effect of muscle characteristics on the relation between shear force and taste panel assessments of tenderness. (Shear force differences for similar samples varied between 0.2 and 2.2 kg, ○, similar muscles; ●, different muscles.)

repeating the test as necessary. Comparisons were made between the same or similar muscles, and between widely different muscles. In addition to level of tenderness (shear force of taste panel samples varied from 1.7 to 7.3 kg) and kind of muscle, the effect of cooking method was also studied.

RESULTS & DISCUSSION

SAMPLES differing in shear force by 0.5 kg or more were readily detected by the taste panel regardless of the level of tenderness or the method of cooking (Table 1). The lack of an effect of level of tenderness on the detectable differences appears to indicate that factors other than shear force which may interfere in the judgement of tenderness, become less important at high shear force values.

The taste panel discriminated more readily between samples from different muscles than samples from the same or similar muscles (Fig. 1). This might suggest that mouth becomes more sensitive to tenderness when there are texture differences as well as shear force differences. Thus, texture differences would appear to reinforce shear force differences.

The results show that there can be a very close and definite relation between shear force measurement and the results of taste panel tests. Insufficient precision in the sampling and experimental techniques appears to have been the cause of the conflicting and poor correlations between shear force and taste panel scores reported in the literature. Factors such as the use of the same sample for shear force and taste panel assessments and minimization of sample variability for taste panel studies appear to be important in obtaining meaningful results. Proper attention to these factors and the use of the sampling technique described here would also appear to increase the usefulness of taste panel tests employing a hedonic rating scale, both for training the taste panel in quantifying tenderness differences and for obtaining better correlations.

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A Research Note
ACTIVITY OF ANTIOXIDANTS IN FRESH FISH

INTRODUCTION

GENERALLY, antioxidants are not used to help stabilize frozen seafood products even though the shelf life of these products is shortened by oxidative rancidity. In a recent review, Labuza (1971) discusses oxidation processes in fish and other foods. During frozen storage, fatty fish such as herring, mackerel and salmon are prone to oxidize even at temperatures as low as $-20^{\circ}C$. Lean fish such as haddock and cod also oxidize in the frozen state and are substantially less stable than beef or chicken at low temperatures. This difference between the stabilities of fish and beef or chicken is probably due to the high degree of unsaturation in the fish lipids (Olcott, 1962) and to the unusually high concentrations of metals in seafood (Watt and Merrill, 1963).

Attempts have been made to stabilize seafood with antioxidants such as ascorbic acid (Andersson and Danielson, 1961), tocopherols (Brown et al., 1957), and BHA-BHT combinations (Yu et al., 1969); but, as yet, no treatment has been fully effective against the development of oxidative rancidity. Stuckey (1968) reported that phenolic antioxidants applied to frozen fish fillets have generally been found ineffective in retarding oxidative rancidity. This may be attributed to either inadequate distribution of these water-insoluble antioxidants on the fish samples or the antioxidants may simply have lacked sufficient potency in this particular application.

Before antioxidants can be successfully applied to frozen fish, more information is needed concerning the relative potencies of commercially important antioxidants and combinations of these antioxidants with chelators in a variety of fish. The purpose of the work reported here was to determine the relative effectiveness of various stabilizer formulations in stabilizing fresh, ground fish flesh against oxidation at low temperatures.

MATERIALS & METHODS

THE FOLLOWING antioxidants were tested: butylated hydroxyanisole, butylated hydroxytoluene, and propyl gallate (Tenox® BHA, Tenox® BHT, and Tenox® PG, respectively, Eastman Chemical Products, Inc.), thiodipropionic acid (Evans Chemetics, Inc.), dl-α-tocopherol (as prepared by Nelan, 1969), d-δ-tocopherol (Eastman Organic Chemicals), and tert-butylhydroquinone (as prepared by Sherwin and Thompson, 1967). The chelating agents used were citric acid (Chas. Pfizer & Co.) and disodium EDTA (Ciba-Geigy Corp.). Chlortetracycline hydrochloride (Sigma Chemical Co.) was used as a bactericide and 2-thiobarbituric acid (Eastman Organic Chemicals) was used in determining rancidity. All other materials were reagent grade.

Silver salmon steaks, haddock and flounder (marine species) as well as crappie, bass and fresh trout (fresh-water species) were trimmed of as much skin, bone, fat and connective tissue as possible. Then, all discolored and exterior portions of the flesh were removed (1/4 to 1/8 in. deep). After the addition of 30 ppm chlortetracycline hydrochloride to inhibit bacterial growth, the flesh was ground once in an aluminum meat grinder. The antioxidants (16 ppm) and chelators (100 ppm) dissolved in a small amount of propylene glycol or water were then added, and the flesh was reground five times to ensure thorough mixing and incorporation of oxygen. Aliquots (10g) were stored at 4–5°C in individual glass jars closed with low-density polyethylene (oxygen permeable, water impermeable). After 1–10 days of storage, rancidity of each aliquot was determined by using the thiobarbituric acid (TBA) test (Tarladgis et al., 1960). Samples were tested every other day, and the number of days required for the samples to give a TBA number of 1.0 mg malonaldehyde per 1000g sample was determined. This time was considered to be an induction period before the onset of oxidative rancidity. Except for induction periods of less than 1 day or more than 10 days, the data are usually the average values for duplicate runs. The experiments were terminated after 10 days because mold growth usually became visible at this point.

Table 1–Effect of antioxidants on oxidation in salmon and trout

Antioxidant[a]	Induction period[b] (days) Salmon	Trout
Control	< 1	<1
TBHQ	2–3	6
BHA	< 1	2
PG	< 1	2
BHT	< 1	1
α-Tocopherol	< 1	–
δ-Tocopherol	< 1	–
Thiodipropionic acid	< 1	–
Ascorbic acid	< 1	–

[a] Added at a level of 16 ppm (except for ascorbic acid, 100 ppm)
[b] Induction period is the number of days (at which time a sample is stored at 3–5°C) required to produce a TBA number of 1 mg malonaldehyde/1000g sample.

Table 2–Effect of antioxidants and chelators on oxidation in fresh fish

Stabilizer[a]	Induction period[b] (days) Salmon	Haddock	Flounder	Crappie	Bass
None	< 1	< 1	< 1	< 1	< 1
EDTA	5	–	4–5	6	3–4
Citric acid	< 1	1	< 1	< 1	< 1
TBHQ	2–3	1	1	8	3–4
TBHQ + EDTA	>10	>10	>10	>10	5
TBHQ + Citric acid	>10	1	10	>10	5
BHA + EDTA	>10	–	–	–	–
BHA + Citric acid	5–6	–	–	–	–

[a] Phenolic antioxidants added at a level of 16 ppm and chelators added at a level of 100 ppm
[b] Induction period is the number of days (at which time a sample is stored at 3–5°C) required to produce a TBA number of 1 mg malonaldehyde/1000g sample.

RESULTS & DISCUSSION

IN WHOLE FISH fillets or steaks, oxidation is expected to occur only at the exposed surfaces. In fact, we found that the interior flesh of salmon steaks was not oxidized, as determined by the TBA test, even though the surface flesh was already quite rancid. Similarly, Yu and Sinnhuber (1957) reported TBA values for the surface flesh of rock fish fillets which were much higher than those from the whole sample. The methods used in the work reported here, i.e., removing the surface flesh and grinding the sample, make it possible to obtain a fresh starting material with a large surface area in intimate contact with oxygen. This type of sample provides a model for the surface tissue of an intact fillet or steak.

The data in Table 1 show the effectiveness of various antioxidants in maintaining the TBA value of salmon and trout samples below 1 mg of malonaldehyde/1000g of sample [which indicates the onset of rancidity (Labuza, 1971)]. When no antioxidant is added, high TBA values (5–15 mg malonaldehyde/1000g of sample) are produced in less than 24 hr. Under these conditions, only TBHQ has a significant stabilizing effect in salmon. In general, most antioxidants (TBHQ, BHA and PG) were more effective in trout.

Due to the high metal ion content in marine fish, the use of chelating agents (citric acid and EDTA) in addition to the antioxidants improved the oxidative stability of the fish samples. In Table 2, the data for oxidative stability of some marine and fresh-water fish with antioxidants and chelators are shown. EDTA, which is a more effective chelator than citric acid, was effective even when no phenolic antioxidant was present; but the most potent inhibitors were combinations of EDTA or citric acid and TBHQ or BHA. The most effective stabilizer combination in salmon (the most unstable fish tested) was TBHQ and citric acid.

The relatively high potencies of the best stabilizer systems identified in this study suggest that, if methods can be developed for adequate distribution of these stabilizers onto the fish flesh, the shelf life of frozen fish might be substantially increased.

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A Research Note
A QUALITATIVE AND QUANTITATIVE STUDY OF SUGAR-ALCOHOLS IN SEVERAL FOODS

INTRODUCTION

SUGAR-ALCOHOLS are formed by reduction of the corresponding carbohydrates and thus classified as tetritols, pentitols, hexitols and so on.

Galactitol was isolated by Hünefeld from *Melampyrum nemorosum* as long ago as 1837. Since that time there has been a number of similar investigations. Sugar-alcohols have been found not only in branches (Monteverde, 1892), leaves (Plouvier, 1956), bark (Kubel, 1862; Monteverde, 1892; Rogerson, 1912) and seaweed (Haas and Hill, 1931, 1932; Hassid, 1933, 1936) but also in varieties of *Melampyrum* and *Rhinanthus* (Braecke, 1923, 1925; Bridel and Braecke, 1922) and in many other foods which have long been in common use.

Work by Frérejacque (1943) and Kratzl et al. (1963a,b) has suggested the presence of arabitol in an edible Boletus mushroom (Boletus bovinus, L. ex Fr.) and of xylitol in champignons.

The work reported in this paper proves that the sugar-alcohols (sorbitol, mannitol, xylitol and galactitol) can, indeed, be found in a very wide range of foods. The importance of these investigations rests on the fact that (for example) xylitol is sold under various trade names to people suffering from diabetes mellitus as a substitute for sugar. Sorbitol is also used by diabetics as a sweetening agent. It is also employed as an antidrying agent in foods and as an antihardening agent in confectionery.

MATERIALS & METHODS

THE EXTRACTION of the sugars and the sugar-alcohols was accomplished using a mixture of acetone and methanol (1:1) together with 3% water. The solvent was removed by means of a rotavapor and the residue dissolved in water. After purification and fermentation the yeast was centrifuged and the clear supernatant concentrated to a small volume (Washüttl, 1973).

Qualitative and quantitative determinations were made in all cases using thin-layer chromatography and when needed, results were confirmed by gas-liquid chromatography.

Direct thin-layer chromatography of the fermented extracts was carried out in the absence of fermentable sugars on phosphate-buffered silicagur G (n-butanol-acetone-phosphate buffer, pH 5, 40:50:10). Sugar-alcohols were identified with sodium periodate-benzidine reagent.

The sugar-alcohols were determined semiquantitatively by measuring of the spot-area. The results based on external and internal standards showed good agreement. After eluation of the sugar-alcohols from the plates we also used the microchemical determination by the method of Rappaport et al. (1937). Gas chromatography was carried out after concentrating the extracts and acetylation and purification of the residue (Washüttl, 1973).

RESULTS

RESULTS shown in Table 1 are the average values of three determinations. Values are expressed as milligrams per 100 grams of dry matter, or as milligrams in a 100 grams of wine or juice.

The presence of pentitols and hexitols has been sought in a wide range of foods, using analytical methods based on thin-layer chromatography and gas-liquid chromatography. Xylitol (and, very occasionally, arabitol) was found in most of the vegetables and in a few of the fruits and fruit products examined; for example, in cherries, morello cherries and morello cherry jam. As expected, we found sorbitol in many fruits and fruit products; also—to our surprise—we found galactitol, although only on rare occasions. Neither of these two hexitols was found in any of the vegetables examined, except in trace amounts; the sugar-alcohol predominantly present was mannitol.

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Table 1—Occurrence of sugar-alcohols in food[a]

Product	Arabitol	Xylitol	Mannitol	Sorbitol	Galactitol
Bananas (*Musa sapientum* L.)	–	21	–	–	–
South African grapes (*Vitis vinifera* L.)	–	–	–	52.5	–
Pears (*Pyrus communis* L.)	–	–	–	4,600	–
Raspberries (*Rubus Idaeus* L.)	–	268	–	–	–
Strawberries (*Fragaria var.*)	–	362	–	–	–
Canned pineapple (*Ananas sativus*)	–	21	8	–	–
Peaches (*Prunus Persica Stokes*)	–	–	–	960	–
Reineclaudes (*Prunus domestica* subspec. italia)	–	935	–	935	–
Carrots, fresh (*Daucus carota*, L.)	–	86.5	–	–	–
Endives (*Cichorium Endivis*, L.)	–	258	334	–	–
Parsley (*Petroselinum crisoum* (Mill)Nym.	–	–	334	–	–
Onions (*Allium cepa* L.)	–	89	47.5	–	–
Artichokes (*Cynara Scolymus* L.)	–	–	183	–	–
Celery (*Apium graveolens* var. dulce Miller)	–	–	4,050	–	–
Lettuce (*Lactuca sativa*)	–	131	–	–	–
Chinese cabbage (*Brassica pekinesis*)	–	–	100	–	–
Cauliflower (*Brassica oleracea* L. var. botrytis)	–	300	–	–	–
Pumpkins (*Cucurbita pepo* L.)	–	96.5	200	–	–
Spinach (*Spinacia oleracea* L.)	–	107	–	–	–
Kohlrabi (*Brassica oleracea* L. var. gongylodes L.)	–	94	–	–	–
Red cabbage (*Brassica oleracea* L. var. capitata L.f.rubra	250	–	–	–	–
Egg plant (*Solanum Melon gena* L.)	–	180	270	–	–
Asparagus (*Asparagus officinalis* L.)	–	–	170	–	–
Leeks (*Allium Porrum* L.)	–	53	–	–	–
Fennel (*Foeniculum vulgare* Mil.)	–	92	–	–	–
Lamb's lettuce (*Valeria–nella olitoria* L.)	–	273	190	–	–
Beetroot (*Beta vulgaris* L. var, cruenta Alef)	–	–	192	77	–
Lentils (*Lens esculenta* Moench)	3	–	–	–	–
White mushrooms (*Boletus edulis* Bull.)	340	128	476	–	48
Parasol mushrooms (*Macro-lepiota procera* Sing)	–	–	1,390	–	–
Chanterelles (*Cantharellus cibarius* Fries)	107	–	374	–	–
Bakers' yeast	–	–	–	–	195
Brewers' yeast	10.5	4.5	–	–	–
Liquorice	15.5	4.5	–	–	–
Juniper berry electuary	–	–	–	–	75.5
Manna of pine	–	–	17,000	–	–
Black salsify, dried (*Scorzonera hispanica* L.)	–	–	–	–	32
Chestnuts edible (*Castanea vesca*)	–	14	20	11	–
Cornmeal	1	0.5	–	2	1
Prunes, dried (*Prunus domestica* L.)	–	–	–	2,420	–
Fig coffee[b]	–	–	–	–	124
Cherry preserve	–	–	–	645	–
Morello cherry preserve	–	–	–	965	11.5
Red cherry jam	43	56	–	1,100	–
Morello cherry jam	42	54.5	–	900	–
Elderberry jelly	–	–	–	27	–
Fig jam	–	–	–	–	9
Quince jam	–	–	–	125	–
Rose-hip jam	21	–	–	27	15
Black currant jam	42	34	–	27	–
Plum jam	–	–	–	108	22.5
Black currant wine	–	–	–	10	13
Apple wine	–	120	–	220	–
Red wine	–	–	–	14.5	–
White wine	–	–	–	8	–
Pear juice	–	–	–	394	–
Grape juice	–	–	–	4.5	–
Apple juice	–	–	–	203	–
Elderberry juice	–	–	–	133	–
Hawthorn juice	–	–	–	665	–
Carrot juice	–	12	–	–	–
V-8 vegetable juice cocktail	–	–	16.5	–	–
Vegetable five-juice cocktail	–	–	36	–	–
Artificial honey[c]	–	–	119	123	159
Yoghurt	–	–	–	–	893

[a] Expressed as mg per 100g of dry matter, or as mg per 100g wine or juice
[b] Surrogate prepared of figs
[c] Man made honey prepared from inverted saccharose

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A Research Note

DEPENDENCE OF FRUIT-BERRY WINES' STABILITY ON THE CONTENT OF MINERAL AND NITROGENOUS COMPONENTS

INTRODUCTION

THE INCREASED USE of metallic apparatus and capacities in wine-making result in richer content of iron salts, both in wine materials and in the finished product. Iron in wines is found in an ionic state, as in ferric (Fe^{+++} and ferrous (Fe^{++}) salts (Arjun, 1966; Bergner and Lang, 1970). The formation of precipitate (Cass) does not depend solely on its iron content. Acids in wines are known to form soluble complexes with trivalent iron which prevents precipitate (Cass) formation. Conversely, iron precipitate (Cass) is intensively formed in a medium of sufficient protein content. Thus in fruit-berry wines, there are substances promoting both the decrease and disturbance of stability.

Since fruit-berry juices contain few nitrogenous substances, it was very important to find the dependence of fruit-berry wines' stability on the content of iron, organic acids and proteins (Gorinstein et al., 1971). In apple juice the natural nitrogen content is hardly sufficient for sugar fermentation which yields 5–6% vol alcohol. Wine-making experience elaborated modes of incorporating additional nitrogenous nourishment to complete the process of fermentation. It is quite natural that additional incorporation of nitrogenous substances results in the disturbance of stability. Thus the necessity arose to establish an optimum rate of additional nitrogenous nourishment which would cause no wine stability disturbance but would assist completion of the fermentation process. A series of experiments were carried out with this purpose in view.

EXPERIMENTAL

THE OBJECTS of this investigation were fruit-berry wines. The fruit-berry wines apple, strawberry, cherry, currant, fruit wines, red sweet wine, white sweet wine, Yantarnoye and Yubileynoye, produced by the experimental wine-making factory "Anyksciu Vynas," were prepared from high acidic juices diluted with water by adding 27 gr/100 ml sugar, with fermentation lasting 70–100 days. All analyzed wine samples, except apple-wine, were prepared by combination.

Standards for comparison of wines were the same drinks clarified by filtering masses "Kineshma" (control) and "Evlakh" (test) (Gorinstein, 1970). Filtering masses "Kineshma"/6.6% turbidity of standard solution and "Evlakh"/ 1.9% turbidity of standard solution, distinquished by its filtering ability, were manufactured from raw materials of different compositions. "Evlakh" filtering mass was made at the Lvov Brewery Firm "Kolos" (Ear) by Gorinstein (1968; 1970). Iron was determined spectroscopically in wines by methods of Steiner and Oliver (1963); Bukharov and Mekhuzla (1964). Common nitrogen was found by the micromethod of Duma and Kjeldahl; (Gorinstein et al., 1971; Bulgakov, 1959; Klimova, 1967; Strukova and Fedorova, 1966; Fedoseev and Osadchii, 1969); protein nitrogen by deposition with hydrate of copper oxide; amino ni-

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Table 1—Physico-chemical indices of fruit-berry wines

Qualitative I indices	Apple wine				Cherry wine					Currant wine		
	Before clarification	Control	Test	Bentonite	Before clarification	Control	Test	Bentonite	Before clarification	Control	Test	Bentonite
Iron, mg/liter	48.9	42.3	31.4	46.5	38.3	27.6	27.3	27.9	28.5	23.4	16.5	24.8
Nitrogen, mg/liter												
Common	86.5	75.4	75.1	75.5	134.4	118.3	118.1	118.5	93.4	86.3	81.4	85.7
Organic	50.4	46.8	46.5	46.6	81.3	75.6	79.3	80.2	67.3	52.7	49.6	51.0
Protein	38.9	33.5	33.3	33.4	45.4	36.2	30.3	32.4	48.0	41.7	39.8	43.3
Amine	15.7	11.8	11.7	11.9	16.8	14.3	11.7	12.0	15.7	12.6	10.7	11.2
Colloids, mg/liter	520.3	400.1	399.9	400.2	1103.3	872.4	851.0	879.4	400.7	354.7	572.1	280.5
Tannic & Dyeing substances, mg/liter	430.70	364.70	364.60	364.81	749.00	644.50	638.25	641.00	146.10	138.4	140.2	116.75
Dry substances mg/liter	578.20	503.42	481.03	499.10	872.02	794.52	775.40	789.31	344.00	331.14	305.00	318.40
Pectinic acid mg/liter	0.410	0.365	0.362	0.364	0.280	0.250	0.220	0.246	1.472	1.398	1.372	1.348
Insoluble ash, mg/liter	7.70	6.69	6.67	6.70	8.00	7.35	7.21	7.42	33.09	32.14	32.96	27.41
Soluble ash, mg/liter	19.2	18.1	18.2	18.3	20.9	17.6	17.1	17.4	25.5	23.6	24.8	18.3
Acidity, %	–	4.9	4.7	5.8	–	9.1	9.1	9.1	–	7.4	7.2	7.3
Stability, months	–	5.0	5.1	4.9	–	4.0	6.0	5.4	–	5.0	5.0	4.8

Table 2—Dependence of the process of fermentation (alcohol) of apple juice and wine stability from additional nitrogenous nourishment

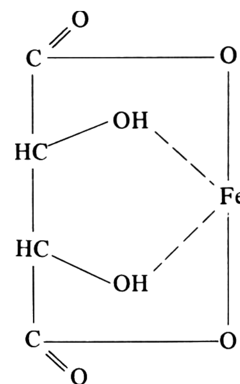
	Common nitrogen, mg/liter						Wine		
Composition of nourishment	Juice	Beginning of fermentation	Additional nitrogeneous nourishment	After addition of nitrogen	At end of fermentation	Wine material	Common nitrogen mg/liter	Alcohol %	Stability months
KC + $(NH_4)_2HPO_4$	218.6	98.6	100	198.6	142.3	91.4	77.4	12.7	4.3
	218.6	98.6	200	298.6	150.4	93.5	80.3	13.1	4.4
	218.6	98.6	300	398.6	162.7	95.6	85.9	13.5	4.7
	218.6	98.6	400	498.6	169.5	99.7	89.8	13.7	4.9
KC + $(NH_4)_2SO_4$	218.6	98.6	100	198.6	143.5	93.6	79.4	11.8	4.3
	218.6	98.6	200	298.6	151.7	95.8	82.1	12.0	4.6
	218.6	98.6	300	398.6	164.2	97.3	87.9	12.4	4.7
	218.6	98.6	400	498.6	174.8	101.3	91.5	12.7	4.9
KC + NH_4Cl	218.6	98.6	100	198.6	142.0	90.5	74.9	12.8	4.7
	218.6	98.6	200	298.6	149.3	91.8	76.5	12.9	4.8
	218.6	98.6	300	398.6	161.5	94.3	81.3	12.1	4.9
	218.6	98.6	400	498.6	167.6	98.5	84.5	13.9	5.0
KC + $(NH_4)_2HPO_4$ + $(NH_4)_2SO_4$	218.6	98.6	100	198.6	140.5	87.9	73.5	13.1	5.4
	218.6	98.6	200	298.6	147.3	89.8	75.4	15.2	6.0
	218.6	98.6	300	398.6	157.3	93.5	79.7	14.3	5.3
	218.6	98.6	400	498.6	162.9	97.6	81.5	14.5	5.1
KC	218.6		0	98.6	85.1	63.7	57.3	9.3	3.0

trogen by means of copper; and organic nitrogen by $K_2S_2O_8$ (Gertner and Gidinic, 1965). Colloids were determined according to GSE (Dumanskii et al., 1936); dry, pectic substances and insoluble and soluble turbidity gravimetrically; tannic, dyeing substances and acidity titrimetrically; and colloid-protein stability and alcohol by conventional methods (Fertman and Gorinstein, 1970; Nilov and Skurikhin, 1967).

RESULTS & DISCUSSION

THE CONTENTS of iron, common, organic and amine nitrogen protein, alcohol, colloids, dry, tannic, dyeing, pectinic, ashen substances and acidity were studied. The data obtained show that before clarification three groups of wines could be distinguished according to the concentration of the main components underlying the terms of stability: (1) wines with high content of iron; (2) common nitrogen; and (3) wines with mean numbers both of iron and common nitrogen. Examples of these groups are apple, cherry and currant wines, respectively (Table 1).

It was interesting to follow the dynamics of indices studied after the clarification of fruit-berry wines by filtration through filtering masses "Kineshma," "Evlakh" and bentonite. The data are given in Table 1. Analyses of these data show, that after clarification, typical distinctions of qualitative indices of three groups of wines continue to change: in apple-wine the highest concentration of iron is retained; in cherry-wine that of common nitrogen. Interesting data were obtained by analysis of the relation of stability terms of wines containing iron and common nitrogen. According to the laws of formal logic it could be expected that in wine with high iron content the stability would be the least. However, the data obtained experimentally contradicted this. In the sample of apple-wine clarified by bentonite, the iron content (true to statistics) is higher than in sample controls and tests (in both cases $P < 0.001$; P = probability of distinction). With approximately the equivalent quantity of common and protein nitrogen, it is in the apple-wine sample, clarified with bentonite, that the least stability could be expected. However, in our experiment, wines clarified by different means have the same stability. This implies that other factors affecting the stability exist (Nilov and Skurikhin, 1967; Rodonulo, 1971; Ogorodnik and Dranovskaya, 1970). Indeed, despite the high concentration of iron in the apple wine clarified by bentonite, the presence of considerable amounts of organic acids (acidity, 5.8% and iron 46.5 mg/liter) prevents stability decrease. This is explained by the fact that malic and citric acids readily form soluble complexes preventing the formation of iron precipitates (Cass). According to Nilov and Skurikhin (1967), tartaric acid in wine with Fe^{++} oxidizes COOH–CHOH–CHOH–COOH^{-2H} COOH–COH=COH–COOH. But this process is weaker, because iron (II) is oxidized to iron (III) and has no catalytic force. But iron in wine forms the following complex (Nilov and Skurikhin, 1967):



This complex is possessed with a catalytic force and prevents transition of Fe^{II} to Fe^{III} (Nilov and Skurikhin, 1967).

It is known that the velocity of redox reactions depends upon the acidity of the media. In acid media, that is in wine, oxidizing processes took place more slowly than in neutral and alkaline media. It is obvious that while the oxidation process is necessary for the ripening of wine, results may also be undesirable. For example, in oxidation the possibility of the formation of tannin-protein combinations falling out in the sediment and the acquisition of stable transparence in wine was stronger, so the bitter taste of tannides was softer. But simultaneously the oxidizing of the amino acids results in deterioration of aroma and taste.

According to Table 1 the largest amount of common nitrogen is found in cherry wine. In samples of this wine clarified with different types of filtration masses and bentonite, essential distinctions in content of iron, common nitrogen and in acidity, indices were not found. With equivalent values of indices influencing the terms of stability, one could expect it to be identical for all samples of cherry wine. Experiments show, however, that the highest stability is found in cherry wine test (6.0 months), somewhat less in the wine clarified with bentonite (5.4 months), and still less in wine control (4.0 months). By qualitative analysis it was found that in wines with equal amounts of common nitrogen there were different contents of protein nitrogen (Table 1). Thus it can be concluded that wine stability is in reverse dependence from the contents of protein (not common) nitrogen. Concluding the analysis of Table 1, it must be noted that the best qualitative indices are possessed by wines clarified with the filtration mass "Evlakh," with wines clarified with bentonite somewhat worse.

The protein content of bentonite-treated wines decreases considerably while stability increases (Table 1). But in comparing wine test to wine bentonite, wine bentonite has a little more protein. The adsorption ability of the filtering mass "Evlakh" is better than bentonite.

The amount of common nitrogen was determined in apple, cherry and red currant juices. Qualitatively and quantitatively different protein nourishment was incorporated into each juice. Again common nitrogen was determined and repeated at the end of fermentation in wine material and in finished wine. The data were compared with alcohol content and terms of stability (Table 2). Analysis of data obtained enable us to establish optimum additional nitrogenous nourishment both quantitatively and qualitatively. Optimum nitrogenous nourishment was considered the one which in minimum amounts ensured completion of the fermentation process (according to alcohol) and the longest terms of stability (Table 2).

CONCLUSION

THUS WE CAME to the conclusion that the addition of nitrogenous nourishment to the high nitrogenous cherry juice, is superfluous. Optimum nitrogenous nourishment for apple juice is a mixture of phosphate and ammonium sulfate in the amount of 200 mg/liter and for currant juice, ammonium phosphate in the amount of 100 mg/liter which is considerably below conventional rates.

The results of the investigation show that fruit-berry wines, like beer, are subject to common regularites of retaining colloid-protein stability of ethanol media (Gorinstein, 1968).

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A Research Note
USE OF FORCEPS IN STERILITY TESTING: A POSSIBLE SOURCE OF CONTAMINATION

INTRODUCTION

THE USE OF large-bore sterile glass pipettes in sterility testing for the inoculation of subculture media with foods is routinely practiced (Denny, 1970; Denny, 1972). Because of the viscosity of some products and the total lack of liquid in others, however, pipettes cannot always be used. Also, there are times when garnish too large to be subcultured with a pipette must be examined for sterility. In these instances, forceps have proven satisfactory for manipulating food particles which cannot be satisfactorily handled with a pipette. When large numbers of samples must be examined, the forceps cannot be autoclaved between samples and must be sterilized by other means. Alcohol flaming is a common method used for "sterilizing" such implements, but Doyle and Ernst (1969) reported that bacterial spores in the alcohol could survive the flaming technique and thereby contaminate. In several instances in this laboratory where contamination occurred during sterility testing, the forceps were suspected of being the cause. This study was initiated to determine what role forceps play in contamination during sterility testing and how this contamination could be significantly reduced or eliminated.

EXPERIMENTAL

Forceps

Eight inch, highly polished, chrome-plated steel forceps with straight, medium points were used throughout this study. Like almost all forceps, the inside points of these forceps were file-cut. On one pair, these file-cuts were removed and the inside tips made smooth by grinding and polishing. A piece of rubber tubing was placed over the handles so that the forceps did not become too hot to the touch during flame-sterilization. In this way, the tips could be heated more thoroughly to insure sterility. Forceps were flamed longer (about 10–15 sec) than just allowing the alcohol to burn off and product sizzled when touched with the sterilized forceps. After flaming and before handling the subculture sample, the forceps were cooled by touching them to a part of the sample not being subcultured.

Subculture medium

Brom Cresol Purple (BCP) broth (16g of Difco Purple Broth Base, 5g of glucose and 1,000 ml of distilled water) was used as the subculture medium. The lot used was tested and found to support the growth of microorganisms. The medium was dispensed in 18 × 150 mm tubes, 10 ml per tube, and autoclaved for 15 min at 15 pounds pressure (121°C). Incubation of subculture tubes was at 35°C for 7 days.

Experimental design

Three experiments were conducted in this study. All were performed in a positive pressure inoculating room under the conditions outlined by Evancho et al. (1973) so that contamination during subculturing could be minimized. The forceps, after subculturing, were immersed in a beaker of hot, soapy water and wiped with a paper towel to remove food particles before being alcohol flamed.

In experiment 1, pasta forms were subcultured from 50 cans of product known to be sterile (product had been incubated for several weeks and examined to assess sterility) using both the file-cut tip and the smooth tip forceps. Both forceps were thoroughly cleaned before use.

In experiment 2, no product was subcultured. Tubes of medium were inoculated by touching the tips of the forceps into the medium. Thoroughly cleaned file-cut tip forceps, file-cut tip forceps with food particles "baked" in the file-cuts, and smooth tip forceps were dipped into a sporulated culture of *Bacillus licheniformis* (isolated from a spoilage incident and sporulated on Nutrient Agar) and allowed to air dry. To insure viability of the organism, two control tubes of medium were inoculated with each of the unflamed forceps. 25 tubes of medium were then inoculated with each of the forceps which were alcohol flamed between tubes. The experiment was repeated three times.

In experiment 3, pasta forms from 1,456 cans were subcultured with smooth tip forceps.

RESULTS & DISCUSSION

IN EXPERIMENT 1, 14 of 25 subcultures (56%) made with the file-cut tip forceps were positive, whereas all subcultures made with the smooth tip forceps were negative. The organisms encountered in the positive subcultures were *Bacillus* sp. Close examination of the file-cut tip forceps revealed food particles had become embedded in the file cuts and could not be removed by cleaning in hot soapy water. The food particles became "baked" into the file cuts during flame sterilization and when the hot forceps were touched to the product. The absence of positive subcultures when using the smooth tip forceps indicated the source of contamination had been eliminated by removing the file cuts.

All subculture tubes inoculated with unflamed forceps in experiment 2 showed growth of the *Bacillus*. Subculture tubes inoculated using smooth tip forceps showed a contamination rate of 0–17%, those inoculated using thoroughly cleaned file-cut tip forceps showed a rate of 0–17%, whereas 38–67% of the subculture tubes inoculated using file-cut forceps with "baked-on" food particles were positive. The results incriminate food particles in the file cuts as the major contributor to contamination rather than the presence or absence of file cuts on the forcep tip. Removal of the file cuts facilitates cleaning and consequently reduces the chance of contamination resulting from the use of forceps.

Pasta from 1,456 cans of product were subcultured in experiment 3 using smooth tip forceps. A contamination rate of 0.07% was encountered.

Removal of file cuts from the inside points of forceps results in a significant reduction in contamination caused by the use of forceps. The presence of file cuts hinders thorough cleaning of embedded food particles and allows bacteria to survive the flame sterilization, thus resulting in contamination.

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Institute of Food Technologists

The Institute of Food Technologists is a professional society of scientists, engineers, educators and executives in the field of food technology. Food technologists are professionals who apply science and engineering to the research, production, processing, packaging, distribution, preparation, evaluation and utilization of foods. Individuals who are qualified by education, special training or experience are extended an invitation to join in professional association with the select group of the food industry's scientific and technological personnel who are Institute members. Membership is worth many times its modest cost, reflecting positive benefits, stimulation and opportunities for the individual in his business or profession.

OBJECTIVES

The Institute, as a non-profit, professional, educational society, has several major aims: to stimulate investigations into technological food problems; to present, discuss and publish the results of such investigations, to raise the educational standards of Food Technologists; and to promote recognition of the scientific approach to food and the basic role of the Food Technologists in industry. All of these activities have the ultimate objective to provide the best possible foods for mankind.

ORGANIZATION AND PROGRESS

Organized July 1, 1939, at Cambridge, Mass., with a membership of less than 100, the Institute has grown to more than 11,000. It is worldwide in scope with almost 2,000 of its membership overseas.

QUALIFICATIONS FOR MEMBERSHIP

Professional Members. Any person who meets the following minimum requirements by training and experience in food technology: (1) Bachelor's degree or higher from a college or university in which he has majored in one or more of the sciences or branches of engineering associated with food technology; (2) Five years of professional experience in food technology, for which a master's degree may be presented as the equivalent of one year's experience; a doctor's degree, the equivalent to three years' experience.

Members. Any person active in any aspect of the food industry and who evidences interest in supporting the objectives of IFT.

Student Members. Any person who is registered as a student in an educational institution and who is actively pursuing candidacy for an Associate Degree or higher in one or more of the sciences or branches of engineering associated with food technology shall be eligible for membership as a Student Member. He may remain a Student Member until the end of the calendar year in which he completes his schooling.

Fellows. Any Professional Member who has been active for at least ten years and who has been nominated by the IFT Committee on Fellows for outstanding contributions to the field of food science/technology is eligible to be elected a Fellow of the Institute by the IFT Council.

DUES

Professional Members and Members–$20 a year; includes subscription to *Food Technology* and *IFT World/Directory & Guide;* option to subscribe to the *Journal of Food Science* at members' special rate of $10. Student Members–$5 a year; includes subscription to one IFT journal, *IFT World/Directory & Guide,* and option to subscribe to the other journal at $5. Emeritus Members–no dues; includes *IFT World/Directory & Guide;* option to subscribe to either or both journals at $5 each.

PUBLICATIONS

The Institute publishes two journals. *Food Technology*, issued monthly, is the official journal of the Institute. The *Journal of Food Science*, issued bimonthly, is devoted to basic and applied research papers on fundamental food components and processes. In addition, an *IFT World/Directory & Guide* is published annually.

REGIONAL SECTIONS

Where 25 or more members live within commuting distance of a given point, a regional section may be established. Meetings can be held at more frequent intervals by such groups. Presently, there are 41 regional sections.

DIVISIONS

Where 50 or more members of the Institute have a common interest in a particular broad-based discipline of food technology, they may form a division. There are presently eight divisions serving the areas of Carbohydrates, Food Service, Quality Assurance, Refrigerated and Frozen Foods, Sensory Evaluation, Microbiology, Nutrition, and Student.

STUDENT CHAPTERS

An IFT Student Chapter certificate may be granted to a group of students enrolled in the food science and technology curriculum in a particular school who have organized to form a student club. There are 19 student chapters.

AFFILIATE ORGANIZATIONS

Affiliate certificates may be granted to food technology organizations outside the U.S.A. There are currently nine chartered affiliate organizations.

ANNUAL MEETINGS

An Annual Meeting of the Institute provides a specially-organized technical program, awards program, and industrial exhibit of equipment, services, processes and ingredients. The program is designed to emphasize current trends and technological developments. Special guest speakers are invited.

AWARDS

The Institute administers the following awards:

NICHOLAS APPERT AWARD. Purpose of this award (Medal furnished by the Chicago Section, and $1,000) is to honor a person for pre-eminence in and contributions to Food Technology.

BABCOCK-HART AWARD. Purpose of this award ($1,000 and Plaque; sponsored by the Nutrition Foundation) is to honor a person for contributions to Food Technology that have improved public health through some aspects of nutrition or more nutritious food.

IFT INTERNATIONAL AWARD. Purpose of this award (Silver Salver sponsored by Australian Institute of Food Science and Technology, and $1,000) is to recognize an IFT Member for promoting international exchange of ideas in Food Technology.

IFT FOOD TECHNOLOGY INDUSTRIAL ACHIEVEMENT AWARD. Purpose of this award (Plaques to company and individuals) is to recognize and honor the developers of an outstanding new food process and/or product representing a significant advance in the application of Food Technology to food production, successfully applied in actual commercial operation.

WM. V. CRUESS AWARD FOR EXCELLENCE IN TEACHING. Purpose of this award ($1,000 and Medal sponsored by the Northern California Section) is to recognize the teacher's role in Food Technology advances.

SAMUEL CATE PRESCOTT AWARD FOR RESEARCH. Purpose of this award ($1,000 and Plaque; sponsored by IFT) is to recognize a research scientist 35 years of age or younger who has demonstrated outstanding ability in Food Science or Technology research.

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Purpose of IFT-administered Fellowships is to encourage graduate work in the field of Food Science and Technology directed to extending or improving knowledge in some phase of food conservation, food production, or food processing. Available to graduate students.

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- Institute of Food Technologists (donor: IFT)–Five (each $1,000) and plaque
- Alexander E. Katz Memorial (donor: F. Ritter & Co.)–$1,000 and plaque

Purpose of IFT-administered Scholarships is to focus attention on the need for more young people in Food Science and Technology, and to encourage deserving and outstanding students to take undergraduate work leading to a Bachelor's Degree in Food Science, Food Engineering, or Food Technology. Available to Juniors and Seniors who have completed at least one term of study at the institution from which they expect to earn a bachelor's degree.

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- IFT Scholarships–Thirty, each valued at $500 plus a complimentary subscription to *Food Technology* during the tenure of the scholarship.

Purpose of the IFT Scholarships is to attract and encourage worthy students to enter the fields of Food Technology, Food Engineering, or Food Science. Up to 10 available to incoming freshmen; balance to sophomores, juniors, and seniors.

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A

Aberle, E.D., 536
Acott, K.M., 316
Acton, J.C., 421, 571, 1228
Addis, P.B., 313, 354, 355, 995
Adhikari, H.R., 486
Agarwala, O.P., 917
Ahmed, E.M., 230, 356, 362, 1145
Åkesson, C., 386, 682
Alaniz, I., 431
Allen, C.E., 652
Amundson, C.H., 519
Andersen, L.D., 690
Anderson, J.C., 1256
Anderson, L.L., 1205
Ang, C.Y.W., 791
Anglemier, A.F., 59
Antonio, A.A., 915
Appledorf, H., 79
Araujo, P.E., 1255
Arnold, R.G., 129, 450
Ashby, B.H., 254, 258
Ashkenzai, Y., 1247
Ashton, D.H., 185, 764, 1267
Ayres, J.C., 889

B

Babbitt, J.K., 1089, 1101
Badenhop, A.F., 471
Bakal, A., 623
Baker, K.A., 507
Baldwin, R.E., 179, 528
Ball, H.R. Jr., 978
Ballantyne, W.W., 710
Ballinger, W.E., 909
Balmaceda, E., 905, 1169
Bancher, E., 1262
Banwart, G.J., 133
Baraldi, D., 108
Barbour, B.C., 1224
Barnhart, H.M., 1238
Barrera, D., 580
Barylko-Pikielna, N., 524
Bates, R.P., 362, 783, 1145
Baxter, R.I., 932
Beauchat, L.R., 1063
Beavers, H.C., 1087
Beelman, R.B., 951
Behnke, J.R., 275, 539
Bell, T.A., 499, 504
Bembers, M., 1122
Ben-et, G., 546
Ben-Gera, I., 14
Bengtsson, N., 560
Benjamin, N.D., 799
Bennett, M.E., 536
Berkowitz, D., 1093
Berridge, L., 43
Berry, B.W., 1074
Berry, R.E., 340, 1047, 1244
Beuchat, L.R., 531
Biemuller, G.W., 261
Birch, G.G., 665, 1179
Bishov, S.J., 1093
Bissett, O.W., 1047
Black, L.T., 618
Blessin, C.W., 602
Board, R.G., 303
Boggess, T.S. Jr., 531, 1190, 1194
Bomben, J.L., 590
Bookwalter, G.N., 618
Booren, A., 63
Booth, A.N., 496
Borton, R.J., 1087
Bothast, R.J., 75
Bourne, M.C., 84, 720
Bouton, P.E., 404, 816, 932
Bowers, J.A., 553
Bramblett, V.D., 536
Breene, W.M., 99, 210, 334
Breeze, J.E., 965
Breslaw, E.S., 1016
Brewster, L.C., 1153
Briskey, E.J., 185, 764
Brown, A.H., 496
Brown, H.E., 512
Brown, W.D., 813, 971, 1104
Bruemmer, J.H., 225
Brusco, V., 484
Bryan, W.L., 1047
Buelow, F.H., 542
Bullerman, L.B., 129, 1238
Burgess, J.W., 507
Burr, H.K., 87

C

Cahill, V.R., 1087
Campos, J. Jr., 116
Carpenter, J.A., 261
Carpenter, Z.L., , 182, 849, 929, 998, 1074
Carroll, D.E., Jr., 21, 909
Carroll, F.D., 404, 816
Carse, W.A., 1124
Cassens, R.G., 539, 1085, 1128, 1220
Cash, J.N., 807
Catalano, E.A., 338
Cater, C.M., 126, 516, 849
Cavins, J.F., 602
Chang, C.M., 901
Chang, P.K., 239
Chang, S.S., 345, 393, 788
Chang, Y.S., 646
Charles, R.J., 456
Cheftel, C., 11
Chen, T.C., 155, 860
Chen, T-S., 363
Chenchin, E.E., 40
Chew, V., 903
Childs, E.A., 718, 1009
Chipley, J.R., 566
Chiriboga, C.D., 464
Chirife, J., 671, 768
Chism, G., 1022
Chou, H-E., 210, 316
Chu, N.T., 1038
Chudgar, A.S., 354
Chung, J.I., 29
Clary, B.L., 234
Cliplef, R.L., 408
Cloninger, M.R., 528
Clydesdale, F.M., 1038, 1051, 1056
Cobb, B.F. III, 431
Coffelt, R.J., 89
Coffey, D.L., 189
Collins, J.L., 189, 489
Colosie, S.S., 965
Corson, L.M., 1267
Costello, W., 1076
Coulter, S.T., 1182, 1186
Cox, N.A., 679
Crawford, D.L. 575, 1089, 1101, 1197
Cross, H.R., 998
Culotta, J.T., 155, 860
Cumming, D.B., 320
Cuq, J.L., 11
Currie, R.W., 696, 700, 981, 987

D

Dagbjartsson, B., 165, 242
Dagher, S.M., 424
Dahle, L., 484
Dainius, B., 949
Dalvi, R.R., 453
Daly, C., 426
Darby, J., 1257
Daun, H., 1247
Davey, C.L., 1124
Davidson, C.M., 303
Davis, D.W., 210
Davis, K.C., 442
Davis, P.L., 225, 871
Dawson, L.E., 161, 1232
Dayton, A.D., 412
Deatherage, W.L., 602
Deck, R.E., 345
Deethardt, D., 1076
De La Mora, R.A., 580
Del Valle, F.R., 246, 580
De Man, J.M., 320
De Marchena, E.S., 590
Deng, J.C., 299
Deobald, H.J., 338
de Vos, L., 656
Dietrich, W.C., 590
Dill, C.W., 4
Dimick, P.S., 1080
Dincer, B., 489
Doerr, R.C., 1084
Dolev, A., 546
Donovan, W.P., 79
Dooley, G.J., 1096
Dougherty, M.H., 659, 913
Dowdell, M.J., 303
Drake, B., 524
Dravnieks, A., 34, 1024
D'Souza, S.V., 519
Du, C.T., 810
Dubrow, D.L., 1012
Dugan, L.R. Jr., 791
Duong, T.B., 369, 374
Durkee, E.L., 507
Dutra De Oliveira, J.R., 116
Dwivedi, B.K., 450

E

Eagerman, B.A., 1051, 1056
Edwards, V.H., 1070
Eggling, S.B., 215
Eheart, M.S., 202, 954
Ehmann, J.D., 1115, 1119
Eino, M.F., 45, 51
Elkins, E.R. Jr., 595
Elliker, P.R., 426
Elliot, J.I., 1091
Ellis, D.E., 919
Erdman, J.W. Jr., 447
Erickson, J.A., 1158
Erlandson, J.A., 460
Escher, F.E., 889
Eskin, N.A.M., 408
Etchells, J.L., 499, 504
Ettrup Peterson, E., 119
Evancho, G.M., 185, 764, 1267

F

Fair, J.G., 189
Fan, T.Y., 1067
Farkas, D.F., 590
Farrow, R.P., 595
Fellers, D.A., 879
Fennema, O., 275, 539, 1128
Fett, H.M., 1097
Fiddler, W., 714, 1084
Field, R.A., 63
Fields, M.L., 1166
Filppi, J.A., 133
Filsoof, M., 945
Fisher, A.L., 816
Fitzhugh, H.A. Jr., 264
Fleming, H.P., 499, 504
Fleming, S.E., 468
Flink, J., 119, 671, 1174
Flores, S.C., 575
Fluck, R.C., 230
Fox, H.M., 637, 1211
Francis, F.J., 25, 464, 649, 810, 1035, 1038, 1043, 1051, 1056
Frederick, D.P., 215
Free, B., 306
Freeman, L.R., 1093
Friedly, J.C., 826
Froning, G.W., 279
Fry, J.L., 175

G

Garcia, W.J., 602
Garibaldi, J.A., 1241
Gejl-Hansen, F., 1174
Giddings, G.G., 705
Gilbert, S.G., 1247
Gillis, A.T., 408
Goll, D.E., 69
Gomez, R., 1004
Gorinstein, S., 1264
Govindarajan, S., 675
Graham, P.P., 75
Grunden, L.P., 712
Guadagni, D.G., 493
Guilleux, F., 11

H

Haard, N.F., 331, 639, 642, 907, 1022
Hackler, L.R., 471
Hadziyev, D., 772
Hagenmaier, R., 516
Hagyard, C.J., 1124
Haller, R.W., 275
Halvarson, H., 310
Hansen, L.J., 286
Hansen, S.P., 1104
Haq, A., 271, 1224
Hargus, G., 484
Harmon, L.G., 474
Harms, R.H., 175
Harper, W.J., 477, 481
Harrington, R.B., 536
Harris, P.V., 404, 816, 932
Harrison, D.L., 412, 1205
Hartung, T.E., 129, 1238
Hasegawa, S., 1153
Hasling, V.C., 338
Haugh, C.G., 158
Hawrysh, Z.J., 7
Hay, J.D., 696, 700, 981, 987
Hayakawa, K-i., 623
Haydar, M., 772
Heaton, E.K., 531, 1190, 1194
Hegarty, G.R., 398
Hegge, R.I., 903
Heideibauer, R.J., 885
Heidelbaugh, N.D., 129
Heldman, D.R., 282
Henig, Y., 1247
Herberg, L.S., 856
Herring, H.K., 398
Herz, K.O., 393
Hill, R.M., 1122
Hill, T.K., 1194
Hinnergardt, L.C., 831, 834
Hinojosa, J., 580
Hinton, C.F., 175
Hirai, C., 393
Hoeft, R., 362
Hoekstra, W.G., 1085, 1220
Hofferber, L.M., 66
Hofmann, R.C., 871
Hoge, H.J., 841
Holt, C., 92
Homnick, D., 192
Hoover, M.W., 96
Horgan, D.J., 821
Hostetler, R.L., 264
Hou, S-H.F., 447
Hourihan, D., 1255
Howell, A.J., 1224
Huang, F., 1169
Hudson, C.A., 1241
Hudson, J.S., 590
Hudspeth, J.P., 145
Hultin, H.O., 424, 1115, 1119
Humphrey, J., 595

I

IFT Expert Panel on Food Safety & Nutrition, 367, 729
Ijichi, K., 1241
Inglett, G.E., 602
Ioannou, J., 1022
Iwasaki, T., 1162
Ivey, F.J., 271

J

Jackson, H., 1091
Jacobs, R.A., 168
Jadhav, S.J., 453, 1099
Jakobsson, B., 560
James, G.M., 254, 258
Janave, M.T., 1149
Jelen, P., 99, 1182, 1186
Jeon, I.J., 334
Johansson, B., 524
Johnson, F., 279
Johnson, H.C., 437
Johnson, L.A., 21
Johnson, M.G., 1228
Johnston, M.R., 189
Jones, K.A., 177
Josephson, S.A., 69
Juhn, H. 849
Juliano, B.O., 915

K

Kaji, M., 1139
Kaneko, T., 350
Karel, M., 671, 756, 768, 896, 1174
Karmas, E., 735, 736
Kaufman, V.F., 1241
Keeney, P.G., 611, 1158
Keenan, T.W., 43
Keller, J.D., 1200
Kelling, R.E., 499, 504
Kelly, R.F., 75
Kempe, L.L., 168
Kemper, K., 595
Khan, A.W., 56, 710, 1258
Khayat, A., 716
Kielsmeir, E.W., 1220
Kies, C., 637, 1211
Kilbuck, J.H., 949
Kimura, H., 668
King, R.L., 938, 941
Kinsella, J.E., 1200
Kirton, A.H., 1124
Klement, J.T., 1128
Kleyn, D.H., 1016
Klose, A.A., 903
Koburger, J.A., 356
Koehler, P.E., 889
Koizumi, C., 813
Kon, S., 215, 493, 496
Korschgen, B.M., 179
Korslund, M., 637
Kosaric, N., 369, 374
Kosikowski, F.V., 945, 1070, 1162
Kossovitsas, C., 901
Köster, E., 524
Kraft, A.A., 1108
Kramer, A., 14, 254, 258, 1012
Kropf, D.H., 412
Kuhn, G.D., 951
Kumar, M., 877
Kunert, G.F., 1078
Kunsman, J.E. Jr., 63
Kushnir, I., 714
Kuykendall, J.R., 874

L

Labuza, T.P., 177, 316
La Chance, M., 426
Lachance, P.A., 447, 607
Lamb, F.C., 595
Landmann, W.A., 4, 264
Law, D.K., 1089, 1101
Law, S.E., 102
Lee, S-C., 788
Lee, W.T., 1232
Lentz, C.P., 56, 81, 1258
Lenz, M.K., 630
Levin, E., 268, 306
Libbey, L.M., 450
Lillard, D.A., 299
Lillard, H.S., 151, 903
Lima, D.C., 358
Lindley, M.G., 665, 1179
Lindsay, R.C., 528
Lineweaver, H., 1241
Link, B.A., 264
Lisberg, G., 363
Liston, J., 437
Livingston, G.E., 901
Locker, R.H., 1124
Lopez, A., 195
Lorentzen, J., 119
Lorenz, K., 173
Lovell, R.T., 679
Lovett, J., 1094
Low, N., 595
Lowe, E., 507
Lu, J., 837
Luh, B.S., 29, 544
Lumry, R., 744
Lund, D.B., 519, 542, 630
Lycometros, C., 971
Lyon, B.G., 145
Lyon, C.E., 145

M

Ma, R.T-I., 313, 995
Maagdenberg, H.J., 192
Mabee, M.S., 566
MacDonald, B.D., 919
MacFarland, J.J., 294
NacNeil, J.H., 712, 1080
Maga, J.A., 173, 898, 1251
Magaudda, G., 1253
Maier, V.P., 1153
Mamers, H., 856
Mandigo, R.W., 831, 834, 1078
Maness, E.P., 909
Mannheim, C.H., 959
Marion, W.W., 1082
Markakis, P., 550, 705
Martin, F.G., 230
Masai, H., 350
Mast, M.G., 1080
Mattil, K.F., 126, 516, 849, 864
Mayer, J., 1255
McArdle, F.J., 951
McCaskill, L.H., 421
McCoy, B.J., 633
McCrae, S.E., 66
McCready, S.T., 175
McKay, L.L., 796
McPhee, A.D., 1012
Mehran, M., 945
Meinke, W.W., 864
Mendenhall, V.T., 356
Meo, D. III, 826
Mercer, W.A., 192
Mercuri, A.J., 145
Merson, R.L., 633
Middaugh, P.R., 885
Miller, C.F., 493
Miller, D.G., 919
Milone, N.A., 168
Miner, B.E., 690, 1214
Misaki, M., 668
Moerck, K.E., 978
Molina, M.R., 607
Monge, L.E., 633
Monte, W.C., 898
Montgomery, M.W., 59, 799
Møller, A.J., 824
Morey, K.S., 1104
Moritaka, S., 668
Morr, C.V., 324
Morris, H.A., 675
Moshonas, M.G., 360
Mossman, A.P., 879
Munson, S.T., 334

N

Nakamura, R., 1113
Navab, M., 901
Nelson, A.I., 583
Nelson, F.E., 18
Nelson, P.E., 1136
Nesbitt, W.B., 909
Newbold, R.P., 821
Niketić-Aleksić, G.K., 84
Nonaka, J., 813

O

Oblinger, J.L., 1108
Ockerman, H.W., 1087
Odland, D., 202, 954
Ohanneson, J.G., 496
Ohmori, S., 350
Olson, A.C., 215
Olson, D.G., 251, 1214
Onayemi, O., 173
Osman, S., 1096
Ostovar, K., 611, 663

P

Padilla, M., 246
Palnitkar, M.P., 282
Panasiuk, O., 586
Pangborn, R.M., 524
Pantos, C.E., 550
Park, Y.K., 358, 1004
Parrett, N.A., 1087
Parrish, F.C. Jr., 69, 690, 1214
Parvin, D.W., 1190
Passy, N., 959
Paul, P.C., 66
Paulson, R.J., 1082
Pearson, A.M., 1124
Peeler, J.T., 1094
Peleg, M., 959
Penner, K.K., 553
Pensabene, J.W., 714, 1084
Pereira, R.R., 893
Peri, C., 135, 867
Perkins, E.G., 112
Persson, T., 377, 386, 682
Petropakis, H.J., 59
Petrus, D.R., 659, 913
Philip, T., 18, 874, 1032
Pigott, G.M., 917
Pilnik, W., 656
Pilsworth, M.N. Jr., 841
Piotrowski, E.G., 714, 1084
Plitman, M., 1004
Podebradsky, E.V., 1220
Pokorny, J., 345, 393
Pompei, C., 135, 867
Porter, V.L., 583
Powers, J.J., 724
Provansal, M., 11
Purcell, A.E., 548
Purchas, R.W., 556
Purohit, K.S., 726

Q

Quinlan, M.C., 724
Quinn, J.R., 289

R

Raab, C.A., 544
Ralls, J.W., 192
Ramakrishnan, T.V., 25
Ranadive, A.S., 331
Rand, A.G. Jr., 1132
Randall, J.M., 1047
Raymond, J.E., 181
Rea, R.H., 182
Reagan, J.O., 929

Reddy, B.R., 788
Reeves, M.P., 365
Reidy, G.A., 282
Reilich, H.G., 34
Reineccius, G.A., 355, 796
Resmini, P., 867
Restrepo, F., 779
Reynolds, A.E., 261
Rha, C.K., 905, 1169
Rhee, K.C., 126
Richter, G.A., 218
Richter, R.L., 324, 796
Riederer, P., 1262
Riha, W.E., 1
Rockwell, W.C., 879
Rodriguez, P., 246
Roe, B., 225
Rossi, F., 135
Roy, R.B., 896
Russell, G.F., 1028
Rust, R.E., 251
Ruz, A., 246

S

Sabir, M.A., 468
Saffle, R.L., 181, 551, 968
Saio, K., 1139
Salata, E.B.Z.M., 116
Saldana, G., 512
Salunkhe, D.K., 453, 1099
Sanders, E.J., 700, 981
Sandine, W.E., 426
Sanshuck, D.W., 590
Sapers, G.M., 586
Sato, K., 398
Satterlee, L.D., 268, 306, 418, 1122
Schantz, E.J., 764
Scheusner, D.L., 474
Schneider, K.C., 1076
Schoenemann, D.R., 195
Schroder, D.J., 1091
Schweigert, B.S., 544
Sebranek, J.G., 1085, 1220
Setser, C.S., 412
Shaffer, T.A., 1205
Shaw, P.E., 360
Shaw, R.L., 586
Shemer, M., 112
Sheneman, J.M., 206
Shewfelt, A.L., 1190
Shorthose, W.R., 404, 816, 932
Shrikhande, A.J., 649
Shults, G.W., 991
Shrikhande, A.J., 1035
Simon, S., 919
Singh, R.P., 542
Sinnhuber, R.O., 1197
Sinskey, A.J., 1004
Sirrine, K.L., 218
Sison, E.C., 161
Sistrunk, W.A., 807, 1060
Skalski, C., 1060
Skelley, G.C., 1228
Smit, C.J.B., 646
Smith, G.C., 182, 849, 929, 998, 1074
Snell, R.L., 1214
Snyder, H.E., 779
Solberg, M., 1, 165, 242
Somorin, O., 911
Sosulski, F.W., 468
Spata, J., 722
Stadelman, W.J., 158
Stamer, J.R., 84
Stanley, D.W., 45, 51, 320
Starr, M.S., 1043
Steinberg, M.P., 583, 722
Stephens, T.S., 512
Stine, C.M., 7
Stoewsand, G.S., 1256
Strandberg, K., 69
Stumbo, C.R., 726
Surles, T., 1257
Suter, D.A., 234
Suzuki, J.I., 949
Svrcek, W.Y., 369, 374
Sweat, V.E., 158
Sweet, C.W., 1260
Swenson, P.E., 324

T

Talley, F.B., 586
Tannenbaum, S.R., 1067
Tappel, A.L., 486
Tarky, W., 917
Tatarsky, D., 546
Tatum, J.H., 340, 1244
Thomas, P., 1149
Thompson, C.A. Jr., 431
Thompson, E.H., 652
Thompson, R.L., 499, 504
Timbie, D., 642
Toledo, R.T., 141, 924
Tollefson, C.I., 218
Townsend, W.E., 837
Trejo-González, A., 342
Tume, R.K., 821
Tung, M.A., 456
Tuomy, J.M., 831, 834
Tybor, P.T., 4

U

Ukai, N., 29

V

Vacinek, A.A., 924
Vajdi, M., 893
Valaris, M., 477, 481
VanBuren, J., 656
Van Den Berg, L., 81, 1258
Veek, M.E., 1028
Vestergaard, T., 824
Von Sydow, E., 377, 386, 682

W

Wagner, J.R., 215
Waldman, R.C., 919
Walter, W.M. Jr., 548
Wang, C.F., 938, 941
Wang, W.S., 155
Wardlaw, F.B., 421, 1228
Warner, K.A., 618
Washuttl, J., 1262
Wasserman, A.E., 1084, 1096
Watanabe, T., 1139
Watson, C.A., 34, 1024
Webb, N.B., 271, 1224
Wei, L.S., 112, 583, 722
Weissberger, W., 1158
West, R.L., 182
Westerberg, D.O., 919
Wetlaufer, D.B., 740
Whitfield, J., 34
Whitfield, J.K., 271, 1224
Wierbicki, E., 991
Wierzbicki, L.E., 1070
Wild-Altamirano, C., 342
Williams, M.P., 1136
Wilson, J.M., 14
Winder, W.C., 1220
Winter, F.H., 89
Wiskus, K.J., 313
Wismer-Pedersen, J., 824
Wolf, I.D., 652
Wolfe, F.H., 696, 700, 981, 987
Worthington, R.E., 1194
Wright, D.G., 1132
Wrolstad, R.E., 460
Wu, L.C., 783
Wyse, R.E., 453

Y

Yacoub, N.L., 192
Yang, H.Y., 1156
Yoo, Y., 1145
Young, C.T., 123
Young, R.B., 690, 1214
Yamamoto, H.Y., 40
Yu, T.C., 1197

Z

Zachariah, N.Y., 268, 418, 1122
Zak, J.M., 92
Zimmerman, G.L., 779
Zinnecker, M., 192

Journal of Food Science
Subject Index–Volume 38, 1973

A

AAS (atomic absorption spectrophotometry)
examination of bone content in mechanically-deboned poultry meat by, 712
absorption
UV method for evaluating citrus essences, 1047
acceptability
of phosphate-treated and precooked chicken pieces microwave reheated, 161
acceptance, large scale
pilot plant production, with quick-salted fish cakes, 246
acidification
of canned tomatoes: effect on physical and chemical characteristics of heat processed, 195
acidity, titratable
changes in during berry development of Thompson seedless grapes, 874
acids, acetic
biogenic and synthetic: discrimination between with a LSC, 350
acids, ascorbic
content of artificially ripened tomatoes, 550
acids, chlorogenic
continuous diffusion of from sunflower kernels, 468
measurement in apple juice by chromatographic-fluorometric method, 656
acids, fatty
analysis of antelope and beef fat, 63
composition of bovine fat as related to breed and sex, 408
flavor thresholds for, in buffered solutions, 528
formation in Swedish fermented sausage, 310
of chicken bone marrow, 978
acids, hydrocyanic
in canned sweet cherries, 1256
acids, lactic
fermentation: preservation of carrots by, 84
acids, malic
quantitative estimation of, in grape juice, 18
acids, β-nitropropionic
production in foods, 1162
acids, nonvolatile
in strawberries, 807
acids, organic
contents of Scuppernong grapes during ripening, 21
quantitative estimation of in grape juice, 18
production by S. lactis in low-lactose skim milk, 796
acids, tartaric
quantitative estimation of in grape juice, 18
acidulation, chemical
control of S. aureus in sausage by starter cultures and, 426
adsorption
contribution to volatile retention in a freeze-dried food model containing PVP, 768
aerobes, gram negative
properties of, isolated from meats, 303
aflatoxin
effect of roasting on content of artificially contaminated pecans, 889
modified method for determination in spices, 949
tissue distribution and metabolism of B_1-^{14}C in layer chickens, 566
use of gamma irradiation to prevent production of in bread, 1238
aging
influence of on quality and yield of frozen raw beef, 560
vs. palatability of pork loin chops, 536
aging, postmortem
effect on chicken muscle fibrils, 981
effect on chicken muscle lipids, 696
effect on chicken breast muscle sarcoplasmic reticulum, 700
of broiler meat: effect on pH, WHC and extractability of myofibrillar proteins, 421
aglycones
effect of sensory properties of simple glycoside structures; structural functions of taste in the sugar series, 665
air blast
chilled poultry: heat transfer, organoleptic quality changes and moisture exchange in, 924
alkaloids, solanum
biosynthesis and inhibition by chemicals, 453
ammonium bicarbonate
quality of frozen green vegetables blanched in four concentrations of, 954
ammonium compounds
use for chlorophyll retention in frozen green vegetables, 202
amylase, alpha
in sweet potatoes: comparison between amyloclastic and chromogenic starch methods of analysis, 548
modified method for analysis of sweet potatoes, 338
amylglucosidase-resin complex
continuous conversion of starch to glucose, 358
amylolytic degradation, beta
effects on pastes of waxy maize starch, 484
amylose
content and puffed volume of parboiled rice, 915
animal protein concentrate
preparation and properties of low temperature extracted, 141
anthocyanins
factors influencing color degradation in Concord grape juice, 1060
ion exchange purified pigments as colorant for cranberry juice cocktail, 464
of black grapes of 10 clones of Vitis rotundifolia, Michx., 909
of Roselle (Hibiscus sabdariffa, L.), 810
pigments of sour cherries, 649
antioxidants
activity of in fresh fish, 1260
effect on oxidative rancidity in dry-cured hams, 251
APC (animal protein concentrate)
preparation and properties of low temperature extracted, 141
apples
electronic redness meter, 965
apple juice
effect of gamma radiation on D-glucose in, 108
measurement of chlorogenic acid and flavonol glycosides by a chromatographic-fluorometric method, 656
Armour tenderometer
relationship of measurements to objective, subjective and organoleptic properties of bovine muscle, 1214
aroma, canned beef
GC and MS analysis of the volatiles, 377
models for correlation of instrumental and sensory data, 682
sensory properties, 386
arthrobacter globiformis
limonoate dehydrogenase of, to prevent or remove limonin bitterness in citrus juices, 1153
ascorbic acid
content of artificially ripened tomatoes, 550
atomic absorption spectrophotometry
examination of bone content in mechanically-deboned poultry meat by, 712
ATR (attenuated total reflectance)
quantitative determination of fat, protein and carbohydrates of soy products, 14
autolysis
as a factor in production of protein isolates from whole fish, 864
autoxidation
of oxymyoglobins: effect of copper binding on, 1122
or porcine, ovine and bovine myoglobins at freezing temperature: effect of light, pH and buffer strength, 418
avocado
compounds contributing to heat-induced bitter off-flavor in, 546

B

baby foods
Vitamin E content in, 442
Bacillus
effect of DPA on vegetative cells of, 258
bacteria
aerobic, mesophilic, in dehydrated onion products, 206
influence on carbonyl compounds of ground porcine muscle, 75
oxidation-reduction potential and growth of Salmonella and P. fluorescens, 1108
reduction on pork carcasses, 261
bacteriology
bacterial counts and rancidity estimates of stored quick-salted fish cakes, 580
HTST processing of suspensions containing bacterial spores, 168
immobilization of microbial lactases by covalent attachment to porous glass, 1070
bags
oven film: effect of end point and oven temperature on beef roasts cooked in, in open pans, 1205
plastic: use for concentration of dilute solutions in conventional freeze dryers, 1093
bananas
chilling injury in green: changes in peroxidase isozymes in soluble and particulate pools, 642
chilling injury in green: kinetic anomolies of IAA oxidase at chilling temperatures, 907
inhibition of ripening and indole-3-acetic acid oxidase by p-CPIB, 639
physical, rheological and chemical properties during ripening, 456
storage quality of, packaged in selected permeability films, 1247
bean products
cooked, canned and instant powders: vitamin retention in, 493
beans
green snap: storage behavior of artificially waxed, 542
Lima: effect of heat processing on retention of vitamin B_6 in, 544
Phaseolus volgaris: effect of treatments on phytate and soluble sugars in, 215
split peeled: preparation and some properties, 496

Trinidad cacao: isolation and characteristics of microorganisms involved in the fermentation of, 611
beef
adipose tissue: growth of two genera of psychrotrophs on, 1074
aging: influence of antemortem glycolysis and dephosphorylation of high energy phosphates on, 56
boiled: isolation and identification of volatile flavor compounds in, 393
bullock: increasing tenderness by antemortem enzyme injection, 182
canned aroma: GC and MS analysis of the volatiles, 377
canned, aroma: models for correlation of instrumental and sensory data, 682
canned: sensory properties of aroma, 386
catheptic activity, textural properties and surface ultrastructure of postmortem, 45
comparison of protein nutritional value of soybean TVP, methionine-enriched TVP and, for adolescent boys, 637
composition of patties containing defatted corn germ flour, 602
effects of end point and oven temperature on roasts cooked in oven film bags and open pans, 1205
effects of gamma-irradiation on odor intensity and rancidity of fat, 374
effects of varying vegetable protein nitrogen ratio on protein nutritive value for humans, 1211
emulsions: utilization of high protein tissue powder as a binder/extender in, 306
freeze dried, low and intermediate moisture contents: texture stability during storage, 282
freeze drying: theory and experiment, 841
freezing of raw: influence of aging, freezing rate and cooking method on quality and yield, 560
heat-induced changes in extractability of collagen, 66
phospholipid changes and lipid oxidation during cooking and frozen storage of raw ground, 1200
post-slaughter pH variation in, 710
quality changes in prerigor, at $-3°C$, 539
relation between shear force and tenderness of, 1258
skin hydrolyzates: utilization as a binder or extender in sausage emulsions, 268
statistical approach to subjective and objective measurements of odor induced by gamma irradiation of fat, 369
surface ultrastructure and tensile properties of cathepsin and collagenase treated fibers, 51
systematic variation in toughness and some of its implications in, 286
use of UV for extending retail caselife of, 929
beverages
dark colored: comparison of color scales for, 1051
dark colored: development of new transmission color scales for, 1056
qualitative and quantitative study of sugar-alcohols in, 1262
BHA (butyl hydroxyanisole)
stability in water under stresses of sterilization, 898
binders
rheological properties of hydrocolloids, 1169
biochemistry
changes in citrus fruits during controlled-atmosphere storage, 225
parameters of textural change in frozen-stored cooked lobster, 242
volatile N and amino N analysis in shrimp, 431
biosynthesis
of solanum alkaloids by chemicals, 453
biosystems
protein interactions in, 735
protein interactions in: nature of interactions between proteins and liquid water, 744
protein interactions in: protein-lipid interactions, 756
protein interactions in: protein structure and stability, 740
water in, 736
blanching
evaporative cooling of vegetables, 89
hot gas, of spinach: in-plant, continuous, 192
in NH_4HCO_3: effect on quality of frozen green vegetables, 954
levels of DDT isomers in turnip greens after, 189
blood protein concentrate
effect of decolorization and lactose incorporation on the emulsification capacity of spray-dried, 4
blood system
of poultry: contamination with C. perfringens during water scalding, 151
bovine
changes in low molecular weight N compound of excised muscle, 59
effect of altering ultimate pH on tenderness, 816
effect of carcass suspension method on sensory panel scores for, 264
effect of decolorization and lactose incorporation on the emulsification capacity of spray-dried BPC, 4
effect of feeding protected safflower oil supplemented on postmortem changes in, 821
effect of postmortem conditions on certain chemical, morphological and organoleptic properties of, 690
fatty acid composition of intramuscular and subcutaneous fat as related to breed and sex, 408
influence of pH and fiber contraction state upon factors affecting tenderness of, 404
myofibril fragmentation in L. dorsi, as an index of tenderness, 824
myoglobin: conversion into multiple, charge-heterogeneous subfractions, 289
radiant energy-induced changes in pigment, 412
relationship of tenderness measurements made by Armour tenderometer and objective, subjective and organoleptic properties of, 1214
tenderness: effect of intramuscular collagen and elastin on, 998
botulinum toxin
standardized reversed passive hemagglutenation technique for determination of, 764
BPC (blood protein concentrate)
effect of decolorization and lactose incorporation on the emulsification capacity of spray-dried, 4
bread
fresh: application of low dose irradiation of, for space flights, 129
making: functionality of yeast protein dried at two temperatures, 177
use of gamma irradiation to prevent aflatoxin production in, 1232
brine, spent pickle
field tests of salt recovery system for, 507
brining
effect on texture profiles on cucumber varieties, 210
Brix, degrees
changes in during berry development of Thompson seedless grapes, 874
broilers
microbiological comparison of steam- and immersion-scalded, 903
browning
of mango: induced by gamma irradiation; polyphenol oxidase activity, 1149
Browning reaction
fluorescent products in a glucose-glycine, 486
buffer solutions
flavor thresholds for fatty acids in, 528
buffer strength
effect on autoxidation of porcine, ovine and bovine myoglobins at freezing temperatures, 418

C

caffeine
spectrophotometric determination of in Niegerian kola nuts, 911
calcium
in chicken breast muscle: postmortem increase of extractable, 1113
influence on glycolysis, tenderness and shortening of sheep muscle, 1124
calcium chloride
effects of different submergence times in, on peeling efficiency of tomatoes, 512
caloric content
of male turkey breast and thigh, 1082
calorimetry
for measuring heat of respiration of small samples of biological materials, 234
cans
storage stability of dehydrated potato granules packed in, 363
capsaicin
method for determination in capsicum fruits, 342
capsicum fruits
method for determination of capsaicin in, 342
carbohydrate matrices
pea lipids and their oxidation on, 772
carbohydrate solutions
microscopic investigations of the freeze drying of volatile-containing model food solutions, 1174
carbohydrates
quantitative determination of soy products with ATR, 14
carbon dioxide
production in the fermentation of brined cucumbers, 504
carbonyls, aliphatic
analysis of antelope and beef fat, 63
carbonyl compounds
of ground porcine muscle: influence of bacteria on, 75
carboxymethyl cellulose
effect on proteolysis of α_S-casein by immobilized pepsin, 477
effect on proteolysis of α_S-casein by immobilized trypsin, 481
carotenoids
changes in heated paprika, 25
nature of esterification in tangerines, 1032
carrageenan
scientific status summary, 367
carrots
preservation of by lactic acid fermentation, 84
cartons
storage stability of dehydrated potato granules packed in, 363
casein
alpha: effect of CMC on proteolysis of by immobilized pepsin, 477
alpha: effect of CMC on proteolysis of by immobilized trypsin, 481
oxidation of methionine residues of by H_2O_2, 11
catfish, channel
preservation of with selected chemicals, 531
quality comparisons of albino and regular (gray), 1194
techniques for stunning and their effects on product quality, 1190
cathepsins
activity of postmortem beef muscle, 45
effect of curing agents, pH and temperature on the activity of porcine muscle, 299
treated muscle fibers: surface ultrastructure and tensile properties, 51

cells, stone
from pear fruit: chemical nature of, 331
centrifugation
to improve quality of mechanically deboned fowl meat, 279
cheese
pink discoloration in Cheddar, 675
production of β-NPA in, 1162
chemical properties
of bananas during ripening, 456
of bovine muscle: effect of postmortem conditions on, 690
chemistry
preservation of channel catfish with selected chemicals, 531
studies of three processed soybean foods, 112
cherries
anthocyanin pigments of sour, 649
flavonol glycosides of sour, 1035
pit detection by IR radiation scatter, 102
polyphenol oxidase of Royal Ann: purification and characteristics, 799
sweet, canned: hydrocyanic acid in, 1256
chicken
effect of binding on GAP dehydrogenase activity, 424
effect of NaCl and condensed phosphates on WHC, pH and swelling of, 991
effect of postmortem aging on fibrils, 981
effect of postmortem aging on lipids, 696
effect of postmortem aging on sarcoplasmic reticulum, 700
effect of water and microwave energy precooking on microbiological quality, 155
franchise dinners: protein, fat and mineral analyses, 79
fresh and aged: polyacrylamide disc gel electrophoresis of proteins in sodium dodecylsulfate, 987
hot water and microwave energy for precooking: effect on yield and organoleptic quality, 860
lactate dehydrogenase: substrate inhibition of as a function of temperature, 1115
lactate dehydrogenase: temperature dependence of the Michaelis constant, 1119
layer: tussue distribution and metabolism of B_1-^{14}C in, 566
lipids and fatty acids in bone marrow, 978
lipid changes during cooking in fresh and reused cooking oil, 1232
postmortem increase of extractable Ca in breast muscle, 1113
stability and acceptability of phosphate-treated and precooked pieces reheated with microwave energy, 161
thermal conductivity of meat at temperatures from −75 to 20°C, 158
weight of broiler parts as related to carcass weight and type of cut, 145
chilling injury
of citrus fruits: reduction of in cold storage by intermittent warming, 871
of green banana fruit: changes in peroxidase isozymes in soluble and particulate pools, 642
of green banana fruit: kinetic anomolies of IAA oxidase at chilling temperatures, 907
chlorophyll
retention in frozen green vegetables: use of ammonium compounds for, 202
chocolate, dark and milk
survival of S. aureus in, 663
citrus fruits
biochemical changes during controlled atmosphere storage, 225
damaging stresses to fresh and irradiated, 230
reduction of chilling injury in cold storage by intermittent warming, 871
UV absorption method for evaluating essences, 1047
citrus products
limonin bitterness in: prevention or removal using limonoate dehydrogenase of A. globiformis, 1153
Clostridium perfringens
contamination of poultry during water scalding, 151
standardized RPHA technique for determination of toxin, 764
CMC (carboxymethyl cellulose)
cocoa beans
tocopherols in the unsaponifiable fraction of, 1158
coconuts
aqueous processing for recovery of oil and skim milk, 516
coconut flour, defatted
new enzymic-chemical method for fiber-free protein extraction of, 607
coconut meal
studies on utilization of: new enzymic-chemical method for fiber-free protein extraction of defatted flour, 607
cod, true
quantitative changes in whole myofibrils and myofibrillar proteins during frozen storage of, 718
coffee
influence of freeze-drying parameters on retention of flavor in, 119
collagen
beef muscle: heat-induced changes in extractability of, 66
effect of intramuscular on bovine muscle tenderness, 998
collagenase
treated muscle fibers: surface ultrastructure and tensile properties, 51
color
changes in heated paprika, 25
colorimetric characteristics of egg yolk and egg yolk products, 175
comparison of scales for dark colored beverages, 1051
development of new transmission scales for dark colored beverages, 1056
electronic apple redness meter, 965
factors influencing degradation in Concord grape juice, 1060
influence of, on potato chip sensory preferences, 1251
measurement of canned tuna, 716
of cranberry juice cocktail: effect of metallic ions on, 1043
of strawberry puree: effect of metal ions on, 460
red, of fresh meat: oxidative changes in oxymyoglobin during interaction with arginine linoleate, 813
stability of cured hams in accelerated processing, 1078
colorants
ion exchange purified anthyocyanin pigments as, for cranberry juice cocktail, 464
colorimetry
to characterize egg yolk and egg yolk products, 175
compaction
behavior of ground corn, 877
conductivity, thermal
of chicken meta: at temperatures from −75 to 20°C, 158
contamination
by forceps in sterility testing, 1267
controls
feedback: experimental study of optimal, of a freeze dryer, 826
cookies
composition of, containing defatted corn germ flour, 602
cooking method
influence of on quality and yield of frozen raw beef, 560
vs. palatability of pork loin chops, 536
copper
effect of binding on autoxidation of oxymyoglobins, 1122
corn
ground: compaction behavior of, 877
muffins: composition of, containing defatted corn germ flour, 602
odor: classification by statistical analysis of GC patterns of headspace volatiles, 34
odor: classification from low-resolution to GC profiles of headspace volatiles, 1024
sweet: distribution and heat inactivation of peroxidase isoenzymes in, 40
sweet: effect of hybrids and processing on the dimethyl sulfide potential of, 1136
whole kernel: nutritive content of, 595
containers
use of plastic bags for concentration of dilute solutions in conventional freeze dryers, 1093
p-CPIB (p-2,4-dichlorophenoxy-isobutyric acid)
inhibition of ripening and indole-3-acetic acid oxidase of banana fruit by, 639
crab
canned: blueing discoloration in, 1101
dungeness: phenolases and blue discoloration in whole cooked, 1089
crabmeat
sensitivity of V. parahaemolyticus to cold in, 437
cranberries
isolation and identification of some fluorescent phenolic compounds in, 1038
cranberry juice cocktail
effect of metallic ions on color and pigment content, 1043
ion exchange purified anthocyanin pigments as a colorant for, 464
crayfish (Procambarus clarkii, Girard)
identification and characteristics of microflora and spoilage bacteria in fresh water, 679
cream, freeze-dried
absorption of volatile methyl ketones by a natural model system, 7
cucumbers, brined
bloater formation in fermented, by L. plantarum, 499
CO_2 production in the fermentation of, 504
effect of brining on objective texture profiles of, 210
texture: correlation of instrumental and sensory measurements, 344
cultures, starter
control of S. aureus in sausage by, and chemical acidulation, 426
curing
effect of frankfurter ingredients on N-nitrosodimethylamine formation in a model system, 714
curing agents
effect on activity of porcine muscle cathepsins, 299
cyclohexane polyols
as sweet analogues of the sugars, 1179
cystine
loss from alkaline soaking procedure: methionine supplementation of soy milk to correct, 471
cytochrome c
interaction of myoglobin and hemoglobin with molecular oxygen and its lower oxidation states and with, 705

D

DDT
levels of isomers in turnip greens after blanching and thermal processing, 189
decay
of potatoes and onions: effect of RH, temperature and storage on, 81
deformation
use of nenetrometer for testing of foods, 720
dehydration
process for producing pumpkin flakes, 96
dehydrogenase, GAP
effect of binding to muscle particulate fractions on activity, 424
demineralization
of untreated cottage cheese whey by electrodialysis, 519

dephosphorylation
of high energy phosphates on beef aging and tenderness, 56
dieldrin
distribution of in milk fractions, 791
diets
bean-based: manioc flour as a methionine carrier to balance, 116
diffusion, continuous
of chlorogenic acid from sunflower kernels, 468
discoloration
pink, in Cheddar cheese, 675
DPA (dipicolinate)
effect on vegetative cells of Bacillus, 1166
drying
changes in components of summer sausage during, 1228

E

EDTA
examination of bone content in mechanically-deboned poultry meat by, 712
egg
albumen: effect of polyphosphates on functional properties of spray dried, 239
liquid: determining microbial quality using filtrate release test, 133
yolk: colorimetric characterization of, 175
yolk: identification of gangliosides as constituents of, 43
yolk: microbiology of a modified procedure for cooling pasteurized salt, 1241
elastin
effect of intramuscular, on bovine muscle tenderness, 998
electrodialysis
demineralization of untreated cottage cheese whey by, 519
emulsifier
FPC as mayonnaise-type, 179
emulsions, water/tallow
batch dry rendering: an investigation of heat transfer to boiling, 856
enterotoxin
growth and production by S. aureus strains in foods, 474
enzyme(s)
antemortem injection to increase tenderness of bullock beef, 182
catheptic: activity of postmortem beef muscle, 45
direct conversion of lactose to acid by lactose dehydrogenase, 1132
proteolytic: a new source from ginger rhizome, 652
epinephrine
influence upon glycolysis, tenderness and shortening of sheep muscle, 1124
Escherichia coli
on pecans: survival under various storage conditions and disinfection with propylene oxide, 1063
essences
citrus: UV absorption method for evaluating, 1047
ethyl caprylate
flavor detection threshold values for, 724
ethylene oxide
effects on selected spices, 893
evaporation
cooling of blanched vegetables, 89
extrusion
texturized poultry meat: composition and properties of, 571
thermoplastic: fate of water soluble protein during, 320

F

fat
analyses of franchise chicken dinners, 79
carbonyl and fatty acid analyses of antelope, 63
carbonyl and fatty acid analyses of beef, 63
nature of in nondairy imitation milks, 945
quantitative determination of soy products with ATR, 14
fermentation
bloater formation in brined cucumbers by L. plantarum, 499
CO_2 production in, of brined cucumbers, 504
changes in components of summer sausage during, 1228
lactic acid preservation of carrots, 84
fiber contraction
of bovine muscle: influence upon factors affecting tenderness of, 404
film
polymeric: storage qualities of bananas packaged in, 1247
protein-lipid: influence of ingredients upon edible characteristics, 783
firmness
of fermented sausage: association of protein solubility with, 1128
fish
autolysis as a factor in production of protein isolates from whole, 864
canned: blueing discoloration in crabmeat, 1101
fresh: activity of antioxidants in, 1260
in vitro: interaction of formaldehyde with muscle, 1009
protein hydrolysate from waste, 917
sensitivity of V. parahaemolyticus to cold in fillets, 437
fish cakes
quick salted: bacterial counts and rancidity estimates of stored, 580
quick-salted: pilot plant production of and large scale acceptance trials with, 246
flavor
absorption of volatile methyl ketones by a natural model system: freeze-dried cream, 7
and chemical characteristics of conventionally and microwave reheated pork, 553
detecting threshold values for ethyl caprylate and phenyl ethyl alcohol and estimate of % of population having greater sensitivity, 724
isolation and identification of volatile compounds in boiled beef, 393
of coffee: influence of freeze-drying parameters on retention of, 119
potato chip-like: formation of from methionine under deep-fat frying conditions, 788
quality of potato flakes: effect of raw material and processing, 586
thresholds for fatty acids in buffered solutions, 528
warmed over: inhibition of in cooked meats, 398
Flavonoid(s)
glycosides of sour cherries, 1035
flour, corn germ, defatted
composition of cookies, corn muffins and beef patties containing, 602
flow properties
of some food powders, 959
Fluorimetry
determination of vitamin A in foods, 447
foods
canned: effect of DPA on vegetative cells of Bacillus, 1166
canned: sterility testing of heat processed, 185
convenience: inhibition of staphylococcal enterotoxin production in, 885
fluorometric determination of vitamin A in, 447
growth and enterotoxin production by various strains of S. aureus in selected, 474
mercury in: scientific status summary, 729
model solutions: microscopic investigations of the freeze drying of volatile-containing, 1174
models, freeze-dried: studies on mechanisms of retention of volatile in: the system PVP-n-propanol, 671
prepared: chilled-holding vs. frozen storage; effect on quality and wholesomeness of, 901
processing: potential application of polysaccharide 13140 in, 668
qualitative and quantitative study of sugar alcohols in, 1262
ready-to-eat: chicken lipid changes during cooking in fresh and reused cooking oil, 1232
rheological properties of hydrocolloids used as binders in, 1169
use of penetrometer for deformation testing of, 720
water activity determination in range 0.80–0.99, 1097
formaldehyde
interaction with fish muscle in vitro, 1009
FPC (fish protein concentrate)
as an emulsifier, 179
effect of temperature on lipid extraction and functional properties of, 1012
frankfurters
effect of cure ingredients on n-nitrosodimethylamine formation in a model system, 714
efficacy of protein additives as emulsion stabilizers in, 849
packaged: influence of nitrite and nitrate curing ingredients on quality, 919
use of NaAsc or NaEry to inhibit formation of n-nitrosodimethylamine in, 1084
freeze dryer
experimental study of the optimal feedback control of a, 826
freeze drying
contribution of adsorption to volatile retention in a food model containing PVP, 768
influence on retention of flavor compounds of coffee, 119
of beef: theory and experiment, 841
of volatile-containing model food solutions: microscopic investigations of, 1174
use of plastic bags for concentration of dilute solutions in conventional, 1093
freezer burn
of hams in frozen storage: effects of freezing and packaging methods on, 258
freezing
cryogenic: of tomato slices, 362
effects on shrinkage of hams in frozen storage, 254
effects on freezer-burn of hams in frozen storage, 258
of raw beef: influence of aging, freezing rate and cooking method on quality and yield, 560
freezing rate
influence of, on quality and yield of frozen raw beef, 560
frozen foods
green vegetables: quality of blanched in NH_4HCO_3, 954

G

Gangliosides
identification of as constituents of egg yolk, 43
GAP (glyceraldehyde-3-phosphate)
effect of binding to muscle particulate fractions on dehydrogenase activity, 424
gas chromatography (GC)
aroma of canned beef, analysis of volatiles of, 377
classification of corn odor by statistical analysis of GC patterns of headspace volatiles, 34
gas-liquid chromatography (GLC)
rapid analysis of moisture in meat by, 355
ginger rhizome
new source of proteolytic enzyme, 652

glucose
continuous conversion from starch by an amylglucosidase-resin complex, 358
glycine: fluorescent products in browning reaction, 486
D-glucose
in apple juice: effect of gamma radiation on, 108
glucosylation
enzymatic, of solanidine, 1099
glycolysis
antemortem, and dephosphorylation of high energy phosphates on beef aging and tenderness, 56
association of struggle during exsanguination to, in turkey pectoralis muscle, 995
influence of epinephrine and Ca on, of sheep muscle, 1124
glycosides
flavonol: measurement in apple juice by chromatographic-fluorometric method, 656
structures: effect of aglycones on sensory properties of simple; structural functions of taste in the sugar series, 665
grape juice
Concord: factors influencing color degradation in, 1060
quantitative estimation of malic and tartaric acids in, 18
grapefruit juice
method for determining naringin content of, 340
method for estimating limonin content of, 1244
grapes
black: anthocyanins of Vitis rotundifolia, Michx., 909
S. pombe: effect of pH on activity of, 1156
Scuppernong: organic acid and sugar contents of, during ripening, 21
Thompson seedless: changes in titratable acidity, °Brix, pH, potassium content, malate and tartrate during berry development of, 874
gums
CMC and xanthan: rheological properties of syrups containing, 489

H

hams
cured color stability of accelerated pork processing, 1078
dry cured: oxidative rancidity in: effect of low pre-oxidant and antioxidant salt formulations, 251
effect of freezing and packaging methods on freezer burn in frozen storage, 258
effect of freezing and packaging methods on shrinkage in frozen storage, 254
heat processing
changes in components of summer sausage during, 1228
heat transfer
investigation of, to boiling water/tallow emulsions in batch dry rendering, 856
organoleptic quality changes and moisture exchange in air-blast chilled poultry carcasses, 924
hemagglutination, reversed passive
standardized technique for determination of botulinum toxin, 764
hemoglobin
interaction with molecular oxygen and its lower oxidation states and with cytochrome c, 705
quantitative determination of combined, with myoglobin in poultry, 968
histidine
reaction products with autoxidized methyl linoleate, 896
HTST (high temperature-short time)
processing of suspensions containing bacterial spores, 168
hydrocolloids
rheological properties of, 1169
hydrogen peroxide
oxidation of methionine residues of casein by, 11
reaction with myoglobins, 1104
hydrolyzates, beef and pork skin
utilization as a binder or extender in sausage emulsions, 268
hyperfiltration
improved reverse osmosis permeation by heating, 633

I

inhibition, chemical
of solanum alkaloids, 453
instrumental measurements
texture of cucumbers: correlation with sensory measurements, 334
instruments
electronic apple redness meter, 965
ions, metal
effect on color of strawberry puree, 460
IQB (individual quick blanching)
pilot plant evaluation of, for vegetables, 590
IR (infrared)
radiation of cherries for pit detection, 102
iron
assimilation of from iron-fortified milk by baby pigs, 941
chemical and sensory evaluation of iron-fortified milk, 938
irradiation
damaging stresses to fresh citrus fruits, 230
gamma: effect on D-glucose in apple juice, 108
gamma: effect on myoglobin, 971
gamma: effects on odor intensity and rancidity of beef fat, 374
gamma: effects on selected spices, 893
gamma: polyphenol oxidase activity and browning of mango fruits induced by, 1149
gamma: statistical approach to subjective and objective measurements of beef fat odor induced by, 369
gamma: use to prevent aflatoxin production in bread, 1238
low dose: application to a fresh bread system for space flights, 129
of potatoes: recognizing, or nonirradiation by weight loss, 1253
isoenzymes
distribution and heat inactivation of peroxidase in sweet corn, 40
isozymes, peroxidase
changes in, in chilling injury in green banana fruit, 642

J

juices, citrus
method for estimating limonin content of, 1244

K

ketones, methyl
absorption of by a model system: freeze-dried cream, 7
kinetics, thermal destruction
effect of pH on, of patulin in aqueous solution, 1094

L

lactases, microbial
immobilization by covalent attachment to porous glass, 1070
lactate dehydrogenase
chicken muscle: substrate inhibition of as a function of temperature, 1115
chicken muscle: temperature dependence of the Michaelis constant of, 1119
lactic acid
formation in Swedish fermented sausage, 310
Lactobacillus plantarum
bloater formation in brined cucumbers fermented by, 499
lactose
direct enzymatic conversion of to acid, by lactose dehydrogenase, 1132
effect of certain salts and other whey substances on the growth of crystals, 1182
effects of supersaturation and temperature on growth of crystals, 1186
effects on emulsification capacity of spray-dried blood protein concentrate, 4
for texture improvement of fresh-pack dill pickles, 99
lactose dehydrogenase
direct enzymatic conversion of lactose to acid, 1132
light
effect on autoxidation of porcine, ovine and bovine myoglobins at freezing temperature, 418
limonin
method for estimating content of citrus juices, 1244
limonoate dehydrogenase
of A. globiformis for prevention or removal of limonin bitterness in citrus products, 1153
linoleate, arginine
oxidative changes in oxymyoglobin during interaction with, 813
lipid(s)
and fatty acids of chicken bone marrow, 978
effect of postmortem aging on chicken muscle, 696
effect of temperature on, and functional properties of FPC, 1012
oxidation during cooking and frozen storage of raw ground beef, 1200
oxidation: sorption hysteresis and chemical reactivity, 316
pea, and their oxidation on carbohydrate and protein matrices, 772
protein interactions in biosystems, 756
relationship between dieldrin and different classes in milk systems, 791
tocopherols in the unsaponifiable fraction of cocoa, 1158
lipoxygenase, soybean
calcium activation of, 779
lobster (Homarus americanus)
parameters of textural change in frozen-stored cooked, 242
textural changes in precooked resulting from irradiation followed by refrigerated storage, 165
LSC (liquid scintillation counter)
improved method for discrimination between biogenic and synthetic acetic acid with, 350

M

malate
changes in during berry development of Thompaon seedless grapes, 874
mango
polyphenol oxidase activity and browning induced by gamma irradiation, 1149
manioc flour
as a methionine carrier to balance common bean-based diets, 116
mathematics
new computational procedure for determining apparent thermal diffusivity of a solid body approx. with an infinite slab, 623
temperature distribution during heat/hold processing of food, 630

measurement
continuous a-c of sausage emulsion stability, 1224
some aspects of raw meat tenderness: factors affecting change with cooking and a new means of, 556
subjective and objective of odors induced by gamma irradiation of beef fat: statistical approach, 369
meat
aspects of tenderness: factors affecting change with cooking and measurement of raw, 556
changes in components during fermentation, heat processing and drying of a summer sausage, 1228
comminuted: ^{15}N tracer studies of nitrite added to, 1220
cooked: inhibition of warmed-over flavor in, 398
freeze-dried: feasibility of adding, in preparation of fermented dry sausage, 837
influence of nitrite and nitrate curing ingredients on quality of packaged frankfurters, 919
rapid analysis of moisture in by GLC, 355
rapid analysis of moisture in by refractometry, 354
red color: on the interaction of myoglobin and hemoglobin with molecular oxygen and its lower oxidation states and with cytochrome c, 705
UV spectrophotometric determination of protein in, 1087
viability of S. aureus in intermediate moisture, 1004
meat emulsions
use of squid in, 551
utilization of high protein tissue powders as a binder/extender in, 306
meat loaf
effect of postmortem muscle changes on properties of poultry, 421
meat products
partial recovery of nitrite N by the Kjeldahl procedure in, 1085
properties of gram negative aerobes isolated from, 303
UV spectrophotometric determination of protein in, 1087
media, bacteriology
instability of Na nitrite in, 1
merchandising
use of UV for extending retail caselife of beef, 929
mercury
in food: scientific status summary, 729
metallic ions
effect on color and pigment content of cranberry juice cocktail, 1043
methionine
formation of a potato chip-like flavor from, under deep-fat frying conditions, 788
manioc flour as a carrier to balance common bean-based diets, 116
oxidation of casein residues by H_2O_2, 11
supplementation of soybean foods, 112
supplementation of soy milk to correct cystine loss resulting from an alkaline soaking procedure, 471
methods
enzymic-chemical for fiber-free protein extraction of defatted coconut flour, 607
examination of bone content in mechanically deboned poultry meat by EDTA and AAS, 712
for making quick-cooking beans, 496
for measurement of chlorogenic acid and flavonol glycosides in apple juice by combined chromatographic-fluorometric, 656
simple shear press for measuring tenderness of whole soybeans, 722
methyl linoleate
reaction products of histidine with autoxidized, 896
microbiology
comparison of steam- and immersion-scalded broilers, 903
identification and characteristics of microflora and spoilage bacteria in fresh-water crayfish, 679
instability of Na nitrite in chemically defined microbiological medium, 1
of a modified procedure for cooling pasteurized salt yolk, 1241
quality of chicken parts: effect of water and microwave energy precooking on, 155
volatile N and amino N analysis in shrimp, 431
microorganisms
isolation and characteristics of, involved in fermentation of Trinidad's cacao beans, 611
microwave energy
effect of reheating with, on stability and acceptability of phosphate-treated and precooked chicken pieces, 161
effect on microbiological quality of chicken parts, 155
effects on selected spices, 893
finish drying of potato chips, 583
for precooking chicken parts: effect on yield and organoleptic quality, 860
reheating of pork: flavor and ehcmical characteristics of, 553
milk
distribution of dieldrin in fractions, 791
iron-fortified: assimilation of iron from, by baby pigs, 941
iron-fortified: chemical and sensory evaluation of, 938
milk, imitation
nature of fats and fatty components in nondairy, 945
mineral analyses
of franchise chicken dinners, 79
minerals
dependence of fruit-berry wines' stability on content of, 1264
moisture
content of male turkey breast and thigh, 1082
diffusion of in thawed frozen French fried potatoes and its relation to performance during finish frying, 87
exchange in air-blast chilled poultry carcasses, 924
low and intermediate contents: texture stability during storage of freeze-dried beef, 282
rapid analysis of in meat by GLC, 355
rapid analysis of in meat by refractometry, 354
monocarbonyl compounds
neutral, volatile: formation in Swedish fermented sausage, 310
morphological properties
of bovine muscle: effect of postmortem conditions on, 690
MS (mass spectrometry)
aroma of canned beef, analysis of volatiles of, 377
confirmation of a contaminant in n-nitrosodimethylamine by high resolution, 1096
MSG (monosodium glutamate)
ingestion and thirst production, 1255
mushrooms, canned
influence of post-harvest storage and soaking treatments on yield and quality, 951
mutton
comparison of the effects of aging, conditioning and skeletal resistraint on tenderness of, 932
myofibrils
fragmentation in bovine L. dorsi as an index of tenderness, 824
quantitative changes in whole during frozen storage of true cod, 718
myoglobin(s)
bovine: conversion into multiple, charge-heterogeneous subfractions, 289
effects of gamma irradiation on, 971
interaction with molecular oxygen and its lower oxidation states and with cytochrome c, 705
porcine, ovine, bovine: effect of light, pH and buffer strength on autoxidation of, at freezing temperatures, 418
quantitative determination of, combined with hemoglobin in poultry, 968
reaction of H_2O_2 with, 1104
myosin B
molecular properties of postmortem muscle: effect of SH reagents and postmortem storage on changes in, 69

N

naringin
method for determining content in grapefruit juice, 340
nitrate
effect of N nutrition and day temperature on, in tomato fruit, 29
influence on quality of packaged frankfurters, 919
nitrite
influence on quality of packaged frankfurters, 919
natural inhibitors of nitrosation reactions: concept of available, 1067
N: partial recovery by Kjeldahl procedure in meat products, 1085
^{15}N tracer studies of, added to comminuted meat products, 1220
sodium: instability in chemically defined microbiological medium, 1
nitrogen
dependence of fruit-berry wines' stability on content of N-components, 1264
^{15}nitrogen
studies of nitrite added to a comminuted meat product, 1220
nitrogenous compounds
low molecular weight: changes in of excised bovine muscle, 59
n-nitrosamines
confirmation of a contaminant in n-nitrosodimethylamine by high resolution Ms, 1096
nitrosation
natural inhibitors of reactions: concept of available nitrite, 1067
n-nitrosodimethylamine
confirmation of contaminant in by high resolution MS, 1096
effect of frankfurter cure ingredients on formation of, in a model system, 714
use of NaAsc or NaEry to inhibit formation in frankfurters, 1084
β-NPA (nitropropionic acid)
production of in foods, 1162
nutrition
content of canned tomato juice and whole kernel corn, 595
effect of hot air toasting and rolling of whole wheat on quality, 879
human: effect of varying ratio of beef and textured begetable protein nitrogen on protein nutritive value, 1211
iron-fortified syrup blends: preparation, characteristics, application, 618
N: effect on composition, color and nitrate content in tomato fruit, 29
protein: comparison of value of TVP, methionine-enriched TVP and beef for adolescent boys, 637
studies of three processed soybean foods, 112
studies on soybean curd produced by $CaSO_4$ precipitate of soybean milk, 1091
nuts, Nigerian kola
spectrophotometric determination of caffeine in, 911

O

odor
- beef fat: effect of gamma irradiation on intensity of, 374
- beef fat: statistical approach to subjective and objective measurements of, induced by gamma irradiation, 369
- corn: classification by statistical analysis of GC patterns of headspace volatiles, 34
- corn: classification from low-resolution GC profiles of headspace volatiles, 1024

off-flavor, bitter
- in avocado: compounds contributing to head induced, 546

oil
- coconut: aqueous processing of coconuts for recovery of, 516
- pimento leaf: chemical and sensory properties of, 1028
- safflower: effect of feeding on composition and properties of sarcoplasmic reticulum and on postmortem changes in bovine skeletal muscle, 821

onions
- dehydrated: survey of aerobic mesophilic bacteria in, 206
- effect of RH, temperature and storage time on decay and quality of, 81

orange essence
- newly found components including trans-2-pentenal, 360

orange juice
- method for estimating limonin content of, 1244
- spectral characteristics of three varieties of Florida, 659
- spectrophotometric analyses of, 913

orange pulp washes
- spectrophotometric analyses of, 913

organoleptic properties
- of bovine muscle: effect of postmortem conditions on, 690

organoleptic quality
- changes and moisture exchange in air-blast chilled poultry carcasses, 924

osmosis, reverse
- permeation by heating, improved, 633

ox muscle
- prerigor pressurization of: effects on pH, shear value and taste panel assessment, 294

oxidation, lipid
- in model system: as affected by sorption hysteresis, 316

oxygen, molecular
- interaction with myoglobin and hemoglobin, 705

oxymyoglobin(s)
- effect of copper binding on the autoxidation of, 1122
- oxidative changes in during interaction with arginine linoleate, 813

oysters
- sensitivity of V. parahaemolyticus to cold in, 437

P

packaging
- effect on freezer burn of hams in frozen storage, 258
- effect on shelf life of frozen silver salmon steaks, 1197
- effect on shrinkage of hams in frozen storage, 254
- storage quality of bananas packaged in selected permeability films, 1247

palatability
- of pork loin chops: muscle quality, cooking method and aging vs., 536

paprika
- color and carotenoid changes in heated, 25

parameters f_h/U:g
- refinement and extension of for process calculation, 726

patulin
- effect of pH on thermal destruction kinetics in aqueous solution, 1094

peaches
- characteristics of pectins isolated from soft and firm-fleshed varieties, 646

peanut(s)
- aqueous process for pilot plant-scale production of protein concentrate, 126
- influence of drying temperature at harvest on volatiles released during roasting, 123

pears
- chemical nature of stone cells from, 331

pecans
- E. coli on: survival under various storage conditions and disinfection with propylene oxide, 1063
- effect of roasting on aflatoxin content of artificially contaminated, 889

pectins
- characteristics of, isolated from soft and firm-fleshed peach varieties, 646

peeling
- of tomatoes: effect of different submergence times in hot $CaCl_2$, 512

pentenal, trans-2
- newly found orange essence components, 360

pepsin, immobilized
- effect of CMC on proteolysis of alpha$_s$-casein by, 477

peroxidases
- distribution and heat inactivation of isoenzymes in sweet corn, 40

pet foods
- simple determination of phosphorus in, 1257

pH
- changes in during berry development of Thompson seedless grapes, 874
- effect of altering on bovine muscle tenderness, 816
- effect of NaCl and condensed phosphates on, of chicken muscle, 991
- effect of, on activity of porcine muscle cathepsins, 299
- effect of prerigor pressurization of muscle on, 294
- effect on activity of S. pombe, 1156
- effect on autoxidation of porcine, ovine and bovine myoglobin at freezing temperature, 418
- effect on thermal destruction kinetics of patulin in aqueous solution, 1094
- influence upon factors affecting tenderness of bovine muscle, 404
- postslaughter, variation in beef, 710

phenolases
- and blue discoloration in whole cooked dungeness crab, 1089

phenolics
- in cranberries: isolation and identification of some fluorescent compounds, 1038

phenyl ethyl alcohol
- flavor detection threshold values for, 724

phosphates
- effect on WHC, pH and swelling of chicken muscle, 991
- high energy: influence of antemortem glycolysis and dephosphorylation on beef aging and tenderness, 56

phospholipids
- changes during cooking and frozen storage of raw ground beef, 1200

phosphorus
- simple determination of in pet foods, 1257

phosphorylase
- molecular species: in postharvest potato tubers, 1022

physical properties
- of bananas during ripening, 456

phytate
- effect of treatments on, in California small white beans, 215

pickles, dill
- fresh-pack: texture improvement by addition of lactose and sucrose, 99

pigment, bovine muscle
- radiant energy-induced changes in, 412

pimento, leaf oil
- chemical and sensory properties of, 1028

polypeptides
- changes in, of excised bovine muscle, 59

polyphenol oxidase
- activity and browning of mango fruits induced by gamma irradiation, 1149
- of Royal Ann cherries: purification and characteristics, 799

polyphosphates
- effect of on functional properties of spray-dried egg albumen, 239

polysaccharide 13140
- a new thermo-gelable polysaccharide, 668

polyvinylpyrrolidone (PVP)
- contribution of adsorption to volatile retention in a freeze-dried food model containing, 768
- n-propanol: studies on mechanisms of retention of volatile in freeze-dried food models, 671

pork
- effect of curing agents, pH and temperature on activity of muscle cathepsins, 299
- effect of electronic, convection and conventional oven roasting on acceptability of loin roasts, 1076
- flavor and chemical characteristics of conventionally and microwave reheated, 553
- freeze-dried: accelerated processing of, 834
- fresh-frozen: accelerated processing of, 831
- influence of bacteria on carbonyl compounds of muscle, 75
- muscle quality, cooking method and aging vs. palatability of loin chops, 536
- processing, accelerated: cured color stability of hams, 1078
- reduction of bacteria on carcasses, 261

pork emulsions
- utilization of high protein tissue powders as a binder/extender in, 306

pork, skin hydrolyzates
- utilization as a binder or extender in sausage emulsions, 268

potassium content
- changes in during berry development of Thompson seedless grapes, 874

potatoes
- chips: microwave finish drying of, 583
- chips: influence of freshness and color on sensory preferences of, 1251
- chips: isolation and identification of volatile compounds from, 345
- conditioning and disposal of solids from waste water treatment, 218
- dehydrated granules: stability of, packed in cans and cartons, 363
- effect of RH, temperature and storage time on decay and quality of, 81
- flakes: flavor quality and stability; effects of raw material and processing, 586
- French fried: effect of finish-frying conditions on quality, 92
- frozen French fries: diffusion of moisture within the thawed product and its relation to performance during finish frying, 87
- recognizing irradiated and nonirradiated by weight loss, 1253
- tubers: molecular species of phosphorylase in post-harvest, 1022

poultry
- composition and properties of extruded, texturized meat, 571
- contamination of with C. perfringens during water scalding, 151
- deboned: use of chemical compounds and rosemary spice in quality maintenance of, 1080
- effect of postmortem muscle changes on meat loaf properties, 421
- heat transfer, organoleptic quality changes and moisture exchange in air-blast chilled carcasses, 924
- improving quality of mechanically deboned meat by centrifugation, 279

mechanically deboned meat: examination of bone content by EDTA and AAS methods, 712
quality changes in prerigor at −3°C, 275
quantitative determination of combined hemoglobin and myoglobin in, 968
weight of broiler parts as related to carcass weight and type of cut, 145

powders
food: flow properties of, 959
high protein tissue: utilization as a binder/extender in meat emulsions, 306

PPC (peanut protein concentrate)
aqueous process for pilot plant-scale production of, 126

preservation
of carrots by lactic acid fermentation, 84

processing
accelerated: of freeze-dried pork chops, 834
accelerated: of fresh-frozen pork chops, 831
heat: effects on physical and chemical characteristics of acidified canned tomatoes, 195
heat: effect on retention of vitamin B_6 in Lima beans, 544
heat/hold of food: temperature distribution during, 630
HTST: of suspensions containing bacterial spores, 168
microbiological: of a modified procedure for cooling pasteurized salt yolk, 1241
thermal, calcluations: refinement and extension of f_h/U:g parameters, 726

prooxidant
effect on oxidative rancidity in dry-cured hams, 251

propylene oxide
E. coli on pecans: survival under various storage conditions and disinfection with, 1063

proteins
analyses: of franchise chicken dinners, 79
animal concentrate: preparation and properties of low-temperature extracted, 141
association of solubility with physical properties in a fermented sausage, 1128
autolysis as a factor in production of isolates from whole fish, 864
blood concentrate: effect of decolorization and lactose incorporation on emulsification capacity of spray dried, 4
chicken muscle: polyacrylamide disc gel electrophoresis of, in sodium dodecylsulfate, 987
digestive acceptability measured by initial rate of in vitro proteolysis, 173
efficacy of additives as emulsion stabilizers in frankfurters, 849
hydrolysate from fish waste, 917
in vitro digestibility in yogurt at various stages of processing, 1016
interactions in biosystems: introduction, 735
interactions in biosystems: nature of interactions between proteins and liquid water, 744
interactions in biosystems: protein-lipid interactions, 756
interactions in biosystems: protein structure and stability, 740
interactions in biosystems: water, 736
myofibrillar: quantitative changes in during frozen storage of true cod, 718
pea lipids and their oxidation on matrices, 772
peanut concentrate: aqueous process for pilot plant-scale production of, 126
quantitative determination of soy products with ATR, 14
skim milk: process optimization for recovery and purification by ultrafiltration, 135
skim milk: recovery and purification by ultrafiltration; influence of temperature on permeation rate and retention, 867
soy, water soluble: fate during thermoplastic extrusion, 320
soybean: food use of 7S and 11S, 1139
textured vegetable: comparison of nutritional value of soybean, methionine-enriched TVP and beef for adolescent boys, 637
UV spectrophotometric determination in meat and meat products, 1087
varying beef: vegetable ratio: effect on human nutrition, 1211
whey concentrate: functional characteristics of, 324
yeast: functionality in bread making, 177

proteolysis, in vitro
digestive acceptability of proteins as measured by the initial rate of, 173

Pseudomonas fluorescens
oxidation-reduction potential and growth, 1108

psychrotrophs
growth of two genera on beef adipose tissue, 1074

public health
survey of aerobic mesophilic bacteria in dehydrated onion products, 206

pumpkin flakes
dehydrated: process for producing, 97

Q

quality
prerigor beef muscle at −3°C, 539
changes in prerigor poultry at −3°C, 275
effect of polyphosphates in improving acceptability of reheated chicken pieces, 161
in storage of bananas packed in selected permeability films, 1247
maintenance of deboned poultry meat using chemical compounds and rosemary spice, 1080
microbiological, of chicken: effect of water and microwave energy precooking on, 155
microbiological, of liquid eggs: determination using a filtrate release test, 133
muscle vs. palatability of pork loin chops, 536
of channel catfish: comparison of albino and regular (gray), 1194
of French fried potatoes: effect of finish-frying conditions on, 92
of frozen catfish: effect of stunning technique on, 1190
of mechanically deboned fowl: improving by centrifugation, 279
of prepared foods: comparison of chilled-holding vs. frozen storage on, 901
of potatoes and onions: effect of RH, temperature and storage on, 81
organoleptic: hot water and microwave energy for precooking chicken parts, effects on, 860
organoleptic, physical and nutritional: effect of hot air toasting and rolling whole wheat on, 879
postmortem changes in iced Pacific shrimp (Pandalus jordani), 575

quality control
modified method for analysis of sweet potato alpha-amylase, 338

R

rabbit muscle
molecular properties: effect of SH reagents and postmortem storage on changes in myosin B, 69

radiation
IR: scatter for pit detection in cherries, 102

radurization
of precooked lobster meat: textural changes in, 165

rancidity
estimates of in stored quick-salted fish cakes, 580
of beef fat: effect of gamma irradiation on, 374
oxidative, in dry-cured hams: effect of low prooxidant and antioxidant salt formulations, 251

rendering, batch dry
investigation of heat transfer to boiling water/tallow emulsions, 856

refractometry
rapid analysis of moisture in meat by, 354

relative humidity
effect on decay and quality of potatoes and onions, 81

respiration
calorimeter for measuring heat of, in biological materials, 234

reticulum, sarcoplasmic
effect of feeding protected safflower oil supplement on composition and properties of, 821
effect of postmortem aging on chicken breast muscle, 700

rheological properties
of bananas during ripening, 456
of syrups containing gums (CMC and xanthan), 489

rice
amylose content and puffed volume of parboiled, 915
consistency of aqueous soybean mixtures, 1145

ripening
inhibition of, in banana fruit by p-CPIB, 639

roasting
effect of electronic, convection and conventional oven on acceptability of pork loin roasts, 1076
effect on aflatoxin content of artificially contaminated pecans, 889

Roselle (Hibiscus sabdariffa, L.)
anthocyanins of, 810

rosemary spice
use in quality maintenance of deboned poultry meat, 1080

RPHA (reversed passive hemagglutination)

S

salmon
effect of packaging on shelf life of frozen silver, 1197

salmonella
oxidation-reduction potential and growth of, 1108
reduction of on pork carcasses, 261

salts
effects on growth of lactose crystals, 1186
prooxidant and antioxidant: effect on oxidative rancidity in dry-cured hams, 251
recovery from spent pickle brine: field tests, 507

sausage
control of S. aureus by starter cultures and chemical acidulation, 426
development and use of laboratory stuffing apparatus, 181
emulsions: utilization of beef and pork skin hydrolyzates as a binder or extender, 268
fermented: association of protein solubility with physical properties in, 1128
fermented, dry: feasibility of adding freeze-dried meat in preparation of, 837
measurement of emulsion stability by electrical resistance, 1224
summer: changes in meat components during fermentation, heat processing and drying of, 1228
Swedish fermented: formation of lactic acid, volatile fatty acids and neutral, volatile monocarbonyl compounds, 310
type emulsions: effect of composition on stability, 271

scalding, water
of poultry: contamination with C. perfringens during, 151

Schizosaccharomyces pombe
effect of pH on activity of, 1156
sensory analyses
effect of carcass suspension method on, for some major bovine muscles, 264
sensory data
aroma of canned beef: models for correlation of instrumental and, 682
sensory evaluation
of iron-fortified milk, 938
sensory measurements
texture of cucumbers: correlation with instrumental measurements, 334
sensory preferences
influence of freshness and color on potato chip, 1251
sensory properties
aroma of canned beef, 386
of pimento leaf oil, 1028
SH (sulfhydryl) groups
effect of post mortem storage on changes in myosin B, 69
shear force
relation between and tenderness of beef, 1258
shear value
effect of prerigor pressurization of muscle on, 294
sheep muscle
influence of epinephrine and Ca upon glycolysis, tenderness and shortening of, 1124
prerigor pressurization of: effects on pH, shear value and taste panel assessment, 294
shelf life
of frozen silver salmon steaks: effect of packaging on, 1197
shrimp
biochemistry and microbial studies: volatile N and amino N analyses, 431
cocktail: modification of texture, 356
Pandalus jordani: postmortem quality changes in iced Pacific, 575
shrinkage
of hams in frozen storage: effects of freezing and packaging methods on, 254
skim milk
acid production by S. lactis in low-lactose, 796
coconut: aqueous processing of coconuts for recovery of oil and, 516
protein recovery and purification by ultrafiltration: influence of temperature on permeation rate and retention, 867
soaking treatments
influence on yield and quality of canned mushrooms, 951
sodium ascorbate
to inhibit formation of n-nitrosodimethylamine in frankfurters, 1084
sodium chloride
difference taste thresholds for among young adults: interlab study, 524
effect on WHC, pH and swelling of chicken muscle, 991
sodium erythorbate
to inhibit formation of n-nitrosodimethylamine in frankfurters, 1084
solanidine
enzymatic glucosylation of, 1099
soy milk
methionine supplementation to correct cystine loss from alkaline soaking procedure, 471
soy products
quantitative determination of fat, protein and carbohydrates of, with ATR, 14
soybeans
consistency of aqueous rice mixtures, 1145
fate of water soluble protein during thermoplastic extrusion, 320
food use of 7S and 11S proteins, 1139
nutritional and chemical studies of processed foods from, 112
nutritional studies of curd produced by $CaSO_4$ precipitate of soybean milk, 1091
whole: simple shear press for measuring tenderness, 722
soybean lipoxygenase
calcium activation, 779
space technology
application of low dose irradiation to a fresh bread system for space flights, 129
spectrometry
aroma of canned beef: analysis of volatiles with MS, 377
confirmation of a contaminant in n-nitrosodimethylamine by high-resolution mass, 1096
spectral characteristics of three varieties of Florida orange juice, 659
spectrophotometry
analyses of orange juices and orange pulp washes, 913
atmoic absorbance: examination of bone content in mechanically-deboned poultry meat, 712
determination of caffeine in Nigerian kola nuts, 911
UV determination of protein in meat and meat products, 1087
spices
comparative effects of ethylene oxide, gamma irradiation and microwave treatments on selected, 893
modified method for aflatoxin determination in, 949
spinach
in-plant, continuous hot-gas blanching of, 192
spoilage
identification and characteristics of microflora and bacteria in freshwater crayfish, 679
spray drying
of egg albumen: effect of polyphosphates on functional properties of, 239
squid
use in meat emulsions, 551
stability
of phosphate-treated and precooked chicken pieces microwave reheated, 161
of potato flakes: effects of raw materials and processing, 586
of sausage-type emulsions: effect of composition on, 271
stabilizers, emulsion
efficacy of protein additives as, in frankfurters, 849
staphylococcal enterotoxin
inhibition of products in convenience foods, 885
Staphylococcus aureus
growth and enterotoxin production in foods, 474
in sausage: control by starter cultures and chemical acidulation, 426
survival in dark and milk chocolate, 663
viability of in intermediate moisture meats, 1004
starch
continuous conversion to glucose by an amylglucosidase-resin complex, 358
waxy maize: some effects of beta amylolytic degradation of pastes of, 484
statistical analysis
aroma of canned beef: models for correlation of instrumental and sensory data, 682
classification of corn odor of GC patterns of headspace volatiles, 34
subjective and objective measurements of odor induced by gamma irradiation of beef fat, 369
sterilization
stability of BHA in water under stresses of, 898
storage
behavior of artificially waxed green snap beans, 542
biochemical changes in citrus fruits during controlled-atmosphere, 225
effect on decay and quality of potatoes and onions, 81
frozen: effects of freezing and packaging methods on shrinkage of hams in, 254
frozen: effects of freezing and packaging methods on freezer burn of hams in, 258
frozen, of true cod: quantitative changes in whole myofibrils and myofibrillar proteins during, 718
post-harvest: influence on yield and quality of canned mushrooms, 951
postmortem: effect of SH reagents on changes in myosin B, 69
quality of bananas packaged in selected permeability films, 1247
refrigerated: textural changes in precooked lobster resulting from radurization, 165
stability of dehydrated potato granules packed in cans and cartons, 363
texture stability of freeze-dried beef at low and intermediate moisture contents, 282
strawberries
nonvolatile acids of, 807
strawberry puree
effect of metal ions on color of, 460
Streptococcus lactis
acid production by, in low-lactose skim milk, 796
structure
of postmortem beef muscle, 45
surface and tensile properties of cathepsin and collagenase treated muscle fibers, 51
sucrose
for texture improvement of fresh-pack dill pickles, 99
sugar
contents of Scuppernong grapes during ripening, 21
soluble: effect of treatments on in Calif. small white beans, 215
structural functions of taste: effects of aglycones on the sensory properties of simple glycoside structures, 665
sugar-alcohols
qualitative and quantitative study of in several foods, 1262
sugar series
structural functions of taste: cyclohexane polyols as sweet analogues of, 1179
sulfide, dimethyl
effect of hybrids and processing on potential of sweet corn, 1136
sunflower kernels
continuous diffusion of chlorogenic acid from, 468
supersaturation
effect of on growth of lactose crystals, 1182
suspension methods
for major bovine muscles: effect on sensory panel scores, 264
sweet potatoes
alpha-amylase in: comparison between amyloclastic and chromogenic starch methods of analysis, 548
alpha-amylase: modified method for analysis of, 338
syrup
rheological properties of, containing gums, 489
syrup blends
iron fortified: preparation, characteristics and application, 618

T

tangerines
nature of carotenoid esterification in, 1032
tartrate
changes in during berry development of Thompson seedless grapes, 874
taste
panel assessment of: effect of prerigor pressurization of muscle on, 294
structural functions of, in the sugar series: chclohexand polyols as sweet analogues of the sugars, 1179
structural functions of, in the sugar series: effect of aglycones on the sensory properties of simple glycoside structures, 665

thresholds for NaCl among young adults: interlab study, 524
temperature
chilling injury of green banana fruits: kinetic anomolies of IAA oxidase at, 907
dependence of the Michaelis constant of chicken breast muscle lactate dehydrogenase, 1119
distribution during heat/hold processing of food, 630
effect on activity of porcine muscle cathepsins, 299
effect on beef roasts oven cooked in oven film bags and open pans, 1205
effect of day, on composition, color and nitrate in tomato fruit, 29
effect on decay and quality of potatoes and onions, 81
effect on growth of lactose crystals, 1182
effect on lipid extraction and functional properties of FPC, 1012
freezing: effect of light, pH and buffer strength on the autoxidation of porcine, ovine and bovine myoglobins, 418
influence of drying at harvest on volatiles released during roasting of peanuts, 123
influence on permeation rate and retention in skim milk protein recovery and purification by ultrafiltration, 867
low: preparation and properties of extracted APC, 141
substrate inhibition of chicken muscle lactate dehydrogenase as a function of, 1115
thermal conductivity of chicken meat from −75° to 20°C, 158
tenderness
beef: influence of antemortem glycolysis and dephosphorylation of high energy phosphates, 56
beef: relation between shear force and, 1258
bovine muscle: effect of altering ultimate pH, 816
bovine muscle: effect of intramuscular collagen and elastin, 998
bovine muscle: influence of pH and fiber contraction state upon factors affecting, 404
bovine muscle: measurements by Armour tenderometer; relationship to objective, subjective and organoleptic properties of, 1214
bovine muscle: myofibril fragmentation in L. dorsi as an index of, 824
bullock beef: increasing by enzyme injection, 182
chicken breast muscle: postmortem increase of extractable Ca, 1113
mutton: comparison of the effects of aging, conditioning and skeletal restraint on, 932
raw meat: factors affecting change with cooking and measurement of, 556
sheep muscle: influence of epinephrine and Ca on, 1124
soybeans: simple shear press for measuring, 722
tests/testing
alpha-amylase in sweet potatoes: comparison between the amyloclastic and chromagenic starch methods of analysis, 548
examination of frozen vegetables by two sample preparation procedures, 365
filtrate release: to determine microbial quality of liquid eggs, 133
for determination of capsaicin in capsicum fruits, 342
for discrimination between biogenic and synthetic acetic acid with a LSC, 350
method for determining naringin content in grapefruit juice, 340
microdiffusion procedure improvement: for TVN and TMN analysis in shrimp, 431
modified method for aflatoxin determination in spices, 949
rapid analysis of moisture in meat by GLC, 355
rapid analysis of moisture in meat by refractometry, 354
simple determination of phosphorus in pet foods, 1257
sterility: forceps as a possible source of contamination in, 1267
sterility: of heat processed canned foods, 185
use of penetrometer for deformation, of foods, 720
texture
changes in precooked lobster resulting from radurization followed by refrigerated storage, 165
modification of cocktail shrimp, 356
of cucumbers: correlation of instrumental and sensory measurements, 334
of fresh-pack dill pickles: improvement by addition of lactose and sucrose, 99
of lobster tail muscle: parameters of change in frozen-stored cooked, 242
properties of postmortem beef muscle, 45
stability during storage of freeze-dried beef at low and intermediate moisture content, 282
texture profiles
of cucumber varieties: effect of brining on, 210
thermal diffusivity
of a solid body: new computational procedure for determination, approximated with an infinite slab, 623
thiamine
some minor volatile components from thermally degraded, 450
tocopherols
in unsaponifiable fraction of cocoa lipids, 1158
tomato juice, canned
nutritive content of, 595
tomatoes
ascorbic acid content of artificially ripened, 550
canned: heat processing effects on physical and chemical characteristics of acidified, 195
cryogenic freezing of slices, 362
effects of different submergence times in hot $CaCl_2$ on peeling efficiency, 512
effects of N nutrition and day temperature on composition, color and nitrate in, 29
toughness
of beef LD: systematic variations in and its implications, 286
trypsin, immobilized
effect of CMC on proteolysis of alpha$_S$-casein, 481
tuna
canned: color measurement of, 716
greening: reaction of H_2O_2 with myoglobins, 1104
turkey
male breast and thigh moisture and caloric content, 1082
pectoralis muscle: association of struggle during exsanguination to glycolysis, protein solubility and shear in, 995
postmortem changes in dark muscle, 313
turnip greens
levels of DDT isomers after blanching and thermal processing of, 189
TVP (textured vegetable protein)

U

ultrafiltration
process optimization in skim milk protein recovery and purification by, 135
skim milk protein recovery and purification by: influence of temperature on permeation rate and retention, 867
ultraviolet light
for extending retail caselife of beef, 929
UV (ultraviolet) energy
radiant energy-induced changes in bovine muscle pigment, 412

V

vegetables
blanched: evaporative cooling of, 89
frozen: examination by two sample preparation procedures, 365
green, frozen: quality of, blanched in NH_4HCO_3, 954
green: use of ammonium compounds for chlorophyll retention in frozen, 202
pilot plant evaluation of IQB for, 590
Vibrio parahaemolyticus
sensitivity to cold in oysters, fish fillets and crabmeat, 437
vitamin(s)
retention in bean products: cooked, canned and instant bean powders, 493
vitamin A
fluorometric determination of, in foods, 447
vitamin B_6
effect of heat processing on retention of in Lima beans, 544
vitamin E
content in baby foods, 442
volatile components
from thermally degraded thiamine, 450
of potato chips: isolation and identification of, 345

W

waste disposal
conditioning and disposal of solids from potato waste-water treatment, 218
protein hydrolysate from fish waste, 917
water
in biosystems, 736
liquid: nature of the interactions between proteins and, in biosystems, 744
precooking: effect on microbiological quality of chicken parts, 155
water activity
in foods: determination in range 0.80–0.99, 1097
WHC (water-holding capacity)
effect of NaCl and condensed phosphates on, of chicken muscle, 991
wheat, whole
hot air toasting and rolling: effect on organoleptic, physical and nutritional quality, 879
whey
cottage cheese: demineralization of untreated, by electrodialysis, 519
effect of substances on growth of lactose crystals, 1186
protein concentrate: functional characteristics of, 324
wines
berry: dependence of stability on content of mineral and nitrogenous components, 1264
grape: effect of pH on activity of S. pombe, 1156

Y

yeast
functionality of dried, in bread making, 177
yogurt
in vitro digestibility of protein at various stages of processing, 1016

Z

zein
rate of coagulation of, 905