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Letters TO THE SCIENTIFIC EDITOR

Letter to the Editor

February 13, 1974

Dear Sir:

The recent publication – "Monosodium glutamate ingestion and thirst production" by P.E. Araujo, D. Hourihan and J. Mayer [*J. Food Sci.* 38(7): 1255] – yields interesting information, in that glutamate was found to be no different from sodium chloride in increasing water consumption by the rat.

Regrettably, the levels of test compounds administered were far in excess of that used in food technology or by the consumer. For example, in Experiment 1 pretreatment was with test solutions of 0.15–1.2M concentration. Glutamate, commonly used in foods in concentrations of 0.1–0.5% (as consumed basis) is therefore ingested at molar concentrations of only 0.005–0.027M. On a body weight basis, the doses studied ranged from 0.561–3.74g/kg or 467–3733% of the Acceptable Daily Intake established by the FAO/WHO (1973). That the doses studied were high is illustrated by the similarity to the oral emetic dose of glutamate (0.5–1.0g/kg) in dogs (Unna and Howe, 1945).

The 0.17 molar solution used in the ad lib experiments is equivalent to a 3.18% solution or five times greater than the maximum application levels of glutamate. Humans would find all the concentrations used (2.8–22.4%) to be organoleptically unacceptable.

Similarly, the concentrations of NaCl equivalent to the doses given (0.15–1.2M) are 0.87–7.0%. Only the lower concentration (0.15M) approaches the NaCl content of physiological saline solution or typical use levels of table salt.

One wonders if completion of similar experiments using concentrations of MSG and NaCl common to use in foods by humans would yield similar results. We know of no information in the extensive literature on glutamate where water intake was measured in any species following ingestion of glutamate at rates and routes common to its use by man.

A.G. EBERT, Chairman
International Glutamate Technical Committee
85 Walnut Street
Watertown, MA 02172

REFERENCES

FAO/WHO. 1973. List of additives evaluated for their safety — In use in food. CAC/FAL 1-1973 FAO/WHO Rome.
Unna, K. and Howe, E.E. 1945. Toxic effects of glutamic and aspartic acids. Fed. Proc. 4: 138.

Letter to the Editor

April 24, 1974

Dear Sir:

Regarding the recent publication "Monosodium glutamate ingestion and thirst production" by P.E. Araujo, D. Hourihan and J. Mayer [*J. Food Sci.* 38(7): 1255], Dr. Ebert appears to be in agreement with our findings.

We state that this investigation was attempted after several informal reports to us that eating meals containing MSG causes severe thirst. The original hypothesis was that the low levels of MSG common in food preparation could produce such a response only if MSG had a peculiar effect on the thirst mechanism. Failure to elicit a response that could be distinguished from that of NaCl at any of the concentrations tested led to the suggestion that MSG would have its reported effect only at high concentrations. I cannot understand from Dr. Ebert's calculations how he would conclude that MSG would cause unpleasant sensations at low concentrations. We, of course, cannot make any claim for the organoleptic sensibilities of the rats. We had no trouble with emesis during the experiments with delivery by intubation, nor did the rats fail to drink the 0.17M MSG solution.

P.E. ARAUJO
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Institute of Food & Agricultural Sciences
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Gainesville, FL 32611

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ABSTRACTS:
IN THIS ISSUE

EFFECT OF THE FAT CONTENT OF GROUND BEEF ON THE HEAT INACTIVATION OF POLIOVIRUS. J.A. FILPPI & G.J. BANWART. *J. Food Sci.* 39, 865–868 (1974)–An indicator virus, polio type 1, was inoculated into ground beef containing 3, 27 and 47% fat, and heated at 50, 60, 70 and 80°C for different time intervals to determine the thermal resistance of the virus. The resistance of the virus to inactivation at the various temperatures tested was found to increase as the fat content of the meat samples increased. The results of this investigation provide evidence that poliovirus in ground beef containing fat levels approaching 30% are resistant to levels of heating commonly employed during cooking in the home or restaurant.

CHANGES IN THE MECHANICAL PROPERTIES OF VEAL MUSCLES PRODUCED BY MYOFIBRILLAR CONTRACTION STATE, COOKING TEMPERATURE AND COOKING TIME. P.E. BOUTON, P.V. HARRIS, W.R. SHORTHOSE & D. RATCLIFF. *J. Food Sci.* 39, 869–875 (1974)–Adhesion values of muscle samples, from 1-month and 6–8-month old calves, cooked at 60°C were significantly lower than for samples cooked at 50°C regardless of myofibrillar contraction state. Shear force values were positively and linearly related to sarcomere length for samples cooked at 50°C whereas at 60°C they were negatively and exponentially related to sarcomere length for values $> 1.2–1.3 \mu m$. Shear force values obtained for muscle samples, from the older calves, which were cooked at 90°C for 1 and 3 hr, showed that even prolonged heating of highly contracted muscles did not overcome the deleterious effects of cold shortening.

LIPID AUTOXIDATION IN MECHANICALLY DEBONED CHICKEN MEAT. K.E. MOERCK & H.R. BALL JR. *J. Food Sci.* 39, 876–879 (1974)–Lipid autoxidation was determined in mechanically deboned chicken meat over a 15-day storage period at 4°C by thiobarbituric acid (TBA) reaction and fatty acid analyses. Hexaenoic, pentaenoic, tetraenoic and trienoic fatty acids of the phospholipid fraction were the major substrates of autoxidative deterioration. Major oxidative changes occurred after 6 days of storage. No apparent oxidation occurred in the less unsaturated triglycerides even though this fraction comprised 92.6% of the total lipids. Minimal autoxidation occurred when a mixture containing 20% butylated hydroxyanisole, 6% propyl gallate and 4% citric acid in propylene glycol (Tenox II) was added at 0.01% by weight of fat present. Addition of 1% Aureomycin (chlortetracycline) decreased total bacterial counts and increased TBA values. There was, however, no acceleration in fatty acid oxidation, suggesting that the microorganisms present removed malonaldehyde and possibly other dicarbonyl compounds formed during autoxidation.

FISH PROTEIN CONCENTRATE FOAM. R.E. BALDWIN & S. SINTHAVALAI. *J. Food Sci.* 39, 880–882 (1974)–Ten percent fish protein concentrate (FPC) foams had greater specific volume than foams from lower or higher concentrations. Regardless of concentration, 20 or 24 min of whipping resulted in more stable foams with greater specific volume than those whipped 16 min. Adjusting pH to 8 or 10 before whipping favored stability in foams. Solubilization (adjusting to pH 12 and heating) of 10% FPC followed by adjusting to pH 6 before whipping improved both volume and stability of foams and reduced the optimum whipping time to 4 min, but none of the foams was stable to heat.

EVALUATION OF FISH PROTEIN CONCENTRATE AS A REPLACEMENT FOR DRY SKIM MILK IN LAUBINA WEANING FOOD MIXTURES. A. MARINOU, D.Y.C.L. CO & G.E. LIVINGSTON. *J. Food Sci.* 39, 883–886 (1974)–Fish protein concentrate (FPC) was evaluated nutritionally and organoleptically as a replacement for the dry skim milk in Laubina mixtures which are blended foods containing parboiled wheat and chick-peas (Laubina 104) or lentils (Laubina 106) as the legume, respectively. On the basis of amino acid analysis, the limiting essential amino acid in milk-free Laubina was found to be methionine. Amounts of FPC required to be added to the milk-free Laubina mixtures to raise their methionine level to that of hen's eggs were determined to be 14.05% for Laubina 106, and 2.91% for Laubina 104. The Protein Efficiency Ratio (PER) for Laubina 104 with fish protein was 2.27, compared to 2.42 for Laubina 104 with milk. PER for Laubina 106 with fish protein was 2.53 compared to 2.15 for Laubina 106 with milk and 2.50 for casein. Organoleptically, Laubina mixtures containing fish protein or milk protein showed no statistically significant differences to be present when they were used in preparing soups or cookies; but when mixtures were made up into semisolid infant food, difference was detected at the 5% level of significance only in Laubina 106.

ELEMENTAL ANALYSIS OF PROTEIN-CONTAINING FOOD MATERIALS FROM VARIOUS SOURCES. A.K. FURR, F.V. KOSIKOWSKI, C.A. BACHE & D.J. LISK. *J. Food Sci.* 39, 887–891 (1974)–12 samples of protein and other food constituents derived from marine fish, seaweed, algae, petroleum gas oil, waste sulfite liquor, fermented sheeps milk, fermented rice, soybeans and dog food were analyzed for 64 elements mainly by neutron activation analysis. Of most concern were concentrations of 9.4 ppm of mercury in swordfish protein, up to 1210 ppm of zinc in yeast protein food material produced from petroleum and elevated levels of cadmium and lead in several other samples. It is suspected that the zinc in petroleum yeast protein could possibly derive from the use of zinc oxide in the desulfuration of gas oil prior to protein production or from contact with metal processing equipment. Elevated levels of arsenic, barium, bromine, cadmium, cobalt, copper, iodine, lead, selenium, silver, strontium and tin were present in various of the marine foods.

SOME FACTORS INFLUENCING GELATION AND STABILITY OF SOY PROTEIN DISPERSIONS. J.N. EHNINGER & D.E. PRATT. *J. Food Sci.* 39, 892–896 (1974)–Factors which influence gelation and stability of soy protein dispersions were investigated. The viscosity tended to increase exponentially as the concentration increased. The exponential change in viscosity was pH dependent with little thickening occurring below pH 5.5. At pH 6.5 and 6.0, smooth consistent gels were obtained. Stability of the dispersions increased as the viscosity increased. The addition of 5% sucrose tended to slightly increase viscosity of SSP dispersions. However, dextrose increased viscosity of SSP dispersions when 10% was used. The influence of sucrose and dextrose on viscosity of dispersions was markedly affected by pH. The higher level of NaCl (0.2M) decreased the viscosity and stability of both isolates.

LEGUME POWDERS: PREPARATION AND SOME NUTRITIONAL AND PHYSICOCHEMICAL PROPERTIES. S. KON, J.R. WAGNER & A.N. BOOTH. *J. Food Sci.* 39, 897–899 (1974)–Two types of powders

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(moisture content of 5%) were prepared by the use of a double drum drier from different bean and pea varieties. One of the powders with all its cells intact retained the bean flavor and texture, the other with most of its cells broken and with the free starch fully gelatinized had low flavor intensity. Bulk density and consistency for the two powders and for slurries prepared from them were found to be quite different. The two processes described had no detrimental effect on the growth rate, PER and digestibility as tested on rats.

VOLATILE COMPONENTS OF CANNED ALPHONSO MANGO. G.L.K. HUNTER, W.A. BUCEK & T. RADFORD. *J. Food Sci.* 39, 900–903 (1974)–The volatile flavor components of Alphonso Mango (*Mangifera indica L.*) were isolated. Analysis by gas chromatography, infrared and mass spectroscopy led to the identification of 41 components, one of which is 2,5-dimethyl-4-methoxy-2-H-furan-3-one not reported heretofore in natural products.

EFFECT OF FLAVONOLS ON ASCORBIC ACID AND ANTHOCYANIN STABILITY IN MODEL SYSTEMS. A.J. SHRIKHANDE & F.J. FRANCIS. *J. Food Sci.* 39, 904–906 (1974)–Model systems were prepared in citrate phosphate buffer at pH 2.8 with added ascorbic acid, anthocyanins and flavonols. Flavonols in concentrations of 3–9 mg/100 ml retarded the oxidation of ascorbic acid, while cranberry anthocyanins promoted the oxidation of ascorbic acid. The protective effects of flavonols (quercetin and quercitrin) on ascorbic acid and an indirect protection for anthocyanins was shown. Any treatment which reduced the oxidation of ascorbic acid, also reduced the loss of anthocyanins, if both are simultaneously present. Flavonols added to cherry juice did not protect anthocyanins upon storage because pasteurization of the juice caused the added flavonols to crystallize out.

INFLUENCE OF SPECTRAL QUALITY OF LIGHT ON PIGMENT SYSTEMS OF RIPENING TOMATOES. J.J. JEN. *J. Food Sci.* 39, 907–910 (1974)–White fluorescent light and color filter combinations with maxima at 650, 570 and 500 nm, corresponding to red, green and blue, respectively, were compared at the same radiant energy level with dark controls for their effect on the pigment systems of tomatoes ripened under controlled environments. Degradation of chlorophylls was faster in tomatoes illuminated with light than the dark control, with red light yielding the highest degradation rate. No change in the chlorophylls a/b ratio was observed. The carotenoid levels after 10 days illumination, in $\mu g/g$, were 136, 117, 81, 72 and 42 for blue, red, green, white and dark control, respectively. Illumination of red and yellow lutescent tomatoes produced similar results.

THERMAL DECOMPOSITION OF SOME AMINO ACIDS. Valine, Leucine and Isoleucine. Y.C. LIEN & W.W. NAWAR. *J. Food Sci.* 39, 911–913 (1974)–The effect of heat on three branched amino acids heated individually for 1 hr at temperatures varying from 180–270°C, was studied. The volatile decomposition products of valine, leucine and isoleucine were detectable in samples heated at 220°C or higher. Each of

the amino acids gave rise to a characteristic set of decomposition products which consisted mainly of ammonia, CO, CO_2, certain hydrocarbons, ketones, aldehydes, primary amines, imines and secondary amines. Hydrocarbons are formed by decarboxylation and deamination of the amino acids; the primary amines by decarboxylation; and the aldehydes by decarbonylation and deamination. The imines can result from reaction of the aldehydes with the primary amines and may give rise to secondary amines by hydrogenation. The primary amines and olefins may also react to produce secondary amines. In addition, reaction of the primary amines with imines could produce the secondary amines.

THERMAL DECOMPOSITION OF SOME AMINO ACIDS. Alanine and β-Alanine. Y.C. LIEN & W.W. NAWAR. *J. Food Sci.* 39, 914–916 (1974)–Certain differences were observed between thermal decomposition reactions of alanine and β-alanine and those of valine, leucine and isoleucine. In the case of alanine, the secondary amine (typical of the three latter branched amino acids) was absent. In addition, 2-methyl-5-ethylpyridine, propionamide, and N-ethylpropionamide were identified. The heating temperature necessary for detection of the volatile decomposition products of β-alanine was lower than that required for amino acids having no terminal amino groups. The major decomposition products from β-alanine were pyridine derivatives which probably arise from the polymerization of aldehydes and ammonia. A linear polymer, poly-β-alanine, was the major component of the residue from heated β-alanine.

THERMAL INTERACTION OF AMINO ACIDS AND TRYGLYCERIDES. Valine and Tricaproin. Y.C. LIEN & W.W. NAWAR. *J. Food Sci.* 39, 917–919 (1974)–A model system consisting of a mixture of valine and tricaproin was used to study the interaction of fats and amino acids during heat treatment. New decomposition products, not formed originally when each compound was heated alone, were identified from the heated mixture. These were caproic amide, N-isobutylcaproic amide and caproic nitrile. Mechanisms involving the interaction between the amino acid and the triglyceride are introduced to explain the formation of these products. In addition, certain compounds normally produced when the triglyceride or the amino acid are heated separately were absent when the mixture of both was similarly treated. This is explained on the basis of reactions which take place preferentially when both compounds are heated together.

PECTIC ENZYMES: INDIVIDUAL AND CONCERTED KINETIC BEHAVIOR OF PECTINESTERASE AND PECTINASE. S. DAHODWALA, A. HUMPHREY & M. WEIBEL. *J. Food Sci.* 39, 920–926 (1974)–Selected kinetic properties of pectin esterases obtained from *A. niger* and tomato and pectinase from *A. niger* are presented. Integral, steady-state analysis demonstrates that the depolymerizing enzyme, pectinase, used in this study operates predominantly by endo cleavage and exhibits an unusual form of substrate inhibition. The influence of temperature, pH and ionic strength upon the kinetic behavior of both pectinase and pectin esterase is discussed relative to their use in fruit juice clarification. Concerted kinetic behavior of pectinesterase and pectinase in pectin degradation is presented and optimal ratios of enzyme activities are described for specific processing needs.

PREPARATION AND STABILITY OF TRYPSIN IMMOBILIZED ON POROUS GLASS. E.C. LEE, G.F. SENYK & W.F. SHIPE. *J. Food Sci.* 39, 927–929 (1974)–To prepare glass-bound trypsin with optimum enzymatic activity, silanization solvents, types of linkage and washing procedures were investigated. Acetone was better than toluene or water as the silanization solvent. Glutaraldehyde-linked derivatives were easy to prepare and very reproducible. Azo-linked preparations had better activity but poorer reproducibility than their glutaraldehyde-linked counterparts. Protein content of azo and glutaraldehyde-linked preparations ranged from 10–18 and 25–33 mg per gram, respectively. Exhaustive washing was necessary to remove loosely bound or entrapped enzyme from the glass support. Phosphate buffer was the preferred washing solution. Glass-bound trypsin had excellent storage stability at 5°C. Good operational stability was also obtained when microbial growth was controlled.

IMMUNOCHEMICAL STUDIES ON HUMAN AND BOVINE MILK LYSOZYMES. R.R. EITENMILLER, B.A. FRIEND, K.M. SHAHANI & E.M. BALL. *J. Food Sci.* 39, 930–933 (1974)–Comparative immunological studies of the human and bovine milk lysozymes and egg white lysozyme (mucopeptide N-acetylmuramylhydrolase, EC 3.2.1.17) (muramidase), utilizing immunodiffusion, enzyme inhibition, and intragel specific absorption techniques, revealed them to be antigenically and serologically different from each other. Acetylation of the lysine residues or nitration of the tyrosine residues weakened and diffused the precipitin band between modified lysozymes and native antisera. Conformational changes brought about by modification of the cystine or methionine residues through complete oxidation or reduction of the lysozyme molecules destroyed their antigenic activity. Modification of the histidine residue had no effect on the precipitin reaction.

TREATMENT OF SOYBEAN SPENT SOLUBLES BY MEANS OF YEAST CULTIVATION. H. SUGIMOTO. *J. Food Sci.* 39, 934–938 (1974)–The yeast cultivation on soybean spent solubles derived during the preparation of soybean proteins by means of a combination of isoelectric precipitation and heat-coagulation, was investigated in order to eliminate biological or chemical oxygen demand (BOD or COD) and to produce single-cell proteins (SCP) simultaneously. Yeasts especially suitable for this purpose, *Candida guilliermondii* OUT 6005 and *Debaryomyces kloeckeri* AHU 3932 were employed. In the application of the latter strain, for instance, 97% of carbohydrates and nearly 90% of organic acids in the solubles were consumed after 60 hr of aerobic cultivation. In this case, the yeast cells, containing about 34% crude protein, were harvested with a recovery of 29% (yeast wt per solubles wt on a dry base). However, the reduction of nitrogenous substances was limited to 56%, and a large part of the proteins or peptides remained in the supernatant of the culture broth. 84% of BOD or 80% of COD of the spent solubles was removed through this process. The results indicate the possibility of a practical preliminary treatment of the spent solubles because of reducing a conventional biological treatment such as an activated sludge method, and simultaneously producing SCP from a cost-free raw material.

SYNERGISTIC EFFECT OF ETHANOL AND SODIUM CHLORIDE ON AUTOLYSIS OF BAKER'S YEAST FOR PREPARING FOOD-GRADE YEAST EXTRACTS. H. SUGIMOTO. *J. Food Sci.* 39, 939–942 (1974)–It is known that the addition of sodium chloride to compressed baker's yeast induces strong plasmolysis of the cells. This paper shows that the simultaneous addition of ethanol and sodium chloride activated some of the intracellular proteinases, which accelerated autolysis following plasmolysis. The addition of ethanol alone caused a lesser activation of these enzymes. However, an unpleasant bitterness developed. Utilization of the synergistic effect of ethanol and sodium chloride made possible the preparation of food-grade yeast extracts having meaty flavor without bitterness. A high recovery (62.1% as dry matter or 86.9% as Kjeldahl-N) was also obtained. The optimum concentrations of ethanol and sodium chloride, and other incubation conditions for most effectively autolysing baker's yeast are discussed. The microbial aspects of the process are also described.

USE OF RESPONSE SURFACE METHODOLOGY IN THE DEVELOPMENT OF ACCEPTABLE HIGH PROTEIN BREAD. M.R. HENSELMAN, S.M. DONATONI & R.G. HENIKA. *J. Food Sci.* 39, 943–946 (1974)–Information generated from Response Surface computer techniques and Descriptive Flavor Panel analyses were used in the development and selection of a protein-rich bread rated high in acceptance when judged by 79 bread consumers. Proteins from milk, soy and fish were incorporated and examined for the optimum combination. The resultant bread containing 20% protein, as compared to 8% protein for standard white bread, was significantly preferred over a standard white bread and a 6% soy protein-enriched bread.

STRUCTURAL FUNCTIONS OF TASTE IN THE SUGAR SERIES: SENSORY PROPERTIES OF DEOXY SUGARS. G.G. BIRCH & C.K. LEE. *J. Food Sci.* 39, 947–949 (1974)–A number of mono- and dideoxy derivatives of the analogous and conformationally stable and well-defined models, α,α-trehalose and methyl α-D-glucopyranoside, were tested. Their sensory properties are described and compared with those of some previously known monodeoxy sugars.

GIBBERELLIC ACID IN MALTING OF OATS. Y. POMERANZ & H.L. SHANDS. *J. Food Sci.* 39, 950–952 (1974)–Three oat cultivars, grown with and without 112 kg/hectare (100 lb/acre) N as KNO_3 fertilizer, were malted on a laboratory scale with 0–2.5 ppm gibberellic acid (GA_3). The cultivars ranged from 12.9–17.8% protein (Kjeldahl–N × 6.25) and also varied widely in their malting characteristics and response to GA_3. High protein content decreased malt extract and ratio of wort N:malt N. GA_3 treatment increased solubility of proteins, diastatic power and alpha-amylase activity. Total extract and diastatic power in oat malt were much lower than in barley malt. Alpha-amylase activity in GA_3-treated 'Dal' oats approached that found in ordinary barley malt; diastatic power was low.

METAL PROTEIN COMPLEXES IN ETHANOL MEDIA. S. GORINSTEIN. *J. Food Sci.* 39, 953–956 (1974)–By emission spectroscopy, the presence of metal-protein complexes in beer, represented mainly by ferro- and cupro-proteins (compounds of iron and copper with globulins and hordeins) was established. Thermochemical investigations revealed that metals (Fe and Cu) of metal-protein complexes form stable bonds with organic ligands. This was confirmed by endoeffects obtained. An additional introduction of ions of metals (Fe^{3+}) decreases the stability of metal-protein complexes, whereby the stability of the colloid-protein system of beer is destroyed.

INFLUENCE OF WINE INTAKE ON MOUSE GROWTH, REPRODUCTION AND CHANGES IN TRIGLYCERIDE AND CHOLESTEROL METABOLISM OF OFFSPRING. G.S. STOEWSAND & J.L. ANDERSON. *J. Food Sci.* 39, 957–961 (1974)–The objectives of this study were to feed a varietal wine to two generations of laboratory mice for 26 wk each and compare growth, body weight changes, reproduction and blood cholesterol and triglyceride levels with those of mice fed a 12%-ethanol solution or distilled water. Wine intake had no observed effect on development in either generation; however, a slower rate of maturity occurred. Effects were observed on cholesterol and triglyceride metabolism: (1) Wine fed to parents and continued as the exclusive liquid fed to offspring caused elevated levels of blood and liver triglyceride and liver cholesterol; (2) On the other hand, wine-fed offspring born from control (water-fed) parents showed lowered levels of blood triglyceride. In addition, males from this treatment (wine-fed mice from control parents) exhibited lowered levels of liver cholesterol and triglyceride. No changes in blood cholesterol were observed.

ABSTRACTS:
IN THIS ISSUE

FACTORS INFLUENCING AIR-BORNE CONTAMINATION OF FOODS. A Review. D.R. HELDMAN. *J. Food Sci.* 39, 962–969 (1974)—The extent of air-borne contamination of a food product is directly related to the population of air-borne microorganisms in enclosed space near the exposed product surface. The population of air-borne microorganisms in an enclosed space is dependent on: (a) sources of microbial particles within the enclosed air space; (b) diffusion of microbial particles within enclosed air space; (c) transport of air-borne microbial particles from adjoining spaces; and (d) the extent to which control measures are introduced near exposed product surface. Typical sources of air-borne microorganisms in food processing facilities include ventilation systems, floor drains, employees and dust-collecting surfaces. The influence of ventilation system design on diffusion and transport of air-borne microbial particles in enclosed spaces is related to both rate and inlet design. An analysis of techniques utilized to control air-borne contamination of foods indicates that localized control at the source of contamination would be most effective.

AN APPARATUS FOR MEASURING PLUCKING FORCE OF PIG HAIRS. M. MOWAFY & R.G. CASSENS. *J. Food Sci.* 39, 970–971 (1974)—An apparatus was developed to measure the force in grams required to pluck a single hair. The device was used on two groups of pigs which were kept for 4–5 wk in two different controlled climatic conditions that simulated the summer and early winter conditions in Wisconsin. Plucking force was related to the stage of growth of a given hair follicle, and was greatest during the growing phase.

CURED HAM PROPERTIES AS AFFECTED BY NITRATE, NITRITE AND FRESH PORK QUALITY. J.D. KEMP, J.D. FOX & W.G. MOODY. *J. Food Sci.* 39, 972–976 (1974)—Normal and PSE hams were dry cured with salt and sugar only, salt, sugar and potassium nitrate or salt, sugar and sodium nitrite. PSE hams lost more weight during curing and aging but were generally more tender than normal hams although normal hams were more flavorful. Hams cured with nitrate or nitrite had more desirable color and general appearance scores than controls. Aroma was similar for all groups. Flavor was more desirable in either the nitrate or nitrite groups than in controls, with no difference between nitrate and nitrite groups. Saltiness was not affected by either quality or cure. In general, dry-cured hams cured with a curing mixture containing either nitrate or nitrite were superior to those cured with salt and sugar only although many of the latter were highly acceptable.

CHARACTERISTICS OF CURED HAM AS INFLUENCED BY LEVELS OF SODIUM NITRITE AND SODIUM ASCORBATE. C.L. BROWN, H.B. HEDRICK & M.E. BAILEY. *J. Food Sci.* 39, 977–979 (1974)—Hams cured with varied levels of sodium nitrite and sodium ascorbate were evaluated for flavor and texture desirability by a sensory panel and residual nitrite was determined. Hams cured with 91 and 182 ppm nitrite were similar in flavor but those cured with nitrite were scored as having more intense cured meat flavor than nonnitrite cured hams. The concentration of nitrite injected into the hams was directly related to residual nitrite in the cured hams. Hams treated with sodium ascorbate had lower residual nitrite than nonascorbate-treated hams. Higher levels of ascorbate resulted in increased nitrite depletion.

FACTORS INFLUENCING COOLING OF POULTRY CARCASSES. C.H. VEERKAMP & G.J.P. HOFMANS. *J. Food Sci.* 39, 980–984 (1974)—Cooling of broiler carcasses means removing energy from them while the temperature does not fall below $-1°C$ at any point of the carcass. Water and air are the coolants used for the experiments described. The heat-transfer coefficient, broiler weight and cooling time changed during the experiments. The energy removal under defined conditions was determined by accurate calorimetric measurements. With the use of these experimental results an equation was elaborated for calculating the cooling time under different conditions and with different cooling equipment. An example of cooling broiler carcasses of extremely different weights shows the validity of the model. The experiments with air demonstrate that the model equation is only valid for processes in which water is the coolant used.

EVALUATION OF SUCCINIC ACID AND HEAT TO IMPROVE THE MICROBIOLOGICAL QUALITY OF POULTRY MEAT. N.A. COX, A.J. MERCURI, B.J. JUVEN, J.E. THOMSON & V. CHEW. *J. Food Sci.* 39, 985–987 (1974)—Legs of freshly processed broilers were immersed into an unheated (24°C) or heated (60°C) succinic acid solution (1, 3 or 5%) for 1 or 3 min, followed by a 1-min immersion in unheated or heated (60°C) tap water. When unheated succinic acid was used, the mean log reductions in total aerobic count for the 1, 3 and 5% solutions were 0.53, 0.42 and 0.78 respectively, whereas when heated succinic acid was used, the mean log reductions were 0.64, 1.12 and 1.21, respectively. Shelf life of the hot acid-treated legs was extended as much as 6 days at 4.4°C. *Pseudomonas* was found to be the predominant type of bacteria (about 80%) at the time of spoilage on both untreated and acid-treated samples.

SIMPLIFIED METHODOLOGY FOR MEASURING MEAT COLOR. E.D. STRANGE, R.C. BENEDICT. R.E. GUGGER, V.G. METZGER & C.E. SWIFT. *J. Food Sci.* 39, 988–992 (1974)—Instruments which measure reflectance can be used to predict consumer acceptability (H) of meat color. Two separate types of reflectance instruments were evaluated: color difference meters and reflectance attachments for spectrophotometers. A linear correlation coefficient (r) of 0.91 was obtained for H vs. "a" from the Gardner Color Difference Meter (GCDM). Selected wavelengths from the diffuse reflectance spectra of meat samples were also used to predict H. For H vs. %R 630 nm – %R 580 nm, "r" was 0.86 and for H vs. ln (%R 580/%R 630 nm), "r" was −0.86. Statistical analysis of these "r" values indicated that the "a" value from the GCDM was the most effective prediction for consumer acceptability.

BOLOGNA PRODUCT CHARACTERISTICS AS INFLUENCED BY VARIOUS SOURCES AND LEVELS OF COTTAGE CHEESE WHEY. K.L. NEER, R.F. PLIMPTON JR. & H.W. OCKERMAN. *J. Food Sci.* 39, 993–996 (1974)—The sausage segment of the meat industry has been considered as a possible market outlet for various protein sources (such as cottage cheese whey) which might otherwise be difficult to merchandise in their natural form. This project evaluates four different forms of whey as it affects bologna characteristics at four levels of incorporation. Organoleptic observations, in conjunction with various objective measurements, showed beef bologna containing whole, spray-dried whey at 3.5% of the protein block to be equal to, and in some observations, superior to

the all beef control. This work shows that whole, spray-dried whey, a direct by-product from cheese manufacture, can be added at the allowable 3.5% (for approved fillers) to bologna or bologna-type products.

promoters of the twisted strip type were found to be very effective in improving permeate flux of tubular ultrafiltration equipment. With whey protein concentrate of 11% DS and 6.5% protein, permeate flux of 0.8 m^3/m^2 day (~20 gfd) was achieved at 25°C.

FATTY ACID COMPOSITION OF MUSCLE PHOSPHOLIPIDS FROM CALVES, AND GROWING AND MATURE STEERS FED PROTECTED SAFFLOWER OIL.

W.I. KIMOTO, R. ELLIS, A.E. WASSERMAN, R. OLTJEN & T.R. WRENN. *J. Food Sci.* 39, 997–1001 (1974)–Calves, and growing and mature steers with increased linoleic acid (18:2) content in the phospholipids were reared by the Animal Physiology and Genetics Institute and the Nutrition Institute, USDA. Rump roasts from animals fed protected and unprotected safflower oil showed average 18:2 levels from 38.3–40.3% and from 19.8–29.0%, respectively. Tocopherol levels for the rump roasts varied from 4.2–7.3, 2.7–4.9 and 2.7–3.1 µg/g of sample for the calves, and growing and mature steers, respectively. Lipid oxidation of ground portions from the rump roasts stored at 3°C for 3 days was followed by the 2-thiobarbituric acid (TBA) method. Variation in the TBA numbers was minor for mature steers, and not significant for the growing steers fed protected and unprotected safflower oil.

MICROFLORA OF FERMENTED KOREAN SEAFOODS.

A. SANDS & E.V. CRISAN. *J. Food Sci.* 39, 1002–1005 (1974)–52 strains of bacteria and three yeasts were isolated from 10 samples of Korean fermented seafoods. Species and strains of *Bacillus* were most common and occurred in all samples. *Micrococcus* and *Pediococcus* were the second and third most frequently isolated genera and were commonly isolated from samples containing few bacilli. Yeasts were found only in fish roe pastes. The possible sources of inoculum and the interrelationships between the characteristic microflora and the habitats of the animals used in the various fermentations are discussed. Further studies are suggested to evaluate fermentation as a tool for developing new food products.

COMPUTATION OF INSULATION EFFICIENCIES OF FISH TRANSPORT CONTAINERS.

P. CHATTOPADHYAY, B.C. RAYCHAUDHURI & A.N. BOSE. *J. Food Sci.* 39, 1006–1010 (1974)–Insulation efficiency of fish containers was compared in terms of their thermal time constants. Thermal time constant values of a plywood box lined inside with polyethylene film, a plywood box lined inside with 1 cm thick expanded polystyrene in polyethylene bag and a 3-ply moisture proof corrugated double-walled board with wood-wool insulation were determined experimentally and found to be close to the mathematically predicted thermal time constant values of the containers.

ANALYSIS OF A VAPOR PRESSURE MANOMETER FOR MEASUREMENT OF WATER ACTIVITY IN NONFAT DRY MILK.

V.C. SOOD & D.R. HELDMAN. *J. Food Sci.* 39, 1011–1013 (1974)–Rapid and accurate water activity measurement is needed to assure the use of water activity as a feasible quality control parameter for dry foods. The purpose of this investigation was to evaluate the use of a vacuum manometer for rapid measurement of water activity of nonfat dry milk. The apparatus utilized in this investigation included a U-tube manometer, a vacuum pump and a modified three-way ground glass stopcock. The bulb of the stopcock was modified to allow attachment of a sample container. The samples were exposed to vacuum and one arm of the manometer in order to measure vapor pressure of the sample directly. The investigation involved evaluation of the influence of apparatus design parameters on measurement time and accuracy of measurement. The results indicated that measurements were within ± 0.01 water activity of conditions used to equilibrate nonfat dry milk samples. In general, the water activity measurements required 30 min or less.

TURBULENCE PROMOTERS IN ULTRAFILTRATION OF WHEY PROTEIN CONCENTRATE.

P. DEJMEK, B. FUNETEG, B. HALLSTRÖM & L. WINGE. *J. Food Sci.* 39, 1014–1017 (1974)–Turbulence

GROWTH OF LACTIC ACID BACTERIA IN SOY MILKS.

B.K. MITAL, K.H. STEINKRAUS & H.B. NAYLOR. *J. Food Sci.* 39, 1018–1022 (1974)–Soy milks with protein content similar to cow's milk were prepared from (1) soaked, raw soybeans ground with boiling water and (2) ground raw soybeans defatted with 95% ethanol and chloroform. Sucrose and refined soy oil were added to the latter milk. Growth of seven species of lactic acid bacteria in the soy milks was determined by plate count, acid production and pH changes during 16-hr incubation and compared with similar changes in cow's milk. *Streptococcus thermophilus* attained greater numbers and showed greater acid production in cow's milk. However, significant increases in population and acid production were also observed in both the soy milks. Lactobacilli, with the exception of *Lactobacillus bulgaricus*, attained maximum population and showed greater acid production in soy milks than in cow's milk. Among the soy milks, soy milk 1 proved to be a better substrate for lactobacilli than soy milk 2. In general, mixed cultures of *S. thermophilus* and lactobacilli showed similar growth and acid production in all the three milks. Microscopic examination of the mixed cultures revealed that *S. thermophilus* outgrew the lactobacilli. The ability of the organisms to ferment oligosaccharides present in soybeans was determined by growing them in broth containing 0.5% sucrose, melibiose, raffinose or stachyose as the only energy source. The ability of *S. thermophilus, Lactobacillus acidophilus, Lactobacillus cellobiosis* and *Lactobacillus plantarum* to produce substantial amounts of acid in soy milk was related to their ability to utilize sucrose, the major fermentable sugar. *L. cellobiosis, Lactobacillus fermenti, Lactobacillus buchneri* and *L. plantarum* also utilized melibiose, raffinose and stachyose.

PHYTIC ACID IN SOY AND ITS HYDROLYSIS DURING BREADMAKING.

G.S. RANHOTRA, R.J. LOEWE & L.V. PUYAT. *J. Food Sci.* 39, 1023–1025 (1974)–Several commercial soy preparations were tested for phytase activity and phytate phosphorus content. Compared to wheat, all soy preparations tested showed negligible phytase activity but were high in phytate phosphorus. Phytate phosphorus was 40–75% of the total phosphorus present. All the phytate in wheat bread and more than 3/4 of that in soy-fortified wheat flours (soy, 10%; wheat, 90%) was hydrolyzed during the process of breadmaking, apparently, due to phytases in wheat and/or yeast. Corresponding increases occurred in the levels of available (inorganic and residual) phosphorus. Addition of whey to soy drastically inhibited phytate hydrolysis. Increasing addition of soy to wheat flour increased, in some cases, phytate hydrolysis during breadmaking.

INFLUENCE OF VACUUM SOAKING ON YIELD AND QUALITY OF CANNED MUSHROOMS.

F.J. McARDLE, G.D. KUHN & R.B. BEELMAN. *J. Food Sci.* 39, 1026–1028 (1974)–Canned mushroom yield was significantly increased by applying vacuum to the raw product submerged in water and subsequently holding the product in water for 10 min at atmospheric pressure. Combination effects occurred between vacuum soaking and cold storage of the mushrooms. Yield increases, compared to stored mushrooms, were 12% when vacuum soaking was applied to previously stored mushrooms or when the process was applied to fresh mushrooms which were subsequently stored before processing. Vacuum soaking alone resulted in yield increases of 5%, but when vacuum soaking and storage treatments were combined yields were greater than those obtained with the PSU-3S Process. Vacuum soaking offers a more significant advantage to commercial processors by reducing the time required for optimum water absorption by the product from 2 hr to 10 min. Solids analysis of canned mushrooms indicated that increased yields were the result of greater water retention by mushroom tissues. Color and organoleptic quality factors of treated products were comparable to controls.

ABSTRACTS:
IN THIS ISSUE

INFLUENCE OF POST-HARVEST STORAGE AND CANNING ON THE SOLIDS AND MANNITOL CONTENT OF THE CULTIVATED MUSHROOM AND THEIR RELATIONSHIP TO CANNED PRODUCT YIELD. G.K. PARRISH, R.B. BEELMAN, F.J. McARDLE & G.D. KUHN. *J. Food Sci.* 39, 1029–1031 (1974)—An investigation was conducted to determine the interrelationships among solids content, mannitol content and canned product yield as influenced by post-harvest storage of two strains of the cultivated mushroom, *Agaricus bisporus*. Treatments consisted of 0, 1, 2 and 3 days of storage at 2°C and 90% RH. Canned product yields increased with post-harvest storage treatments. An increase in the water-holding capacity of the mushroom tissues was shown to occur during the storage period. A decrease in mannitol content of the fresh mushrooms occurred during post-harvest storage apparently due to the utilization of mannitol as a carbon source. The mannitol content of the mushrooms was decreased 57–66% by the canning process while the solids content increased by 7.2–19.3%.

USE OF RECYCLED SALT IN FERMENTATION OF CUCUMBER SALT STOCK. E.L. DURKEE, E. LOWE & E.A. TOOCHECK. *J. Food Sci.* 39, 1032–1033 (1974)—Cucumber salt stock was prepared with recycled salt reclaimed by submerged combustion and incineration and compared with salt stock prepared with fresh kiln-dried salt. Brine made from the recycled salt was prepared in one of four different ways: (1) as received; (2) pH adjusted; (3) clarified; and (4) pH adjusted and clarified. Test results indicate substantially no difference in fermentation or in the quality of the salt stocks. There were no advantages in clarifying or adjusting the pH of the starting brines. Eliminating these two operations naturally reduces the cost of salt recovery.

EFFECT OF PROCESSING AND STORAGE ON PROVITAMIN A AND VITAMIN C IN APRICOTS. H.R. BOLIN & A. E. STAFFORD. *J. Food Sci.* 39, 1034–1036 (1974)—Drying method influenced vitamin A retention in apricot halves and sheets, with little or no losses occurring during dehydration, but up to 30% during sun drying. Exposure to sunlight during drying also increased the loss of vitamin C. Sulfuring exhibited a protective action on the vitamins during storage. Sulfured dried apricot sheets retained 13% more vitamin A and 300% more vitamin C, after 13 wk storage at 32°C, compared to the unsulfured samples.

CLOUD LOSS DURING STORAGE OF PASTEURIZED CITRUS JUICES AND CONCENTRATES. G. ROTHSCHILD & A. KARSENTY. *J. Food Sci.* 39, 1037–1041 (1974)—Loss of turbidity during storage of pasteurized citrus products was found to be due to either high acidity or residual pectinesterase activity or both. The extent of cloud loss was correlated with increasing acidity or increasing enzyme activity, and was also influenced by juice concentration and the variety of product tested. Cloud loss could be considerably reduced and the period of cloud stability extended by keeping juices and concentrates in cold storage.

INFLUENCE OF HOLDING TIME BEFORE PASTEURIZATION, PASTEURIZATION AND CONCENTRATION ON THE TURBIDITY OF CITRUS JUICES. G. ROTHSCHILD & A. KARSENTY. *J. Food Sci.* 39, 1042–1044 (1974)—The turbidity of freshly extracted citrus juices was measured. The influence of duration of holding time before pasteurization, of pasteurization and of juice concentration on cloud retention was determined. Initial turbidity differed for the different varieties and was lowest in shamouti juice. With prolonged holding times, cloud content in unpasteurized juice remained unchanged in lemon and grapefruit but increased in valencia and shamouti oranges. Whereas the turbidity increase was considerable in unpasteurized shamouti juice, holding times of up to 6 hr before pasteurization had no influence on cloud content in the pasteurized juice. On pasteurization, cloud increased in all juices and markedly in the shamouti variety. Sixfold concentration caused additional cloud increase in shamouti but had no further effect on valencia juice. The considerable differences in initial turbidity in shamouti and valencia orange, grapefruit and lemon juices diminished or disappeared completely after pasteurization or concentration.

EXTRACTION OF A HIGH-PROTEIN LAYER FROM OAT GROAT BRAN AND FLOUR. V.L. YOUNGS. *J. Food Sci.* 39, 1045–1046 (1974)—Oat groats were milled into flour and bran. Each mill stream was slurried in water and fractionated by centrifugation. A protein-rich layer containing 49.9–57.3% (dry basis) protein was obtained from both flour and bran. In oat groat flour, nearly 70% of the total recovered protein was obtained in this layer; in bran, more than 35%. Other layers obtained were water-solubles, residue bran and starch. In addition to protein analysis, ash and lipid concentrations of each layer were measured.

AN ANALYSIS OF HIGH TEMPERATURE/SHORT TIME STERILIZATION DURING LAMINAR FLOW. S.G. SIMPSON & M.C. WILLIAMS. *J. Food Sci.* 39, 1047–1054 (1974)—Rational design and optimization of continuous shell and tube (or plate) sterilizers operating on non-Newtonian fluids in the laminar flow regime is investigated. The intent of the work is the achievement of a "safe" sterilization, while maximizing nutrient retention and minimizing investment and operating costs. It is demonstrated computationally that properly designed sterilizers operating in the laminar regime perform safely and most efficiently when sterilization is completed during passage through the heating and cooling sections, without recourse to a holding section. Further, costs are reduced and nutrient retention significantly increased when the tubes of the sterilizer are reduced in diameter (or the plates reduced in spacing) and correspondingly increased in number, and the average processing velocity of the fluid reduced (again, increasing the number of tubes to maintain the total production rate). The practical limit to the diameter reduction will be established by the probability of tube blockage. Finally it is shown that the rheology of the fluid should be accurately characterized for the most satisfactory design. Computations are made for model systems represented by the nutrient thiamin and the bacterium *Clostridium botulinum*, with inlet temperature 20°C and tube wall temperatures up to 145°C. Tube diameters investigated range from approximately 1/4–1 in. It is shown that when tube diameter is doubled, the physical size of the whole equipment must be increased by a factor of four, which in turn is the primary factor increasing operating and investment costs. All calculations have been based here upon fluids of interest primarily to the food processing industry. However, methods developed and results are of general application in the biochemical industry wherever sterilization of the medium is achieved in laminar flow.

CAFFEINE DISTRIBUTION IN C. acuminata, T. cacao and C. arabica. O. SOMORIN. *J. Food Sci.* 39, 1055–1056 (1974)—The purpose of this investigation was to determine the concentration of caffeine in the bark, beans, leaves, roots and pods of *C. acuminata, C. arabica* and *T. cacao.* Results showed that kolanuts, coffee beans and cocoa beans are the main sites of caffeine storage in *C. acuminata, C. arabica* and *T. cacao* respectively. The leaves of *C. acuminata, C. arabica* and *T. cacao*, and pods of *C. acuminata* and *T. cacao* may contain traces of caffeine.

THE DETERMINATION OF HYPOGLYCIN A IN ACKEE. P.M. SCOTT, H.G. BOTTING, B.P.C. KENNEDY & J.E. KNIPFEL. *J. Food Sci.* 39, 1057–1058 (1974)—A rapid method for the determination of hypoglycin A in ackee has been developed in order to monitor the commercial product for this toxin. An aqueous extract of the ackee fruit (arillus) is deproteinized by adding 5% trichloroacetic acid. Hypoglycin A in the extract is measured after separation from L-leucine and other amino acids using an amino acid analyzer. Mean concentrations of hypoglycin A found in seven samples of commercially canned ackee ranged from 108–260 ppm in the arillus. Concentrations in the brines were a little higher and correlated with the concentrations in the fruit.

SOME OBSERVATIONS ON THE OCCURRENCE OF CHLOROPHYLL AND SOLANINE IN POTATO TUBERS AND THEIR CONTROL BY N^6-BENZYLADENINE, ETHEPHON AND FILTERED LIGHTS. R.B. JEPPSEN, M.T. WU, D.K. SALUNKHE & S.J. JADHAV. *J. Food Sci.* 39, 1059–1061 (1974)—Ethephon and N^6-benzyladenine (N6BA) were applied as preharvest foliar sprays to potato plants. Upon illumination of the harvested tubers, N6BA significantly reduced both chlorophyll and solanine syntheses, while Ethephon significantly inhibited solanine synthesis. Chlorophyll formation was inhibited in tubers exposed to red, orange, yellow and violet filtered lights. Orange and yellow were the most effective colors of light in lowering the formation of chlorophyll. Solanine formation was inhibited to the greatest degree under green light, while red and violet filtered lights were moderately effective. In untreated tubers, deposits of solanine and other glycoalkaloids could be identified and pinpointed on microscopic sections of the peridermal and cortical cell layers.

THE QUANTITATIVE DETERMINATION OF GLUCOSE, FRUCTOSE AND SUCROSE IN FRUITS AND POTATOES. E.S. DELLA MONICA, M.J. CALHOUN & P.E. McDOWELL. *J. Food Sci.* 39, 1062–1063 (1974)—Various combinations of existing methods for the determination of the sugar content of fruits or vegetables were evaluated in an effort to develop a procedure that would be generally applicable for determining the major sugars in these products, initially and at any stage of processing. Factors considered were extraction of sugars from processed products as well as determination of the individual sugar concentration.

SEPARATION OF COMMERCIAL SOYBEAN-MILK WHEY PROTEIN BLENDS BY ELECTROPHORESIS AND ISOELECTRIC FOCUSING. S. BADUI & R.V. JOSEPHSON. *J. Food Sci.* 39, 1064–1065 (1974)— Soybean protein and milk whey proteins and caseins were resolved and identified in commercial products and blends by acidic and alkaline urea starch gel electrophoresis and urea polyacrylamide gel isoelectric focusing. No one method was effective in resolving all protein components simultaneously, but combining results from the three methods allowed for identification of the proteins in complex blends. Soy globulins and milk whey proteins in commercial blends exhibited similar patterns to those of nonblended samples, although some change in β-lactoglobulin and α-lactalbumin was observed in the processed blends. Isoelectric focusing and alkaline electrophoresis methods developed for milk proteins provided new means of resolving and characterizing soy globulins.

IMPORTANCE OF SURFACE HEAT TRANSFER DURING HEAT AND HEAT/HOLD PROCESSES. G.E. BROWN. *J. Food Sci.* 39, 1066–1067 (1974)—Equations were developed for heat and heat/hold processes with surface heat transfer. Using published data for food pieces, it was shown that surface heat transfer is important during steam heating of food. Surface heat transfer coefficients as low as $100BTU/hr-ft^2-°F$ were found. Neglecting these effects could lead to serious underestimates of heating times.

USE OF CARBON DIOXIDE PELLETS FOR SHIPMENT AND STORAGE OF LAMB CARCASSES. G.C. SMITH, Z.L. CARPENTER, R.D. SIMMONS & W.H. MARSHALL. *J. Food Sci.* 39, 1068–1069 (1974)—24 lamb carcasses were placed in polyethylene bags with 1.4 kg of CO_2 pellets, boxed in cardboard containers and shipped 1287 km by common carrier. The boxes were unloaded at a distribution center, stored for 3 days at 1°C, loaded, shipped 322 km in a refrigerated trailer, unloaded and stored in a 2°C cooler. Six lambs were evaluated at each of four postmortem intervals (7, 11, 15 and 19 days following packaging). Differences in freshness, surface appearance, odor and bacterial counts were evident on the external surfaces of the intact carcasses after storage intervals of 15 days. Correspondingly, these data suggest that carbon dioxide chilling can be used to facilitate shipment and storage for periods of 11 days without significantly affecting the freshness, appearance or odor of intact lamb carcasses. Retail cuts from lamb carcasses stored for 15 days were generally less desirable than those from carcasses stored for 7 days in surface discoloration, consumer acceptance, odor, microbial counts and flavor. Use of the CO_2 chilling and associated packaging system resulted in carcasses which maintained acceptable freshness, appearance and odor after 11 days of storage and retail cuts which were acceptable in appearance, odor and palatability. Attempts to use the CO_2 chilling system for storage intervals of 15–19 days resulted in intact carcasses which were unacceptable in appearance and retail cuts with markedly reduced retail caselife and flavor.

THE ROLE OF LEAN AND ADIPOSE TISSUE ON THE FORMATION OF NITROSOPYRROLIDINE IN FRIED BACON. W. FIDDLER, J.W. PENSABENE, J.C. FAGAN, E.J. THORNE, E.G. PIOTROWSKI & A.E. WASSERMAN. *J. Food Sci.* 39, 1070–1071 (1974)—Nitrosopyrrolidine (NO-Pyr) has been found in fried whole bacon and in the cooked-out fat. When lean and adipose tissue were fried separately under the same conditions of frying, NO-Pyr was found in the adipose tissue residue and in the cooked-out fat, but not in the lean tissue or the Crisco in which it was fried. Therefore, the NO-Pyr precursor is present primarily in the adipose portion of bacon. No NO-Pyr was found in ham, Canadian bacon, or beef bacon-like products. The collagen present in bacon adipose tissue is postulated as a source of precursors responsible for NO-Pyr production.

AN IDEA FOR PRECISELY CONTROLLING THE WATER ACTIVITY IN TESTING CHAMBERS. E. LOWE, E.L. DURKEE, D.F. FARKAS & G.J. SILVERMAN. *J. Food Sci.* 39, 1072–1073 (1974)—Precise control of humidity in biological testing chambers is possible in a system involving the addition of exactly the right amount of moisture to air that has been preconditioned to a stabilized dew point. The added moisture is metered into the system not as a vapor but as a liquid, using a syringe-type infusion pump. The water is vaporized prior to its addition to the air stream.

Errata Notice

- *J. Food Sci.* 39(2): 264–266. N.L. DERISE, H.A. LAU, S.J. RITCHEY and E.W. MURPHY: "Yield, proximate composition and mineral element content of three cultivars of raw and roasted peanuts." On page 266, change the formula to read:

$$\frac{\text{batch wt (g) of kernels } after \text{ roasting} \times \text{ nutrient content/g roasted kernels} \times 100}{\text{batch wt (g) of kernels } before \text{ roasting} \times \text{ nutrient content/g raw kernels}}$$

- *J. Food Sci.* 39(2): 396–401. D.R. BUEGE and J.R. STOUFFER: "Effect of pre-rigor tension on tenderness of intact bovine and ovine muscle." On page 399, reproduction of Figures 2 and 3 was insufficiently clear to differentiate between tensioned and nontensioned rib and loin samples. Delete and substitute new figures reproduced herewith.
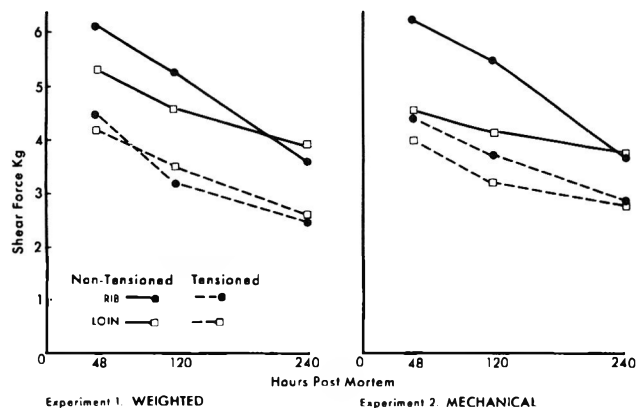
Fig. 2—Effects of tension, muscle location and postmortem aging on shear force values in experiment 1 and 2.

Fig. 3—Effects of tension, muscle location and postmortem aging on shear force values in experiment 3 and 4.

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EFFECT OF THE FAT CONTENT OF GROUND BEEF ON THE HEAT INACTIVATION OF POLIOVIRUS

INTRODUCTION

THE IMPORTANCE of food-borne viral disease has often been underestimated, since it is known that viruses do not multiply in foods. However, a hazard can exist if the viruses are still infective at the time of food consumption. Unfortunately, little is currently known about the effectiveness of presently employed methods of food processing, cooking procedures and food components on the inactivation rates of viruses present in food products.

A survey conducted by Sullivan et al. (1970) indicated that three of 12 packages of ground beef purchased at different times from different retail markets contained enterovirus contamination. Aizen and Pille (1972), reported that enteroviruses in meat are inactivated during cooking. However, their study did not encompass what effect the fat content of the meat would have on virus inactivation. Hamburger can contain fat percentages as high as 30% and has been found by an independent consumer group to often have fat levels in excess 30% (Anonymous, 1971). Studies have shown that the fat content of milk has a protective effect towards viruses during pasteurization (Sullivan et al., 1971). This may also be true for viruses in ground beef. Since ground beef is often consumed only after limited cooking, studies were conducted to determine the heat sensitivity of an indicator virus (Sabin type 1 poliovirus) suspended in ground beef portions containing various levels of fat.

EXPERIMENTAL

Preparation of meat samples

Meat used in the study was derived from a bottom round cut purchased at a local retail supermarket. Manipulations of the meat were conducted in a laminar flow hood. Gloves and sterile apparatus were used at all times during meat processing to decrease extraneous contamination. Superficial fat was removed from the cut. After trimming, portions of the meat were diced into 3-in. cubes and forced three times through a grinder plate containing 1/4-in. openings. The fat content of 10% of the total weight of ground beef was then assayed in 9-g replicate portions using a modified Babcock technique obtained from Griffith Laboratories, Chicago, Ill. The fat level for the meat was found to be 3%. A calculated amount of fat was then ground together with the remaining aliquots of meat to achieve fat levels of 27% and 47%. Equal weights of the meat at each fat level were inoculated with poliovirus type 1 suspended in minimal essential media containing Hanks' balanced salt solution (MEM-H). The titer of the inoculated virus had been determined previously using the agar overlay method of Dulbecco and Vogt (1954). The virus was suspended in the meat by mixing at low speed with an electric mixer for 10 min.

The samples to be tested were delivered into 16 × 150 mm screw cap test tubes in 10-g portions using a modified cookie press to facilitate packing, then samples were stored at –20°C until tested. In order to determine if the virus had been suspended uniformly in the samples, seven unheated samples were tested at each fat level for viral content. Additionally, 30g of each of the three batches were monitored for viral or bacterial contamination prior to inoculation.

Thermal treatment of meat samples

Thermal inactivation studies were conducted at temperatures of 50, 60, 70 and 80°C on viruses suspended in meat samples containing 3, 27 and 47% fat content. The temperatures utilized were below 100°C, therefore, a magni whirl water bath was used to thermally treat the samples. After calibrating the bath, trials were conducted to determine the reproducibility of heating times, heat penetration at different fat levels and reliability of the temperature monitoring apparatus. All studies were monitored for temperature values using a model 42 SC telethermometer, model 4002 switch box and model 423 thermistor probes (Yellow Springs Instruments, Yellow Springs, Ohio). A single-point recorder (Perkin-Elmer, Hitachi Ltd., Tokyo, Japan) was connected to the telethermometer in order to visualize and monitor temperature changes. Span adjustments of the recorder were made to accommodate the arithmetic scale of the recorder to the logarithmic scale of the telethermometer.

Samples of meat to be thermally treated and three uninoculated controls were removed from the –20°C freezer and allowed to thaw. The internal temperatures of the three uninoculated samples were monitored via internal placement of thermistor probes. The controls had been at room temperature the same time as the samples. The samples and controls were placed simultaneously in the water bath which was set one degree above the desired processing temperature. The water bath temperature was also monitored with a thermistor probe. The bath was filled so that the entire samples were covered with water to insure uniform heat penetration. The processing time was determined using a stop watch and the chart speed control on the recorder. Timing was commenced when the internal temperature of the controls reached 50, 60, 70 or 80°C. After the processing time elapsed, the treated samples were immediately placed in ice water and the temperature was again monitored until the samples reached room temperature or below. They were then stored at –20°C or tested immediately for viral content. Three runs were performed at each fat level and each processing time and temperature to determine thermal resistance of the virus.

Elution and concentration of virus from samples

Elution of the virus was accomplished using a modified method of Sullivan et al. (1970). 40 ml of phosphate buffered saline (PBS), pH 8.5, was added to the sample and the resulting mixture shaken by hand to suspend the meat. The pH was then re-adjusted to 8.0 with 1N NaOH. To control bacterial contamination, 3.0 ml of antibiotics was added to obtain concentrations of penicillin G of 1000 units/ml, streptomycin sulfate of 1000 µg/ml and tetracycline hydrochloride of 50 µg/ml. The resulting slurry was agitated on a reciprocal shaker for 1 hr at room

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Table 1—Recovery of poliovirus from 10-g meat samples containing a determined number of viruses[a]

Original PFU/g	Fat content of samples					
	3%		27%		47%	
	Recovered PFU/g	% Rec[b]	Recovered PFU/g	% Rec	Recovered PFU/g	% Rec
2.0×10^6	1.6×10^6	80	1.8×10^6	90	1.75×10^6	88
1.4×10^3	1.1×10^3	72	1.27×10^3	90	1.23×10^3	87
1.4×10^1	9.8	70	1.24×10^1	88	1.2×10^1	85

[a] Values are averages of seven samples
[b] Percent recovery

Table 2—Recovery values for poliovirus present in unheated 10g meat samples inoculated with 2.2×10^7 PFU/g and stored at −20°C

Storage time (days)	Fat content of samples					
	(3%)		(27%)		(47%)	
	PFU/ml ($\times 10^7$)	% Rec[b]	PFU/ml ($\times 10^7$)	% Rec	PFU/ml ($\times 10^7$)	% Rec
3	1.9	86	1.9	86	2.0	90
5	1.8	80	2.1	95	2.1	95
10	1.6	71	2.0	90	1.9	86
15	2.0	90	1.9	86	1.8	80
26	1.9	86	2.2	100	2.0	90
39	2.1	95	2.0	90	2.1	95
47	2.0	90	2.0	90	2.1	95
58	1.9	86	1.8	80	2.1	95
71	1.8	80	1.9	86	2.2	100
84	1.7	77	2.1	95	2.0	90
93	1.8	80	2.0	90	2.0	90
Average	1.86[a]	84	1.99[a]	90	2.0[a]	90
Standard deviation	±0.018		±0.08		±0.01	

[a] Values with this letter are not significantly different at the 5% level
[b] Percent recovery

Table 3—Summary of D values for poliovirus in 10-g meat samples containing three different fat levels, calculated using data from ultrafiltration titrations

Temp (°C)	% Fat	Observations (no.)	Runs (no.)	D value (min)	R^2[a]
50	3	15	3	25.10[b]	0.99
50	27	15	3	26.21[b]	0.99
50	47	15	3	28.70[b]	0.98
60	3	12	3	2.80[c]	0.99
60	27	12	3	4.12[b]	0.93
60	47	12	3	5.18[b]	0.93
70	3	12	3	2.68[c]	0.98
70	27	12	3	4.42[c]	0.95
70	47	12	3	5.00[b]	0.94
80	3	12	3	1.23[b]	0.93
80	27	12	3	1.73[b]	0.93
80	47	12	3	2.31[b]	0.91

[a] Indicates the amount of correlation between effect of time on virus numbers.
[b] Formula used in calculations: $Y = B_0 + B_1 \sqrt{X} + e$.
[c] Formula used in calculations: $Y = B_0 + B_1 X + e$

temperature and then stored at 4°C for at least 16 hr. The samples were again agitated at room temperature for 1 hr. Gross meat particles were removed by passing the 40 ml slurry through fiberglass partially enclosed in cheesecloth. The bottles which had contained the meat samples were rinsed with 50 ml of PBS and the rinse added to the slurry. The material was allowed to filter for 45 min and the filtrate centrifuged at 2500 rpm for 30 min in a refrigerated unit. Following centrifugation 2 ml of the antibiotic mixture was added to the supernate to further eliminate bacterial contamination.

The supernatants of the eluted samples were concentrated using a modification of methods reported by Kostenbader and Cliver (1972; 1973). 75 ml of the sample was concentrated in a model 202 Diaflo unit fitted with a XM 100 cellulose acetate membrane (Amicon Corp., Lexington, Mass.). The suspension was allowed to filter under nitrogen pressure with constant stirring. Following concentration to a volume of 9 ml, the filter was rinsed with 3.0 ml of PBS. This volume was added to the harvest and the resultant 12 ml stored at −20°C until tested.

Titration of virus

The number of viral particles present in the stock suspension and the treated meat samples following concentration was determined using a procedure first introduced by Dulbecco and Vogt (1954). 0.3 ml of concentrated sample was placed on confluent HEp 2 cell monolayers in 60 × 150 mm disposable tissue culture dishes. A total of six monolayers per sample was utilized and 1.8 ml of the original 12 ml was tested. The 0.3 ml inoculum on each dish was gently spread over the cell surface by rocking, and then incubated for 45 min at 36°C in a humidified atmosphere of 5% of CO_2 and 95% air. Following adsorption, the cell layers were overlayed with 5.0 ml of an equal mixture of double strength MEM-H and 1.8% ionagar (Difco) containing 20% calf serum and 75 units penicillin G and 50 μg streptomycin sulfate per ml (GIBCO). After solidification, the infected cells were re-incubated for 36 hr. Viral plaques were observed using 0.05% neutral red dye (vital dye) pipetted onto the surface of the cell layers. Plaques were seen as unstained clear areas. The number of plaques present on each of the six plates were counted and the average multiplied by the dilution factor and a factor of 9.5 to account for the concentration procedure to determine the plaque forming units per milliliter (PFU/ml).

Statistical evaluation

Results from the thermal inactivation studies were analyzed for linearity (Snedecor, 1956). A step-wise regression determination was performed on the data through the courtesy of the Statistics and Computer Science Dept., The Ohio State University, employing polynomial regression formulae.

Following analysis of the data for statistical validity, D values were determined employing the "end point" technique (Stumbo et al., 1950). The D values were calculated using estimated values from the polynomial regression formulae.

RESULTS & DISCUSSION

THE EFFICIENCY of virus recovery and the effect of storage on viruses in the

meat samples are summarized in Tables 1 and 2. The recovery of viruses from samples was acceptable even when numbers as low as 14 PFU/g were inoculated into the meat. The ultrafiltration method used in this study provided high recovery values over several ranges of virus levels. This method was previously reported as being as effective as ultracentrifugation in concentrating viruses present in oyster suspensions by Kostenbader and Cliver (1972). Moreover, it is less expensive and time consuming.

Storage of the samples had no effect on virus numbers. The viruses were assumed to be uniformly suspended in the samples since the standard deviations for recovery values from meat samples (Table 2) were similar to those observed for stock virus titrations conducted on a similar number of samples (± 0.04, ± 0.026).

A summary of D value determinations for various times and temperatures at each fat level is presented in Table 3. The effect of fat content on inactivation is seen particularly at temperatures of 60, 70 and 80°C. This relationship was dependent upon fat content since the processing times were initiated only after the center of the sample reached the desired temperature. The effect of fat content was the least when a processing temperature of 50°C was employed and the most pronounced at 80°C where the range in viral PFU/g was the greatest after 5 min of processing between samples containing different fat levels.

Studies conducted on thermal inactivation rates of poliovirus and other viruses in tissue culture and other fluids consistently have indicated the lack of a first-order reaction when viruses were subjected to thermal treatments. A majority of the thermal inactivation curves found in this study also indicated more than one slope occurred during the inactivation treatments Figures 1, 2, 3 and 4. Most previous studies have been concerned with viruses suspended in a medium which was assumed to provide only minimal heat protection. Nonhomogeneous populations were attributed to the presence of viruses which were more genetically stable than others or to clumping (Younger, 1957; Berg, 1966).

In this study, viruses in the 3% fat content samples were found to derive the least amount of protection from the effects of heat. A straight line relationship between heat and time was found at 3% fat content until virus levels reached 32 PFU/g at 80°C. At this point, a "tailing" effect was observed. More than one change in slope was found for samples containing 27 and 47% fat content at temperatures of 50, 60, 70 and 80°C. This indicated the protective effect of the fat towards the viruses. It appeared that the fat content of the meat provided different levels of thermal resistance for the viruses in the meat. Whether this resistance in the samples was due to the direct protection of the viruses by the fat or due to viral clumping as a result of the fat content cannot be ascertained from this study. It is obvious however, that fat does provide protection for the viruses, thus decreasing the possibility of inactivation of the particles.

Virus numbers inoculated into the meat in this study were high in terms of the reported levels of 39 PFU/g isolated
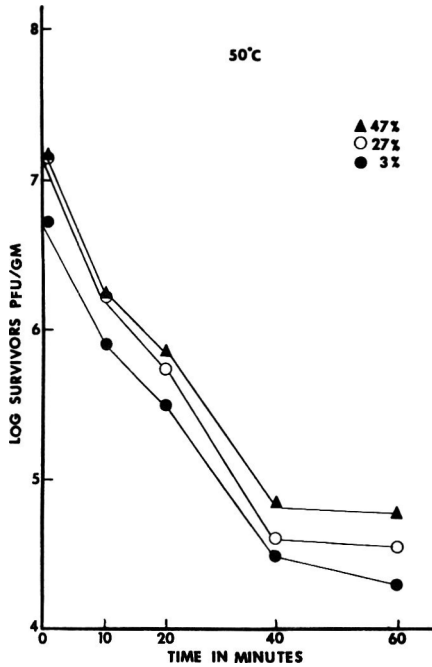
Fig. 1—Thermal inactivation curve for poliovirus heated at 50°C in 10-g meat samples containing 3, 27 or 47% fat.

Fig. 2—Thermal inactivation curve for poliovirus heated at 60°C in 10-g meat samples containing 3, 27 or 47% fat.

Fig. 3—Thermal inactivation curve for poliovirus heated at 70°C in 10-g meat samples containing 3, 27 or 47% fat.
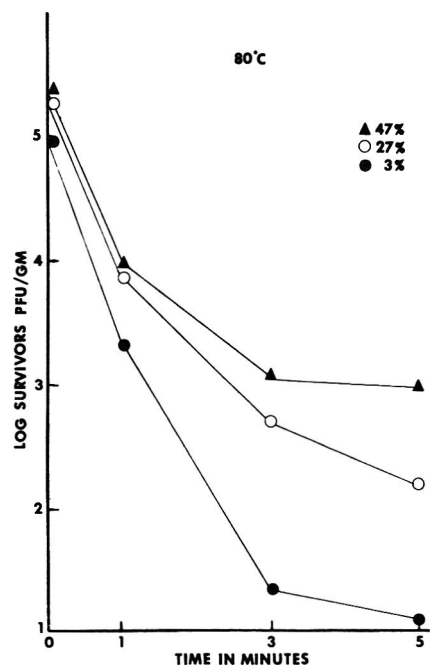
Fig. 4—Thermal inactivation curve for poliovirus heated at 80°C in 10-g meat samples containing 3, 27 or 47% fat.

from ground beef in supermarkets (Sullivan et al., 1970). However, analogies derived using D values calculated in this study from regression coefficients (Table 3) indicate that a 100g hamburger (approximately 1/4 lb) made from meat containing 30% fat content and 39 PFU/g would have to be cooked at 70°C at least 10 min 4 sec to insure inactivation of all virus particles. Moreover, meat heated to an internal temperature of 80°C would have to be heated for 6 min 55 sec to insure virus inactivation. The possibility of this being accomplished in the home or restaurant where doneness is judged by color seems unlikely. Studies of meat cooking indicate denaturization of pigment globin begins at 65°C in beef. At 70°C the meat appears medium rare and by the time the meat reaches 77°C the beef appears well done with small quantities of brown juice (Lawrie, 1966).

CONCLUSION

COMPLETE INACTIVATION of virus particles is important. Studies have indicated the minimal infective dose for the SM strain of poliovirus is only 2 PFU/ml of tissue cluture fluid (Koprowski et al.,

1956) and between 30–80 TCD_{50} of the Fox strain of poliovirus 3 given in milk to infants is sufficient to cause infection (Plotkin et al., 1959). The results of this investigation clearly indicate that poliovirus in ground beef may be quite resistant to the levels of heating commonly employed during cooking especially if the meat contains fat levels approaching 30%.

Further information regarding the effects of food components on thermal inactivation rates of viruses in food is needed. Without such information, the present standards for heat processing and cooking cannot be considered adequate.

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CHANGES IN THE MECHANICAL PROPERTIES OF VEAL MUSCLES PRODUCED BY MYOFIBRILLAR CONTRACTION STATE, COOKING TEMPERATURE AND COOKING TIME

INTRODUCTION

MEAT IS usually cooked before it is eaten so it is of interest to study the effect of heat on the myofibrillar and connective tissue proteins. The work of Locker (1960), Locker and Hagyard (1963), Marsh and Leet (1966) and Herring et al. (1965, 1967a), showed that there was a close relationship between myofibrillar contraction state and the tenderness of cooked meat. The possible influence of connective tissue has been relegated to that of background toughness although it is still held responsible for animal to animal, or muscle to muscle, differences (Marsh, 1972).

It has been shown, using Warner-Bratzler (WB) shear force measurements (Machlik and Draudt, 1963; Draudt et al., 1964; Draudt, 1972) and WB shear force, compression and tensile measurements (Bouton and Harris, 1972a), that values obtained from objective measurements of meat tenderness were usually lower for meat samples cooked at 60°C than for those cooked at 50°C. The magnitude of this effect of cooking temperature was dependent on animal age (Bouton and Harris, 1972a; Schmidt et al., 1970) and was believed to be due to changes in connective tissue strength (Bouton and Harris, 1972a; Draudt, 1972). However, it has been shown (Draudt et al., 1964) that the changes in WB shear force, occurring between 50 and 60°C, were much less marked for muscles such as the longissimus dorsi (LD) and semimembranosus (SM) than for the semitendinosus (ST). The LD and SM muscles can both cold shorten because they are not restrained by their skeletal attachments (on carcasses hung from the Achilles tendon) unlike the ST muscles, which are restrained (Bouton et al., 1973a, b). The results of Draudt et al. (1964) suggest that myofibrillar contraction state could influence the differences observed in tenderness occurring in the 50–60°C range. Purchas (1973) has also shown that the toughening associated with cold

shortening develops as the meat is heated from 50–60°C while Paul et al. (1966) has demonstrated that there are large changes in the solubility of the myofibrillar and sarcoplasmic proteins occurring at temperatures up to 60°C. There is also evidence (Bouton and Harris, 1972b) that WB shear force values are less affected by variations in connective tissue strength than by changes in the myofibrillar structure.

In the present paper the work of Bouton and Harris (1972a) has been extended by an investigation of the effects of myofibrillar contraction state on the shear force and adhesion values of muscles from young cattle, cooked at 50 and 60°C.

More extreme cooking conditions were also used on muscles from young animals to reduce the strength of the connective tissue to low values, and hence minimize its effective contribution to toughness, in samples of varying myofibrillar contraction state.

MATERIALS & METHODS

Experimental material

Twelve animals were used in each of the two main experiments reported in this paper. Six calves, aged about 1 month and six calves, 6–8 month old, (vealers), were used in the first experiment and in the second experiment 12 vealers (aged 6–8 months) were used. In the first experiment the carcasses of two animals from each age group were subjected to one of the three different post-slaughter treatments. The treatments used were:

(a) selected muscles removed within 1 hr of slaughter;
(b) carcass hung from the Achilles tendon; and
(c) carcass hung from the pelvis, using the method of Hostetler et al. (1970).

The carcasses and those muscles which were removed pre-rigor were stored at 0–1°C for 2 days before dissecting and/or cooking the selected muscles. The following muscles were used: longissimus dorsi (LD), biceps femoris (BF), semimembranosus (SM), gluteus medius (GM), vastus lateralis (VL), adductor (A), semitendinosus (ST), rectus femoris (RF), triceps brachii (TB), deep pectoral (DP) and psoas major (PM). Muscles from both sides of each carcass were used. One of each pair of muscles from a carcass was cooked at 50°C for 1 hr while the contralateral muscle was cooked at

60°C for 1 hr. The BF and SM muscles from the 1-month old calves were large enough to provide samples for cooking at 90°C for either 1 or 3 hr. The effects of cooking at 50, 60 and 90°C for 1 hr and at 90°C for 3 hr could thus be compared directly using these muscles viz BF and SM.

In the second experiment the 12 animals were slaughtered in pairs. The carcasses were then split and the sides assigned, according to the statistical plan 11.1 of Cochran and Cox (1957), to their appropriate post-slaughter treatment. The four post-slaughter treatments were:

(a) selected muscles removed within 1 hr of slaughter and allowed to cold shorten at 0–1°C;
(b) side hung from Achilles tendon;
(c) side placed flat on a horizontal plane with legs placed in walking position (Herring et al., 1965); and
(d) side hung from pelvis (Hostetler et al., 1970).

As in the first experiment, the sides and those muscles which were removed pre-rigor, were stored for 2 days at 0–1°C before dissection and/or cooking. The muscles used were the LD, BF, SM, GM, VL, ST, DP and PM. Each muscle was cut into two approximately equal sized pieces. One piece was cooked at 90°C for 1 hr while the other was cooked at 90°C for 3 hr.

Mechanical measurements

A Warner-Bratzler shear device and an Instron Universal Testing Machine (type TM-M) were used. The latter was used for tensile measurements of adhesion between meat fibers. Methods of sample preparation for adhesion and shear force measurements and for the measurement of adhesion values have been described elsewhere (Bouton and Harris, 1972a, b).

Cooking methods

Samples from the selected muscles, weighing 130–150g, were cooked at the required temperature and time using the method described by Bouton et al. (1971). Calf PM muscles samples weighed only 80–100g. These differences in the weight of samples cooked at 50 or 60°C have been shown by Bouton and Harris (1972a) to have no significant effect on the mechanical measurements. Samples cooked at 90°C were weighed before and after cooking to determine cooking losses.

Measurement of sarcomere length

Sarcomere lengths of samples from all muscles were measured using a modification of the light diffraction method first proposed by Ranvier (1874). The modified method, using a He/Ne laser as the light source, has been de-

scribed by Bouton et al. (1973a, b). Measurements were also made on the cooked meat samples using Voyle's (1971) method in which cooked fibers were placed in a 0.2M sucrose in phosphate buffer, pH 7.1. The sarcomere length values quoted in this paper are means of values measured between the centers of the first order diffraction bands. The variability of values within a given muscle sample could be estimated from the width of each diffraction band and was found to be within ± 0.1 μm of the mean value. Light diffraction patterns were obtainable even from samples cooked for 3 hr at 90°C so that the sarcomere pattern remains intact even after appreciable changes in myofibrillar and connective tissue proteins and after appreciable shrinkage and moisture losses.

pH measurement

Measurements of ultimate pH were made at room temperature, 20°C, on the LD and ST muscles using a Phillips C64/1 combined glass electrode with a Phillips PW 9408 Digital pH meter. These two muscles were considered sufficiently representative of the other muscles used to determine the presence of high pH samples (Bouton et al., 1972). The pH values measured were between 5.5 and 5.7, so that the results reported in this paper were obtained on muscles of 'normal' pH.

Statistical methods

In order to show the effect of cooking at temperatures of 50, 60 and 90°C on muscles from the 1-month-old calves, a split plot design with 6 animals (main plots) and two muscles per animal (sub-plots) was used. To compare the effect of cooking at 50 and 60°C on 1-month and 6–8-month old animals, a split plot design was again used but with 12 animals (6 × 1-month old and 6 × 6–8-month old) as main plots and 11 muscles (sub-plots) per animal. The three post-slaughter treatments were allocated at random to the 6 animals within each age group. The muscles were divided into approximately two equal pieces and the temperature treatments allocated at random.

For the experiment with 90°C cooking a split-plot design with 2 sides (main plots) per animal and 6 muscles (sub-plots) per side was used. A balanced incomplete block design (Cochran and Cox, 1957 – Plan 11.1) was used to assign the four post-slaughter treatments to the sides. Significance tests were based on an analysis of variance of all data for each variate. The exponential curves showing the Warner-Bratzler shear force-sarcomere length relationship were fitted using the Gauss iterative procedure. The standard errors of the estimated parameters are based on the inverse matrix of the linear approximation and are not unbiased.

RESULTS & DISCUSSION

THE RESULTS obtained for the calf BF and SM muscles, cooked at 50, 60 and 90°C for 1 hr and at 90°C for 3 hr, are presented first to allow a direct comparison of the effect of these temperature-time treatments on muscles of different myofibrillar contraction state. The results of the more detailed investigations of the effect of myofibrillar contraction state on (a) the effects of cooking at 50 and 60°C and (b) the effects of cooking at 90°C for 1 or 3 hr on mechanical properties are then presented and discussed separately.

Table 1—Mean WB shear force, adhesion and sarcomere length values obtained for BF and SM muscles (1-month old calves) obtained from carcasses subjected to three post-slaughter treatments then cooked at 50, 60 or 90°C for 1 hr and 90°C for 3 hr

| Measurement | Temperature treatment | Post-slaughter treatment | | |
		Removed pre-rigor[a]	Achilles tendon hung[a]	Pelvic hung[a]
Adhesion kg/cm²	50	1.15	1.25	0.93
	60	0.22	0.27	0.20
	90-1hr	0.24	0.23	0.16
	90-3hr	0.06	0.04	0.03
Sarcomere length μm	50	1.19	1.53	3.20
	60	1.17	1.57	2.89
	90-1hr	0.97	1.22	1.86
	90-3hr	0.97	1.13	1.92
WB shear force kg	50	5.53	5.13	8.06
	60	10.31	9.01	3.20
	90-1hr	14.37	10.69	4.45
	90-3hr	12.05	10.01	3.56

	Temperature treatment		Post-slaughter treatment
LSD (5%) for Sarcomere length	0.27	(0.20)[b]	0.15
WB shear force	1.80	(2.42)	2.90
Adhesion	0.19	(0.06)	0.29

[a] Held at 0–1°C for 2 days before cooking
[b] Figures in parentheses represent LSD (5%) to test for significance of cooking time for the samples cooked at 90°C.

Effects of cooking temperature and time on the properties of calf BF and SM muscles

Table 1 illustrates the effects of cooking temperature and time on the SM and BF muscles from the 1-month old calves. The results for the two muscles were combined since they were not statistically different. Large and apparently contradictory effects were produced on the WB shear force and adhesion values by the different cooking regimes. For the muscle samples contracted by their post-slaughter treatment, the WB shear force and adhesion values for the samples cooked at the 60°C are significantly greater than the shear force values obtained for the samples cooked at 50°C. For the muscles prevented from shortening the WB shear force values are much lower for the samples cooked at 60°C, which agrees with the results reported by Machlik and Draudt (1963), Draudt et al. (1964) and Bouton and Harris (1972a). The results in Table 1 indicate that both the magnitude and direction of the difference between the WB shear force values obtained for samples of calf muscle cooked at 50 or 60°C depends on myofibrillar contraction state. The results reported here are not directly comparable with the earlier results of Bouton and Harris (1972a) because not only were the immediate post-slaughter and pre-rigor processing temperature conditions different but the muscles used by Bouton and Harris (1972a) were held at 0–1°C for 6–7 days before cooking, i.e., they had been aged. Other work (Bouton et al., unpublished data) has shown that aging reduces shear force values in calf muscles cooked at 60°C to a much greater extent than in muscles cooked at 50°C. Aging thus increases the differences in shear force values found for calf muscles cooked at 50 and 60°C.

WB shear force values (see Table 1) increase significantly as cooking temperature is increased from 60° to 90°C. However, prolonged cooking at 90°C (3 hr) produces only a small decrease in shear force values even though there is a further large decrease in adhesion values. Cooked meat, which could be pulled apart into individual fiber bundles or fibers with great ease, still yielded high shear force values. This result indicates that adhesion between the meat fibers has comparatively little effect on shear force values in contracted and cooked muscles.

Adhesion values (which are considered a measure of connective tissue strength, Bouton and Harris, 1972a, b) obtained for samples cooked at 50°C are nearly five times the values obtained for the samples cooked at 60°C, regardless of

Table 2—Mean sarcomere length and adhesion values for LD, BF, SM, GM, VL, A, ST, RF, DP, TB, and PM muscles from 1-month and 6—8 month-old beef animals subjected to different post-slaughter treatments.

Animal age (months)	Temperature treatment °C	Post-slaughter treatments		
		Pre-rigor removed[a]	Achilles tendon hung[a]	Pelvic hung[a]
Adhesion values				
6—8	50	0.98	0.83	0.69
0—1	50	1.11	0.85	0.68
6—8	60	0.51	0.38	0.30
0—1	60	0.26	0.22	0.21
Sarcomere length in μm		1.22	2.04	2.61
LSD (5%) for Post slaughter treatment			0.18	
Temperature treatment			0.10	
Animal age			0.14	
LSD (5%) for Sarcomere lengths			0.28	

[a] Held at 0—1°C for 2 days before cooking

Table 3—Mean adhesion values for groups of muscles from 1-month and 6—8-month old beef animals cooked at 50 or 60°C for 1 hr.

Animal age mths	Cooking temperature °C	Muscle groups[a]		
		1	2	3
1	50	1.18a	0.71b	0.28c
	60	0.29a	0.20b	0.10c
6—8	50	1.16a	0.63b	0.20c
	60	0.55a	0.30b	0.11c

[a] Different letters on the same line indicate mean values are significantly different at P < 0.05 level. Group 1 comprises DP, BF, SM, VL and ST muscles, Group 2 the LD, GM, A, RF and TB, while Group 3 contains the PM muscles.

myofibrillar contraction state. Adhesion values decreased by nearly 5 to 1 when the cooking time at 90°C was increased from 1 to 3 hr. Thus the adhesion values obtained for the samples cooked at 50°C were about 20 times the values obtained after cooking for 3 hr at 90°C.

On the basis of the results shown in Table 1 adhesion and WB shear force values cannot be affected by the same structural parameter.

Differences in the mechanical properties of muscles cooked at 50 or 60°C for 1 hr

Adhesion measurements. Table 2 shows the mean adhesion values obtained for the muscles from the 1-month and 6—8-month old animals. Samples cooked at 60°C had much lower adhesion values (P < 0.001) than obtained for samples cooked at 50°C. No animal age effect is evident for the samples cooked at 50°C but for the samples cooked at 60°C the younger animals have significantly (P < 0.05) lower adhesion values. The post-slaughter treatments which produced longer sarcomeres also yielded muscles with lower adhesion values.

Differences in adhesion values between muscles within both animals and age groups were highly significant (P < 0.001). It was possible to classify the muscles into three groups, viz.: Group 1 (DP, BF, SM, VL and ST); Group 2 (LD, GM, A, RF and TB); and Group 3 (PM), which differed significantly (P < 0.05) in mean adhesion values but within which between-muscle differences were not significant. Table 3 lists the mean adhesion values obtained for the muscles within each of these groups.

There was no significant treatment × temperature interaction since adhesion

values decrease with the increase of cooking temperature (viz. 50—60°C) irrespective of the myofibrillar contraction state.

Shear force values obtained for samples cooked at 50°C. Figure 1 shows the shear force values obtained for the muscles, from all three groups, cooked at 50°C as a function of sarcomere length. There was a positive and significant (P < 0.001) relationship between shear force values and sarcomere lengths for muscle groups 1 and 2 from both the 1-month and 6—8-month old animals. Correlation coefficients were 0.68 (n = 60) and 0.73 (n = 60), respectively, for the two different age groups. However, when the mus-

cles from groups 1 and 2 are considered separately it is evident (see Fig. 1) that the regression line fitted to the results obtained for the muscles in Group 1, which have higher mean adhesion values (see Table 3), show a greater change in shear force values with increasing sarcomere length than do the Group 2 muscles. For the younger animals the appropriate regression coefficients are 1.38 (SE 0.22) and 0.81 (SE 0.29), respectively, for the muscles of Groups 1 and 2 while for the older animals the coefficients are 1.73 (SE 0.25) and 0.91 (SE 0.21) for Groups 1 and 2, respectively. These coefficients are significantly different for the two

Fig. 1—The relationship between WB shear force and sarcomere length values for samples from 1- and 6—8-month old calves cooked at 50°C for 1 hr. The regression line for the combined results for Group 1 and 2 muscles is shown as a full line (———) while that for Group 1 muscles is (– – –) and for Group 2 muscles (·····).

muscle groups at the $P < 0.05$ level for the older animals and are approaching significance at the $P < 0.05$ level for the younger animals. The separate regression lines for the two muscle groups are shown in Figure 1.

The WB shear force results obtained for the samples cooked at 50°C show no sign of the increased toughness usually associated with cold shortening. The greatest change in WB shear force values with increase in sarcomere length occurred for the muscle group with greatest adhesion values. Stretching muscle decreases fiber diameter (Herring et al., 1967a) so there should be more fibers per unit area in stretched than in contracted muscle. If degree of actin-myosin overlap is not a major contributor to shear strength of the samples cooked at 50°C, or lower, then other factors, such as the number of muscle fibers and surrounding connective tissue sheaths in a given cross sectional area, could assume greater importance. An explanation of the relationship between shear force and sarcomere length found for samples cooked at 50°C or less requires a greater knowledge than is presently available of the mechanics of the WB shear force method, and of the relative contributions of the connective tissue and myofibrillar structures to the values obtained.

It is of interest to note that it has been demonstrated (Bouton and Harris, 1972a; Purchas, 1973; Machlik and Draudt, 1963) that there is no significant difference between shear force values obtained for raw meat and for meat cooked at 50°C.

Shear force values of samples cooked at 60°C. The relationship between WB shear force values and sarcomere length for the samples cooked at 60°C was complicated since there appeared to be a maximum in WB shear force values at sarcomere lengths of 1.2–1.3 μm. The results for the 60°C samples were considered as a function of sarcomere length for (a) values $< 1.30 \mu$m; and (b) values $> 1.20 \mu$m.

Regression equation for sarcomere length values $< 1.30 \mu$m. A positive significant ($P < 0.001$) linear regression was fitted to the combined results from Group 1 and 2 muscles for both age groups (correlation coefficient 0.57 n = 47). The regression equation found is W = 21.7L − 15.2 where W represents WB shear force values in kg and L the sarcomere length in μm for values $< 1.30 \mu$m. Standard errors for the parameters are 5.9 and 4.9 kg, respectively.

Regression equations for sarcomere lengths $> 1.20 \mu$m. For the WB shear force values obtained for samples of Group 1 and 2 muscles with sarcomere lengths $> 1.20 \mu$m there was an exponential relationship between shear force and sarcomere length. An exponential curve

of the type W = b_0 + b_1 exp (− b_2 (L−d)) was fitted to the two sets of data from the 1-month and 6–8-month old animals. W represented WB shear force values, L the sarcomere lengths in μm and d the sarcomere length in μm at which WB shear force values would be expected to be maximum. b_0 defines the lower limit for shear force while $b_0 + b_1$ represents the shear force corresponding to a sarcomere length of d. The value chosen for d was 1.2 μm. The equations fitted to the results for Groups 1 and 2 muscles from the younger and older animals respectively are:

(a) W = 2.67 + 10.63 exp (− 2.13 (L − 1.2)) Multiple correlation coefficient R = 0.92 ($P < 0.001$)

(b) W = 4.57 + 7.42 exp (− 2.35 (L − 1.2)) Multiple correlation coefficient R = 0.84 ($P < 0.001$)

In the above equations parameters b_0 (LSD 1.42) and b_1 (LSD 1.98) are significantly different.

Figure 2 shows the above curves adapted to the results obtained for the two age groups. In the case of the 1-month old animals there is no evidence of any difference between shear force values obtained for muscles from any of the three muscle groups. In the older animals however the Group 1 muscles generally have a higher shear force (above the curve) than the Group 2 muscles (under the curve). Separate curves for the two different groups, however, are not statistically different.

Comparison with results obtained by other workers. The curves are very similar to the classic curve Marsh and Leet (1966) and Davey et al. (1967) used to relate shear value to percentage contraction. Assuming a value for rest length of 2.2–2.3 μm then the peak force should

occur at 40% contraction which would be at a sarcomere length of ca. 1.3 μm. The present results indicate peak values occur at 1.2–1.3 μm. 60% contraction (relative to a rest length of 2.2–2.3 μm) would be equivalent to a sarcomere length of ca. 0.9 μm. At 0.9 μm shear force values (estimated from the regression equation obtained for sarcomere lengths $< 1.3 \mu$m) are of the same order as those obtained for samples with sarcomere lengths of 2.2–2.3 μm. These results thus agree with those of Davey et al. (1967) except that they believed that rest length was 2.5 μm with the peak force at ca. 1.5 μm.

The sarcomere lengths used in the regression equations are those found for the samples cooked at 60°C. However, there is no significant difference between the values obtained for raw meat and those obtained for the samples cooked at 60°C. (Giles, 1969 and this paper).

Also shown in Figure 2 is the regression line obtained for the samples cooked at 50°C (shown in more detail in Fig. 1). It is readily apparent that the difference between the shear force values at 50°C and the shear force values at 60°C changes sign at sarcomere lengths of ca. 1.6–1.8 μm for the younger calves and ca. 1.8–2.0 μm for the older calves. Only for the muscles with sarcomere lengths $> 2.0 \mu$m do the shear force and adhesion measurements change concomitantly (see Table 1 and Fig. 2) as both shear force and adhesion values obtained for samples cooked at 50°C are higher than those obtained for samples cooked at 60°C.

Changes in mechanical properties of muscle samples cooked at 90°C

The mean shear force, adhesion, cooking loss and sarcomere length results obtained for the LD, BF, SM, VL, ST and GM muscles from 6–8-month old vealers
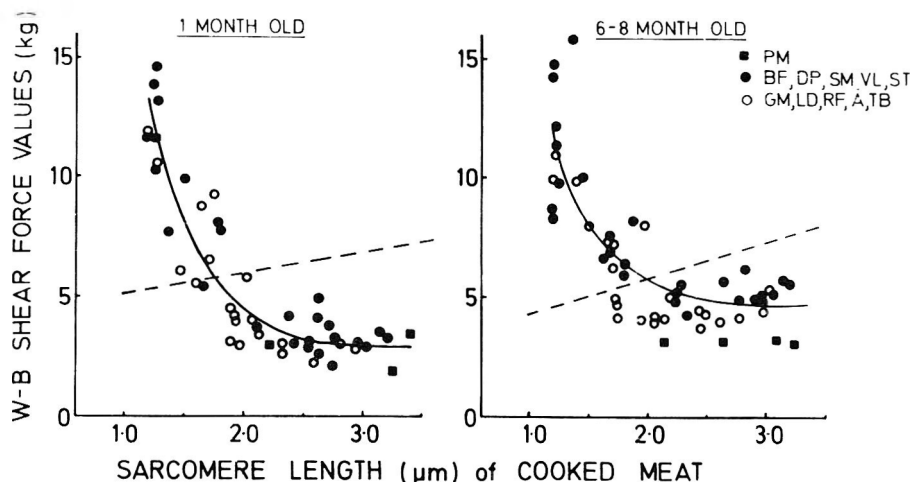
Fig. 2—WB shear force values as a function of sarcomere length (for sarcomere lengths > 1.2 μm) for muscles from 1- and 6–8-month old calves cooked at 60°C for 1 hr. Regression for samples of Group 1 and 2 muscles cooked at 50°C is shown as dotted line.

(cooked at 90°C for 1 and 3 hr) are shown in Table 4. The selected post-slaughter treatments produced a wide range of mean sarcomere length values. Cooking losses decreased significantly with increase in sarcomere length – a result which agrees with other published results (Bouton et al., 1972).

Adhesion values are greatest for the most contracted muscles and least for the most stretched muscles after both 1 and 3 hr cooking. The extra 2 hr cooking at 90°C reduced adhesion values significantly (P < 0.001) by a factor of nearly four. WB shear force values decreased with increasing sarcomere length although no significant decrease was found once sarcomere lengths were greater than 2.0 μm. The longer cooking time at 90°C significantly reduced shear force values but even the extended cooking period did not reduce the shear force values obtained for the contracted muscles to the values obtained for the stretched muscles. There are similarities between the effects of aging (Davey et al., 1967, Bouton et al., 1973b) and the effects of heating in as far as neither aging nor cooking will make contracted muscles shear as easily as stretched muscles.

Regression equations were fitted to the data for all of the muscles (those in Table 4 together with the DP and PM muscles) cooked at 90°C. The regression equations fitted are of the same form as those used to describe the results obtained for the muscles cooked at 60°C.

$$W = b_0 + b_1 \exp(-b_2 (L - 1.20))$$

where W represents WB shear force values, L the sarcomere length of raw meat in μm.

The equations obtained for shear force values of samples cooked at 90°C for 1 and 3 hr are respectively:
(a) W = 4.22 + 6.25 exp (− 2.28 (L − 1.20)) Multiple correlation coefficient, R, = 0.68 (P < 0.001)
(b) W = 3.18 + 5.20 exp (− 2.49 (L − 1.20)) Multiple correlation coefficient, R, = 0.59 (P < 0.001)
Only the b_0 coefficients are significantly different (LSD 5% 0.92).

Effect of cooking on sarcomere length

Sarcomere lengths measured on the raw meat and on that cooked at 50 and 60°C were not significantly different. However, the sarcomere lengths measured for the samples cooked at 90°C were significantly (P < 0.001) shorter than the corresponding values from raw meat. The regression equation, for relating sarcomere 1 ngth of raw samples to sarcomere length obtained after cooking at 90°C for 1 hr, was Y = 1.19 x − 0.15 x^2 − 0.13 (correlation coefficient R = 0.99); Y represents sarcomere length (μm) of raw meat and x the sarcomere length (μm) of

the cooked meat. The regression equation derived for the samples cooked for 3 hr was not significantly different from the equation derived for the samples cooked for only 1 hr. As can be seen in Figure 3 the longer sarcomeres decrease more in length than the shorter sarcomeres during cooking at 90°C. These results agree with earlier results, on thermal shortening (Bouton et al., 1973c).

Table 4—The effect of cooking LD, BF, SM, VL, ST, and GM muscles from 6–8-month old vealers at 90°C for 1 or 3 hr on sarcomere length, adhesion, cooking loss and shear force values

Measurement	Cooking time (hr)	Post-slaughter treatment			
		Removed pre-rigor[a]	Achilles tendon hung[a]	Herring et al. (1965) method	Pelvic hung[a]
Adhesion	1	0.67	0.54	0.49	0.40
kg/cm^2	3	0.20	0.14	0.08	0.11
WB shear	1	10.03	8.67	5.08	4.61
force kg	3	8.63	6.33	3.43	3.18
Sarcomere	Raw	1.18	1.62	2.03	2.43
length in μm	1	1.05	1.39	1.60	1.94
	3	1.03	1.35	1.56	1.84
% Cooking	1	36.5	35.7	34.4	33.1
loss	3	42.2	40.7	41.2	39.1

		Cooking time	Post-slaughter treatment
LSD (5%) for	Adhesion	0.05	0.03
	Shear force	0.71	2.17
	Sarcomere length	0.07	0.08
	Cooking loss	0.8	1.2

[a] Held at 0–1°C for 2 days before cooking
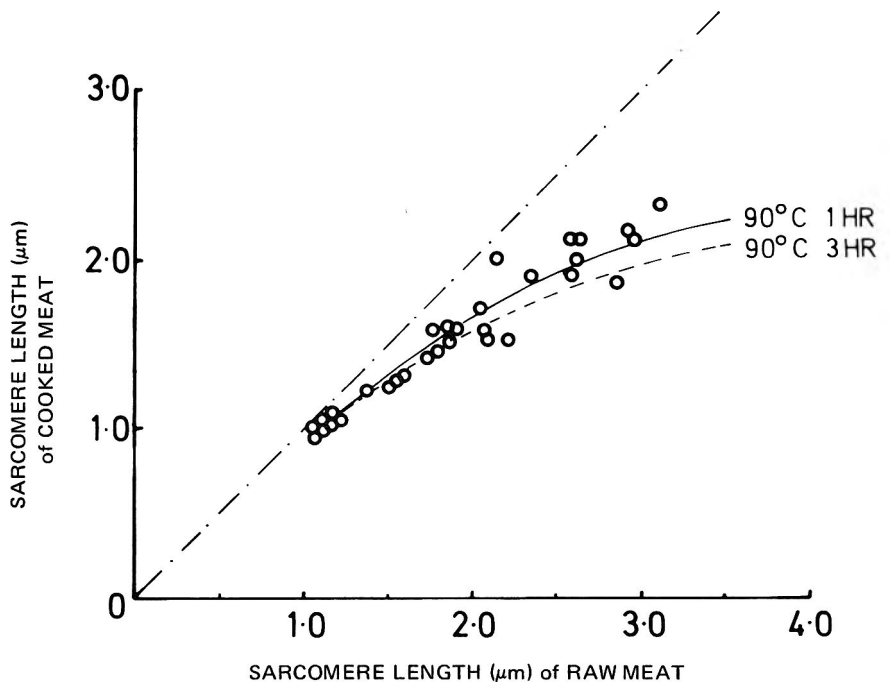
Fig. 3—Raw sarcomere length vs cooked sarcomere length samples cooked at 90°C for 1 and 3 hr. The points shown are the mean values obtained for all the muscles cooked at 90°C for 1 hr.

DISCUSSION

Implications and possible interpretation of results

In the results of the various experiments reported in this paper the changes in adhesion values with myofibrillar contraction state, animal age, cooking temperature and cooking time have been consistent. Increasing cooking temperature from 50–60°C or increasing cooking time at 90°C from 1 to 3 hr significantly reduces adhesion values regardless of myofibrillar contraction state. Increasing myofibrillar contraction state increases adhesion values. Animal age also increases adhesion values although only for the samples cooked at 60°C. The effects of both temperature and animal age on adhesion values relates to the work of others on collagen solubility (Hill, 1966; Herring et al., 1967b; Goll et al., 1964a, b; Carmichael and Lawrie, 1967) and reinforces the contention that adhesion values are a good index of connective tissue strength. The decrease in adhesion values associated with an increase in sarcomere length might be explained in terms of spatial rearrangement. Assuming connective tissue in muscle forms a network round and between each fiber, which holds the fibers together and connects with the tendons at each end of the muscle, then this network must be able to adjust to accommodate dimensional changes which occur when muscles contract or stretch. If the network contains a certain number of cross connections per sarcomere length between adjacent fiber bundles then it would not be unreasonable to expect the number of cross connections per unit length to increase as the sarcomere shortens and the number of sarcomeres in a unit length increases.

Samples for adhesion measurements are cut to a standard size with a constant cross-sectional area (1.5 × 0.67 cm) so that the fibers or fiber bundles of both contracted and stretched muscles are of approximately constant length D (= 0.67 cm). Samples cut from muscles with shorter sarcomeres will thus have more sarcomeres per unit length D with a consequent increase in the number of possible cross connections between adjacent fiber bundles. However, since muscle contraction is a constant volume process the fiber bundle diameters increase when sarcomeres shorten. Samples of contracted muscle, cut to standard dimensions for adhesion measurement, should contain more cross connections per unit length between adjacent fiber bundles but with fewer fiber bundles than would be the case in samples of stretched muscle. The mechanical strength of such a network will be a complex function of the interaction between these two effects. The increase in number of possible cross connections between adjacent fiber bundles

obviously has the greatest effect since adhesion values increase with decrease in sarcomere length even though there are fewer fiber bundles involved.

It is known that shortening during cooking can increase adhesion values (Bouton and Harris, 1972a). Adhesion values are significantly (P < 0.001) lower in muscles, which are physically restrained from shortening during cooking than in muscles free to shorten (Bouton and Harris, 1973 unpublished).

Another possibility is that stretching or contracting the myofibrillar structure could stress the connective tissue network and thus decrease its thermal shrinkage temperature (Rigby, 1964). Changes in thermal shrinkage properties are usually measured on material under zero load and it is known that for collagen fibers under tension during heating the shrinkage temperature could be increased (Verzar, 1964; Lennox, 1949; Gustavson, 1956; Chvapil and Jensovsky, 1963). There is no published evidence known to the authors which shows that intramuscular connective tissue is tensioned in stretched or contracted muscle although Kruggel and Field (1971) and Pfeiffer et al. (1972) have suggested that sarcomere length could be a measure of the amount of stress on the intramuscular connective tissue. It remains to be shown whether the comparatively low forces involved in pre-rigor contraction, viz. 50–60g/cm^2 in normal rigor or up to 500g/cm^2 in thaw rigor (Busch et al., 1972) are sufficient to stress the collagen fibers and to produce in situ the effects found by Rigby (1964) for rat tail tendons in 0.9% saline solution.

In marked contrast to the results obtained for the adhesion values shear force measurements show much more complex changes with cooking temperature and cooking time as well as the myofibrillar contraction state. These results are more difficult to interpret since little is known about the actual relationship between resistance to shear and the relative strength of the connective tissue and myofibrillar structures. It is of interest to note that, while the well established cold shortening effect can be demonstrated by shear force values obtained for samples cooked at 60°C, where coagulation of the myofibrillar proteins is nearly complete (Hamm and Deatherage, 1960; Locker, 1956), shear values of samples cooked at 50°C are positively related to sarcomere length. This result suggests that some stiffening of the myofibrillar structure through protein coagulation is required before toughening due to actin-myosin overlap can be demonstrated. This may also imply that shear or similar measurements on raw meat are unlikely to give a reliable indication of the values obtained for the cooked meat.

It was also found that the differences

in shear force values obtained between samples cooked at 50 and 60°C depend on myofibrillar contraction state as well as on animal age as shown earlier by Bouton and Harris (1972a).

The results presented in this paper have shown that high shear force values can be obtained for samples with very low adhesion values. This, together with the markedly different responses to cooking temperature and myofibrillar contraction treatments, shows that shear force and adhesion measurements are influenced by different structural parameters.

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LIPID AUTOXIDATION IN MECHANICALLY DEBONED CHICKEN MEAT

INTRODUCTION

LIPID OXIDATION is considered to be a major cause of deterioration in mechanically deboned chicken meat (MDM) quality. Oxidation of MDM lipids could also have a significant influence on the stability of finished products containing deboned material. There have been numerous reports on the lipids and fatty acid content of chicken tissues, but very little has been reported on the composition or oxidation of lipids in MDM. Maxon and Marion (1970) determined oxidative changes in deboned turkey meat held at 4°C using the thiobarbituric acid (TBA) reaction. These researchers found that TBA values increased linearly with storage time. Dimick et al. (1972) reported that the keeping quality of MDM as measured by carbonyl concentration and organoleptic evaluation was maintained up to 6 days at 3°C. Froning et al. (1971) showed that deboned turkey with high TBA values produced unacceptable frankfurters when added at a 15% level and held at frozen storage for 90 days. Froning and Johnson (1973) later suggested that heme pigments from bone marrow increased the instability of MDM and products containing MDM during frozen storage.

The purpose of this investigation was to identify and quantitate the lipids and fatty acids of MDM and follow the progress of their oxidation.

EXPERIMENTAL

MDM preparation

Chicken necks and backs were obtained from a local processing plant within 12 hr post-slaughter, packed in ice and ground through a 1/2-in. grinder plate. Bones were separated from the ground material using a Bibun 420 SUM (Food Masters, Inc., Boston, Mass.) meat strainer equipped with a screen with 1 mm holes and cooled with circulating ice water to minimize heat build-up of meat. Approximately 0.5-kg aliquots of the deboned material were placed in plastic bags and stored at 4°C.

Sample treatment

An antioxidant mixture of 20% butylated hydroxyanisole (BHA), 6% propyl gallate, and 4% citric acid in propylene glycol (Tenox II; Eastman Chemical Products, Inc., Kingsport, Tenn.) was added to duplicate 0.5 kg samples of deboned material (0.01% by wt of fat present). Also, duplicate aliquots of meat were treated with 1% Aureomycin (chlortetracycline) and another duplicate set with a combination of 1% Aureomycin and 0.01% antioxidant. Untreated duplicate samples of deboned material served as controls. All samples were placed in plastic bags and held at 4°C.

Lipid analyses

Lipids from treated and untreated samples were analyzed after 0, 2, 4, 6, 7, 9, 11, 13 and 15 days of storage at 4°C. The method of Bligh and Dyer (1959) was used to extract and purify the lipids.

The purified lipid extract was separated into neutral lipid, glycolipid and phospholipid fractions according to the method of Moerck and Ball (1973). The individual neutral lipids were separated using Unisil (Clarkson Chemical Co., Williamsport, Pa.) column chromatography as described by Moerck and Ball (1973) except that triglycerides were eluted with 5% diethyl ether in hexane and free fatty acids were eluted with 8% diethyl ether in hexane. Lipid content of the eluents was quantitated gravimetrically.

The method of Metcalfe et al. (1966) was used to prepare the fatty acid methyl esters from triglyceride and phospholipid fractions. The methyl esters of the fatty acids were separated and identified using gas chromatography (Moerck and Ball, 1973).

Oxidation of fatty acids was determined by following the decrease in the ratio of area percentage data for unsaturated fatty acids to palmitic acid (unsaturation ratio). Palmitic acid was presumed to be a stable component in the phospholipid fraction. This method was used to determine the oxidation of individual unsaturated fatty acids and all polyunsaturated acids grouped together.

Thiobarbituric acid test

A modification of the distillation method of Tarladgis et al. (1960) was used to determine the progress of autoxidation in the lipids of treated and untreated MDM samples. 10g of deboned meat were homogenized in a Waring Blendor with 50 ml of distilled water for 2 min and the mixture transferred to a 500-ml flask by washing with 47.5 ml of distilled water. The mixture was adjusted to pH 1.5 with 2.5 ml of 4N HCl and a small amount of Antifoam B (Sigma Chemical Co., St. Louis, Mo.) was added to prevent foaming. To prevent oxidation of the sample during distillation, Tenox II was added at the rate of 0.01% by weight of fat present. The heating mantle was adjusted to maximum temperature and 50 ml of distillate were collected. 5-ml portions of the distillate were immediately pipetted into equal amounts of TBA reagent (0.02M thiobarbituric acid in 90% glacial acetic acid) in 50 ml glass stoppered tubes. The mixture was heated for 35 min at 100°C and cooled for 10 min under running tap water. A Bausch and Lomb 600 spectrophotometer was used to determine light absorbancy. The absorbance was found to be maximum at 530 nm. The absorbance was converted to mg of malonaldehyde for 1000g of sample by multiplying by a constant of 8.6. This constant was determined by using a 1,1,3,3,-tetraethoxypropane standard curve.

Bacterial colony counts

Colony counts were determined by the pour plate method for treated and untreated MDM samples after 0, 1, 3, 5, 7, 9 and 11 days of storage at 4°C. The initial dilution was made by blending 11g of deboned material with 99 ml of sterile water. Appropriate serial dilutions were plated in duplicate with Plate Count Agar (Difco). The plates were incubated 48 hr at 32°C and all visible bacterial colonies were counted with the aid of a Quebec colony counter.

Statistical analysis

The correlation of TBA values to unsaturation ratios (unsaturated/palmitic) was determined by methods outlined by Snedecor and Cochran (1967).

RESULTS & DISCUSSION

Lipids and fatty acids of MDM

The lipid composition of MDM is reported in Table 1. The total lipid content of MDM was 27% of wet weight. Neutral lipids comprised approximately 98.6% of the total lipid. The "neutral" lipid fraction consisted mainly of triglycerides (92.6%) with small amounts of cholesterol, cholesterol ester, free fatty acids, mono- and diglycerides. Phospholipids comprised approximately 1.4% of the total lipid. Trace amounts of glycolipids were also detected in MDM.

Table 1—Lipid composition of MDM[a]

Lipid fraction	% Total lipids
Neutral lipids	98.6 ± 0.2
Triglycerides	92.6 ± 0.5
Cholesterol	2.1 ± 0.3
Diglycerides	1.1 ± 0.2
Free fatty acids	0.9 ± 0.1
Monoglycerides	0.9 ± 0.2
Cholesterol ester	0.5 ± 0.1
Phospholipids	1.4 ± 0.2
Glycolipids	Trace

[a] Total lipids, 27% of wet weight. Each value a mean and deviation of duplicate determinations from three independent samples

Table 2—Fatty acids and long chain aldehydes (area percent) of MDM phospholipids and triglycerides at 0 day and after 15 days of storage at 4°C[a]

Fatty acid[b]	Triglycerides (%) 0 day[e]	Phospholipids (%)	
		0 day	15 day
14:0	1.3 ± 0.1	f	f
hexadecanal	–	4.00 ± 0.4	4.35 ± 0.3
16:0	21.95 ± 0.5	17.20 ± 1.2	19.55 ± 0.9
16:1	f	1.45 ± 0.3	1.70 ± 0.2
octadecanal	–	2.85 ± 0.3	3.25 ± 0.2
17:0[c]	f	f	f
17:1[d]	–	f	f
18:0	7.85 ± 0.2	14.00 ± 1.2	15.80 ± 1.5
18:1	39.45 ± 1.3	20.25 ± 1.4	25.10 ± 1.4
18:2	21.00 ± 0.6	14.20 ± 1.0	15.60 ± 1.5
18:3	1.60 ± 0.2	0.90 ± 0.1	0.75 ± 0.3
20:0	f	f	f
20:2	f	f	f
20:3	f	1.30 ± 0.2	1.00 ± 0.2
20:4	f	11.60 ± 0.3	6.20 ± 0.4
20:5	f	1.55 ± 0.2	0.60 ± 0.1
22:4	–	2.10 ± 0.1	1.10 ± 0.3
22:5	–	1.90 ± 0.2	0.70 ± 0.2
22:6	–	5.75 ± 0.3	2.60 ± 0.3
24:0	–	f	f
24:2	–	f	f
18:2/16:0	f	0.825	0.797
18:3—22:6/16:0	f	1.460	0.664

[a] Each fatty acid expressed as a percent of total fatty acids. Each value a mean and deviation of duplicate determinations from three independent samples.
[b] Carbon chain length: number of double bonds
[c] Tentatively identified
[d] Tentatively identified
[e] No apparent change after 15 days of storage
[f] Fatty acids present in amounts of 0.5% or less
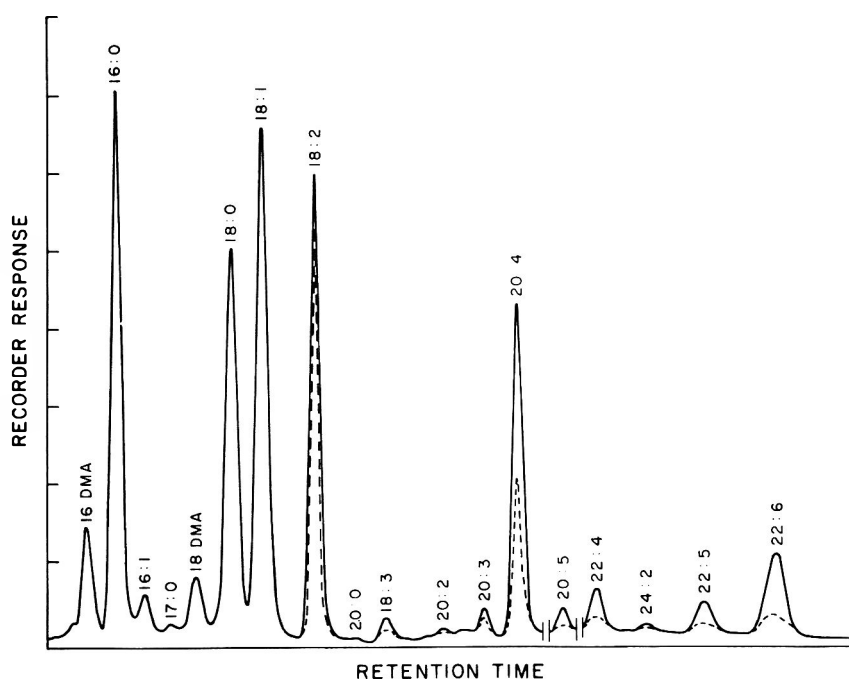
Fig. 1—GLC of the fatty acid methyl esters and dimethyl acetals of MDM phospholipids at 0 day of storage (——) and after 15 days of storage at 4°C (---).

The fatty acid composition of the triglyceride and phospholipid fractions is shown in Table 2. Triglycerides contained primarily 16:0, 18:0, 18:1 and 18:2 carbon fatty acids. Phospholipids contained higher levels of 18 carbon saturated and 20:3 to 22:6 carbon polyunsaturated fatty acids than triglycerides. Similar results have also been reported for breast muscle, thigh muscle, skin tissue and bone marrow (Widmer and Holman, 1950; Chang and Watts, 1952; Miller et al., 1962; Marion and Woodroof, 1963, 1965; Katz et al., 1966; and Moerck and Ball, 1973).

Autoxidation of lipids in MDM

No apparent oxidation occurred in the less unsaturated triglycerides, even though this fraction comprised approximately 92.6% of the total lipids in MDM. There was considerable autoxidative deterioration of the highly unsaturated phospholipid fatty acids (Fig. 1). The fatty acid composition of MDM phospholipids before and after 15 days of storage at 4°C is shown in Table 2. Trienoic, tetraenoic, pentaenoic and hexaenoic fatty acids in the phospholipid fraction were the major substrates of autoxidative deterioration in MDM. After 15 days storage, these polyunsaturated fatty acids comprised approximately 13% of the total fatty acids as compared to 25.5% at 0 day. There were no apparent differences in the fatty acid composition of untreated MDM samples and samples treated with 1% antibiotic. The higher levels of microorganisms in untreated MDM (Table 3) did not alter the fatty acid composition of the phospholipids. Samples treated with Tenox II at 0.01% by weight of fat present showed very little autoxidative deterioration. Only slight decreases in the levels of tetraenoic, pentaenoic and hexaenoic fatty acids were detected.

Dimick and MacNeil (1970) reported that arachidonic acid and linoleic acid were the major substrates in autoxidative deterioration of chicken and turkey skin. Using palmitic acid as a stable component of the phospholipid fraction, however, the ratio of area percentage data for 18:2/16:0 did not decrease during that 15 day storage period indicating that very little autoxidation of linoleic acid took place in MDM (Table 2).

The progress of autoxidation of polyunsaturated 18:3 to 22:6 carbon fatty acids of the phospholipid fraction was determined by plotting the decrease in their unsaturation ratios during the storage period (Fig. 2). The major oxidative changes in these fatty acids occurred after 6 days of storage. There appeared to be an induction period of approximately 5—6 days during which the rate of autoxidation was quite slow. This induction period was followed by an accelerated rate of autoxidation which continued to

about the 11–12th day and slowed after the 12th day.

Figure 3 illustrates the progress of autoxidation of dienoic, trienoic, tetraenoic, pentaenoic and hexaenoic fatty acids. Pentaenoic and hexaenoic fatty acids had a shorter induction period and oxidized more rapidly than did dienoic, trienoic or tetraenoic fatty acids. The rate of autoxidation began to slow after the 9th day. These highly unsaturated fatty acids appear to be responsible for the development of oxidative rancidity during the early stages of storage. Tetraenoic fatty acids had a longer induction period (8 days) than hexaenoic or pentaenoic fatty acids. The autoxidation of these acids appears to slow after 13 or 14 days of storage.

As indicated earlier, dienoic fatty acids showed little autoxidative deterioration. Their induction period appears to be extended over the entire 15-day period. Trienoic fatty acids also had a relatively slow rate of autoxidation. These trienoic fatty acids are probably only involved in the development of rancidity near the latter stages of storage.

The progress of autoxidation was also followed using TBA determinations (Fig. 4). TBA values for untreated MDM samples rose to approximately 1.3 in 6 days and to over 22 in 15 days. Values of approximately one have been related to the threshold level for rancidity (Watts, 1962). Samples treated with 1% Aureomycin had similar TBA values after 6

Table 3—Changes in total bacterial counts for MDM[a]

Storage Days at 4°C	Total counts/g	
	Control	1% Aureomycin
0	3.62×10^4	7.53×10^3
1	4.58×10^4	2.10×10^3
3	1.05×10^5	1.54×10^3
5	2.04×10^6	9.20×10^1
7	1.77×10^7	1.31×10^3
9	1.25×10^8	3.56×10^3
11	1.42×10^8	7.65×10^3

[a] Average of duplicate determinations of two independent samples

days but rose to over 50 in 15 days. There was, however, no increase in fatty acid autoxidation as determined by fatty acid analysis. This suggests that the high levels of microorganisms present (Table 3) in untreated MDM samples removed malonaldehyde and possibly other dicarbonyl compounds which were formed during autoxidation. The ability of microorganisms in the genera Pseudomonas and Achromobacter to selectively attack and utilize carbonyl compounds has been demonstrated by Smith and Alford (1968) and Alford et al. (1971). Ostovar et al. (1971) found that the majority of microorganisms present in MDM belonged to the genera of Pseudomonas and Achromobacter. Numerous studies have shown variations in TBA values for poultry and

other meat products. Since most authors failed to inhibit microbial growth prior to TBA determinations, it is possible that these variations are due to the high levels of microorganisms present or changes in the microflora.

There was high correlation between phospholipid fatty acid oxidation and TBA values for both antibiotic treated (r = −0.99) and untreated (r = −0.98) MDM samples. The keeping quality of MDM stored at 4°C as measured by these methods is approximately 5–6 days. Dimick et al. (1972) also found that the keeping quality of MDM as determined by carbonyl concentrations and organoleptic evaluation was 6 days at 3°C. The addition of Tenox II as 0.01% by weight of fat present extended the induction period and keeping quality relative to oxidation of MDM to the final day of storage. TBA values for Tenox II-treated samples remained below the reported rancidity threshold value of one. The addition of antioxidant, therefore, appears to be useful in preventing oxidative rancidity of MDM at refrigerated temperatures. MDM samples treated with a combination of antibiotic and Tenox II showed TBA values slightly higher than those samples treated with only Tenox II.

Although we did not estimate the effect of heme pigments present in bone marrow on the autoxidation of MDM lipids in this study, Froning and Johnson (1973) reported that MDM gained significant quantities of heme pigments from
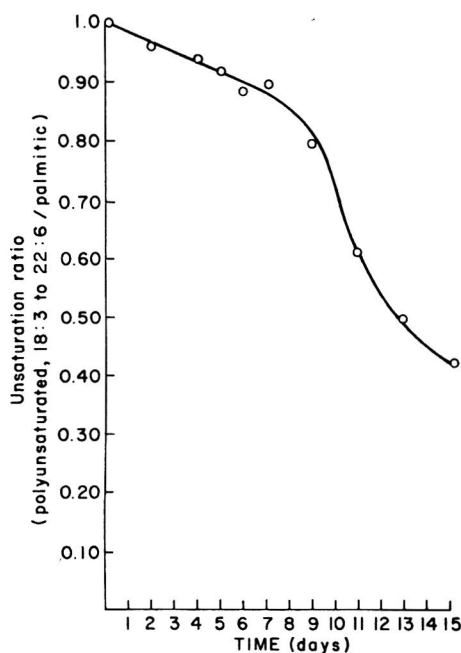
Fig. 2—Oxidation of 18:3 to 22:6 polyunsaturated fatty acids in MDM phospholipids. Unsaturation ratio of 1.0 indicates no oxidation at 0 time.
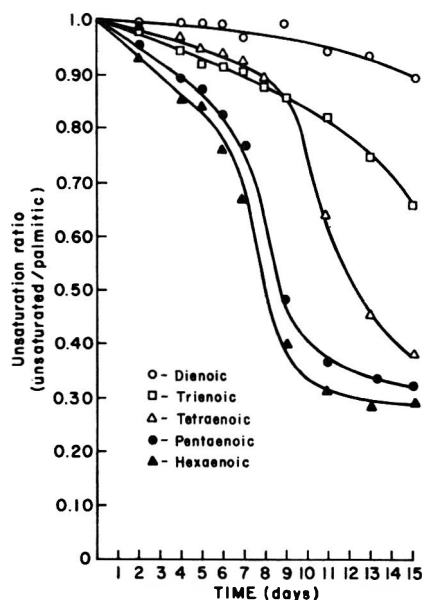
Fig. 3—Oxidation of polyunsaturated fatty acids in MDM phospholipids. Unsaturation ratio of 1.0 indicates no oxidation at 0 time.
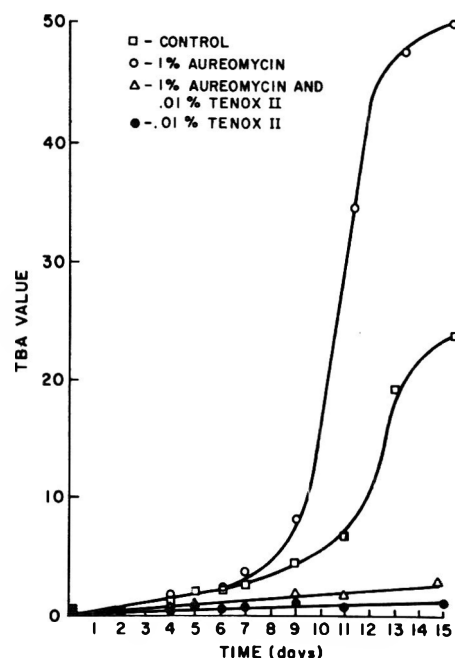
Fig. 4—Measurement of lipid oxidation in treated and untreated MDM samples as determined by the thiobarbituric acid reaction.

bone marrow and that these heme pigments increased the instability of MDM during frozen storage. Froning and Johnson (1973) also showed that by removing hemoglobin from MDM by centrifugation TBA values decreased.

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FISH PROTEIN CONCENTRATE FOAM

INTRODUCTION

FISH PROTEIN concentrate (FPC) has been incorporated successfully as a nutritional supplement into a variety of foods (Learson et al., 1971; Moorjani, 1970; Sidwell, 1967; Woo and Erdman, 1971), but lack of functional properties has been cited as a major factor limiting its use. FPC does not have flavoring, texturizing, or binding properties, nor does it function as a preservative (Holden, 1971). Korschgen and Baldwin (1973), however, utilized FPC as an emulsifier. This study was conducted to investigate foaming of FPC as influenced by concentration, whipping time, solubilization and selected additives.

MATERIALS & METHODS

WHOLE FPC was available only for the experiment on concentration and whipping time. Fillet FPC was used for all other tests. Both whole and fillet FPC were obtained from Viobin Corp., Monticello, Ill. The Viobin process included solvent extraction by ethylene dichloride, followed by alcohol extraction (Levin, 1971). Proximate composition of the fillet FPC was as follows: 3.3% moisture, 85.0% crude protein, 0.1% ether extracted lipid, and 10.5% ash (Missouri Agricultural Experiment Station Chemical Laboratories, Columbia).

Preparation of foams

200g of liquid (distilled water, or distilled water and acid or alkali) were combined with FPC, and 10% concentrations were used except for the investigation on effect of concentration and whipping time. FPC was blended with liquid at 900 rpm for 1 min prior to whipping at 1500 rpm (Kitchen-Aid mixer controlled by voltage regulator) for 24 min unless noted otherwise. Experiments were conducted in triplicate as follows:

(1) Concentrations of 5, 10, 20 and 40% whipped 16, 20 and 24 min;
(2) Addition of 0.3, 0.5, 1.0, 2.0, 4.0 and 8.0% NaCl;
(3) pH levels of 4, 6 (unaltered), 8 and 10 (pH adjusted with 1N HCl or 1N NaOH);
(4) Solubilization by adjusting to pH 12 with 1N NaOH and heating 20 min at 95 ± 3°C (Tannenbaum et al., 1970) followed by adjusting to pH 4, 6, or 8 with 10N H_2SO_4 (75g of this solubilized mixture was whipped 2 or 4 min);
(5) Solubilized, readjusted to pH 6, plus 0.6 or 1.2% guar gum (Jaguar J 25-1, No. 88-2368; Stein, Hall and Co., Inc., NY 10016) (75g of this solubilized mixture with added gum was whipped 2 min).

Specific volume

Specific volume was determined by filling crystallizing dishes (8 cm diam, 4 cm depth) with FPC foam immediately after whipping, and dividing the volume of the dish (ml) by the weight of the foam (g).

Percent drip

Each crystallizing dish of FPC foam from the test for specific volume was inverted on a wire screen (7 wires/cm) and allowed to drain into a preweighed beaker. Weight of liquid released from the foam after 5, 10, 20 and 30 min was determined and calculated as percent drip based on the original weight of the foam.

Stability to heat

Approximately 250 ml of foam from a 10% mixture of FPC, solubilized FPC adjusted to pH 6, and solubilized FPC (pH 6) with guar gum added, as described previously, were heated in a gas oven (177°C) for 15 min. Effects of heating were appraised visually.

Surface tension

Determinations of surface tension were made by the Du Nuoy method (20°C) on untreated and on solubilized FPC mixtures.

Statistical analyses

When significant differences were found by analysis of variance, Duncan's (1955) new multiple range test (P < 0.05) was applied.

RESULTS & DISCUSSIONS

Concentration of FPC and whipping time

Specific volumes of foams from different concentrations of FPC were all significantly different regardless of whipping time. The best foaming, indicated by greatest specific volume, was achieved with 10% concentrations followed in order by 5, 20 and 40%. Regardless of concentration, 16 min of whipping resulted in significantly lower specific volume in foams than did 20 or 24 min (Fig. 1).

Percent drip from foams was inversely related to concentration and no drip was released from the foam made with 40% FPC. The percent drip from foams whipped 20 or 24 min did not differ from each other, but the foams whipped 24 min were significantly more stable than those whipped 16 min. Only at the 20- and at the 30-min intervals were the foams which had been whipped 20 min significantly more stable than those whipped 16 min (Fig. 2).

Since the FPC was denatured during
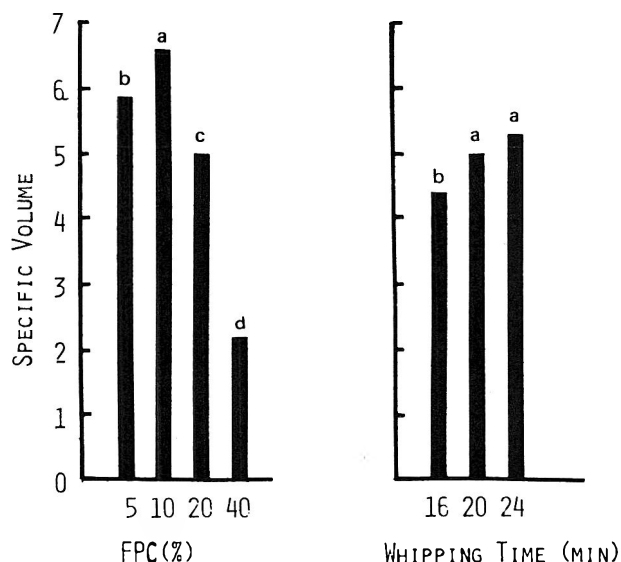
Fig. 1—Mean (n = 3) specific volume of FPC foams representing four concentrations and three whipping times. [Where letters above the bars differ within a graph, the means differ significantly (P < 0.05) from each other (Duncan, 1955).]
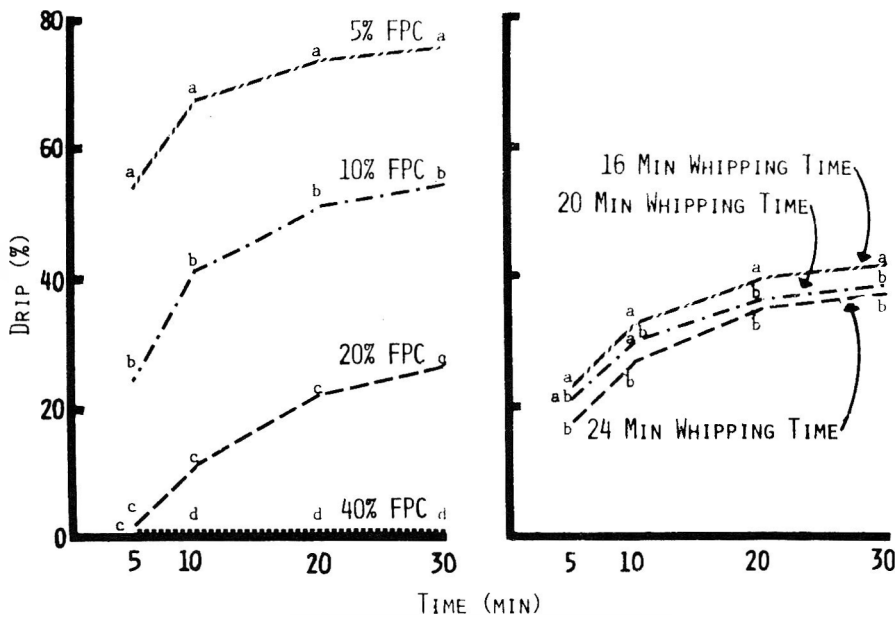
Fig. 2—Mean (n = 3) percent drip at selected time intervals from foams representing four concentrations of FPC and three whipping times. [Where letters differ within a time interval, means differ significantly (P < 0.05) from each other (Duncan, 1955).]

processing, the optimum whipping time cannot be explained on the basis of denaturation of the protein during whipping as is the case with albumen. However, even though FPC is denatured, there may be attractive forces which are favored by whipping 24 min. The character of the foams suggested that the increased stability was due to packing of the FPC powder in the thin layers of liquid surrounding the air bubbles. The foams from FPC mixtures of higher than 10% concentration were coarse and sandy in texture and brownish in color, as was the dry FPC powder.

Effect of NaCl

Addition of NaCl, in amounts suitable for flavor enhancement (0.3–4.0%), to 10% fillet FPC mixtures was neither beneficial nor detrimental when compared to the control foam. However, the specific volume of foam containing 8% NaCl was 7.3 which was significantly greater than for foams with 0.3, 0.5, 1.0 or 2.0% NaCl (specific volumes 6.3–6.6). NaCl is known to be detrimental to foaming of some proteins (Hanning, 1945; Sechler et al., 1959; McDonald and Pence, 1961). In contrast, NaCl in concentrations up to 2% enhanced the whipping ability of soy proteins (Watts, 1937), but stability of soy protein foams was reduced when 5% NaCl was used as the dispersing medium (Eldridge et al., 1963).

Effect of pH

The specific volume of foams was significantly lower at pH 6 than at pH 4, 8 or 10. Mean values for specific volume were 6.8 for pH 6, 8.4 for pH 4, 9.1 for pH 8 and 9.3 for pH 10. Foams were significantly less stable, when the pH of fillet FPC was decreased (pH 4), or unaltered (pH 6) rather than increased (pH 8 or 10) before whipping. Foams from FPC mixtures adjusted to pH 10 were stable for a longer period of time than foams from mixtures at other pH levels (Fig. 3). In addition to improving specific volume and stability, the alkaline pH lightened the color of the foams and reduced the sandiness of the texture. These improve-

ments in the foam were probably due to shifting the pH away from the isoelectric pH of some of the FPC proteins. According to Watts (1937) and Eldridge et al. (1963), the foaming ability of soy proteins was least when the pH was near the isoelectric point. With albumen, however, volume and stability of foams were found to be greatest near the isoelectric pH (Barmore, 1934).
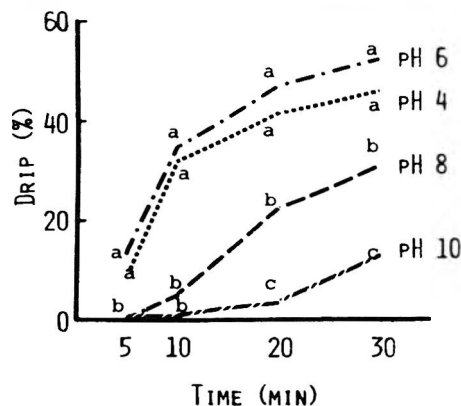
Effect of solubilization

It was necessary to reduce the amount of solubilized FPC mixture to about half for whipping because the volume of foam was so great that the full amount could not be retained in the bowl. Also, a foam with uniform fine texture was formed with whip times of 2 and 4 min, and longer whipping did not improve these characteristics. This improved whipping property of solubilized FPC may have been a result of lowering the surface tension from 58 dynes/cm for untreated mixtures to 48 dynes/cm for solubilized FPC. Also, the foaming property may have been enhanced by a reduction in the size of the protein molecules of FPC due to the solubilization process. Limited electrophoretic analyses of a mixture of 10% FPC, its drip, and solubilized FPC failed to elucidate the foaming phenomenon.

Foams from solubilized FPC readjusted to pH 6 were significantly greater in specific volume than those from pH 4 but were not significantly different from pH 8. Foams from solubilized FPC adjusted to pH 6 or 8 were comparatively white in color and smooth in texture. Specific volume was significantly greater for foams from solubilized FPC whipped 4 min than for those whipped 2 min (Fig. 4).

There was no drip from foams of solubilized FPC readjusted to pH 6 or 8 and whipped for 2 or 4 min. Drip was released from the foams obtained from solubilized FPC mixtures readjusted to pH 4, and significantly more drip was released from these foams after 20 and 30 min of standing when whipping time was 2 rather than 4 min. The percent drip from these foams after 30 min was 39.7 when whipping time was 2 min and 33.0 when whipping time was extended to 4 min.

Influence of guar gum on solubilized FPC

Although 4 min of whipping favored large volume of solubilized FPC foams, beating was limited to 2 min for the foams containing 0.6 and 1.2% guar gum due to the tendency of the foam to fragment and fly out of the bowl during whipping. Specific volume for the control foam was 17.5. Addition of 0.6% guar gum reduced specific volume to 14.5, and 1.2% of this additive reduced it to 12.3.

Fig. 3—Mean (n = 3) percent drip at selected time intervals from foams of 10% FPC, four pH levels, 24 min whipping time. [Where letters differ within a time interval, means differ significantly (P < 0.05) from each other (Duncan, 1955).]
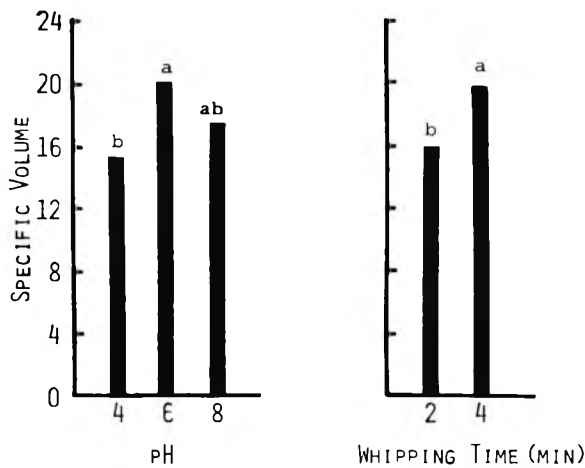
Fig. 4—Mean (n = 3) specific volume of foams representing solubilized FPC readjusted to three pH levels and two whipping times. [Where letters above the bars differ within a graph, the means differ significantly (P < 0.05) from each other (Duncan, 1955).]

No drip was obtained from any of these foams.

Stability of foams to heat

None of the foams responded favorably to heating. The untreated FPC mixture collapsed within 7 min after subjecting it to heat. The solubilized FPC foam and the solubilized foam containing guar gum expanded considerably upon heating, and then collapsed. The residue from the solubilized FPC was dry and crisp as contrasted to the paste-like texture of the residue from the untreated FPC foam. Also, approximately 25 ml of liquid were left in the bottom of the beaker after heating the untreated FPC. All residues were brown in color.

General discussion

The limited use of FPC cannot be attributed solely to the lack of functional properties, since solubilization permitted formation of large volume, fine textured foams. Although foams were not stable to heat, the residues from heated solubilized foams appeared to have some potential as a means of increasing the protein of snack-type products. The aroma of the heated foam residue was mild enough to be considered compatible with cheese-like flavors, whereas unheated foams were characterized by an objectionable aroma. This undesirable aroma was not improved by heating (90–100°C) FPC powder under vacuum for 10 min, 1 hr, or overnight.

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Ms received 11/3/73; revised 5/31/74; accepted 6/3/74.

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EVALUATION OF FISH PROTEIN CONCENTRATE AS A REPLACEMENT FOR DRY SKIM MILK IN LAUBINA WEANING FOOD MIXTURES

INTRODUCTION

THE SHORTAGE of high quality protein, such as animal protein in the diet of preschool children in many developing areas, has resulted in the large scale occurrence of protein malnutrition and its many consequences. For these reasons, attempts have been made to develop in various areas of the world, nutritionally improved blended foods from inexpensive locally available food materials. Protein obtained from cereals such as wheat, corn, or rice can be greatly improved, frequently to the level of animal protein, by increasing the levels of the limiting essential amino acids by direct fortification, by adding protein supplements, or by the admixture with legume in a proportion designed to provide an optimum amino acid balance. In an attempt to meet a need for low cost high-protein weaning foods for use in the areas of Lebanon and Jordan, the cereal mixtures called "Laubina" were developed at the Nutrition Research Laboratory of the American University of Beirut (AUB) in conjunction with Columbia University's Institute of Nutrition Sciences (now the Institute of Human Nutrition).

The principal constituents of the early mixtures were burghul (i.e, parboiled wheat) and chick-peas (Cowan and Pellet, 1969). Later mixtures substituted lentils for the chick-peas. Dry skim milk, bone ash, sucrose, citric acid and vitamins A and D were the minor ingredients used. The compositions of two principal mixtures developed, Laubina 104 and Laubina 106, as reported by Cowan and Pellet (1969), are shown in Table 1.

As shown in Table 2 burghul is lower in lysine and threonine than either chick-peas or lentils. Lentils, on the other hand, are lower in tryptophan and methionine than either chick-peas or burghul.

In view of the declining supply of milk protein for use in feeding programs in developing countries, there has been considerable interest in the potential utilization of fish protein concentrate (FPC) as a new low cost, protein source. The purpose of the present study was to evaluate the feasibility of substituting FPC, made

from hake by the isopropyl alcohol extraction method, for the dry skim milk (DSM) included in the Laubina mixtures. FPC can be made using underutilized species of fish, such as hake as raw material. After the moisture and fat have been removed by solvent extraction, the result is a finely ground powder containing 80–85% animal protein, high in the essential amino acids.

EXPERIMENTAL

Materials

The food materials used in this study were Laubina mixtures No. 104 and No. 106 prepared at the AUB in accordance with the formulation shown in Table 1. In addition, the same mixtures were also prepared without DSM, the original proportions of the remaining ingredients being constant. Details of preparation of Laubina have been published by Cowan and Pellett (1969). FPC was prepared at the Technological Laboratory of the Bureau of Commercial Fisheries in College Park, Md. The sample was described as: FPC, Hake-Isopropyl alcohol extracted, code BH-19/22, date 12/6/67 (sample provided for experimental use only).

Chemical analysis

Proximate analysis. Nitrogen, crude fat (ether extract), and moisture determination were performed according to the methods of the AOAC (1965).

Amino acid determinations. *Acid Hydrolysis:* Potential conditions affecting the destruction of amino acids in preparative acid hydrolysis include duration of the hydrolysis. The destruction of some amino acids may be affected also by the type of protein and the nonprotein components, which occur naturally or which are added to aid in the hydrolysis of the samples.

Block (1956) suggested that the rate of liberation of amino acids during the hydrolysis varies and different conditions of hydrolysis will result in difference in levels of individual amino acid found. The amino acid content in a fish meal sample decreased with the increasing duration of hydrolysis in the case of histidine, threonine, serine, methionine and tyrosine. It increased in the case of glutamic acid. On the other hand, in a sample of FPC, threonine and serine decrease whereas isoleucine increased (Smith et al., 1965). Taking all these factors into consideration, samples corresponding to 7 mg of protein were accurately weighed and hydrolyzed with 2 ml of 6N HCl in vacuum at 110°C for 22, 43 and 72 hr. After hydrolysis, the hydrolysates were transferred into beakers and brought to dryness in a vacuum desiccator in the presence of solid NaOH. The dried residue was washed twice with 1 ml of distilled water and each time it was evaporated to dryness. Finally the residue was diluted to 2 ml with pH 2.2 citrate buffer, then it was filtered

Table 1—Ingredients in Laubina 106 and Laubina 104 when supplemented with dry skim milk (DSM)[a]

Mixture	Composition (%)						
	Burghul	Chick-peas	White-lentil	DSM	Bone ash	Sucrose + Vit. A&D	Citric acid
Laubina 104	62	25	—	10	1	1	1
Laubina 106	60	—	28	10	1	1	—

[a] Cowan and Pellett (1969)

Table 2—Amino acid content of chick-pea, burghul and lentil[a]

	Amino acids (mg/g)						
	Try	Thr	Ileu	Leu	Lys	Met	Cy
Chick-pea	86	248	296	501	463	91	93
Burghul	45	172	195	390	160	90	123
Lentil	36	266	282	485	484	60	60

[a] Jamalian and Pellett (1968)

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Table 3—Composition of experimental diets per 100g

Diet ingredients	Basal diet	106(DSM)[a]	106(FPC)[b]	104(DSM)	104(FPC)
Casein	11.1				
Salt mixture	5.0	5.0	5.0	5.0	5.0
Vitamin mixture	1.0	1.0	1.0	1.0	1.0
Cellulose	1.0	1.0	1.0	1.0	1.0
Water	4.5	0.9	1.9	0.3	0.2
Corn oil	7.9	7.3	7.8	7.1	7.5
Corn starch	69.6	27.3	41.9	16.3	20.9
Laubina 106(DSM)		57.5			
Laubina 106(FPC)			41.5		
Laubina 104(DSM)				69.3	
Laubina 104(FPC)					64.4
TOTAL	100.0	100.0	100.0	100.0	100.0

[a] DSM = dry skim milk
[b] FPC = fish protein concentrate

Table 4—Formula for baby cereal, soup and cookies

Ingredients	Baby cereal (%)	Soup (%)	Cookies (%)
Laubina	25.0	20.0	39.0
Water	62.8	72.5	30.0
Sugar	10.0	—	16.0
Corn oil	2.0	5.0	9.0
Baking powder	—	—	3.8
Cinnamon	—	—	2.0
Vanilla	0.2	—	0.2
Salt	—	1.0	—
Pepper	—	0.5	—
Celery seeds	—	0.5	—
Onion powder	—	0.5	—
TOTAL	100.0	100.0	100.0

and refrigerated until used in the amino acid analyzer.

Tryptophan is destroyed entirely by acid hydrolysis. A method for the analysis of tryptophan in certain samples is the colorimetric determination by Spies and Chambers (1949) and by Wilkening et al. (1947). The technique suggested and described by Lombard and DeLange (1965) was tried several times for tryptophan determination in these mixtures and FPC but the results were not reproducible and reliable. Cystine was also not determined because it did not appear clearly in the graphs of the amino-acid analyzer.

Chromatography: For the amino acid estimation a Beckman Amino-Acid Analyzer, Model 120C was used.

Protein quality test protein efficiency ratio (PER). Male rats of the Windsor colony, 21 days of age, were used as experimental animals. The procedure recommended in the AOAC (1965) was followed in all respects.

The rats were divided randomly into five groups, ten rats in each group. Average weight of rats in any one group, on the day beginning the assay period, did not exceed by 5g the average weight of rats in any other group. Throughout the assay period each rat was kept in an individual cage and provided with the appropriate assay diet and water. Body weight of each rat was reported on the beginning day of assay period, the body weight and food intake of each rat was reported at regular intervals, twice a week, and on the 24th and 28th day, at the end of the assay period. The rats were housed individually in screen-bottomed cages in a room at approximately 24°C, with food and water supplied ad libitum. One of the groups received ANRC reference casein (Sheffield Chemical Co., Norwich, NY), which was incorporated into a basal diet (corn oil, salt mixture, cornstarch, plus complete vitamin mix) (Jansen et al., 1966). Each group of the others received a different diet as shown in Table 3. Laubina 106 and Laubina 104 contain 10% DSM. The supplementation of the DSM-free mixtures with FPC was based on the amino acid determination to raise the limiting amino acids to the level of hen's eggs (NAS-NRC, L963), Laubina 106(FPC) contained 14.05% FPC and Laubina 104(FPC) contained 2.91% FPC.

Table 5—Proximate composition of Laubina mixtures, FPC and casein

Product	Nitrogen (%)	NX6.25 (%)	Ether extract (%)	Moisture (%)
Milk-free				
Laubina 106	2.30	14.37	1.11	7.97
Laubina 106(DSM)	2.78	17.38	1.24	7.09
Milk-free				
Laubina 104	2.06	12.88	1.60	8.69
Laubina 104(DSM)	2.31	14.44	1.24	6.77
FPC	13.96	87.25	0.70	5.11
Laubina 106(FPC)	3.86	24.13	0.50	7.57
Laubina 104(FPC)	2.49	15.54	0.70	7.42
Casein	14.42	90.13	0.78	5.55

Sensory evaluation

The objective of the sensory testing was to evaluate the acceptability of Laubina 106(FPC) and Laubina 104(FPC) by determining whether panelists could distinguish products made with the FPC Laubinas from products made with the DSM Laubinas. The triangular method was thought to be best suited for this purpose. A 12-man panel using laboratory personnel was used.

All the mixtures were prepared in three different forms: baby cereal (semi solid), soup (thin gruel), and cookies. Cooking times and temperatures were standardized for each set of samples which were compared. All samples of compared foods were tested at the same temperature. Also the samples were prepared and made as homogenous and uniform as possible.

The formulae used in preparing the products are shown in Table 4.

Procedures. Baby cereal: Dry ingredients were blended. Oil and water were mixed. The blended dry ingredients were added to the liquid and cooked for 15 min.

Soup: Dry ingredients were blended and added with the oil to the water. The mixture was cooked for 10 min.

Cookies: Dry ingredients were blended, the oil was added and mixed, the water added, mixed and shaped into cookies which were baked for 30 min at 188°C.

RESULTS & CALCULATIONS

Chemical analysis

Table 5 summarizes the results of nitrogen, protein, crude fat and moisture determinations.

The amino acid contents in g/16g of N were calculated by standard methods. The highest value for each amino acid encountered in any of the hydrolyses (i.e., 22, 43, or 72 hr) is reported because it was thought to be the most accurate. Table 6 summarizes the results.

Protein quality test

The supplementation of both Laubinas with FPC was based on the requirement for raising the level of the potentially limiting amino acid methionine. Hen's eggs (FAO, 1965) were used as reference.

Table 6—Essential amino acid content of Laubina mixtures and FPC (g/16g N)

Amino acid	Laubina 106	Laubina 106 (DSM)	Laubina 104	Laubina 104 (DSM)	FPC
Isoleucine	3.97	4.32	4.50	5.36	3.86
Leucine	7.02	7.19	7.71	9.03	6.62
Lysine	5.45	5.81	5.05	6.56	8.37
Methionine	0.59	0.94	0.80	1.03	2.02
Phenyl- alanine	4.54	4.36	5.51	5.95	3.34
Tyrosine	2.02	2.27	2.04	2.60	2.34
Threonine	3.16	3.25	3.21	3.63	3.91
Valine	5.28	5.43	5.57	7.05	4.76

Table 7—Protein efficiency ratio (PER)

Kind of Diet	Experimental				Standardized[a]
	Up to the 24th day		Up to the 28th day		
	Avg	Std Dev	Avg	Std Dev	
Control	3.62	0.28	3.62	0.22	2.50
Laubina 106(DSM)	3.11	0.34	2.95	0.27	2.15
Laubina 106(FPC)	3.67	0.19	3.66	0.20	2.53
Laubina 104(DSM)	3.50	0.33	—	—	2.42
Laubina 104(FPC)	3.28	0.27	—	—	2.27

[a] By reference to casein = 2.50; based on 24 days' growth

Table 8—Results of triangular test

Test	Total No. of respondents	No. of correct identifications	Level of significance
Baby cereal			
106(FPC) vs. 106(DSM)	12	8	0.05
104(FPC) vs. 104(DSM)	12	7	N.S.
Soup			
106(FPC) vs. 106(DSM)	12	7	N.S.
104(FPC) vs. 104(DSM)	12	7	N.S.
Cookies			
106(FPC) vs. 106(DSM)	10	2	N.S.
104(FPC) vs. 104(DSM)	10	3	N.S.

The percentages of FPC needed for the supplementation on this basis were calculated to be 14.05% for Laubina 106 and 2.91% for Laubina 104.

The Laubina mixture supplemented with the above percentages of FPC were used for the rat assays. Because two groups of rats were terminated after the 24th day (as a result of circumstances not involving the health of the animals or quality of the diet), the PER has been calculated for all the five groups up to the 24th day and for three groups up to the 28th day. The PER are given in Table 7.

Organoleptic evaluation

The results obtained in the triangular tests were tabulated and compared with the table of different levels of significance (Hall Ellis, 1961). Table 8 presents the results of the taste panel tests.

As shown, significant difference at p=0.05 was detected only when 106-(FPC) was compared with the control in the triangular test of a baby cereal. Although there is a lack of statistical significance in all other cases, the fewest number of correct panelist selections occurred in the case of the cookies.

DISCUSSION

THE SUPPLEMENTATION of Laubina with FPC was carried out, as shown, on the basis of the limiting amino acid, methionine. Because the methionine content of chick-peas is greater than that of lentils, the Laubina 106 mixture which contains lentils as the legume required a higher level of FPC than did the Laubina 104. Cowan and Pellett (1969) have previously reported that tryptophan is probably the first limiting amino acid in Laubina, and since the object of this study was to evaluate the replacement of DSM by FPC, no particular attempt was made to raise the tryptophan level.

The PER value obtained for Laubina 104 with 10% DSM was higher than that containing 2.91% FPC, but statistical analysis showed that the difference was not significant at the 95% confidence level. In the case of Laubina 106, the PER was significantly higher for the mixture containing 14.05% FPC than for the control with 10% DSM. It should be pointed out, however, that the chick-pea containing control mixtures showed a significantly higher PER than the Laubina 106(DSM). Thus it appears that the protein quality of the Laubina 104(DSM) is superior to that of the Laubina 106 (DSM).

The limitations of the PER method in evaluating protein quality are well recognized by workers in the field. While it was not possible is this study to conduct additional protein quality determinations, it would certainly appear on the basis of the present data, that the replacement of dry skim milk by fish protein concentrate is not likely to reduce the nutritive value of the Laubina weaning foods. Furthermore, the results of the organoleptic tests conducted indicate that acceptance problems for the FPC-containing Laubina mixtures are improbable. Considerable improvements in the processing of FPC prepared by solvent extraction have taken place since the production of the FPC used in these studies. These improvements are particularly related to the problem of particle size. Some of the panelists in our studies, who were able to distinguish between the Laubinas with FPC and those with dry skim milk, indicated some dislike of the texture of the mixtures containing FPC. It is believed that the newer qualities of FPC available would probably overcome such criticism.

CONCLUSIONS

BY REFERENCE to hen's eggs, the limiting amino acid in milk-free Laubina was methionine. Levels of FPC required to raise the methionine levels to that of hen's eggs were: 14.05% FPC in the case of Laubina 106 and 2.91% FPC in the case of Laubina 104. The PER's for the Laubinas 104 and 106 with FPC were determined to be 2.27 and 2.53 respectively, compared to 2.42 and 2.15 for Laubinas 106 and 104 with DSM. Organoleptic evaluation of baby cereals, soups

and cookies made with Laubina-FPC mixtures showed no significant differences from those made with milk, except for Laubina 106 when used in baby cereal.

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ELEMENTAL ANALYSIS OF PROTEIN-CONTAINING
FOOD MATERIALS FROM VARIOUS SOURCES

INTRODUCTION

THE PRESENT world food shortage has stimulated investigations of a number of old and new possible sources of protein. These include land plants (Arehart-Treichel, 1973), marine plants such as algae (Mann, 1973) and seaweed (Guiseley, 1968; Moirano, 1967), marine fish (Lunde, 1973), and even petroleum (gas-oil) (de Pontanel, 1972) and tires (Chem. & Eng. News, 1972) upon which certain microorganisms can grow and proliferate to yield a source of protein. Of necessary concern is the growing practice among poorer classes in this country of supplementing their diet with commercial dog foods. Certain of these sources may be expected to contain various elements some of which are toxic. The present study was undertaken to determine the concentrations of a broad range of elements in such food materials.

METHODS & MATERIALS

A VARIETY of samples were obtained. These are summarized in Table 1. The samples represented commercial products so that the source location of the specific fishes, plants, petroleum, etc., was only generally known. The materials were ground, mixed and subsampled for analysis. About 1g of each sample was weighed into a clear polyethylene vial (1.5 cm i.d. × 2 cm high) and analyzed for a range of elements by nondestructive neutron activation analysis. The samples were irradiated twice, once for a short period (which varied depending on sodium and chlorine content but on the order of 1 min) and again for a period of approximately 4 hr. The neutron flux to which the samples were exposed was about 10^{12} neutrons/cm^2/sec (1.2 × 10^{12} for the short irradiation and 1.3 × 10^{12} for the long irradiation). After the short irradiations the samples were counted within a few minutes on a Ge(Li) counting system for a period of 8 min. Data were acquired using an Intertechnique SA-44 4000 channel analyzer and stored on magnetic tape for later processing. For the long irradiations, the samples were counted as soon as practical, considering the level of the activity due to sodium. This period of time varied from 2–5 days after the end of the irradiation. The samples were recounted later after a minimum of 10 days decay.

After acquisition, the data were processed using an IBM 370 computer and a locally developed program. The main function of this program is to find the areas under preselected peaks in the gamma spectra. The program fits the peaks to a modified Gaussian distribution, assuming a small asymmetry for the peaks, on a background represented by a quadratic polynomial and is capable of subtracting interference due to peaks close to the peak of interest. Computation of parts per million was based on comparison of the peak areas determined as above with peak areas determined for standards run under known circumstances.

The determination of cadmium and lead was performed by dry ashing 5g of the samples (up to 475°C) by the respective AOAC methods (AOAC, 1965) followed by analysis using conventional flame atomic absorption spectrophotometry with a Perkin Elmer Model 303 instrument. Mercury was measured by oxygen flask combustion (Gutenmann and Lisk, 1960) of 1g samples and flameless atomic absorption analysis (Hatch and Ott, 1968). The atomic absorption methods were sensitive to about 0.1, 1 and 0.04 ppm of cadmium, lead and mercury, respectively.

RESULTS

THE NEUTRON activation analysis method was capable of determining 61 elements. The concentrations of elements found in the samples are listed in Table 2. In addition, a number of other elements were analyzed and found to be less than their respective limits of detection by the method. These elements with their respective limits of detection in ppm were as follows: cerium (0.065), dysprosium (0.0005), erbium (0.0023), europium (0.000018), fluorine (6.1), gadolinium (0.048), gallium (0.00093), germanium (0.31), gold (0.0017), iridium (0.00041), lutecium (0.00022), molybdenum (0.073), neodymium (0.084), neon (6.3), nickel (1.3), osmium (0.19), palladium (0.042), platinum (0.20), praseodymium (0.016), rhenium (0.0002), rhodium (0.003), samarium (0.00014), tantalum (0.088), terbium (0.037), thorium (0.036), thulium (0.027) and zirconium (0.49). These detection limits are interference-free limits and hence may be somewhat low. However, the results which were experimentally obtained approached the comparable limits.

The accuracy of the method was within ± 5% when comparing the response of pure standard elements and the response when the elements were present in the sample matrix. The reproducibility of the method when analyzing replicates of the same sample was ± 10% or better.

When examining the data in Table 2

Table 1—Description of samples analyzed

Sample no.	Description
1	Fish protein conc from silver hake (Merluccius bilinearis)
2	Fish protein conc from swordfish (Xiphias gladius)
3	Fish protein conc from assorted marine fish including hake, various species of menhaden (Brevoortea) and others
4	Dried brown seaweed (sample a) from Asia
5	Dried green seaweed (Undaria Spp.) (sample b) from Asia
6	Dried fermented sheeps milk food of nomadic origin (kish) from Southwest Asia
7	Yeast protein from petroleum gas oil (Candida Spp.) from Europe
8	Yeast protein from waste sulfite liquor (Candida utilis) from North America
9	Fermented rice (Tapé), 15% protein
10	Soybean protein isolate
11	Commercial dog food
12	Commercial sodium alginate

Table 2–Elemental content of food materials from various sources (ppm dry wt)

Element	1	2	3	4	5	6	7	8	9	10	11	12
						Sample code[a]						
Aluminum	145	14.5	237	10.9	98.0	119	22.1	17.0	21.6	67.8	272	234
Antimony	0.12	0.16	0.14	0.64	0.48	0.42	0.33	0.070	0.20	0.090	0.16	0.41
Arsenic	<0.00063	0.02	<0.00063	4.3	3.4	<0.00063	0.020	0.036	0.12	0.014	0.025	0.1
Barium	1.4	1.8	3.5	23.2	36	7.2	10.7	9.6	22.3	14.1	25.0	673
Bromine	33.8	5.1	21.9	615	234	37.2	2.4	0.22	13.1	1.0	13.7	25.5
Cadmium	0.59	0.44	0.12	0.21	0.90	0.21	0.26	0.43	0.10	0.17	0.57	0.20
Calcium	21840	1722	37100	4300	5400	2940	2050	416	447	759	19200	5300
Cesium	0.12	0.035	0.05	0.10	0.068	0.085	0.2	0.3	0.10	0.056	<0.0045	0.10
Chlorine	3480	394	2335	11100	16000	17400	382	35.4	170	48.5	1550	690
Chromium	5.14	1.80	6.2	1.4	1.1	1.1	5.2	4.7	0.10	6.6	31.7	6.4
Cobalt	1.70	0.90	0.52	6.5	2.2	0.45	0.29	0.35	0.44	0.21	5.1	0.65
Copper	26	9.3	20.6	53.0	40.0	37	5.2	13.7	7.4	20.1	27.0	14
Hafnium	<0.13	<0.13	<0.13	1.2	0.9	1.0	<0.13	<0.13	<0.13	<0.13	1.3	4.5
Indium	<0.00004	<0.00004	<0.00004	<0.00004	<0.00004	0.13	0.013	0.003	<0.00004	<0.002	<0.00004	0.016
Iodine	4.4	<0.0026	2.5	345	31.4	1.2	<0.0026	0.63	0.16	2.4	2.4	3.0
Iron	892	85	1543	65	148	118	680	408	23	536	705	290
Lanthanum	0.10	0.072	0.15	0.40	0.09	0.09	0.05	0.04	0.01	0.022	0.18	0.24
Lead	7.5	2.3	6.7	11.5	8.2	3.1	11.5	1.0	<1.0	1.2	5.4	1.8
Magnesium	1200	1324	1994	4193	5345	640	2582	1610	815	172	1600	480
Manganese	19.0	1.1	46.6	6.9	7.0	3.2	55	151	27.0	9.2	257	1.47
Mercury	0.32	9.40	0.33	0.04	0.12	0.04	0.20	0.05	0.13	0.02	0.04	0.04
Potassium	974	744	810	2410	2520	537	3144	1850	116	19.0	854	321
Rubidium	<1.1	55	2.5	<1.1	<1.1	<1.1	61	22.2	16.2	<1.1	10.2	<1.1
Ruthenium	<0.15	0.36	<0.15	<0.15	<0.15	<0.15	0.5	0.28	<0.15	<0.15	1.03	21
Scandium	0.056	0.022	0.011	0.015	0.070	0.057	0.014	0.030	0.036	0.029	0.072	0.049
Selenium	1.4	5.0	5.4	<0.26	7.5	<0.26	<0.26	<0.26	1.6	<0.26	<0.26	<0.26
Silver	<0.038	0.07	<0.038	1.6	<0.038	<0.038	0.7	<0.038	1.2	0.10	<0.038	<0.038
Sodium	44/0	765	4594	15930	26200	23800	456	50.5	22.5	73.4	2430	61000
Strontium	81	<0.028	194	<0.028	504	<0.028	0.028	58	<0.028	<0.028	<0.028	103
Sulfur	<245.0	7500	12000	39400	77600	77600	7200	2600	<245.0	8300	<245.0	<245.0
Tin	<0.50	5.2	<0.50	21.6	187	150	<0.50	<0.50	<0.50	<0.50	16	19
Titanium	18.5	6.7	23.0	198	0.74	1.8	2.7	7.4	3.2	3.1	17.8	23.0
Tungsten	0.4	1.5	1.0	<0.0016	<0.0065	<0.0065	0.96	0.72	1.7	1.11	1.34	0.88
Uranium	<0.0065	<0.0065	<0.0065	<0.0065	<0.0065	<0.0065	0.42	<0.0065	0.07	0.13	1.0	0.54
Vanadium	1.0	0.12	1.27	0.50	0.74	0.82	0.13	0.21	0.042	0.63	1.6	0.24
Ytterbium	0.012	0.012	0.012	0.012	0.012	0.012	0.012	0.012	0.012	0.012	0.012	0.24
Zinc	35	95	53	5.0	18.8	5.8	1210	64.0	25	35	60	4.8

a See Table 1.

the presence of appreciable concentrations of the more toxic elements are of most significance. These elements include antimony, arsenic, barium, cadmium, lead, mercury, selenium, silver, strontium, uranium and possibly zinc (Browning, 1969). Also of interest are those more recently suspected of being possibly essential which are chromium (Mertz, 1969; Schroeder, 1970), tin (Schwarz et al., 1970), and vanadium (Lisk, 1972). Hopefully, in order to explain the magnitudes of the concentrations of these elements found in the various samples, Table 3 was prepared. It includes literature values, as many as could be found, of the concentrations of the above-mentioned elements in certain of the types of samples analyzed prior to their processing for food preparation. Where a concentration range is reported (Table 3) it represented the lowest and the highest levels of the element found in samples of the same food or food source often from widely scattered geographical locations.

In comparing the data in Tables 2 and 3 certain observations may be made. Data are sparce on the concentrations of antimony in biological material. The values obtained in this study would indicate that its content is higher in marine than in land plants.

Literature values for arsenic are comparatively high in marine plants especially seaweed and these levels are reflected in the concentrations found in the dried seaweed samples and to a lesser extent in sodium alginate. Similarly, barium appeared at a relatively high level in sodium alginate and in dried seaweed as might be expected from the literature values.

Considering its high toxicity, cadmium was present at appreciable levels in two of the three fish protein samples. Cadmium was not found to be cumulative in a series of lake trout of known ages ranging from 1–12 yr (Lovett et al., 1972) and therefore may not necessarily be expected to be higher in swordfish (sample no. 2) than in smaller fish species such as hake (sample no. 1). Regarding the petroleum protein sample produced in Europe, two other samples of yeast (Candida and Pichia Spp.) protein food material produced in Asia from petroleum n-paraffins contained 0.21 and 0.28 ppm cadmium.

It is difficult to generalize regarding the concentrations of chromium found. Chromium tends to be poorly absorbed from soils by land plants (Allaway, 1968). It should be noted that chromium in brewers yeast contains the element in a highly assimilatable organic form (perhaps as an organic porphyrin complex) (Mertz, 1969). Since chromium now appears to be an essential element, the presence of chromium in yeast grown on gas oil or sulfite liquor may be a valuable and available source of the element.

Lead was notably higher in foods of marine origin than protein from land plants. This situation can easily be reversed by local contamination of land plants from nearby industry, vehicles, power plants and other sources (Lisk, 1972). Lead in protein food from petroleum may also be due to inadvertent contamination from contact with metal processing equipment during production. Two other protein food materials produced from petroleum in Asia were found to contain 2.9 and 4.1 ppm lead.

Mercury, as might be predicted (Henderson et al., 1972; Jernelöv and Lann, 1971; Miller et al., 1972; Friberg, 1971), was much more concentrated in fish protein than other samples. Selenium was notably present in marine foods. It has been shown that the major portion of selenium remains with the protein phase when the oil and fish meal are separated (Lunde, 1973). This is probably due to its close chemical resemblance to sulfur.

Seaweed has been shown to contain appreciable concentrations of silver (Table 3) which would correlate with that found in one of the dried seaweed samples (no. 4). The value of 11 ppm reported for silver in marine fish was listed as questionable (Bowen, 1966) and therefore may not be pertinent. Strontium was found mostly, and at high levels, in foods of marine origin. Tin was found in marine foods which probably reflects the higher concentrations reported for marine organisms in Table 3. Except for the concentration range given for uranium in petroleum which might be of geochemical origin, it is difficult to speculate about the appreciable levels of the element in dog food, sodium alginate, etc. It may be that algae, rice and soybeans are able to concentrate the element to these extents from their water and soil media. Vanadium was detected in all food samples and does not seem to reflect the higher concentrations reported especially in marine plants.

The content of zinc in the foods roughly reflects the concentrations in the respective food sources with the exception of sodium alginate and protein food material from petroleum. It is suspected that the zinc in protein food material from petroleum perhaps may derive from zinc contamination of the gas oil when

Table 3—Literature values[a] for the approximate elemental composition of foods and food sources

	(ppm dry wt)					
Element	Marine fish	Marine algae	Seaweeds	Petroleum	Dairy products	Cereals and grains
Antimony	0.2	–	–	0.03–0.11	–	–
Arsenic	0.3	30	2–58	0.05–1.1	0–0.23	0–2.4
Barium	–	23	2–120	–	–	–
Cadmium	3	0.4	–	0.0015–0.018	0.10–0.14	0.04–0.06[R]
Chromium	0.2	2.4	0.1–3.7	0.002–0.017	0.01	0.04–0.05[R]
Lead	0.5	6.7	0.7–26	–	0–0.79	0–0.1[R]
Mercury	0.16–2.8	0.03	–	0.02–30	0.001–0.027	0.008–0.029[S]
Selenium	0.5–4	0.84	–	0.03–1.4	0.06–1.46	0.02–0.63
Silver	11 ?	0.27	0.1–0.7	0.0002	–	–
Strontium	–	570	77–4000	–	–	–
Tin	3	17	0.03–2.2	–	0.19–0.96	0.28[R]
Uranium	–	–	–	0.004–0.4	–	–
Vanadium	0.14	3.5	0.07–11.9	0.004–0.3	0.01	0.23–0.82[R]
Zinc	80	150	8–136	3.6–85.8	0.14–0.45	0.62–1.61[R]

[a] Generalized from Black and Mitchell (1952); Bowen (1966); Shah et al. (1970a, b); Schroeder and Balassa (1961, 1966); Schroeder et al. (1962, 1963, 1964, 1967, 1970); Tanner et al. (1972); Tkachuk and Kuzina (1972); Williams and Weiss (1973); Young and Langille (1958).
[R] Element concentration in rice
[S] Element concentration in soybeans

the heated petroleum feedstock may, for instance, be desulfurized by passing over a zinc oxide bed to reduce hydrogen sulfide concentrations (Chem. & Eng. News, 1973). Contamination from contact with metal processing equipment is also possible. Concentrations of 358 and 723 ppm of zinc were found in the two samples of yeast (Candida and Pichia Spp.) protein food material made in Asia from petroleum n-paraffins. It is worth noting that zinc is becoming ever more deficient in the human diet (Allaway, 1968). It may be therefore that protein from petroleum, if high in zinc, could be purposely blended with other forms of protein low in zinc to correct the deficiency similar to what is now done with wheat high in selenium (Allaway, 1968).

Other elements such as aluminum, calcium, chlorine, iron, magnesium, manganese, potassium, rubidium, sodium, sulfur and titanium are expectedly high owing either to their essentiality, ubiquitousness, ease of absorption or concentration in the growth media. Bromine and iodine were found particularly high in seaweed which has long been known. Seaweed would appear to also be a good source for the essential elements, cobalt and copper if they are indeed present in assimilatable forms.

It must be assumed that the remaining elements detected at low concentrations including cesium, gold, hafnium, indium, lanthanum, ruthenium, scandium, tantalum, thorium and tungsten were probably present as nonessential trace contaminants. It is interesting to note, however, that a large number of rare elements was recently reported at surprisingly high concentrations in fresh water aquatic plants (Cowgill, 1973).

Some general statements can be made which can affect the presence of foreign elements in the various sample types studied. Virtually all elements are contained in ocean water and marine organisms vary widely in their ability to concentrate or exclude specific elements. For example, the ratio of the concentrations of fluorine and iodine in seaweed to that in the surrounding seawater are, respectively, 1 and 60,000 (Young and Langille, 1958). The ratio of the concentrations of strontium and titanium in algae to that in the surrounding seawater are respectively, 8 and 10,000 (Black and Mitchell, 1952). Marine fish similarly vary in their ability to concentrate different elements (Lisk, 1972).

With the exception of lead and possibly antimony, the dried fermented sheeps milk sample was comparatively low in its content of toxic elements. This may be due to the ability of the mammary gland to exclude these elements. Less than 0.02% of lead fed as lead nitrate to cows was excreted in the milk (Stanley et al., 1971).

The elemental content of petroleum varies greatly depending on the geochemistry of the region of origin. The ability of land plants to absorb and concentrate toxic elements varies greatly. Thus plants may absorb five times the concentration of cadmium that is present in the soil but only 0.05 of the concentration of nickel (Lisk, 1972). Submergence of soil for the growth of rice also alters the availability of soil nutrients by reducing conditions which may lower the valence and change the solubility of the element.

Except for cadmium, protein from waste sulfite liquor did not appear to contain excessively high levels of toxic or unusual elements. Yet trees, especially deep-rooted types, may absorb rarer elements not accessible to shallow rooted plants. Thus nuts have been found to contain a variety of rare earth elements (Robinson, 1943). Also limestone which is used in quantity in the process of paper manufacture contains many toxic elements as impurities (Bowen, 1966). Thus it may be that the microorganisms involved in the production of protein material from waste sulfite liquor (and petroleum gas oil) are able to exclude various toxic elements during their proliferation in the growth medium.

In summary, from the data obtained the main concern regarding toxicity would be the high levels of mercury in swordfish protein concentrate, possibly zinc in protein from petroleum gas oil (Browning, 1969) and elevated levels of cadmium and lead in several of the samples. Beyond this it must be emphasized that it is very difficult to speculate regarding the significance of various elements found in a very limited number of food samples of diverse origin. Elements which may appear to be present at toxic levels may be incorporated in forms which are not absorbed by predators or consumers. This is true of arsenic in shrimp which is high in total concentration but is present in a form which is rapidly excreted (Coulson et al., 1935). Furthermore numerous environmental factors may cause variations in the elemental composition of land plants and aquatic organisms such as soil type and fertility, nearby mineral deposits (Lovett, 1972), plant variety, seasonal variations and contamination resulting from industry, power generation, transportation and food processing.

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SOME FACTORS INFLUENCING GELATION AND STABILITY OF SOY PROTEIN DISPERSIONS

INTRODUCTION

THE STUDY of isolated soy proteins as a gelling medium has been investigated by several workers (Circle et al., 1964; Catsimpoolas and Meyer, 1970, 1971a, b; Aoki, 1965a, b, c; Aoki and Sakurai, 1968, 1969).

The study by Circle et al. (1964) showed that gelation of soybean globulins in heated dispersions was a factor of isolate concentration, pH, length of heating and temperature. In a study by Aoki (1965c) anions were found to have a stronger effect on water-holding capacity than cations. Water-holding capacity was also found to govern the state of the gel. Catsimpoolas and Meyer (1970) explained the gelling phenomenon as a transition of the protein from the sol-progel-gel states which are greatly affected by pH, temperature and ionic strength. Catsimpoolas and Meyer (1971a, b) have also shown that aliphatic chain length of either a water miscible solvent or a triglyceride affected gelation.

The present study is concerned with a continual investigation of gelling at lower concentrations of isolate and at pH values which are more feasible in a food system. The interactions of lower molar levels of sodium chloride on gelling at various concentrations of isolate and pH values were also investigated. In this work the effects of sugars with different molecular weights, sucrose and dextrose, were investigated.

Stability of heated dispersions as a function of freezing and thawing has not been reported in the literature. The influence of NaCl and sugars on the stability of the heated dispersions subjected to freezing and thawing was investigated.

MATERIALS & METHODS

Soy protein isolate

The two soy protein isolates (Promine D and Promine R manufactured by Central Soya Co., Chemurgy Div., 1825 N. Laramie Ave., Chicago, IL 60639) used in the study are commercially available. One is a sodium soy proteinate (Central Soya Co., Promine–An Isolated Soy Protein. Technical Service Bulletin, p. 3) in the neutralized form and water dispersible. It has the following proximate analysis: moisture, 4.7%; protein (N × 6.25) 91.8%; protein (moisture-free basis) 96.5%; water dispersible protein, 75%; crude fiber 0.1%; ash 3.4%;

and pH in aqueous dispersion 7.0. The other isolate is isolated soy protein in the isoelectric form.

Both isolates were made from defatted soybean flakes which are extracted by a slightly alkaline, aqueous solution. The protein extract is then separated, acidified to f rm a curd, and then washed and either neutralized with food grade NaOH and dried or just dried.

Preparation of dispersions

In this study the effect of pH and concentration on the gel viscosity and stability were investigated. The pH of the dispersions was adjusted using a citrate-phosphate buffer. A 0.1M citric acid solution and a 0.2M dibasic

Table 1—pH of the heated dispersions

SSP			ISP		
Distilled H_2O	Buffer pH	Gel pH	Distilled H_2O	Buffer pH	Gel pH
7.0	6.5	6.6	5.35	6.5	6.17
	6.0	6.2		6.0	5.78
	5.5	5.85		5.5	5.42
	5.0	5.4			
	4.5	5.2			

Fig. 1—Effect of pH on apparent viscosity at various concentrations of heated SSP dispersions.

potassium phosphate (Na_2HPO_4) were mixed in varying amounts to obtain the desired pH.

Concentrations of the isolate varied from 6–14% by weight.

The influence of sucrose, dextrose and NaCl were also studied on gel viscosity and stability on both isolates using the citrate-phosphate buffer at 8, 10 and 11% isolate concentrations.

Dispersions of the isolates were made on a weight percentage basis of 200 ml in 1 pt Mason-type jars. These were blended with individual adaptors on an Osterizer for 2 min at high speed and closed with Mason two-piece lids. In this manner the isolate was assumed to be dispersed. Preliminary tests demonstrated that the proteinates could not be adequately dispersed by continual agitation at lower speeds. Under conditions of dispersions, foaming was minimal in that no stable foam was produced.

The dispersions were placed in an agitating water bath kept between 92–96°C and agitated at speed 10 for 60 min. The mean internal temperature reached was 87°C which was adequate for gelation to occur. The lids of the dispersions were removed and the dispersions were placed in a tap water bath kept at 25°C until they reached 25°C. The heating and cooling of dispersions were comparatively slow; heating to the desired temperature required 60 min and cooling to 25°C required approximately 3 hr. This should eliminate any hysteretic effect due to rapid heating and cooling. All dispersions were prepared in triplicate and subsequent measurements were means of these replications.

Measurement of gelation

The viscosities of the dispersions were determined at 25°C on a Brookfield multi-speed RVD model viscometer using the spindles accompanying the viscometer which ranged from 1–7. The measurements were taken at 10 rpm on the second rotation after the film layer was removed from the gel. Since these dispersions are non-Newtonian, "apparent" viscosity was recorded.

Gel stability

The heated and cooled dispersions were stored in 50 ml beakers at −20°C for 48 hr. They were thawed and brought to 25°C to evaluate the effect of freezing and thawing on the stability of the gels.

Both freshly cooked dispersions cooled to 25°C and frozen and thawed dispersions were centrifuged in 15 ml centrifuge tubes for 5 min at speed 7 in an International clinical centrifuge model CL. To show gel stability, the supernatant after centrifuging was decanted and percentage gel was calculated.

RESULTS

Effect of pH and concentration

Figures 1 and 2 show the effect of pH and concentration for the sodium soy

Fig. 2—Effect of pH on apparent viscosity at various concentrations of heated ISP dispersions.

Fig. 4—Relationship of stability of the fresh gel to the apparent viscosity at various pH values for both SSP and ISP.
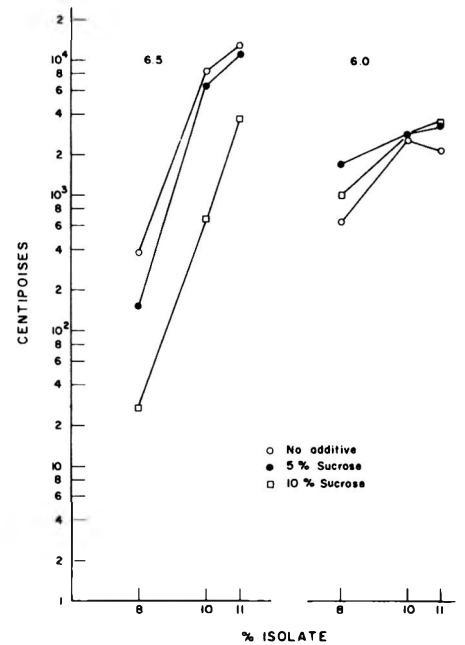
Fig. 6—Effect of 5 and 10% sucrose on apparent viscosity of 8, 10 and 11% ISP buffered at pH 6.5 and 6.0.

Fig. 3—Effect of freezing and thawing on the stability of the heated dispersions varying in concentration and buffered at various pH values. The fresh and frozen-thawed gels of SSP are shown in parts A and B, respectively; of ISP in parts C and D, respectively.

Fig. 5—Effect of 5 and 10% sucrose on apparent viscosity of 8, 10 and 11% SSP buffered at pH 6.5, 6.0 and 5.5.

proteinate (SSP) and the isoelectric soy protein (ISP), respectively. The viscosity tended to increase exponentially as the concentration increased which is a typical response of protein dispersions (Circle et al., 1964). The exponential change in viscosity with concentration was pH dependent. As the pH of the dispersions increased, the viscosity increased. This may be explained by the increased solubility of the protein at the higher pH values. As the pH of the dispersions were lowered, there was less variation in viscosity. At the lower pH values, the lower concentrations had higher viscosity readings than the corresponding concentrations at higher pH values.

Table 1 shows that the pH of the heated dispersions deviated from the pH of the buffer in which they were prepared. In preliminary investigations, other buffering systems also resulted in deviations in pH. SSP dispersions were consistently higher than the pH of the buffer and ISP dispersions were consistently lower. The probability of phosphate and citrate ions affecting soy gelation cannot be overlooked. In some protein systems, these ions influence gelation phenomena.

Since ISP is in its isoelectric form, the pH in distilled water should approximately be the isoelectric point for both isolates. The pH of ISP in distilled water was 5.35. Dispersions of SSP buffered at pH 5.0 and 4.5 thickened but did not form a continuous gel resulting from precipitation of protein. ISP dispersions clumped when the isoelectric point was approached using a buffer with a pH of 5.5. These dispersions were completely unsatisfactory. Due to the wide range of viscosities in food systems, there are no "cutoff" viscosities below which dispersions are not acceptable; other rheological factors must be considered.
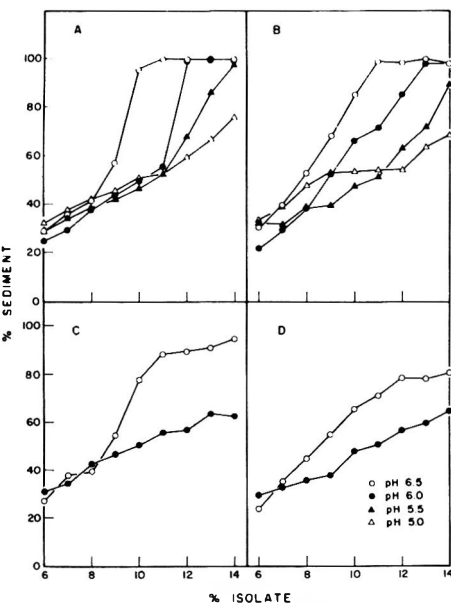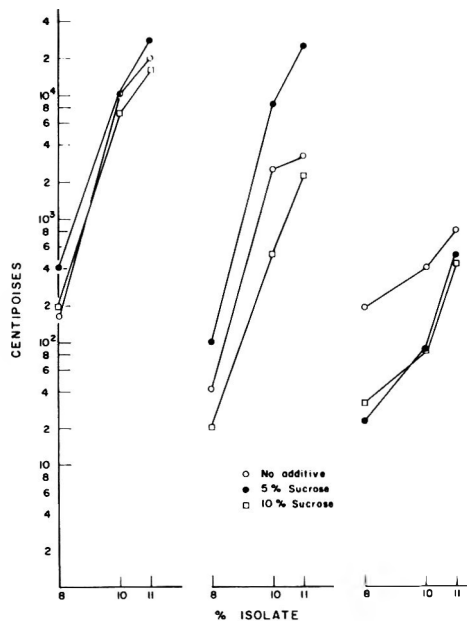
Stability of dispersion

Figure 3 shows the stability of the freshly prepared and frozen-thawed dispersions. Stability is calculated as the percentage sediment remaining after centri-
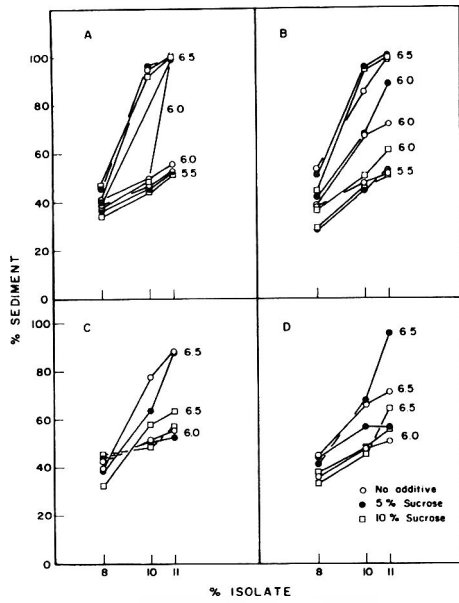
Fig. 7—Effect of freezing and thawing on stability of the heated dispersions with 5 and 10% sucrose at 8, 10 and 11% isolate levels buffered at various pH values. The fresh and frozen-thawed gels of SSP are shown in parts A and B, respectively; of ISP in parts C and D, respectively.
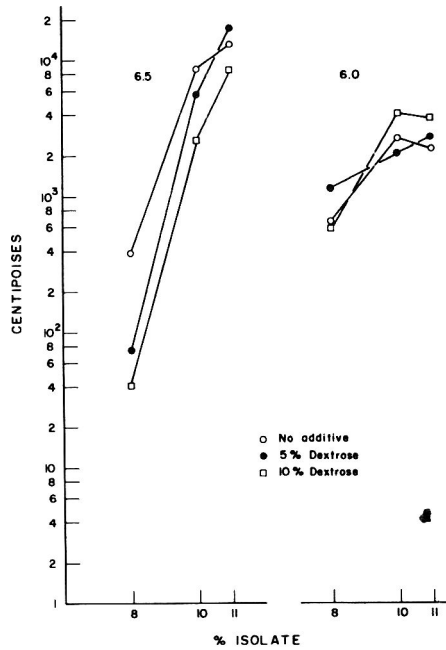
Fig. 9—Effect of 5 and 10% dextrose on apparent viscosity of 8, 10 and 11% ISP buffered at pH 6.5 and 6.0.
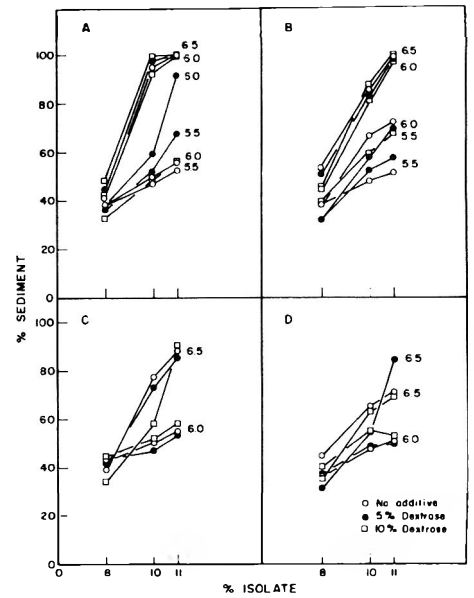
Fig. 10—Effect of freezing and thawing on stability of the heated dispersions with 5 and 10% dextrose at 8, 10 and 11% isolate levels buffered at various pH values. The fresh and frozen-thawed gels of SSP are shown in parts A and B, respectively; of ISP in parts C and D, respectively.

fugation. SSP dispersions exhibited a slight decrease in stability upon freezing and thawing at the different buffered pH levels. With the ISP only the dispersions buffered at pH 6.5 slightly decreased in stability. Both forms seem to remain relatively stable after the freezing-thawing process.

Fig. 8—Effect of 5 and 10% dextrose on apparent viscosity of 8, 10 and 11% SSP buffered at pH 6.5, 6.0 and 5.5.

In Figure 4 the stability of the freshly prepared dispersions was compared to the viscosity of the dispersions measured prior to centrifugation. Stability of the dispersions increased with the increase in viscosity.

Influence of sucrose

In a food system, the isolates would be used with other additives, including sucrose, dextrose and sodium chloride. The effects of 5 and 10% sucrose on viscosity are shown in Figures 5 and 6 for the SSP and ISP dispersions, respectively. These figures demonstrate the pH dependence of the protein gel in the reaction with the two levels of sucrose. Buffered at pH 6.5 and 6.0, the SSP dispersions displayed a consistent increase in viscosity when 5% sucrose was used; however, at pH 6.5 the increase was slight. When 10% sucrose was used, the viscosity decreased for both buffered levels with dispersions at pH 6.0 having the greatest decrease. Buffered at pH 6.0, the 5% sucrose gel had the same viscosity as the 5% sucrose gel buffered at pH 6.5. The dispersions buffered at pH 5.5 demonstrated a large decrease in viscosity for both levels of sucrose.

The addition of 5% sucrose to the ISP dispersions, buffered at pH 6.5, exhibited a slight decrease from the control; however, 10% sucrose showed a large decrease. At pH 6.0, both levels of sucrose displayed similar results which varied slightly from the control.
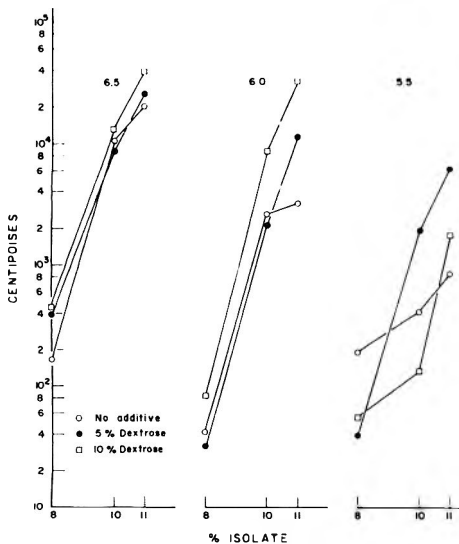
Stability with sucrose

The stability of the heated dispersions with 5 and 10% sucrose is shown in Figure 7. Buffered at pH 6.5 and 5.5, the stability of the SSP frozen-thawed gels at both levels of sucrose remained very similar to freshly prepared gels. However, buffered at pH 6.0, the 5% level of sucrose remained more stable than either the control or the 10% level of sucrose.

In the ISP dispersions, 5% sucrose remained most stable upon freezing and thawing buffered at both pH 6.5 and 6.0.

Influence of dextrose

The viscosity readings of SSP and ISP dispersions with dextrose are shown in Figures 8 and 9, respectively. Buffered at both pH 6.5 and 6.0, the 10% level of dextrose of the SSP dispersions showed a consistent increase in viscosity. These results were similar to the 5% level of sucrose with the same buffered solutions. Buffered at pH 6.5 the increased viscosity was only slight. A difference from sucrose was noted when 5% dextrose was used at pH 6.0. The 5% level abruptly increased when 11% SSP was used. The dispersions buffered at pH 5.5 showed an increase in the range of viscosity with 5% dextrose having the highest viscosity at 10 and 11% SSP.

With the ISP dispersions buffered at pH 6.5, the range of viscosity was increased at both levels of dextrose. The 5% level of dextrose was consistently higher
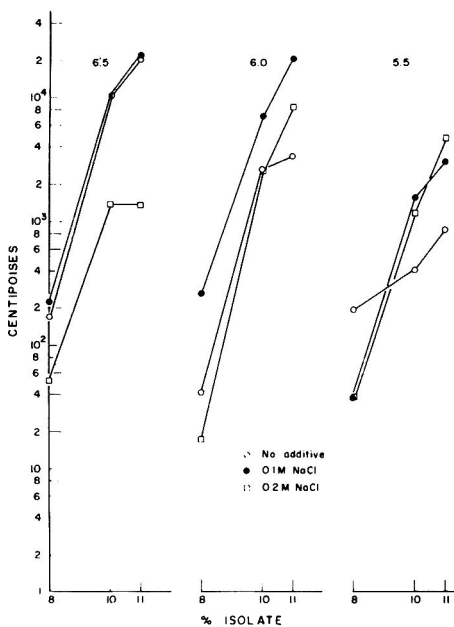
Fig. 11—Effect of 0.1M and 0.2M NaCl on apparent viscosity of 8, 10 and 11% SSP buffered at pH 6.5, 6.0 and 5.5.
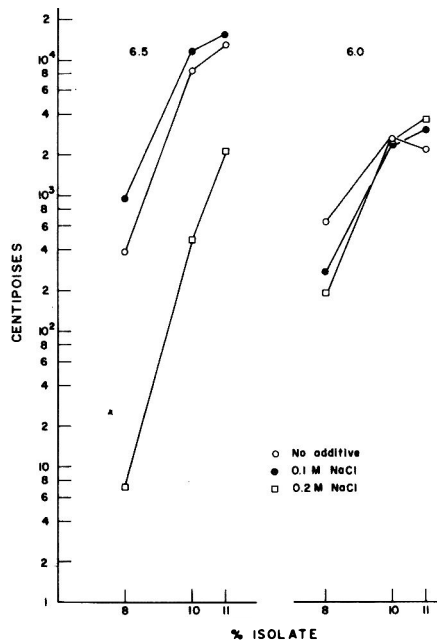
Fig. 12—Effect of 0.1M and 0.2M NaCl on apparent viscosity of 8, 10 and 11% ISP buffered at pH 6.5 and 6.0.
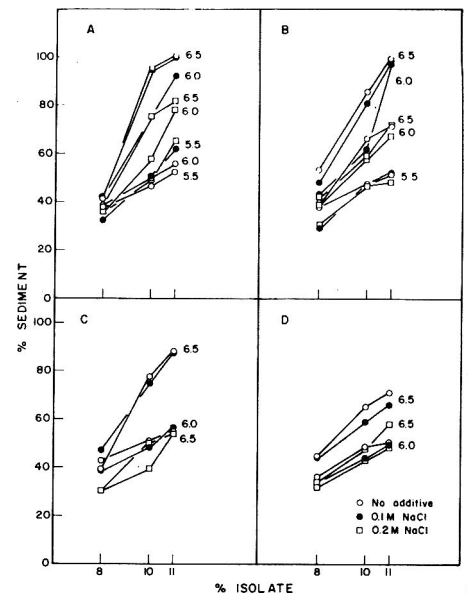
Fig. 13—Effect of freezing and thawing on stability of the heated dispersions with 0.1M and 0.2M NaCl at 8, 10 and 11% isolate levels buffered at various pH values. The fresh and frozen-thawed gels of SSP are shown in parts A and B, respectively; of ISP in parts C and D, respectively.

than the 10% level. However, the controls had higher viscosities than any of the 5% dextrose dispersions except at 11% ISP. At pH 6.0, 10% dextrose dispersions displayed a slight increase in viscosity at the 10 and 11% ISP.

Stability with dextrose

Stability of the heated dispersions with 5 and 10% dextrose is shown in Figure 10. The gels of the SSP form were consistently more stable at 10% dextrose upon freezing and thawing.

Changes in stability of the ISP dispersions at pH 6.5 depended upon the percentage ISP used. At 8 and 10% ISP, the 10% dextrose dispersions remained more stable than 5%; although both dispersions containing dextrose were less stable than the controls. At 11% ISP, 5% dextrose dispersions were more stable upon freezing and thawing than either the controls or 10% dextrose dispersions. Buffered at pH 6.0, 10% dextrose dispersions were the most stable.

The influence of NaCl

The effect of NaCl on the viscosity of SSP and ISP is shown in Figures 11 and 12, respectively. In the higher pH buffered dispersions, the viscosity of 0.1M NaCl was slightly higher or the same as the control. At pH 6.5 there was little difference between controls and dispersions containing 0.1M NaCl but those containing 0.2M NaCl were markedly

lower in viscosity. Buffered at pH 6.0 the 0.1M NaCl had the highest viscosity. As was noted in the addition of dextrose, the viscosity range at pH 5.5 increased for both levels of salt. In addition these two levels were similar in viscosity and were much higher than the control at 10 and 11% SSP.

The ISP dispersions containing 0.2M NaCl at pH 6.5 exhibited much lower viscosities than those of the other samples. Dispersions at pH 6.0 behaved much as the control at 10 and 11% ISP for both salt levels.

Stability with NaCl

Stability of the dispersions with 0.1M and 0.2M NaCl is shown in Figure 13. The SSP dispersions at pH 6.5 and 6.0, containing 0.2M NaCl were less stable than the control or samples containing 0.1M NaCl. At pH 5.5, those containing 0.2M NaCl were similar to the control and 0.1M NaCl dispersions.

The ISP dispersions at pH 6.5 also exhibited less stability when 0.2M NaCl was used. However, at pH 6.0 the stability of 0.2M NaCl dispersions remained similar to the control and 0.1M NaCl dispersions.

DISCUSSION

FERRY (1948) proposed that in the denaturing of a protein, the polypeptide chain unfolds, exposing nonpolar groups, which associate in a three dimensional

network. Crosslinkages which stabilize this network are thought to be sulfhydryl-disulfide, hydrogen, hydrophobic and electrostatic bonds (Catsimpoolas and Meyer, 1971b).

Catsimpoolas and Meyer (1971a) suggested that the soy protein dispersion (sol) is changed to a progel by heating. Upon cooling the gel is formed which has a higher viscosity and is the only step which is reversible. Excessive heat (125°C) causes the progel to form a metasol which does not gel. These researchers theorized that the bonds involved in the progel to gel states are noncovalent bonds since it is a reversible step. They suggested that upon cooling hydrogen and ionic bonds are responsible for the stabilization of the network.

Since ionic bonds are involved in stabilization, the addition of NaCl will decrease the viscosity of the gel if the molarity of NaCl is high enough to neutralize the charges.

The initial reaction of heat and its irreversible consequence is due to the dissociation of the major protein in soybeans, glycinin, into subunits which then aggregate when cooled (Catsimpoolas et al., 1969). Since hydrophobic bonds are preferred by increasing temperatures (Scheraga, 1963), this dissociation into subunits would expose groups capable of hydrophobic bonding. This would stabilize the network and cause it to be ir-

reversible from the sol to progel states (Catsimpoolas and Meyer, 1971a).

The pH dependence of soy gels can be explained by the reduced amount of negative charges on the protein as the isoelectric point is neared. Upon cooling, hydrogen and electrostatic bonds contribute to the increase in viscosity.

The effects of 5 and 10% sugar on the sodium soy proteinate can also be a function of the amount of charges available on the protein. At pH 6.5, there are attractions for hydrogen bonding to the protein as well as the sugar forming a protein-water-sugar complex. At pH 6.0, there are less charges on the protein and less attractions for the water. Some of the water will be bonded only to the sugar causing a weakening of the gel structure. This was evident when both sucrose and dextrose were added. Sucrose has a higher affinity for water than dextrose causing a difference in behavior. With the lower amount of sucrose, there is the formation of the protein-water-sugar complex because the charges are evenly distributed. However, the larger amount of sucrose has a higher attraction for the water producing only a sugar-water complex. At the lowest pH where thickening occurs (5.5), sucrose has more attraction to the water due to the relatively few protein charges available.

Since dextrose has a lower affinity for water, the larger amount is necessary to supply enough bonding sites to form the protein-water-sugar complex. The lower amount can only form this complex when enough protein is added to provide the necessary bonding sites. Charges on sugar and protein molecules may partially or completely explain the characteristics of the soy protein dispersions. Another possibility is that the sugars aid in imbibing water by an osmotic effect. There is a void in the literature concerning the influence of sugars on gelation phenomena of soy protein isolates.

The higher viscosity readings of lower concentrations, at lower pH values, than corresponding concentrations, at higher pH values, may be explained by type of bonding involved. At a lower concentration and lower pH, there are fewer binding sites for hydrogen and electrostatic bonds causing a thin gel. There may be more hydrophobic bonds which interact with the proteins to cause the thickening thus stabilizing the network more than other possible bindings.

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LEGUME POWDERS: PREPARATION AND SOME NUTRITIONAL AND PHYSICOCHEMICAL PROPERTIES

INTRODUCTION

BEANS and other leguminous seeds have high protein contents, are relatively inexpensive and are therefore, a very desirable source of protein and other nutrients. Use of those crops in the United States, except for soybean, is declining (USDA, 1973). The fact that beans take a long time to prepare in the regular fashion contributes to this decline. Normally, beans are soaked overnight and then cooked for an hour or longer. One alternative might be to process beans into precooked powders to be used as an ingredient in convenience foods. Some work on bean powders was reported by Morris (1961).

The flavor of legumes depends largely on cooking procedure and on the retention of the integrity of the cells. Cooked powders prepared so that cellular integrity is preserved retain their flavor and can be substituted for beans in any food recipe.

Legume products with very low flavor intensity, however, could be used as extenders and additives for meat and bakery products. The high protein content coupled with a relatively high lysine content makes legumes especially attractive as extenders to many cereal products.

Grinding treatments that break most or all of the cells and release cell contents of raw legumes prevent the subsequent development of the characteristic cooked bean flavor. Probably because of mixing of the cell contents an off-flavor develops when ground raw legumes are suspended in water. We (Kon et al., 1970) found that this off-flavor was caused by the action of the enzyme lipoxygenase and could be controlled by adjusting the pH of the slurry.

This report describes methods for preparation of two kinds of legume powders using a double drum drier and reports some physicochemical and nutritional properties of those powders.

EXPERIMENTAL

ALL LEGUMES were obtained from the growing areas in either California, Idaho or Michigan and were air-cleaned before use. The detailed study reported here was conducted on two varieties of *Phaseolus vulgaris* – California small white (CSW) and pinto.

Whole cell or "regular" powder

For regular powder the legumes were first cooked until tender either with or without prior soaking. Beans soaked overnight were cooked for 1 hr in about 4 vol of water; unsoaked beans required at least 2 hr. Cooked beans were slurried in a Rietz disintegrater (Rietz Manufacturing Co., Santa Rosa, Calif.) equipped with 1–16 screen, and then were ready to be applied to the drum drier.

Broken cell or acidified powder

To break up cells, raw legumes were ground on a Fitzpatrick mill with final screen size 0.0155. (The powder should pass a 40-mesh screen). Ground beans were added to 8 vol of water which was acidified with HCl to produce a slurry of about pH 3.5. Rapid stirring of boiling acidified water during the addition of the powder prevented the hydrophobic powder from balling. After cooking for 15 min with vigorous stirring to prevent scorching, the slurry was neutralized with NaOH to pH 6–7 and was ready to be applied to the drum drier.

Drum drying

The pilot size double drum drier was equipped with 12 in. × 18 in. chrome plated drums, a variable speed drive, and take-off rolls to facilitate sheet recovery. For best results in preparing both powders the clearance between the drums was set at 0.016 in. which produced sheets of 0.008 in. thickness. On both drums the entering temperature was 127°C and exit temperature 121°C (about 20 lb steam pressure). Rotation of the drums was set at 2 rpm, which gave a drying time of about 25 sec. The trough between the drums was filled with hot slurry using a Moyno pump (Robbins and Myers Inc., Springfield, Ohio). The slurry was kept hot to give good rates of water evaporation and, particularly, to prevent bacterial spoilage of the slurry.

Rat-feeding experiments

Protein efficiency ratio (PER), digestibility and growth rates, were determined by feeding tests with rats as described by Derse (1965) for specially formulated diets in which 10% protein

was supplied entirely by the respective bean samples.

Bulk density

To measure bulk density 150g of flakes and 50g of 20-mesh ground powder were transferred into 1000 ml and 250 ml graduated cylinders, respectively, and the loose volume determined. The 20-mesh ground powder was prepared by grinding the flakes in a Wiley mill using 20-mesh screen.

Consistency determination

Consistency was measured using a Bostwick consistometer (Davis et al., 1954). 25g of the flakes were slurried with different volumes of water at 60°C. The slurry was kept stirring at 60°C for 1/2 hr then put in the Bostwick and the distance traveled in 10 sec recorded (high distances indicated low viscosity).

Vitamin determination

The total content of thiamine, niacin, pyridoxal and folacin was determined as described by Miller et al. (1973).

RESULTS & DISCUSSION

DRY MATERIALS came off the drums as very thin continuous sheets. The acidified material tended to form a better, stronger sheet probably due to the large amount of extra cellular pregelatinized starch in this preparation. The best drying was obtained when the water content of the slurry after cooking was about 90% for the acidified product and about 80% for the regular product. The moisture content of the flakes of both regular and acidified products was around 5%.

Bulk density of products is important economically. Low density products would require a larger package. In Table 1 the bulk densities of the two products are compared before (as flakes) and after (as powder) being ground to 20 mesh with a Wiley mill. Because the acidified product was completely gelatinized and most of its starch was free, stronger flakes formed which were more bulky as they came from the drum drier, than the regular product. After grinding, both powders were more dense and the difference between them diminished considerably. With both products, however, the rate of reconstitution with water decreases after the flakes have been ground.

Readings on a Bostwick consistometer were lower for acidified than for regular slurry (Table 2).

Table 1—Bulk density of flakes and powders (g/cc)

	Pinto	CSW
Regular powder — as is	0.29	0.26
Ground (20 mesh)	0.44	0.43
Acid powder — as	0.14	0.15
Ground (20 mesh)	0.36	0.40

The consistencies of slurries made with the regular powder decreased rapidly with dilution. The acidified slurry, with the free pregelatinized starch, was initially much more viscous and it seemed to retain its high viscosity even at lower concentrations. It would seem that the acidified material, in addition to being a good extender because of its low flavor intensity, could also be used as a thickening agent.

As reported previously (Kon et al., 1971) a short acid treatment does not affect the nutritional qualities of the legumes. The acidified product used in tests reported in Table 3 was prepared as described in the experimental section, but after neutralization the slurry was cooked for an additional 45 min to give both products tested equal cooking time. Nutritional qualities of both bean powders were similar to those of whole cooked beans as determined by weight gain, PER values and digestibilities. As expected, both the weight gain and PER values for all bean products were significantly lower than for casein, but as can be seen from the table both can be raised to the casein level by the addition of 0.6% DL-methionine to the bean products.

The cooking time for the production of the regular powder could not be shortened to less than 45 min, if the cooking of the whole beans is to be done at atmospheric pressure. Shorter cooking times produced material that was too hard and not completely cooked. However, the acid-treated slurry could be cooked for a shorter time without ill effects. Nutritional qualities of acid-treated slurries of pinto beans cooked for only 15 min were similar to those of whole pinto beans cooked for 60 min (Table 4).

Comparison of growth and PER data between Tables 3 and 4 shows that the pinto beans we used were less nutritive than CSW. It has of course been known for many years that different bean varieties differ in their nutritional qualities. From our own experience we know that in some cases those differences are very large. Addition of 0.6% DL-methionine to pinto beans increased animals' weight gains and PER values but not to the levels for animals fed casein.

In addition to being an important source of protein, beans are also a good source of some of the water-soluble vitamins. For this reason we compared the vitamin content of the two products described here with the vitamin content of raw and regularly cooked beans. The results are reported in Table 5. As can be seen niacin was not affected by either of the processes. There was about 10% reduction of thiamine for acid-treated powder and about 15% reduction of pyri-

Table 3—Effect of different treatments of CSW bean products on weight gain, PER and digestibility by rats

Dietary source of protein[a]	Gain ± SD[b]	PER[c] Actual ± SD	PER[c] Corrected[e]	% Digestibility[d] Diet	% Digestibility[d] N
Casein	109 ± 16	3.55 ± 0.16	2.50	95	93
Bean products:					
Cooked whole	46 ± 9	1.98 ± 0.24	1.39	92	74
Regular powder	44 ± 9	2.05 ± 0.14	1.44	93	78
Acid powder (60 min)	48 ± 9	2.09 ± 0.17	1.47	93	81
Bean products + methionine:[f]					
Cooked whole	109 ± 8	3.48 ± 0.10	2.45	91	77
Regular powder	123 ± 21	3.56 ± 0.11	2.51	90	79
Acid powder (60 min)	119 ± 11	3.54 ± 0.26	2.49	92	81

[a] All diets contained 10% protein
[b] Five male weanling rats per group: S-D strain, 21 days old with mean initial wt 53g
[c] PER = gain in wt/g protein consumed
[d] % digestibility = [(feed intake − fecal wt)/feed intake] X 100
[e] Corrected for a standard value of 2.5 for casein
[f] Supplemented with 0.6% DL-methionine

Table 4—Effect of different treatments of pinto bean products on weight gain, PER and digestibility by rats

Dietary source of protein[a]	Gain ± SD[b]	PER[c] Actual ± SD	PER[c] Corrected[e]	% Digestibility[d] Diet	% Digestibility[d] N
Casein	125 ± 41	3.66 ± 0.82	2.50	96	93
Bean products:					
Cooked whole	19 ± 17	1.12 ± 0.25	0.77	92	78
Regular powder	22 ± 18	1.21 ± 0.27	0.83	93	77
Acid powder cooked 15 min	31 ± 20	1.52 ± 0.34	1.04	94	80
Acid powder cooked 30 min	29 ± 19	1.43 ± 0.32	0.98	93	77
Acid powder cooked 60 min	26 ± 18	1.33 ± 0.30	0.91	94	78
Bean products + methionine:[f]					
Cooked whole	106 ± 36	3.08 ± 0.69	2.10	92	79
Regular powder	86 ± 32	2.91 ± 0.65	1.99	94	78
Acid powder cooked 15 min	81 ± 31	2.85 ± 0.64	1.95	94	79
Acid powder cooked 30 min	76 ± 29	2.74 ± 0.61	1.87	94	80
Acid powder cooked 60 min	88 ± 32	2.81 ± 0.63	1.92	93	76

[a] All diets contained 10% protein
[b] Five male weanling rats per group: S-D strain, 22 days old with mean initial wt 56g
[c] PER = gain in wt/g protein consumed
[d] % digestibility = [(feed intake − fecal wt)/feed intake] X 100
[e] Corrected for a standard value of 2.5 for casein
[f] Supplemented with 0.6% DL-methionine

Table 2—Consistency of slurries[a]

ml H₂O added	Cm moved in 10 sec Pinto Regular	Cm moved in 10 sec Pinto Acid	Cm moved in 10 sec CSW Regular	Cm moved in 10 sec CSW Acid
100	7.0		8.7	
125	17.0	2.2	15.5	5.7
150	24.0	7.0	21.7	11.7
175		10.5		16.0
200		13.0		19.0

[a] 25g of powder was used in all measurements and results obtained on slurries at 60°C.

Table 5—Effect of processing on vitamin content (totals) of pinto beans

	Thiamine	Niacin	Pyridoxine	Folacin
	(μg/g dry wt)			
Raw beans — ground	5.4	14.9	6.9	1.9
Cooked beans — slurried	5.1	12.2	6.9	1.5
Regular powder	5.6	11.9	5.7	1.1
Acid powder (cooked 60 min)	4.5	14.7	5.8	0.7

doxine for both powders. The greatest effect of processing was on folacin: 25% destroyed during the regular process, and 50% during the acid treatment and subsequent drying.

Both powders are free-flowing, non-hygroscopic, and very convenient to use. Both powders reconstitute very well in hot water, the regular powder almost instantaneously and the acidified powder somewhat more slowly. The slower rehydration of the acidified powder is probably due to the free gelatinized starch. In cold water the regular powder reconstitutes very well but the acidified powder takes some time and considerable effort.

Storage stability with regard to development of oxidative off-flavors is being determined at the present time. However, it seems that at room temperature the regular powder was stable only for a few months when oxygen was not excluded, and the acidified powder seems to be stable for a year, or possibly longer. When packed under nitrogen, storage life of the regular powder might be extended to about a year.

The two products discussed here were prepared from many different varieties of legumes. In addition to the California small white and the pinto bean products on which the above detailed studies were conducted, powders were prepared from great northern, sanilacs, garbanzo, fava, lima beans, lentils, Austrian winter peas and garden peas. The basic processes described here were adapted to all types of dry beans and peas with some modifications to allow for compositional and functional differences. With all seeds tested, products were of good quality.

During the last 2 yr regular powder from pinto beans and from peas was produced commercially by two processors on a contract basis. Some of the runs were quite large, up to 100,000 lb of product. Only one commercial preparation, of about 2,000 lb, was made of the acidified powder. Results of all the commercial runs were encouraging; the powders were at least as good as the ones made in our laboratory pilot plant.

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VOLATILE COMPONENTS OF CANNED ALPHONSO MANGO

INTRODUCTION

THE MANGO (*Mangifera indica L.*) is a fruit which has been cultivated in India for over 4,000 yr. Production in that country currently exceeds 5 million tons annually, and is expanding commercially in the Philippines, Mexico and Venezuela. The genus contains 41 valid varieties distributed throughout South Asia and the Malay Archipelago with innumerable varieties existing in other tropical areas of the world. However, it is generally found that the Alphonso of West India (Badami of South India) is preferred by the consumer not only in India but in many foreign markets. Since fresh mango does not ship well and long distance frozen shipment is costly, canned hot packed mango puree is accepted as an excellent product with virtually no off-taste when compared to the fresh pulp.

Despite the commercial importance of the mango, no information has been available regarding its volatile flavor composition, with the exception of a recent preliminary report by Angelini et al. (1973).

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EXPERIMENTAL

Isolation of volatiles

The flavor volatiles were isolated from canned Alphonso mango puree (18° Brix) purchased from Kissan Products Ltd., Bangalore, India. 10 gal of the puree were diluted 1:1 with water and distilled under reduced pressure in 3 liter batches using a Rinco rotary evaporator until the volume was reduced by 50%. The distillate was condensed in liquid nitrogen-cooled traps. Evaluation of the residue by aroma and taste indicated that virtually all the flavor had been removed from the juice and the residue tasted flat. However, when the essence was added back to the residue, the flavor was considered restored without noticeable modification.

The combined distillate (22L) was reduced to 2L by freeze concentration in 4L batches using a Virtis 3-100 freeze concentrator. The total freeze concentrate was saturated with sodium chloride and extracted with methylene chloride (4 × 100 ml). The solvent in the combined extracts was removed through a fractionating column to give a residue (2.5g) which still contained solvent. A gas chromatogram of the residue is shown in Figure 1.

The isolated flavor components were separated into 10 fractions by column chromatography on silica gel using pure hexane with gradually increasing polarity, in 25% increments, to pure ethyl ether. Each of the 10 fractions was further separated by gas chromatography and the constituents represented by the individual peaks were collected in glass capillaries cooled in dry ice. Each component was analyzed by mass spectroscopy and, whenever possible, by infrared spectroscopy. The peak identity assignments were further confirmed by enhancement of gas chromatographic peaks with authentic compounds.

Gas chromatography

The gas chromatogram of the flavor isolate shown in Figure 1 was obtained using a Perkin Elmer F-30 instrument equipped with FID and a 300 ft × 0.02 in. stainless steel open tubular column coated with Carbowax 20M. The temperature was programmed from 60° to 200°C at 2°/min. The helium flow rate was 5 ml/min. Fractions obtained from silica gel chromatography were monitored with a Varian 1200 instrument containing an FID and a 20 ft × 1/8 in. stainless steel column packed with 5% Carbowax 20M on 80−100 mesh Chromosorb W. The temperature was programmed from 70° to 200°C at 2°/min. Helium flow was maintained at 30 ml/min. Preparative gas chromatography was performed with an F and M 720 instrument containing a TC detector and a 20 ft × 1/4 in. stainless steel column packed with 15% Carbowax 20M on 60−80 mesh Chromosorb W. The helium flow rate was 75 ml/min.

Mass spectrometry

Mass spectra were obtained using a CEC 21-110B mass spectrometer operating at 70 eV and 100 μA with a source temperature of 150°C. Samples were introduced through a heated glass inlet.
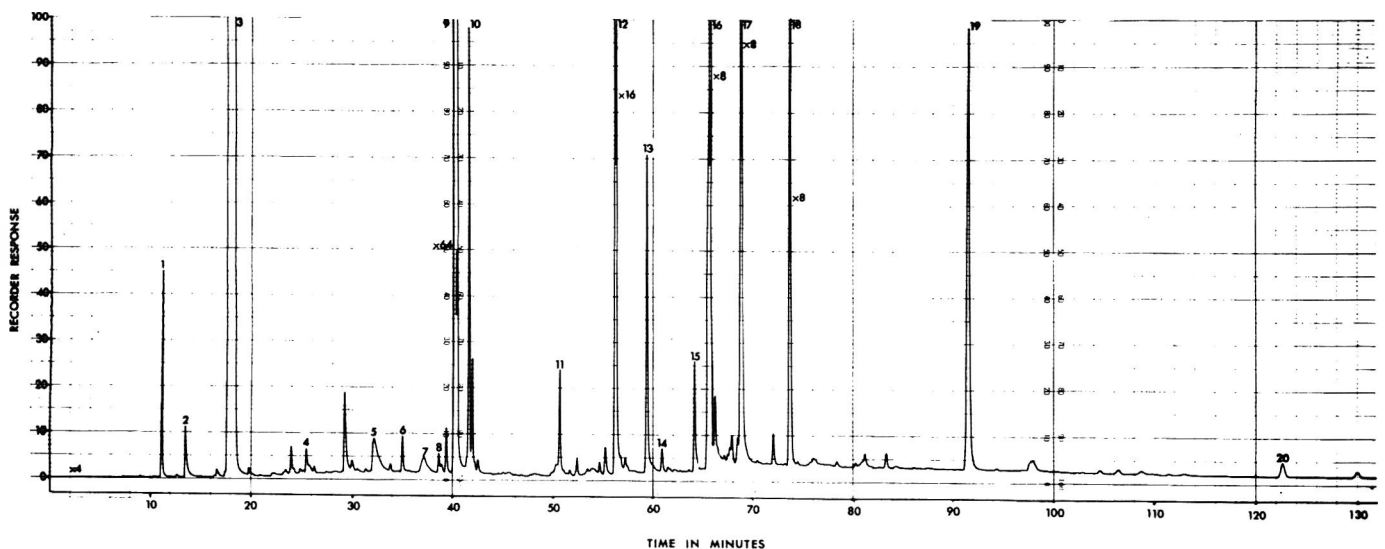
Fig. 1—Gas chromatogram of Alphonso mango essence.

Infrared spectrometry

Infrared spectra were obtained using a Beckman IR-4 with the sample dissolved in carbon tetrachloride.

RESULTS & DISCUSSION

Identification of volatile constituents

Table 1 is a list of 41 compounds identified in Alphonso mango essence. Of the nine hydrocarbons identified, myrcene, limonene, caryophyllene and humulene are well known to one of the authors (Hunter and Brogden, 1965) and identification was based on available spectra. The cis and trans ocimenes were identified by comparison of their mass spectra with material obtained from Bush, Boake and Allen. The Bush, Boake and Allen sample was separated into the two isomers by gas chromatography and their infrared spectra compared with that published by Mitzner et al. (1965). The cis and trans alloocimenes were identified in the same way from samples obtained from Pfaltz and Bauer. The remaining hydrocarbon, cyclopentane, was characterized by comparing its spectrum with one obtained on material from Aldrich Chemical Company. The hydrocarbons identified in mango have been found in nature.

Eight esters have been isolated and characterized by mass spectroscopy and gas chromatographic retention time. The mass spectra of ethyl acetate and ethyl butyrate agreed with that published by Beynon et al. (1961). The three esters, n-butyl butyrate, isobutyl butyrate and isoamyl butyrate found in mango, were compared with esters prepared unequivocally from their respective alcohols and acids. Mass spectra and gas chromatographic retention times of the synthetic esters agreed with those isolated from mango essence. The remaining three esters, ethyl decanoate, ethyl laurate and methyl pyruvate, were identified by comparison of their mass spectra and gas chromatographic retention times with authentic samples obtained from Aldrich Chemical Co. All of these esters have been reported to occur in nature.

The identity of seven of the eight lactones, i.e., butyrolactone, γ-hexalactone, γ-heptalactone, γ-octalactone, Δ-octalactone, γ-nonalactone, and γ-decalactone was confirmed by comparison of their mass spectra with those published by McFadden et al. (1965) and Honkanen et al. (1965). The mass spectral cracking pattern of the remaining lactone, α-methylbutyrolactone, indicated loss of a methyl group (mass 15) from the typical gamma lactone pattern and had a molecular ion at mass 100. Comparison of an authentic sample from Aldrich Chemical Co. with that isolated from mango essence showed them to be identical. All

eight lactones found in mango have been reported to occur in nature.

Of the six alcohols isolated and identified in mango essence, α-terpineol, terpinen-4-ol, and linalool are well known to one of the authors (Hunter and Moshonas, 1966). Two of the remaining alcohols, n-butanol and isoamyl alcohol, gave the typical mass spectral cracking pattern for alcohols as elaborated by Friedel et al. (1956). Comparison of their mass spectra and gas chromatographic retention times with that of authentic samples from Al-

drich Chemical Co. showed them to be identical.

The remaining alcohol has been identified as 2-phenylethanol by comparison of its mass spectra with that published by Gilpin, 1958.

The two terpene oxides, cis and trans-linalool oxide, identified in mango essence, are well known to one of the authors (Hunter and Moshonas, 1966) and identification was accomplished using reference spectra.

Table 1—Compounds identified in Alphonso mango extract

Compound	Basis for Identification	GC Peak #
Solvent	MS,GC	1
Solvent	MS,GC	2
Solvent	MS,GC	3
Hydrocarbons		
Cyclopentane	MS,GC	
Myrcene	MS,GC	6
Limonene	MS,GC	8
cis-Ocimene	MS,GC,IR	9
trans-Ocimene	MS,GC	10
cis-Alloocimene	MS,GC	11
trans-Alloocimene	MS,GC	
β-Caryophyllene	MS,GC	
Humulene	MS,GC	
Esters		
Ethyl acetate	MS,GC	4
Methyl pyruvate	MS,GC	
n-Butyl butyrate	MS,GC	
Isobutyl butyrate	MS,GC	
Isoamyl butyrate	MS,GC	
Ethyl decanoate	MS,GC	
Ethyl laurate	MS,GC	
Alcohols		
n-Butanol	MS,GC	5
Isoamyl alcohol	MS,GC	7
Linalool	MS,GC	14
α-Terpineol	MS,GC	
Terpinen-4-ol	MS,GC	
2-Phenylethanol	MS,GC	
Lactones		
Butyrolactone	MS,GC,IR	17
α-Methylbutyrolactone	MS,GC,IR	
γ-Hexalactone	MS,GC,IR	18
γ-Heptalactone	MS,GC	
γ-Octalactone	MS,GC,IR	19
δ-Octalactone	MS,GC	
γ-Nonalactone	MS,GC	
γ-Decalactone	MS,GC	20
Others		
Acetoin	MS,GC	
Acetic acid	MS,GC	
Furfural	MS,GC	12
2-Acetylfuran	MS,GC	13
2,5-Dimethyl-2-H-furan-3-one	MS	
5-Methylfurfural	MS,GC	15
2,5-Dimethyl-4-methoxy-2-H-furan-3-one	MS,GC,IR	16
β-Ionone	MS,GC	
cis-Linalool oxide (5 membered)	MS,GC	
trans-Linalool oxide (5 membered)	MS,GC	
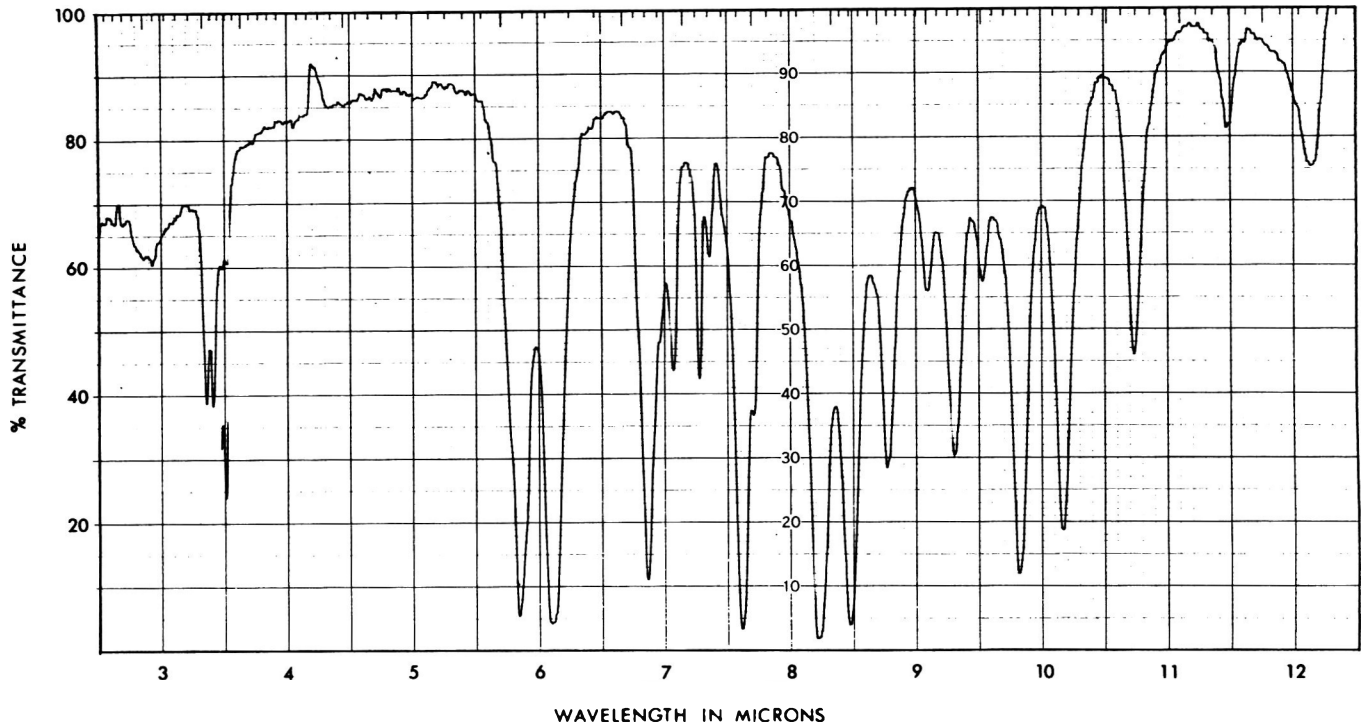
Fig. 2—Infrared spectrum of 2,5-dimethyl-4-methoxy-2-H-furan-3-one.

Of the four ketones listed in Table 1, β-ionone was characterized by comparison of its mass spectral cracking pattern with that published by Biemann (1962), and by comparison with an authentic sample obtained from Fritzsche-D & O. Acetoin was identified by mass spectral comparison with an authentic sample from Aldrich Chemical Co. Of the two ketones, 2,5-dimethyl-2-H-furan-3-one is known to one of the authors (Friedel et al., 1971); the structure of the remaining ketone, 2,5-dimethyl-4-methoxy-2-H-furan-3-one was tentatively deduced from its mass spectral cracking pattern and confirmed by comparison of its mass spectrum and gas chromatographic retention time with material synthesized according to the procedure of Willhalm and Thomas, 1969. Its infrared and mass spectra are reproduced in Figures 2 and 3.

The identification of 2,5-dimethyl-4-methoxy-2-H-furan-3-one as a major volatile component of canned Alphonso mango has not previously been reported in processed foods or in nature, but it is closely related to 2,5-dimethyl-4-hydroxy-2-H-furan-3-one which has been identified in pineapple (Rodin et al., 1965), in strawberry (Willhalm et al., 1965) and in the products from browning processes (Tonsbeek et al., 1968; Underwood, 1971; Hodge, 1967).

The two aldehydes, furfural, and 5-methylfurfural, and 2-acetylfuran, a cyclic ether, were identified by comparison with reference spectra (Stoll et al., 1967)

and confirmed by obtaining mass spectra and gas chromatographic retention times with authentic samples obtained from Aldrich Chemical Co. Scanlan et al. (1973) found these aldehydes to be major

products when L-cysteine·HCl/D-glucose are heated and therefore it is likely they may have been formed during hot packing the mango. In this context, it may be significant that no furan derivatives were
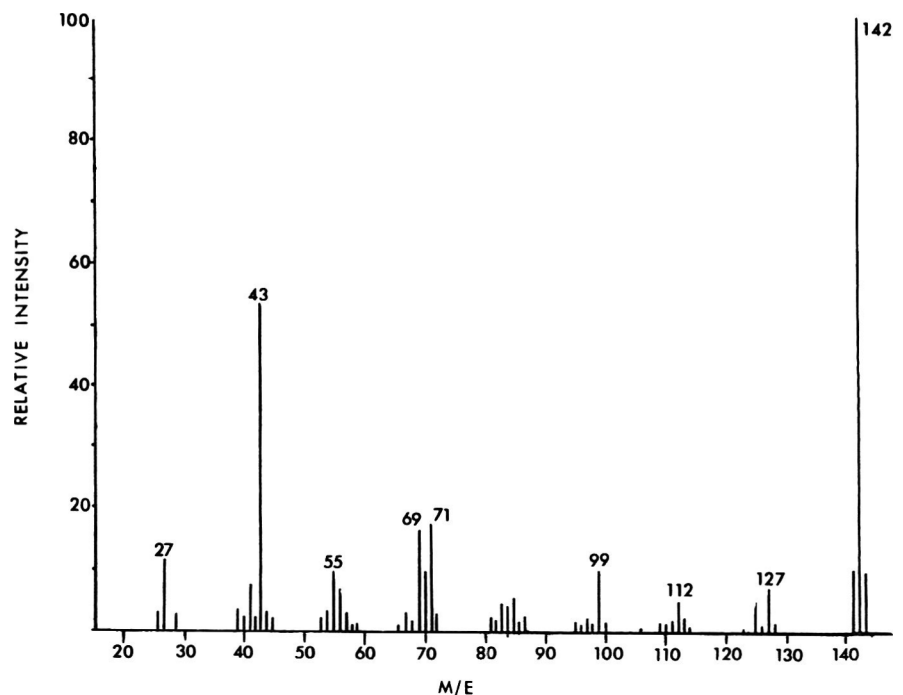
Fig. 3—Mass spectrum of 2,5-dimethyl-4-methoxy-2-H-furan-3-one.

identified in the preliminary study of flavor volatiles isolated from the fresh fruit (Angelini et al., 1973).

Organoleptic evaluations were conducted with individual compounds listed in Table 1 in order to assess their importance to mango flavor. It was concluded that no single compound of those identified is characteristic of mango flavor. The lactones were considered to be important, however, contributing coconut and peach-like notes readily associated with mango. The sweet, caramel-like notes exhibited by furfural and 5-methylfurfural also seem to be essential to the overall flavor. The methoxyfuranone derivative has an aroma characteristic of sherry wine, but appears to make little contribution to mango flavor.

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EFFECT OF FLAVONOLS ON ASCORBIC ACID AND ANTHOCYANIN STABILITY IN MODEL SYSTEMS

INTRODUCTION

THE YELLOW PIGMENTS, flavones and flavonols, always accompany anthocyanins in fruits, probably due to similar biosynthetic pathways (Harborne, 1967). In fruit juices, the flavonol pigments also accompany anthocyanins during extraction procedures (Hooper and Ayres, 1950; Morton, 1968; Chiriboga and Francis, 1973).

Flavonols have strong antioxidant properties for lipid material (Simpson and Uri, 1956; Letan, 1966a, b). This antioxidant property of flavonols has been ascribed to their ability to act as free radical acceptors and also to their metal complexing property. Similarly, the effects of flavonols in retarding the autoxidation of ascorbic acid was noticed by Hooper and Ayres (1950) and others in black currant juice. Recently, Clegg and Morton (1968) and Harper et al. (1969) actually demonstrated the protective action of flavonols on ascorbic acid in model systems. However, anthocyanins from black currant juice had the reverse effect in promoting the oxidation of ascorbic acid. When flavonols and anthocyanins were both present simultaneously, a protective effect on ascorbic acid was noticed. They concluded that the protective action of flavonols was due to the interference in the free radical chain reaction in the autoxidation of ascorbic acid.

In other independent studies, it has been confirmed that ascorbic acid oxidation has a detrimental effect on anthocyanins of fruit juices (Sondheimer and Kertesz, 1953; Starr and Francis, 1968). Earlier, Sondheimer and Kertesz (1952), recognized the formation of hydrogen peroxide from ascorbic acid oxidation, through a free radical chain reaction, which in turn would react with anthocyanins to produce breakdown products.

The present study was undertaken to establish the possible action of flavonols in retarding the oxidation of ascorbic acid and also anthocyanin breakdown, since ascorbic acid oxidation has a detrimental effect on anthocyanins.

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MATERIALS & METHODS

Purification of cranberry anthocyanins

About 14 lb of frozen cranberries (Vaccinium macrocarpon, Ait, var., Early Black) were extracted with 1% HCl in methanol in a Waring Blendor, stored overnight at 4°C and filtered. The residue was remacerated with methanol containing 1% HCl, stored and filtered. The filtrates were combined and evaporated under vacuum at 30°C to an approximate volume of 500 ml. The aqueous solution was extracted with excess petroleum ether to remove chlorophylls, carotenoids and waxy materials. The aqueous layer containing anthocyanins and other flavonols was extracted with excess diethyl ether and ethyl acetate to remove all anthoxanthins. The anthocyanin solution was evaporated under vacuum for 30 min to remove traces of solvents.

The anthocyanin solution was passed through an ion exchange resin (CG 50) to remove water soluble impurities such as sugars, amino acids, etc., by the method of Fuleki and Francis (1968b). The concentrated pigment, after resin purification, was streaked on large sheets of Whatman No. 3 filter paper and developed in 15% acid. A broad band consisting of four major anthocyanins was obtained on each paper. The thick bands were cut and eluted with methanol:acetic acid:water (20:5:75). The eluate was freeze dried in stainless steel trays. The purified anthocyanin preparation was stored over P_2O_5 in a desiccator for further use.

Composition of model systems

Buffer. Citrate phosphate buffer, pH 2.8.

Ascorbic acid solution. 1.5g of crystalline ascorbic acid was dissolved in 100 ml of citrate phosphate buffer. The solution was used within 5 min after mixing.

Flavonol solutions. About 450 mg of crystalline quercetin (Q) (K.K. Laboratories, N.Y.) was dissolved in 40 ml of warm ethanol and made up to 50 ml. This solution gave a concentration of 3 mg/ml. Similarly a solution of quercitrin (Q-3-rhamnoside) was prepared to give a concentration of 9 mg/ml.

Anthocyanin solutions. Sufficient purified cranberry anthocyanins (approx 500 mg) were dissolved in 500 ml of citrate phosphate buffer to give a concentration of 1 mg/ml.

Copper sulphate solution. Standard copper sulphate solution (10 ppm Cu^{++}, Fisher Scientific Co., USA) was used.

Development of model systems

Two model systems were developed at different times, one without copper and one with copper. The following composition was adopted to test the stability of ascorbic acid and anthocyanins:

Composition per 100 ml of solution

No.	Ascorbic acid (mg)	Antho-cyanin (mg)	Quercetin (mg)	Quercitrin (mg)
1	30	–	–	–
2	–	5	–	–
3	30	5	–	–
4	30	5	3	–
5	30	5	6	–
6	30	5	9	–
7	30	5	–	6
8	30	5	–	9

In actual practice, the standard solutions of ascorbic acid, anthocyanins and flavonols were mixed in the desired proportions and the volume was made to 250 ml with buffer. The ethanol concentration was adjusted to 3 ml in all the treatments inclusive of ethanol incorporated with flavonol solutions. The second model system was developed with copper in a concentration of 5 ppm. A higher concentration of anthocyanin (7 mg/100 ml) was used and quercetin was used in the concentrations of 6 and 9 mg/100 ml.

The sequence of additions was anthocyanin, flavonol, ascorbic acid and copper. The copper solution was added just prior to making the volume to 250 ml, otherwise copper would complex with flavonol in the absence of excess citrate ions. In both model systems, when the volume of the flask was made to 250 ml for each treatment, the flask contents were transferred to 500 ml volumetric flasks with glass stoppers. This provided sufficient headspace oxygen. The flasks were stored at room temperature (20–23°C) in the dark. The anthocyanin and ascorbic acid estimations were performed at intervals 4, 6, 10, 24, 48, 72 and 95 hr.

Anthocyanin estimation

The total anthocyanins were determined by the pH differential method of Fuleki and Francis (1968a). This method involves the measurements of the absorbance at 510 nm of diluted anthocyanin solutions at pH 1.0 and pH 4.5.

Ascorbic acid estimation

Ascorbic acid was assayed by the xylene extraction method developed by Pepkowitz (1943). This method was successfully used previously for cranberry products by Servadio and Francis (1963) and Starr and Francis (1968).

RESULTS & DISCUSSION

TO TEST the effect of flavonols on ascorbic acid and anthocyanin stability, model

systems were prepared in citrate phosphate buffer (pH 2.8) utilizing purified cranberry anthocyanins, ascorbic acid and flavonols.

Cranberry anthocyanins were used because their identity and methods of analysis are well known. The major anthocyanin pigments in cranberries were reported as cyanidin-3-galactoside, peonidin-3-galactoside, cyanidin-3-arabinoside and peonidin-3-arabinoside (Zapsalis and Francis, 1965). The precise quantitative methods for the total and individual anthocyanins in cranberry juice were established by Fuleki and Francis (1968a, c).

Citrate phosphate buffer was used to meet two requirements: first, to maintain a pH of 2.8, and second, to chelate metal ions preferentially to ensure no complex formation with the pigments per se. It has been repeatedly reported that excess citrate ions complex with metals (Perry and Dubois, 1952; Harper, 1969). However, oxidation of ascorbic acid also proceeds in the presence of this complex at a slower rate (Huelin and Stephens, 1948; Timberlake, 1960).

Quercetin was used in concentrations of 3, 6 and 9 mg/100 ml of the solution and quercetin was used in concentrations of 6 and 9 mg/100 ml. The quantities of these flavonols were chosen in accordance with the previous studies of Clegg and Morton (1968). Ascorbic acid was added to the model system in concentration of 15 mg/100 ml.

Rate of ascorbic acid oxidation

The rate of ascorbic acid oxidation in the presence of cranberry anthocyanins is presented in Table 1.

Effect of anthocyanins. In the model system at pH 2.8, cranberry anthocyanins accelerated the oxidation of ascorbic acid. For the total period of 72 hr, the rate of oxidation was enhanced by an average of 34% over the pure solution of ascorbic acid without anthocyanins. These results agree with the previous observations of Timberlake (1960); Clegg and Morton (1968) and Starr and Francis (1968). Harper et al. (1969) also observed an acceleration in the oxidation of ascorbic acid in the presence of Cn-3-rhamnosylglucoside and Dp-3-glucoside.

Harper (1968) using polarographic techniques had shown that flavylium salts were able to accept electrons at low negative potential ($-0.3V$) and possibly therefore, anthocyanins may initiate the chain reaction of the ascorbic acid in the same way as copper ions (Silverbatt et al., 1943).

Effect of flavonols. Table 1 shows that both quercetin and quercitrin inhibited the oxidation of ascorbic acid in the presence of anthocyanins. Quercetin in the concentration of 3 and 6 mg/10 ml and quercitrin in the concentration 9

mg/100 ml had almost similar activities and retarded the oxidation by an average of 20% over the period of 72 hr. However, in the initial stages the protective effect was more. Quercetin at 9 mg/100 ml and quercitrin at 6 mg/100 ml were less effective.

These indications on ascorbic acid protection by flavonols closely resemble the results of Clegg and Morton (1968); Harper, et al. (1969) and Stenlid and Samorodova-Blanci (1969). The better antioxidant activity of quercetin over quercitrin may be due to the extra hydroxyl group available at the three position of quercetin. However, when the concentration of quercitrin was three times that of quercetin, the activity was the same. A similar effect was noticed by Letan (1966a) by methylating the three position of quercetin, on the fat oxidation.

Anthocyanin stability

In the system containing flavonols, ascorbic acid and anthocyanins from cranberries, not only was the ascorbic acid protected but the degradation of anthocyanin was slowed down. It is evident from Tables 1 and 2 that flavonols which reduce the oxidation of ascorbic

acid also reduced the loss of anthocyanins. This is expected in view of the indirect involvement of ascorbic acid in degrading pigments (Sondheimer and Kertesz, 1953; Starr and Francis, 1968).

Quercetin in the concentration of 3 and 6 mg/100 ml and quercitrin in the concentration of 9 mg/100 ml had similar protective effects on anthocyanins while quercetin at 9 mg and quercitrin at 6 mg/100 ml had only slight protection, consistent with the results of ascorbic acid oxidation (Table 1). However, no degradation of anthocyanins was noticed in the samples without ascorbic acid.

The stability of anthocyanins in citrate phosphate buffer with ascorbic acid and copper ions (5 ppm) is shown in Table 3. Overall effects of copper ions on anthocyanin degradation were more pronounced as compared to systems without copper (Table 4). This can be attributed to higher rate of ascorbic acid oxidation in the presence of copper (Silverbatt et al., 1943; Harper et al., 1969). Flavonols once again had a protective effect on anthocyanin as shown in Table 3. In the presence of copper ions, quercetin at a concentration of 3 mg/100 ml had the maximum activity. The rate of ascorbic acid oxidation in the presence of copper

Table 1—The effect of anthocyanins and flavonols on ascorbic acid retention in citrate phosphate buffer pH 2.8[a]

Treatments[b]	Time (hr at 20—23°C)					
	0	5	10	24	48	72
	Ascorbic acid conc (μg/ml)					
AA alone	150	120	109	91	73	60
AA + AN[c]	150	74	51	41	23	12
AA + AN + Q (3 mg/100 ml)	150	104	90	64	52	38
AA + AN + Q (6 mg/100 ml)	150	103	90	64	48	36
AA + AN + Q (9 mg/100 ml)	150	87	68	48	29	18
AA + AN + QR (6 mg/100 ml)	150	86	—	43	25	16
AA + AN + QR (9 mg/100 ml)	150	109	98	70	54	40

[a] Each datum represents the average of duplicate samples.
[b] AA = Ascorbic acid; AN = Anthocyanins; Q = Quercetin; QR = Quercitrin.
[c] Anthocyanin conc 5 mg/100 ml

Table 2—The effect of flavonols on anthocyanin retention in citrate phosphate buffer pH 2.8[a]

Treatments[b]	Time (hr at 20—23°C)						
	0	4	6	24	48	72	95
	Anthocyanin conc (mg/100 ml)						
AN alone	4.14	4.14	4.16	4.17	4.14	4.15	4.15
AN + AA[c]	4.14	3.98	3.80	3.25	2.41	2.2	2.0
AN + AA + Q (3 mg/100 ml)	4.14	4.14	4.08	3.94	3.27	3.23	3.17
AN + AA + Q (6 mg/100 ml)	4.14	4.10	4.00	3.61	3.28	—	3.18
AN + AA + Q (9 mg/100 ml)	4.14	4.10	4.00	3.41	2.80	2.40	2.20
AN + AA + QR (6 mg/100 ml)	4.14	4.00	3.98	3.41	2.68	2.25	2.10
AN + AA + QR (9 mg/100 ml)	4.14	4.10	4.00	3.69	3.30	3.22	3.00

[a] Each datum represents the average of duplicate samples.
[b] AN = Anthocyanins; AA = Ascorbic acid; Q = Quercetin; QR = Q-3-rhamnoside.
[c] Ascorbic acid conc 150 μg/ml

was not determined. Similar protection of anthocyanins (pg-3-glucoside) and ascorbic acid was noticed in the presence of thiourea by Sondheimer and Kertesz (1953). They associated this protection with the metal complexing property of thiourea. However, their system did not have any metal ions added. Markakis (1955) observed a slight protection for anthocyanins in the presence of quercetin and rutin. However, his levels were 100 mg/100 ml and probably involve other factors in view of the high concentrations. Such quantities definitely surpass the optimum level found in this study and elsewhere.

The stability of ascorbic acid in fruit and vegetable products is known to vary and several naturally occurring flavonoid substances have been reported to be protective (Davidek, 1960; Samardova-Bianci, 1965). It is quite possible that a similar relationship exists for anthocyanins. Structural variation of anthocyanins has definitely been shown to effect anthocyanin stability (Robinson et al., 1966). The flavonol content and their chemical nature might also influence anthocyanin stability in fruit juices.

We attempted to increase the stability of anthocyanins in cherry juice by adding 9 mg/100 ml quercetin to juice which had been pressed from unblanched frozen cherries. The frozen cherries had to be immersed in boiling water immediately prior to pressing in order to obtain a juice with appreciable anthocyanin content. Without the heat treatment, the enzyme

activity degraded the pigments very rapidly. The cherry juice with added quercetin or quercitrin and ascorbic acid did not show increased anthocyanin stability because the added flavonols could not be maintained in solution. Pasteurization was necessary to store the cherry juice and the flavonols crystallized out immediately after pasteurization. Capacity to exploit the protective effect of flavonols may depend on judicious blending with a juice in which the flavonols are normally in high concentration and are maintained in solution possibly by association with other compounds naturally present. It may also be possible to find a flavonol with a higher solubility in a given juice.

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Table 3—Effect of flavonols on anthocyanin retention in citrate phosphate buffer at pH 2.8 containing Cu++ ions (5 ppm)[a]

Treatments[b]	Time (hr at 20—23°C)							
	0	4	6	10	26	53	101	200
	Anthocyanin conc (mg/100 ml)							
AN alone	5.85	5.85	5.80	5.75	4.43	4.14	3.92	3.50
AN + AA[c]	5.85	5.49	5.05	4.96	2.85	2.13	1.71	1.43
AN + AA + Q (3 mg/100 ml)	5.85	5.78	5.65	5.21	4.38	3.55	3.21	2.91
AN + AA + Q (6 mg/100 ml)	5.85	5.52	5.13	4.87	3.50	3.09	2.90	2.65
AN + AA + Q (9 mg/100 ml)	5.85	5.62	5.10	4.87	—	3.10	2.85	2.49

[a] Each datum represents the average of duplicate samples.
[b] AN = Anthocyanin; AA = Ascorbic acid; Q = Quercetin.
[c] Ascorbic acid conc 150 μg/ml

Table 4—Percent loss of anthocyanin in citrate phosphate buffer at pH 2.8, containing ascorbic acid and flavonols, with and without addition of 5 ppm copper

Treatments[a]	Time (hr at 20—23°C)				
	0	4	6	24	50	95
	Anthocyanin (% loss)					
AN + AA[b]	0	3.9	8.2	21.5	41.8	51.7
AN + AA + Q (3 mg/100 ml)	0	0	0	4.8	21.0	23.5
AN + AA + Cu++	0	6.2	13.6	51.2	63.5	70.7
AN + AA + Cu++ + Q (3 mg/100 ml)	0	1.2	12.3	25.3	39.3	45.1

[a] AN = Anthocyanin; AA = Ascoric acid; Q = Quercetin; Cu++ = Cuprous ions.
[b] Ascorbic acid conc 150 μg/ml

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INFLUENCE OF SPECTRAL QUALITY OF LIGHT ON PIGMENT SYSTEMS OF RIPENING TOMATOES

INTRODUCTION

THE DEGREE of influence that visible light has on the rate of color development in tomato fruits has not been fully explained. Smith and Smith (1931) found that the carotenoid level was lower when light was excluded during the maturation of tomatoes in the field. Denisen (1948) showed that tomatoes would ripen in the dark but McCollum (1954) and Nettles et al. (1955) both observed significantly redder color and higher carotenoid level in tomatoes illuminated with light when compared to tomatoes ripened in the dark. Shewfelt and Halpin (1967) used three different kinds of fluorescent lamps to accelerate the color development of green tomatoes and found the standard Gro-Lux lamp to be most effective in enhancing the color. Boe et al. (1968) studied the effect of light frequency and concluded that red light was nearly as effective as full light for increasing the color of tomato fruit. Worthington et al. (1969) studied the effects of light and temperature on tomato ripening and attributed the increase in red hue in light-treated tomatoes to be a temperature effect.

Biochemically, two sets of pigment systems are involved in the color changes of tomatoes in ripening, the biodegradation of chlorophylls and the biosynthesis of carotenoids. The purpose of this study was to use filtered lights to examine what part of the visible light spectrum was most effective in causing the changes of the two pigment systems in ripening tomatoes.

EXPERIMENTAL

Tomatoes

Field grown tomatoes of the Walter cultivar were harvested at the mature green stage and sorted for uniform size, maturation and with specific gravity between 20–25% aqueous alcohol solutions before the light treatments. In other experiments, red and yellow lutescent tomatoes were grown in the greenhouse and harvested at mature white stage. These fruit, devoid of chlorophylls, were subjected to light treatments shortly after harvest. The analysis of carotenoids in these fruit during the ripening period has been reported recently (Jen, 1974).

Light treatments

For each light treatment, 40 tomatoes were placed, blossom end up, in one of four identical bench-style environators, model XL-3448B (manufactured by Environator Corp., West Des Moines, Iowa), set at 14 hr per day of light exposure. Thermocouples were placed on the surface of the tomatoes and the temperature was monitored by a 24 point potentiometer to ensure the environment was at 70 ± 0.5°F.
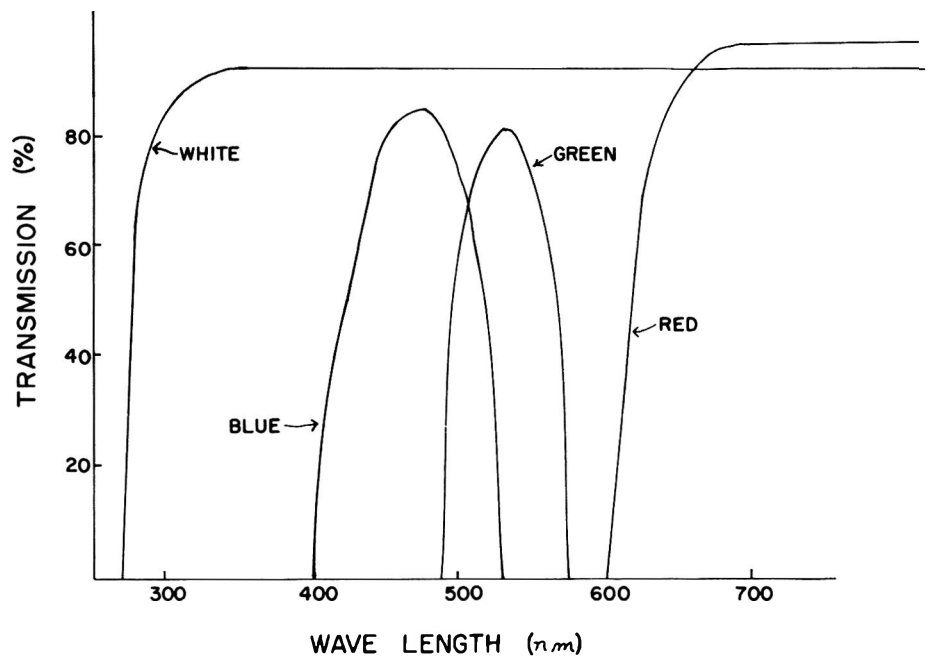
Fig. 1—Absorption spectra of filtered light as scanned in a Perkin-Elmer model 402 spectrophotometer.
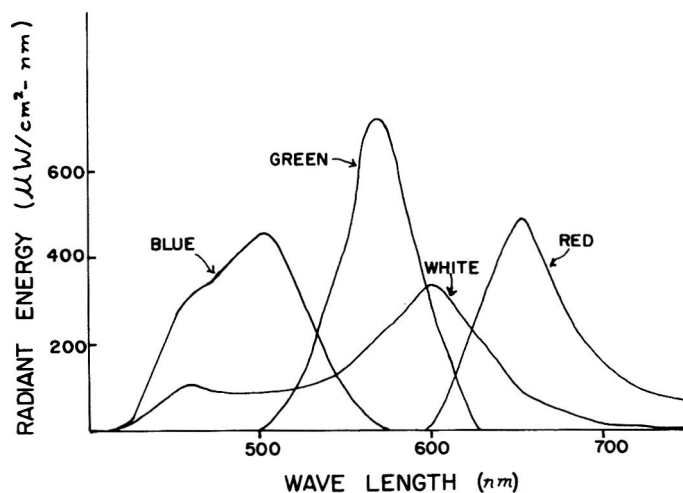
Fig. 2—Radiant energy distribution of the filtered lights.

Each environator was equipped with 12 cold white fluorescent lamps and eight 50 watt incandescent lamps as light source behind a colored filter to produce either red, green or blue light. One environator was equipped with just a thermal barrier to provide white light. The filters acted as a thermal barrier as well to minimize temperature variations. The absorption spectra of the filtered light as scanned in a Perkin-Elmer model 402 spectrophotometer are shown in Figure 1. Additional fruit were placed at the bottom of the environators and excluded from light to serve as dark control. Six switches were located on each environator to adjust the light intensity. Adjustments of light intensity and photo-distance of illumination made it possible for tomatoes in all four environators to receive a radiant energy level at 18,000 microwatt per cm^2. The radiant energy was measured by an ISCO model SR spectroradiometer with a remote probe and an ISCO model SSR recorder. The sensitivity setting was at 30 and the scanning was from 380 nm to 750 nm. The area under the curves was corrected at every 25 nm by the correction factor chart accompanying the instrument and was integrated with a planimeter to obtain the corrected radiant energy. The energy distribution curves of the four filtered lights are shown in Figure 2. The maxima of the red, green and blue lights are at 650, 570 and 500 nm, respectively.

Pigment analyses

Each day, after the illumination period, three tomatoes were removed from each treatment at random for pigment analyses. The tomatoes were weighed and disintegrated in cold acetone and filtered. The residues were re-extracted with acetone:petroleum ether (1:1) until colorless. The combined extracts were washed twice with water and dried over anhydrous sodium sulfate as described by Jen and Mackinney (1970a). The spectrum of chlorophylls in ether was recorded on Perkin Elmer model 402 spectrophotometer. The concentration of chlorophylls *a* and *b* were calculated according to the formula developed by White et al. (1963). The pigment extracts were saponified with methanolic KOH at room temperature overnight in the dark. The carotenoids were transferred to ether, dried, and dissolved in hexane to a suitable dilution to obtain spectra. The concentrations of total carotenoids were calculated according to the formula developed by Zscheile and Porter (1947).

RESULTS & DISCUSSIONS

THE PHOTODESTRUCTION of chlorophylls in green mature tomatoes seems to follow the same pattern as the in vitro experiments on the photodecomposition of chlorophyll solutions (Jen and Mackinney, 1970b). Figure 3 shows that the red light was more effective than blue light in the decomposition of chlorophyll. The green and white lights showed lesser

effects but destroyed chlorophylls faster than tomatoes kept in the dark. Shewfelt and Halpin (1967) showed that the standard Gro-Lux fluorescent lamp was more effective in enhancing the color development of tomatoes than the cool white or wide spectrum fluorescent lamps, although the light energy received by the tomato fruit was not measured. Examination of the energy distribution curve of the Gro-Lux lamp showed that it produced predominantly red light with a maximum very close to 670 nm (Shewfelt, 1970), corresponding somewhat to the red spectral light used in this report. Worthington et al. (1969) showed that incandescent lamps were similar to cool white fluorescent lamps in the production of red hue in tomatoes if temperature inside the tomatoes was maintained as the same. This was probably due to the fact that the energy distribution curves of the cool white fluorescent lamp and the incandescent lamps were similar.

The chlorophyll *a* to chlorophyll *b* ratio in all samples was calculated and showed an average value of 1.55, 1.51, 1.54, 1.58 and 1.54 for the red, blue, green, white lights and dark control, respectively. The ratio was not changed
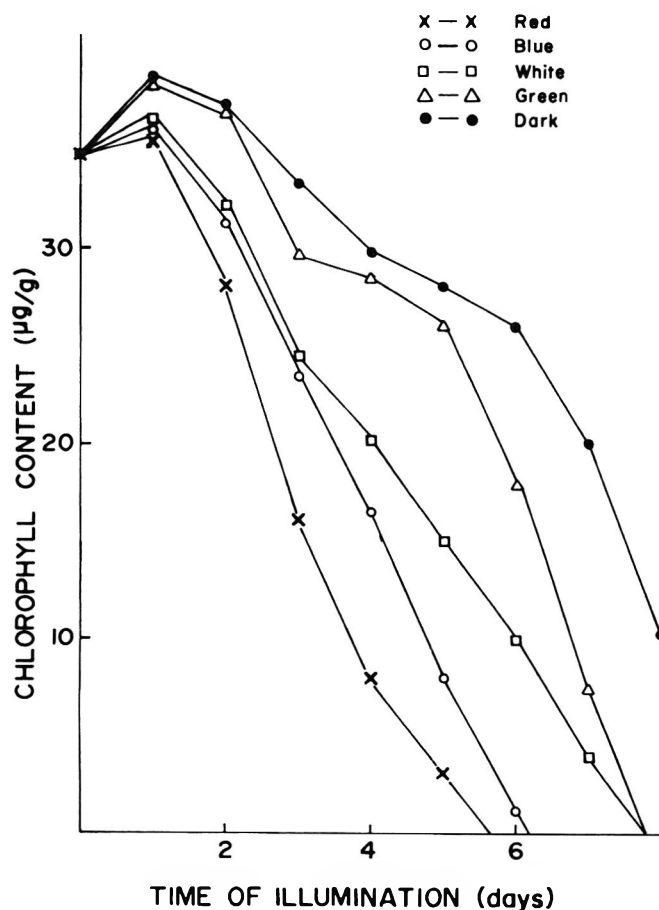
Fig. 3–*Chlorophyll degradation in detached green tomatoes illuminated with spectral lights.*
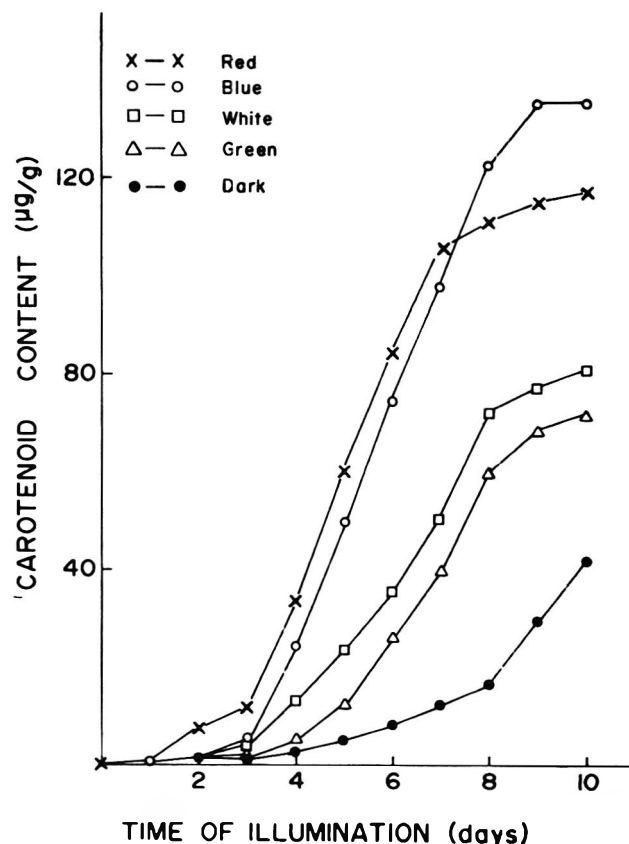
Fig. 4–*Carotenoids synthesis in detached green tomatoes illuminated with spectral lights.*
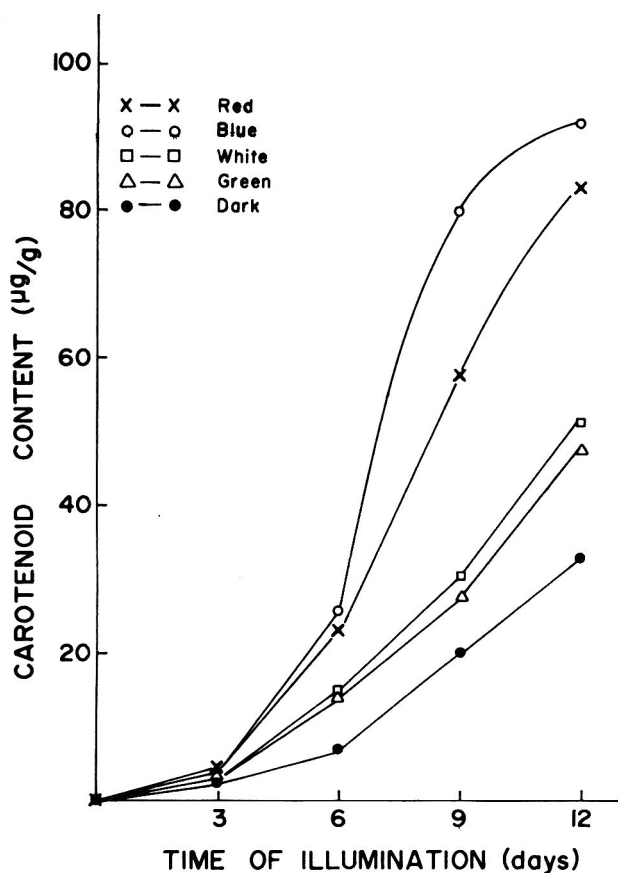
Fig. 5—Carotenoid levels in red lutescent tomatoes illuminated with spectral lights.
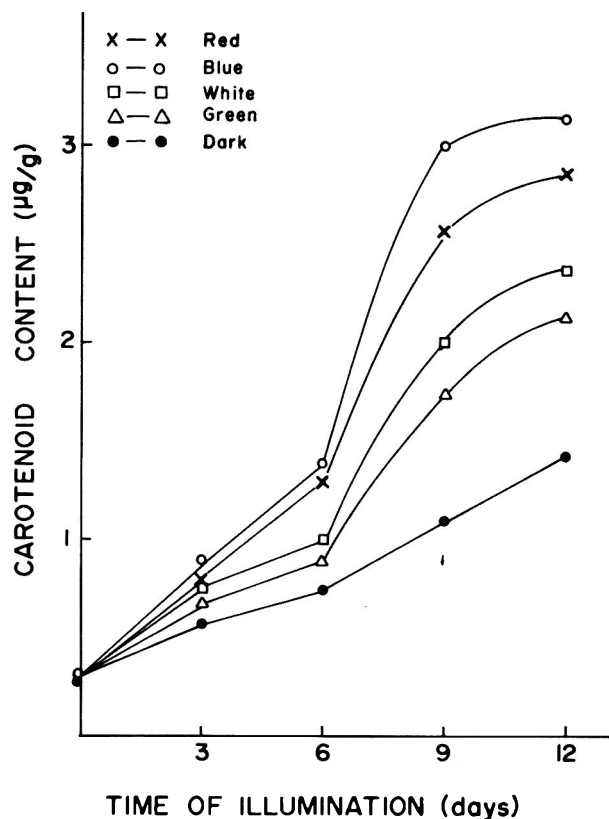
Fig. 6—Carotenoid levels in yellow lutescent tomatoes illuminated with spectral lights.

significantly with the extremes being 1.15 and 2.25 in all samples tested. It was interesting that Jen and Mackinney (1970a) obtained the same pattern in the photodecomposition of chlorophylls *a* and *b* in vitro.

Figure 4 shows that both red and blue lights were more effective than green or white lights in accelerating carotenoid biosynthesis. This agrees with the results obtained by Smith (1936) which stated that light at 540—550 nm had a lesser effect in accelerating carotenoid synthesis than light with either shorter or longer wavelength. Observation of a faster increase in carotenoid levels in red light treated fruit than in fruits treated with blue light in the first four days of illumination was possibly the result of faster chlorophyll destruction in the former fruit. From the sixth day on, the increase in carotenoids in the blue light treated fruit was actually faster than red light treated fruit and eventually the blue light treated fruit produced more carotenoids. Boe et al. (1968) used lights with very broad spectrum and observed the red light gave tomatoes higher respiration rate than green and blue lights.

To eliminate possible interference of

chlorophyll biodegradation, red and yellow lutescent tomatoes were used in further experiments. The mature fruit of these two strains of tomatoes were devoid of chlorophylls and were white in appearance before eventually turning to red and yellow, respectively (Jen, 1974). Figures 5 and 6 show the results of red and yellow lutescent tomatoes, respectively. It is obvious from these data that blue light enhances the biosynthesis of carotenoids in these tomatoes more than red light. Since the absorption maxima of the carotenoids are in the blue light region, the effect of blue light is understandable (Vetter et al., 1971). The greater effect of red light over that of green and white light is, at the present time, obscure. Khudairi (1972) pointed out the possible involvement of phytochrome, which requires red light for activation, in tomato ripening could be a possible explanation for the observation here.

In summary, it can be concluded that the spectral quality of visible light has a marked effect on the acceleration of color development in detached mature green tomatoes. Red light was most effective in accelerating the biodegradation of chlorophylls and blue light was most effective in

enhancing the biosynthesis of carotenoids in tomatoes during the ripening periods.

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THERMAL DECOMPOSITION OF SOME AMINO ACIDS.
Valine, Leucine and Isoleucine

INTRODUCTION

SEVERAL ATTEMPTS have been reported to investigate the feasibility of using pyrolysis-gas chromatography and mass spectrometry as an aid in the identification of proteins and amino acids. In these studies samples were heated in pyrolytic chambers at high temperatures ($500°C->800°C$), and the volatiles swept directly into a gas chromatographic system for analysis. Under certain conditions correlations between the structure of one or more of the thermal fragments obtained and that of the starting material have been demonstrated (Voellmin et al., 1966; Kanomata and Mashiko, 1966; Merritt and Robertson, 1967; Higman et al., 1970).

The present work is part of an overall project designed to investigate the effects of heating on lipid-protein interaction in foods. Amino acids were heated individually at relatively low temperatures under nonoxidative conditions, and their decomposition products studied in some detail. In this report, the three branched compounds valine, leucine and isoleucine are discussed together since they were found to exhibit similar modes of decomposition. The following two communications will deal with additional amino acids and a lipid-amino acid mixture. Experimental work is also underway to study thermal reactions and interactions involving certain amino acids and peptides containing various functional groups, as well as food proteins. It is hoped that the results of such work would provide basis for better understanding of the changes occurring in food components upon exposure to heat treatment, as well as information relevant to the chemistry of flavors and off-flavors.

EXPERIMENTAL

Materials

The amino acids obtained commercially were found to show no detectable impurities when distilled and their distillates analyzed by gas chromatography. Reagents and, when possible, reference standards were purchased in the highest available purity. The four aldimines N-isobutylidene-isobutylamine, N-isobutylidene-isoamylamine, N-isoamylidene-isoamylamine, and N-(2-methylbutylidene)-2-methylbutylamine, were synthesized from the corresponding aldehydes and amines by the method of Campbell et al. (1944). Bis-2-methylbutylamine was prepared by hydrogenation of N-(2-methylbutylidene)-2-methylbutylamine.

Heat treatment

1-g samples of each amino acid were sealed under vacuum (10^{-3} torr) in a pyrex ampoule, 8 in. long X 1 in. o.d., and heated in an oven for 1 hr at temperatures ranging from 180–270°C.

Analytical techniques

The methods used for the isolation and identification of the decomposition products were described in detail previously (Nawar et al., 1969). The lower boiling compounds were recovered on a precolumn while the higher boiling compounds collected by high-vacuum cold-finger distillation. The volatiles were analyzed by gas chromatography (GC) using an alumina column for the hydrocarbons, Penwalt 223 for amines, chromosorb 103 for ammonia, molecular sieve 5A for CO and CO_2, and carbowax 20M for the remaining compounds. Identification of the thermal decomposition products was accomplished by comparing their GC and mass spectra with those of authentic compounds. Whenever feasible, infrared analysis was carried out. The presence of CO_2 and ammonia was also confirmed by introducing the GC effluents into $Ba(OH)_2$ solution and Nessler's reagent, respectively.

RESULTS

THE THERMAL decomposition products identified in this study are shown in Table 1. These compounds were detected in all three amino acids when heated at, or above, 220°C. At 220°C no significant changes in their appearance could be detected. However, when heated at 250°C, they developed a light yellow color, and at 270°C the color changed to orange

Table 1—Thermal decomposition products from valine, leucine and isoleucine

Valine	Leucine	Isoleucine
Ammonia	Ammonia	Ammonia
Carbon dioxide	Carbon dioxide	Carbon dioxide
Carbon monoxide	Carbon monoxide	Carbon monoxide
Propane	Isobutane	Butane
Propene	Isobutylene	Butene
Isobutane	Isopentane	Isopentane
Isobutylene	3-methyl-1-butene	2-methyl-1-butene
Acetone	Acetone	2-butanone
Isobutyraldehyde	Isobutyraldehyde	-methylbutyraldehyde
Isobutylamine	Isovaleraldehyde	2-methylbutylamine
N-isobutylidene-isobutylamine	Isobutylamine	N-(2-methylbutylidene)-2-methylbutylamine
Diisobutylamine	Isoamylamine	Bis(2-methylbutyl)amine
	N-isobutylidene-isoamylamine	
	N-isoamylidene-isoamylamine	
	Diisoamylamine	

or black and the original crystalline appearance was lost.

As can be seen from Table 1, ammonia, carbon dioxide and carbon monoxide were produced in all of the three amino acids while the remaining compounds consisted of hydrocarbons, ketones, aldehydes, primary amines, imines and secondary amines. Gas chromatographic retention times on at least two columns, and the mass spectra of these compounds were identical to those of the reference compounds. Mass spectral fragmentation of the imines are shown in Figure 1, and those of the secondary amines in Figure 2.

The base peak in the mass spectrum of N-isobutylidene-isobutylamine is at m/e 84. This ion can be formed by α-cleavage in the aldehyde component of the molecule or β-cleavage in the amine component (Fischer and Djerassi, 1966):

m/e 84   m/e 127   m/e 84

The next prominent peak is at m/e 57 corresponding to an isobutyl ion. The fragment at m/e 112 results from loss of a

methyl radical from the molecular ion. The molecular ion (m/e 127) is very small.

In the spectrum of N-isobutylidene-isoamylamine (Fig. 1) the peak at m/e 84 may correspond to the ion $\left[CH_3\ \underset{CH_3}{CHCH} = \overset{+}{N} = CH_2\right]$ resulting from α-cleavage in the aldehyde moiety, while β-cleavage in the amine component produces the base peak at m/e 98. In the case of N-isoamylidene-isoamylamine, in addition to the base peak at m/e 98, an important rearrangement ion can be observed at m/e 113. This probably corresponds to a McLafferty rearrangement (Fischer and Djerassi, 1966):

m/e 113

The infrared spectra of the imine compounds showed characteristic C=N stretching vibration at 1670 cm^{-1}.
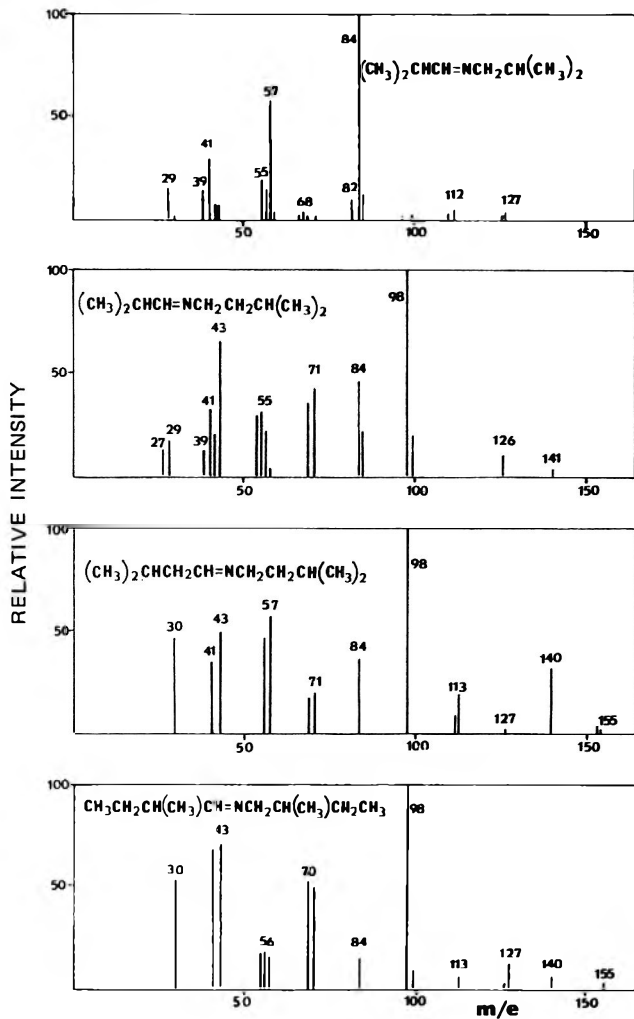
Fig. 1—Mass spectra of compounds identified as imines. From top: N-isobutylidene-isobutylamine, N-isobutylidene-isoamylamine, N-isoamylidene-isoamylamine and N-(2-methylbutylidene)-2-methylbutylamine.
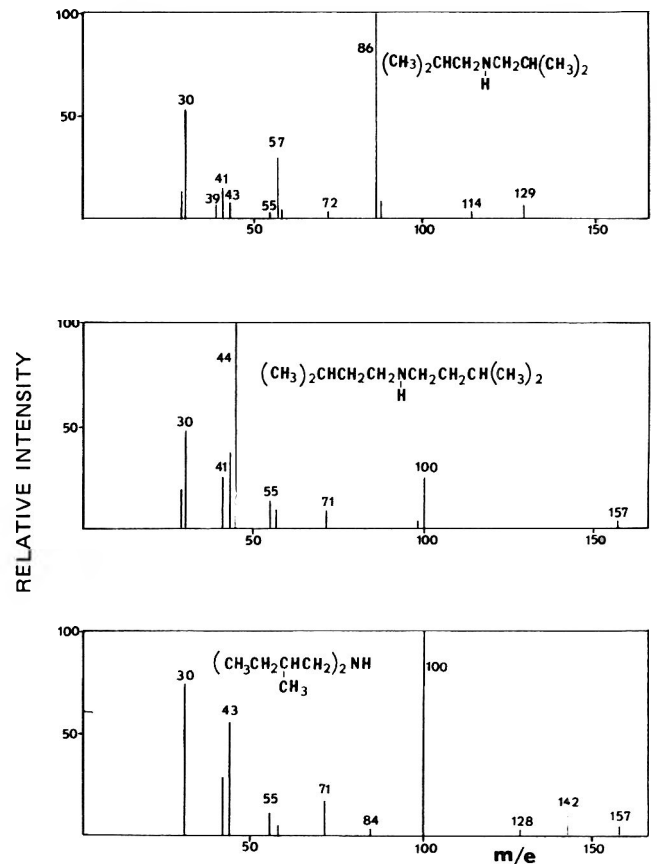
Fig. 2—Mass spectra of compounds identified as secondary amines. From top: diisobutylamine, diisoamylamine, and Bis(2-methylbutyl)-amine.

$$R-\overset{\overset{\displaystyle H}{|}}{\underset{\underset{\displaystyle R'NH_2}{|}}{C}}-\overset{\overset{\displaystyle H}{|}}{\underset{}{C}}-\overset{\overset{\displaystyle O}{||}}{C}-OH$$

$-CO_2$ $-NH_3$	$-CO_2$	$-CO$ $-NH_3$
$R-C=CH_2$ $\quad\;\; \overset{|}{R'}$	$RCHCH_2NH_2$ $\qquad\quad \overset{|}{R'}$	$RCHCHO$ $\qquad \overset{|}{R'}$ NH$_3$
Olefin	Primary Amine	Aldehyde Ammonia

$$RCHCH_2N=CHCHR$$
$$\quad \overset{|}{R'} \qquad\qquad \overset{|}{R'}$$
Imine

$$RCHCH=NH$$
$$\quad\;\; \overset{|}{R'}$$
Imine

2 (H)

$$\left[\overset{RCHCH_2}{\underset{\overset{|}{R'}}{}}\right]_2 NH$$

Secondary Amine
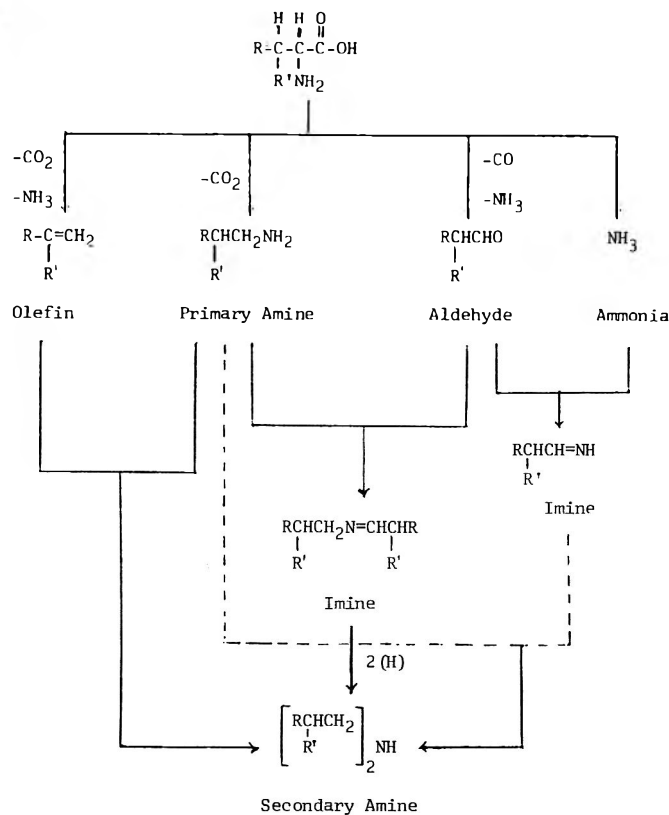
Fig. 3—Summary of thermal decomposition routes for the production of the identified compounds.

DISCUSSION

UPON HEAT TREATMENT, each of the three amino acids gives rise to a set of decomposition products characteristic for the starting amino acid. A general scheme is shown in Figure 3.

Decarboxylation and deamination are responsible for the production of the olefins; the corresponding paraffins result upon reaction with hydrogen. By this process isobutylene and isobutane are produced from valine, 3-methyl-1-butene and isopentane from leucine, and 2-methyl-1-butene from isoleucine. In addition, the formation of propane and propene from valine; isobutane and isobutylene from leucine; and butane and butene from isoleucine, may be explained by cleavage of carbon-carbon bonds beta to the carbonyl group of the amino acid to form a free radical which can then accept or lose a hydrogen atom.

The primary amines are produced by decarboxylation while the corresponding aldehydes result from both deamination and decarbonylation of the amino acid. The reason for the formation in the case of leucine of an additional aldehyde (i.e., isobutyraldehyde) and an additional primary amine (i.e., isobutylamine) is not clear.

Reaction of the carbonyl compounds with the primary amines is probably responsible for the production of the imines:

$$R_2CO + H_2NR'' \rightleftharpoons \left[R-\overset{\overset{\displaystyle OH}{|}}{\underset{\underset{\displaystyle R'}{|}}{C}}-\overset{\overset{\displaystyle}{|}}{\underset{\underset{\displaystyle H}{|}}{N}}-R''\right] \rightleftharpoons \overset{R}{\underset{R'}{}}\!\!>C=N-R'' + H_2O$$
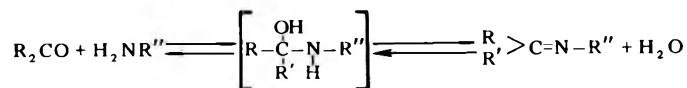
Aliphatic ketones react with amines more slowly than aldehydes to form imines which necessitates the use of higher reaction temperatures and longer reaction times than required for the aldehyde (Layer, 1962). This is probably the reason why only aldimines and not ketimines were found in the present study. Thus the reaction of isobutyraldehyde with isobutylamine, both thermal decomposition products of valine, results in the production of N-isobutylidene-isobutylamine:

$$(CH_3)_2CHCHO + H_2NCH_2CH(CH_3)_2 \xrightarrow{-H_2O} (CH_3)_2CHCH= NCH_2CH(CH_3)_2$$
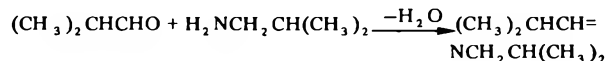
Similarly N-isobutylidene-isoamylamine and N-(2-methylbutylidene)-2-methylbutylamine are produced from thermal decomposition of leucine and isoleucine respectively. Aliphatic aldehydes can also react with ammonia to first form simple addition compounds called "aldehyde ammonias." These compounds are unstable and easily decompose to the original constituents, or they may lose water to form imines (Sprung, 1940).

The secondary amines diisolutylamine, diisoamylamine, and bis (2-methylbutyl)-amine result from the addition of hydrogen to the above mentioned imines. An alternative pathway, however, may be the reaction of the primary amine with the imine to form an additional product which can be reduced to the secondary amine (Schwoegler and Adkins, 1939).

$$RCHO + NH_3 \longrightarrow RCH=NH + H_2O$$

$$RCH=NH + RCH_2NH_2 \rightleftharpoons R\underset{NH_2}{\overset{}{C}}HNHCH_2R \xrightarrow{2[H]} (RCH_2)_2NH + NH_3$$
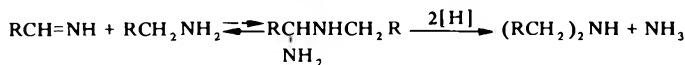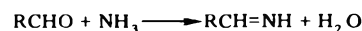
A third possibility may result from the addition of the primary amines to olefins (Walter et al., 1967). Valine, for example, would produce diisobutylamine in the following manner:
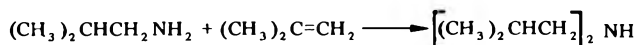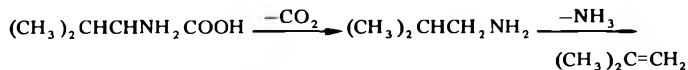
$$(CH_3)_2CHCHNH_2COOH \xrightarrow{-CO_2} (CH_3)_2CHCH_2NH_2 \xrightarrow{-NH_3} (CH_3)_2C=CH_2$$

$$(CH_3)_2CHCH_2NH_2 + (CH_3)_2C=CH_2 \longrightarrow \left[(CH_3)_2CHCH_2\right]_2 NH$$

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Ms received 1/19/74; revised 3/30/74; accepted 4/2/74.

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THERMAL DECOMPOSITION OF SOME AMINO ACIDS.
Alanine and β-Alanine

INTRODUCTION

IN THE PRECEDING paper (Lien and Nawar, 1974) thermal decomposition of valine, leucine and isoleucine was discussed. Although the products were specific for each amino acid, the modes of decomposition were similar for all three. In contrast, thermal decomposition reactions of alanine and β-alanine were found to be significantly different from those of the above mentioned compounds. Hence they are dealt with separately in the present report.

EXPERIMENTAL

METHODS for heat treatment and analysis of the decomposition products were the same as described previously (Lien and Nawar,

1974). N-ethylidene-ethylamine was synthesized by the method of Campbell et al. (1944), and N-ethylpropionamide was prepared by acylation of ethylamine with propionylchloride.

RESULTS & DISCUSSION

THE THERMAL decomposition products from alanine and β-alanine are listed in Table 1. Mass spectra of some of the identified compounds are given in Figures 1 and 2.

As in the case of valine, leucine and isoleucine, compounds typical of decarboxylation, deamination and decarbonylation, were also present in alanine when heated at 220°C for 1 hr. A characteristic imine (N-ethylidene-ethylamine), probably resulting from the reaction of acetaldehyde and ethylamine, was also found. However, the secondary amine which was present in all of the three previously discussed amino acids was absent. Patrick (1952) reported that only tars were produced from the reaction of acetaldehyde and N-ethylidene-ethylamine. It may be that this tendency of the imine to react with acetaldehyde competes with the hydrogenation reaction normally resulting in the secondary amine. On the other hand, the production of a pyridine derivative observed in the case of alanine does not take place when the three previously discussed amino acids are heated. The formation of 2-methyl-5-ethylpyridine may arise from the aldol condensation of acetaldehyde to form croton-aldehyde, followed by dimerization in the presence of ammonia:

$$CH_3CHO + CH_3CHO \xrightarrow{-H_2O} CH_3CH=CHCHO$$

Another possibility is the reaction of acetaldehyde with ammonia to form the imine, followed by aldol-type condensation to produce the pyridine derivative:

$$CH_3CHO \xrightarrow{+NH_3} CH_3CH=NH \xrightarrow{+CH_3CH=NH}$$

Fig. 1—Mass spectra of some compounds identified from thermal decomposition of alanine. From top: N-ethylidene-ethylamine, 2-methyl-5-ethylpyridine, propionamide and N-ethylpropionamide.
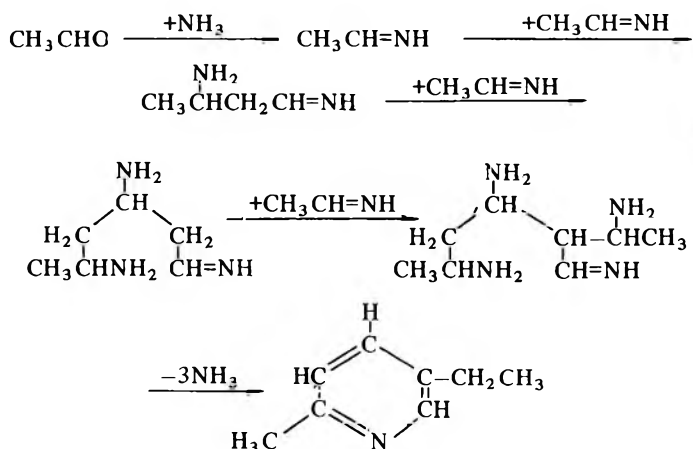
The absence of pyridine derivatives in pyrolyzed valine, leucine or isoleucine is probably due to their branched chain structure which may make the aldol-type condensation difficult (Layer, 1962).

Table 1—Thermal decomposition products from alanine and β-alanine

Alanine	β-Alanine
Ammonia	Ammonia
Carbon dioxide	Carbon dioxide
Carbon monoxide	Carbon monoxide
Ethane	Ethene
Ethene	Propene
Propene	Acetonitrile
2-Butene	Acetone
Acetaldehyde	Pyridine
Ethylamine	3-picoline
N-ethylidene-ethylamine	2,4-lutidine
2-methyl-5-ethylpyridine	3,5-lutidine
N-ethylpropionamide	2,3,5-trimethylpyridine
Propionamide	Poly-β-alanine

Fig. 2—Mass spectra of some compounds identified from thermal decomposition of β-alanine. From top: 3-picoline, 2,4-lutidine, 3,5-lutidine and 2,3,5-trimethylpyridine.

The two compounds, propionamide and N-ethylpropionamide, also not found in the case of the three previous amino acids, were produced in relatively large quantities from alanine. The formation of these two compounds may result from the breakdown of 3,6-dimethyl-2,5-diketopiperazine, which is the dehydration product from two molecules of alanine:

Cleavage at the 1,6 and 2,3 bonds would produce N-ethylpropionamide, while rupture of the 1,6 and 3,4 bonds and abstraction of hydrogen would produce two molecules of propionamide. This mechanism is however in doubt since upon heating authentic 3,6-dimethyl-2,5-diketopiperazine, the compound appeared to be very stable and the amide could not be found.

The beta amino acids decomposed at lower temperatures than those observed for the α-amino acids. β-alanine for example lost its crystalline appearance and developed a yellow color at 200°C. The decomposition reactions of β-alanine were also significantly different from those of the previously discussed amino acids. Pyridine derivatives were the major volatile decomposition products while aldehydes, amines and imines were absent (Table 1).

After cold-finger distillation, the nonvolatile residue was washed with ether and distilled water, and the resulting white powder examined by infrared spectrophotometry. Absorption bands at 3300, 3080, 1640, 1550 and 1280 cm^{-1} which are common to all polypeptides were observed. When this residue was hydrolyzed by sulfuric acid and analyzed by IR again, the spectrum was identical to that of β-alanine. The original compound was therefore identified as poly-β-alanine.

The formation of the pyridine compounds probably occurs via Tschitschibabin reactions (Sprung, 1940). In 1932, Strain reported that pyridine derivatives are formed when heating aldehyde with ammonia. The course of the reactions can be represented as follows:

$$RCH_2CHO + NH_3 \longrightarrow RCH_2CH=NH + H_2O$$

$$3RCH_2CH=NH \longrightarrow RCH_2CH-CH-CH-CHCH=NH \longrightarrow$$
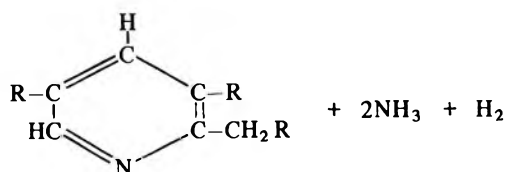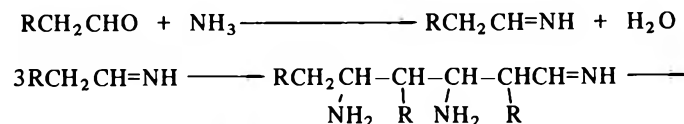
The first step is presumably the formation of an imine, the second step is an aldol-like trimerization of the imine, and the third step is ring closure with concomitant loss of ammonia and hydrogen.

The production of acetonitrile probably occurs as follows (Voellmin et al., 1966):
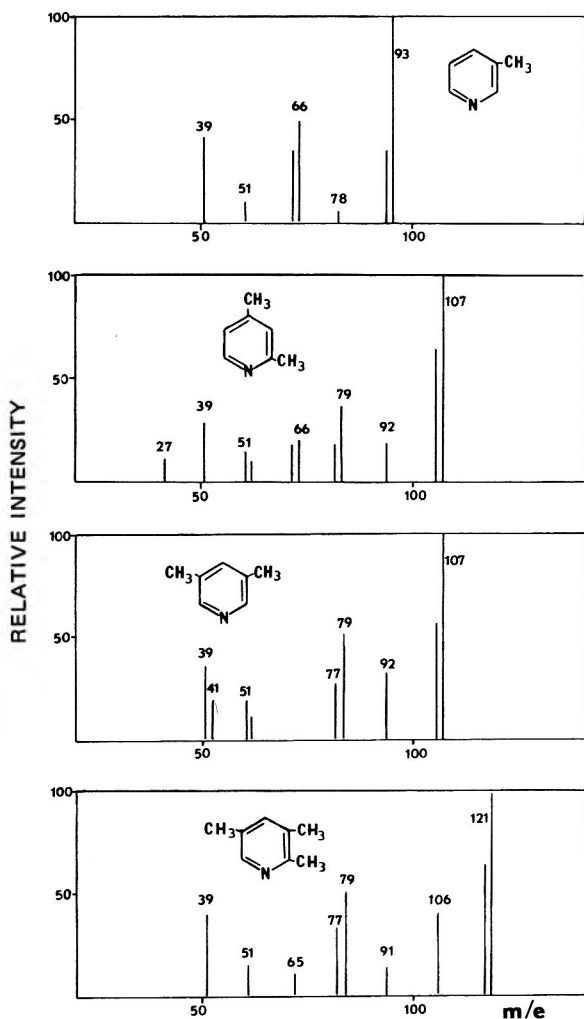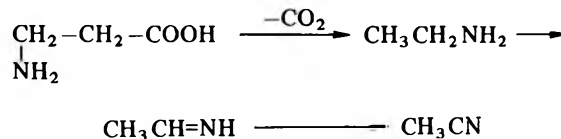
$$CH_2-CH_2-COOH \xrightarrow{-CO_2} CH_3CH_2NH_2 \longrightarrow$$

$$CH_3CH=NH \longrightarrow CH_3CN$$

Amino acids with terminal amino groups polymerize readily when heated to produce the linear insoluble polypeptides.

$$n(H_2 NCH_2 CH_2 COOH) \longrightarrow (-HNCH_2 CH_2 CO-)_n + nH_2 O$$
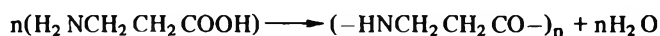
Thus poly-β-alanine (nylon 3) was identified from β-alanine and poly-ϵ-capramide (nylon 6) from epsilon-amino caproic acid. The ease of polymerization in the case of ω-amino acids accounts for their sensitivity to thermal treatment at the lower temperatures, and for the absence of decomposition products typical of α-amino acids.

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THERMAL INTERACTION OF AMINO ACIDS AND TRIGLYCERIDES.
Valine and Tricaproin

INTRODUCTION

ALTHOUGH much attention has been given to the study of protein-carbohydrate interactions during heat treatment of foods, no information is available concerning thermal interactions between lipids and proteins or amino acids under nonoxidative conditions. Previously we reported in detail on the decomposition products of tricaproin (Lien and Nawar, 1973) and amino acids (Lien and Nawar, 1974a, b) when heated separately. In the present study a mixture of one amino acid, valine, and one triglyceride, tricaproin, was selected as a simple model system to investigate reactions arising from the presence of both the fats and the amino acids together during the heating process.

EXPERIMENTAL

VALINE (1g) was mixed with 1g tricaproin, placed into a pyrex ampoule (8 in. long and 1 in. o.d.), and sealed under vacuum. The ampoule was heated at 270°C for 1 hr and the sample analyzed as described previously (Lien and Nawar, 1973). N-isobutylcaproic amide was prepared by acylating isobutylamine with hexanoyl chloride.

RESULTS

THE DECOMPOSITION products obtained from heating a mixture of valine and tricaproin are listed in Table 1. Caproic amide and caproic nitrile were identified by comparison of their GC retention times on two columns and their mass spectra with those of authentic compounds. In the mass spectrum of caproic amide (Fig. 1), the most intense ion has a mass of 59. This rearrangement ion can be correlated with cleavage of the carbon-carbon bond beta to the carbonyl group accompanied by rearrangement of a hydrogen atom to the fragment containing the nitrogen (Gilpin, 1959):

$$H_5C_2\text{—}CH\text{—}\overset{+\cdot}{O} \longrightarrow HO\text{—}C\text{—}NH_2 + C_2H_5\text{—}CH=CH_2$$

m/e 115   m/e 59
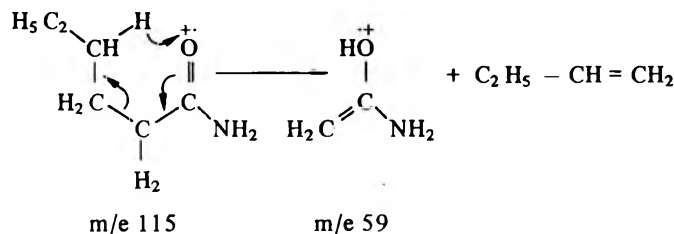
Peaks with m/e 44 and m/e 72 correspond to cleavage of the carbon-carbon bond alpha and gamma to the carbonyl group, respectively. The molecular ion (m/e 115) is present but small.

The base peak in the mass spectrum of caproic nitrile is the ion with mass 41 which probably results from beta cleavage accompanied by rearrangement of a hydrogen atom (McLafferty, 1962):

$$C_2H_5\text{—}CH\text{—}\overset{+\cdot}{N} \rightarrow C_2H_5\text{—}CH=CH_2 + CH_2=C=\overset{+}{N}H$$

m/e 97   m/e 41
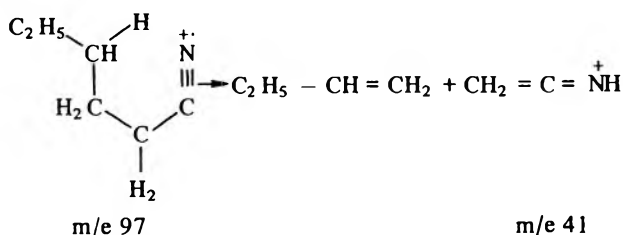
The molecular ion (m/e 97) is very weak, while the M-1 ion is somewhat more abundant. The peaks with mass 54 and 68 correspond to $(M-C_3H_7)^+$ and $(M-C_2H_5)^+$ respectively. Expulsion of 28 mass units from the molecular ion to form the

Table 1—Decomposition products identified from valine-tricaproin mixture heated at 270°C for 1 hr

CO and CO$_2$	Isobutylene
Ammonia	Pentane
Methane	Pentene
Ethane	Acetone
Ethene	Isobutyraldehyde
Propane	Hexanoic acid
Propene	Caproic nitrile
Butane	Caproic amide
Butene	N-isobutylcaproic amide
Isobutane	Dicaproin
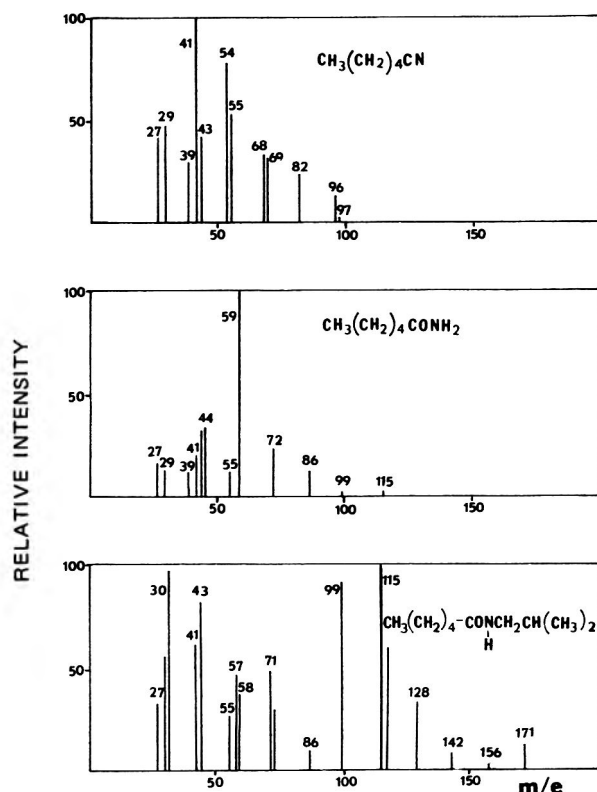
Fig. 1—Mass spectra of compounds formed by thermal interaction of valine and tricaproin. From top: caproic nitrile, caproic amide and N-isobutylcaproic amide.

peak at mass 69 may occur via the following processes (Budzikiewicz et al., 1967):

$$m/e\ 97 \qquad\qquad m/e\ 69$$

$$m/e\ 97 \qquad\qquad m/e\ 69$$
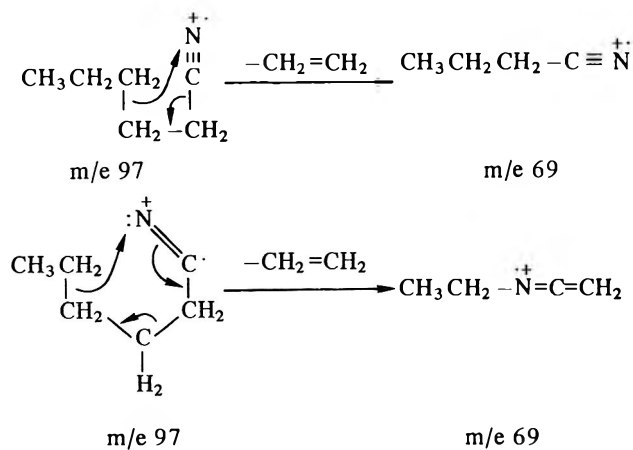
For identification of N-isobutylcaproic amide, the authentic compound was synthesized. Its GC retention time, mass and IR spectra were identical to those of the suspected decomposition product. The most intense peak in the mass spectrum of N-isobutylcaproic amide (Fig. 1) is a rearrangement ion at m/e 115, which can be correlated with cleavage of the carbon-carbon bond beta to the carbonyl group accompanied by the rearrangement of a hydrogen atom (Gilpin, 1959).

$$m/e\ 171 \qquad\qquad m/e\ 115$$
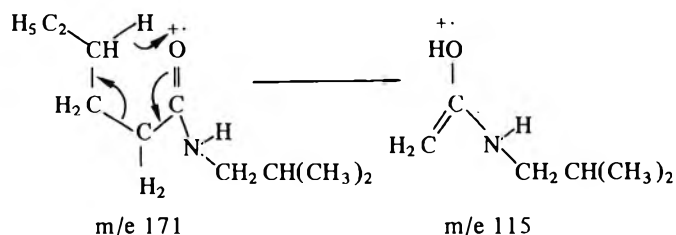
The next intense peak (m/e 30) may result from cleavage of the carbonyl carbon-nitrogen bond and cleavage of the carbon-carbon bond beta to the nitrogen atom, accompanied by a hydrogen rearrangement to the nitrogen-containing fragment (Budzikiewicz et al., 1967):

$$m/e\ 171 \qquad\qquad m/e\ 128 \qquad\qquad m/e\ 30$$
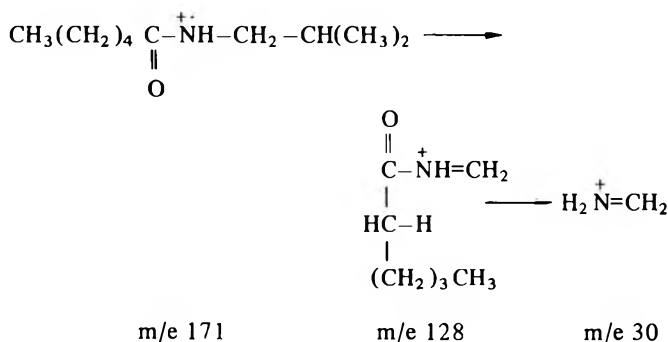
Other important peaks in the spectrum are m/e 99 $[CH_3(CH_2)_4CO]^+$ m/e 71 $[CH_3(CH_2)_4]^+$, m/e 128 [M − 43] and m/e 43 $[(CH_3)_2CH]^+$ or $[CH_3CH_2CH_2]^+$. In its IR spectrum, an N−H stretching band was observed at 3450 cm⁻¹ and a carbonyl stretching vibration at 1680 cm⁻¹.

DISCUSSION

A SCHEME summarizing the reactions proposed for thermal interaction of valine and tricaproin is shown in Figure 2. Propane, propene, CO_2 and CO which were present in the heated triglyceride (Lien and Nawar, 1973) as well as in heated valine (Lien and Nawar, 1974a) were also present when both the
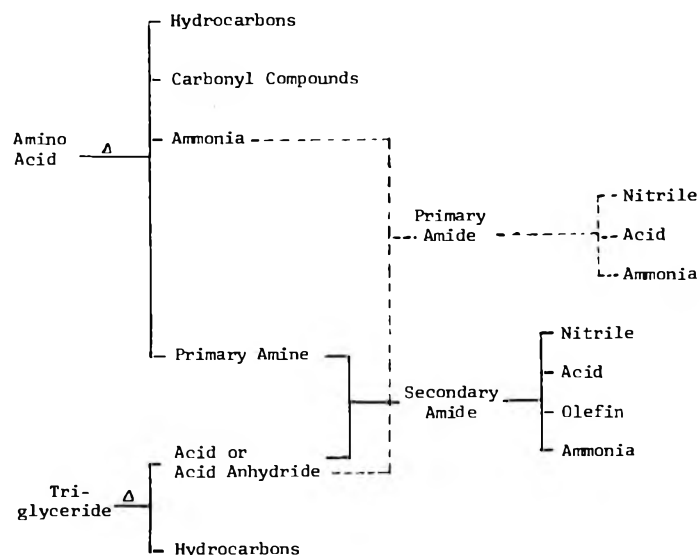
Fig. 2—Scheme for interaction of amino acid and triglyceride upon heat treatment.

amino acid and the triglyceride were heated together.

Methane, ethane, ethene, butane, butene, pentane, pentene, hexanoic acid and dicaproin which were found to form in heated tricaproin were also present in the heated mixture. Also, ammonia, acetone, isobutane, isobutylene, isobutyraldehyde which were produced by heating the amino acid, valine, individually were present when both the amino acid and triglyceride were heated together.

On the other hand, acrolein, methyl hexanoate, hexanal, 6-undecanone, 2-oxopropyl hexanoate, 2-oxoheptyl hexanoate, propanediol dicaproate, propenediol dicaproate and oxopropanediol dicaproate (all observed in heated tricaproin), and isobutylamine, N-isobutylidene-isobutylamine, diisobutylamine (all observed in heated valine), could not be detected when valine and tricaproin were heated together.

In addition, some compounds not previously observed when the individual substrates were heated, have been identified as interaction products formed during the heating of the substrate mixture. These are caproic nitrile, caproic amide and N-isobutylcaproic amide.

Amides can be formed by the acylation of ammonia or the acylation of amines (Beckwith, 1970). Ammonia and amines are produced from thermal decomposition of amino acids (Lien and Nawar, 1974a); acylation agents (i.e. carboxylic acid and acid anhydride) can be formed from pyrolysis of triglycerides (Lien and Nawar, 1973). Therefore, the formation of caproic amide and N-isobutylcaproic amide can result from the following reactions:

(a) Acylation of ammonia and isobutylamine by hexanoic acid:

$$CH_3(CH_2)_4\overset{O}{\overset{\|}{C}}-OH + NH_3 \longrightarrow$$

$$CH_3(CH_2)_4\overset{O}{\overset{\|}{C}}\overset{-}{O}-\overset{+}{N}H_4 \overset{\Delta}{\longrightarrow} CH_3(CH_2)_4CONH_2 + H_2O$$

$$CH_3(CH_2)_4\overset{O}{\overset{\|}{C}}-OH + (CH_3)_2CHCH_2NH_2 \longrightarrow$$

$$CH_3(CH_2)_4CONHCH_2CH(CH_3)_2 + H_2O$$
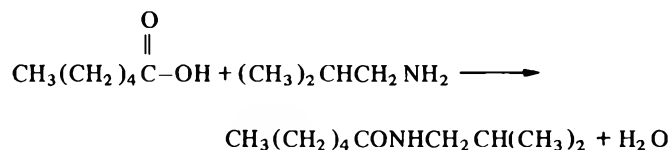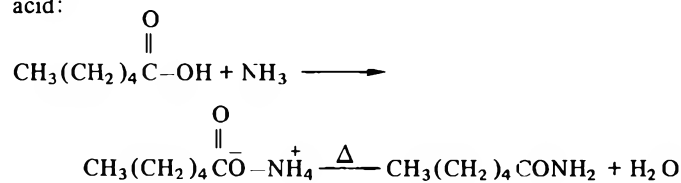
(b) Acylation of ammonia and isobutylamine by hexanoic anhydride:

$$CH_3(CH_2)_4 C=O$$
$$\diagdown$$
$$O + NH_3 \longrightarrow$$
$$\diagup$$
$$CH_3(CH_2)_4 C=O$$

$$CH_3(CH_2)_4 CONH_2 + CH_3(CH_2)_4 COOH$$

$$CH_3(CH_2)_4 C=O$$
$$\diagdown$$
$$O + (CH_3)_2 CHCH_2 NH_2 \longrightarrow$$
$$\diagup$$
$$CH_3(CH_2)_4 C=O$$

$$\overset{O}{\overset{\|}{CH_3(CH_2)_4 C}} \overset{H}{\overset{|}{-N}} -CH_2 CH(CH_3)_2 + CH_3(CH_2)_4 COOH$$
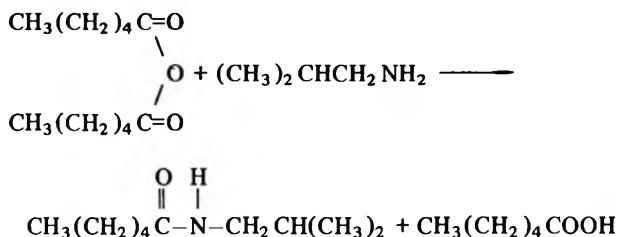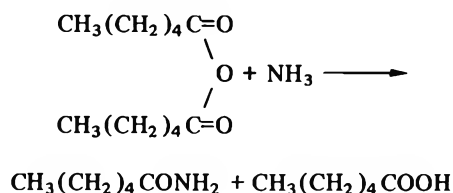
The caproic amide may also react with isobutylamine to form N-isobutylcaproic amide:

$$(CH_3)_2 CHCH_2 NH_2 + CH_3(CH_2)_4 CONH_2 \overset{\Delta}{\longrightarrow}$$

$$\overset{O}{\overset{\|}{CH_3(CH_2)_4 \; C}} \overset{H}{\overset{|}{-N}} -CH_2 CH(CH_3)_2 + NH_3$$
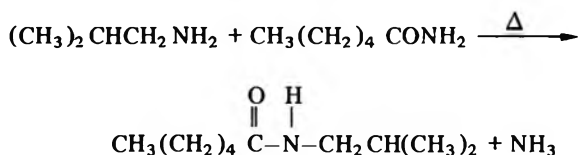
Caproic nitrile is produced from caproic amide which may pyrolyze in two ways:

(a) to nitrile and water:

$$\overset{O}{\overset{\|}{RC}} -NH_2 \longrightarrow RCN + H_2O$$
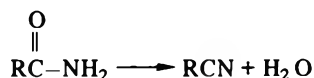
(b) to nitrile, carboxylic acid and ammonia:

$$\overset{O}{\overset{\|}{2RC}} -NH_2 \longrightarrow RCOOH + RCN + NH_3$$

The second of these reactions has been said to result from the direct dehydration of one molecule of amide followed by the hydrolysis of a second molecule (Davidson and Karten, 1956). The caproic nitrile can also result from pyrolysis of N-isobutylcaproic amide. This was verified by heating synthetic N-iso-

butylcaproic amide under the same conditions used for the amino acid-triglyceride mixture. Caproic nitrile, isobutylene and caproic acid were positively identified. The following reactions are probably responsible:

$$CH_3(CH_2)_4 CONHCH_2 CH(CH_3)_2 \overset{\Delta}{\longrightarrow}$$

$$CH_3(CH_2)_4 CONH_2 + (CH_3)_2 C=CH_2$$
$$\downarrow \Delta$$
$$CH_3(CH_2)_4 CN + CH_3(CH_2)_4 COOH + NH_3$$
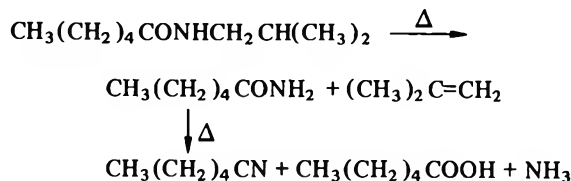
The amount of isobutyraldehyde identified from the valine-tricaproin mixture is greater than the amount of that compound identified from valine alone. This may be due to the presence of hexanoic acid (the major decomposition product of tricaproin) in the mixture, which prevents further reaction of the aldehyde with isobutylamine to produce the imine or the secondary amine. Preferentially the acid reacts with iso-butylamine to produce N-isobutylcaproic amide as discussed above.

6-Undecanone was absent in the heat treated valine-tri-caproin mixture. This may be explained by the fact that its precursor, hexanoic anhydride, reacts with ammonia to produce caproic amide or with isobutylamine to produce N-iso-butylcaproic amide.

It is evident that in a simple system containing only one amino acid and one fatty acid, substantial interaction does occur during heat treatment. It is equally clear that a fundamental knowledge of how individual components decompose when heated separately, can aid significantly in unraveling thermal interaction mechanisms in the more complex mixtures. We are now continuing this investigation to study similar interactions between various peptides and glycerides and between proteins and natural fats.

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Ms received 1/19/74; revised 3/30/74; accepted 4/2/74.

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PECTIC ENZYMES: INDIVIDUAL AND CONCERTED KINETIC BEHAVIOR OF PECTINESTERASE AND PECTINASE

INTRODUCTION

ALTHOUGH PECTIC ENZYMES have been utilized for fruit juice clarification on a commercial scale for several decades, quantitative kinetic information concerning the general behavior of such enzymes is limited. It is now well established that two enzymes which operate in a sequential manner are important in conventional fruit juice clarification processes (Yamasaki et al., 1967). They are pectin esterase (EC 3.1.1.11) and pectinase (polygalacturonase) (EC 3.2.1.5). The commercial enzymes which are obtained from crude protein extracts of fungi such as *Aspergillus niger* contain both enzymes in varying proportions. Tomato is an alternate source of pectin esterase (Nakagawa et al., 1970). We present in this report selected kinetic properties of pectic enzymes obtained from both fungi and tomato.

MATERIALS & METHODS

AN ESTERASE rich commercial enzyme preparation (Pectinol 42-E from *A. niger*) was obtained from Rohm and Haas Co. and used as a crude source of fungal pectin esterase (FPE). Limited purification was achieved by removing a majority of pectinase activity with hydroxylapatite ion exchange chromatography (Harmon and Corden, 1972). Tomato pectin esterase (TPE) and fungal pectinase from *A. niger* (FPG) were obtained from Sigma Chemical Co. in partially purified form. They were used as obtained. Citrus pectin (65% methyl esterified) and polygalacturonic acid (PGA) prepared from citrus pectin were obtained from Sigma Chemical Co.

Pectin esterase activity was measured by a titrimetric method (Vas et al., 1967). The free acid groups produced by hydrolysis of the methyl ester residues of pectin were continuously titrated at constant pH using an automatic titrator module (pHM 25, TT11, ABU 16 and SBR2c) obtained from Radiometer Corp. Initial rates were determined from the slope of the total titrant volume delivered versus time. The standard assay for pectin esterase activity was performed at 30°C and pH 4.5 in solutions containing 0.5% (wt/vol) pectin and 0.1M NaCl. An enzyme unit of esterase activity was defined as that quantity of the enzyme which liberates 1 μmole of H^+ per minute under above stated assay conditions and in the standard assay is uncorrected for fractional dissociation of the resulting uronic acid moiety. Although the optimum pH for TPE lies near pH 8, the enzyme kinetics were investigated at pH 4.5 which reflects a realistic pH environment encountered in fruit juice clarification.
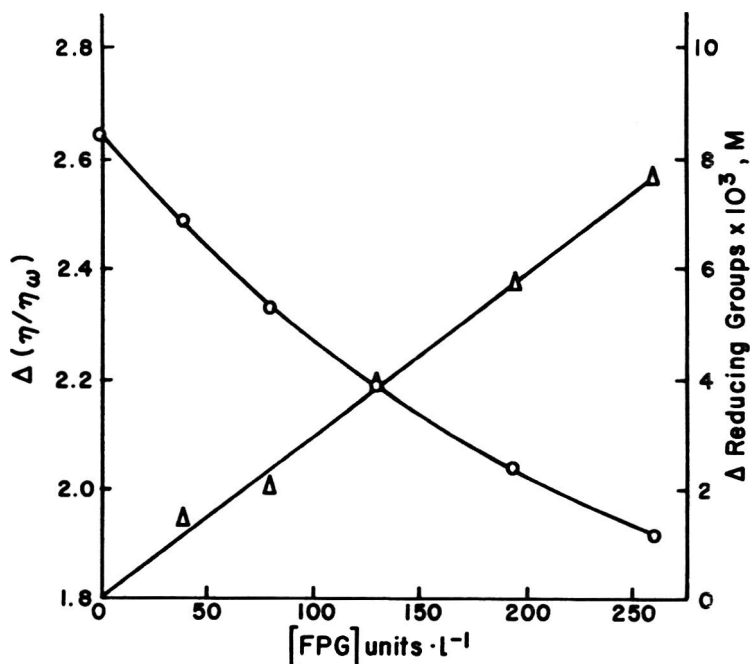
Fig. 1—Change in properties of PGA solutions due to the action of FPG. Change in relative viscosity o——o (left hand ordinate) and relative change in reducing groups △——△ (right hand ordinate) for a 2% PGA solution as a function of FPG concentration. The reaction interval was 30 min. Overnight incubation at 250 units FPG/liter yielded limiting values for relative viscosity and production of reducing groups of 1.1 and 60 mM, respectively. The reaction conditions were 0.1M NaCl and 0.02M acetate buffer at pH 4.5 and 30°C.

Pectinase activity was measured by reducing end group analysis using an iodometric method of Somogyi (1952). A finite reaction time interval of 15 min was used for estimating the reaction velocity. One unit FPE was defined as that quantity of the enzyme which produced 15 μmoles of reducing groups in 15 min for the standard assay described below. The mean of the finite interval velocity measurement corresponds to a standard activity unit of 1 μmole product formed per minute. The viscosity decrease of PGA and pectin solutions which result from depolymerization was used as an estimation of endo hydrolysis by pectinase. An Ubbelohde viscometer was used for the viscosity measurements. The viscosity constants are reported relative to the viscosity of water (n/n_w) at 30°C and pH 4.5. The standard assay solution used for pectinase activity contained 1% PGA (wt/vol), 0.1M NaCl and 0.02M acetate buffer.

TPE, FPE and FPG were assayed for the presence of contaminating pectin lyase which has been reported to be a major component of pectic enzymes produced by *A. sojae* (Ishii and Yokotsuka, 1971). A spectrophotometric assay based upon the detection of the resulting α, β unsaturated galacturonide was used. The assay conditions were 0.5% pectin (wt/vol) pH 4.5 and 30°C. The molar extinction coefficient of the product, 4,5-dehydrogalacturonosyl moiety, at 235 mμ is 3.02 × 10^3 M^{-1} cm^{-1} (Hasegawa and Nagel, 1962) and a unit of enzyme activity is defined as that amount of enzyme which catalyzes the production of 1 μmole product per minute under the above conditions. No detectable pectin lyase activity could be found in both TPE and the purified FPG (the limits of spectrophotometric resolution using a Cary 118C spectrophotometer are approximately 10^{-3} unit/ml). A minor amount of pectin lyase activity was found in the FPE preparation which corresponds to 0.003 unit pectin lyase per unit esterase. This level of contamination (0.3%) is negligible for our purposes.

RESULTS

Mode of action of pectinase

Simultaneous analysis of reducing groups and viscosity were

carried out in order to determine the ratio of endo versus terminal cleavage for FPG. Figure 1 shows the results obtained with 2% PGA when incubated with different enzyme concentrations for 30 min at pH 4.5. While the increase of reducing groups is linear with respect to the enzyme concentration, the viscosity decreases in a nonlinear manner. Approximately 27% of the total decrease in viscosity occurs during the initial 5% hydrolysis as measured by end group analysis. The results are characteristic of extensive endo cleavage.

pH-Rate profile

The pH dependence of enzyme activity under defined assay conditions was studied for TPE, FPE and FPG in the pH range commonly encountered in fruit juices. In Figure 2 it is seen that the fungal enzymes FPG and FPE have similar pH-rate profiles. The optimum for FPG occurs near pH 4.4 while FPE appears to plateau at pH values less than 3.5. These enzymes are effective in the pH range 3.5—5 and could be used in combination with most fruit juice clarification processes. TPE shows only 40% of its maximal activity at pH 4.5.

Effect of NaCl concentration

Figure 3 demonstrates the effect of NaCl concentration on pectic enzyme activities at pH 4.5. FPG appeared quite sensitive to changes in salt concentration. It has a sharp optimum at 0.1M NaCl. FPE was found to be nearly independent of NaCl concentration. TPE displayed extreme sensitivity towards NaCl and was found to be totally inactive at NaCl concentrations below 0.01M. Optimum activity for TPE occurs at 0.17M NaCl and then slowly decreases with increasing NaCl concentration.
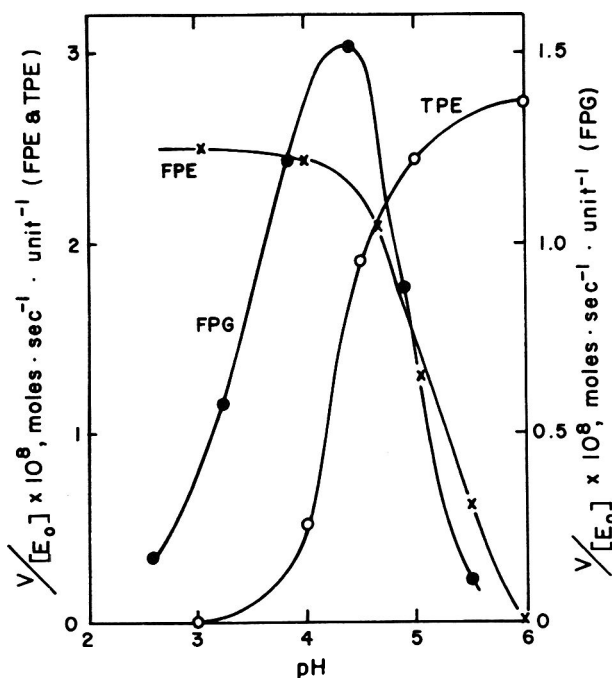
Fig. 2—pH dependence of pectic enzymes. Activities of TPE (o——o), FPE (x——x) and FPG (•——•). The reactions were carried out at 30°C. Specific reaction conditions for each enzyme were as follows: TPE—28 units/liter, 0.5% pectin and 0.1M NaCl; FPE—27 units/liter, 0.5% pectin and 0.1M NaCl; FPG—633 units/liter, 1% PGA, 0.1M NaCl and 0.02M acetate buffer. Reaction velocities for TPE and FPE which are based upon pH-stat measurements are corrected for fractional dissociation of product using a measured pK$_{apparent}$ of 3.9 for the commercial citrus pectin.
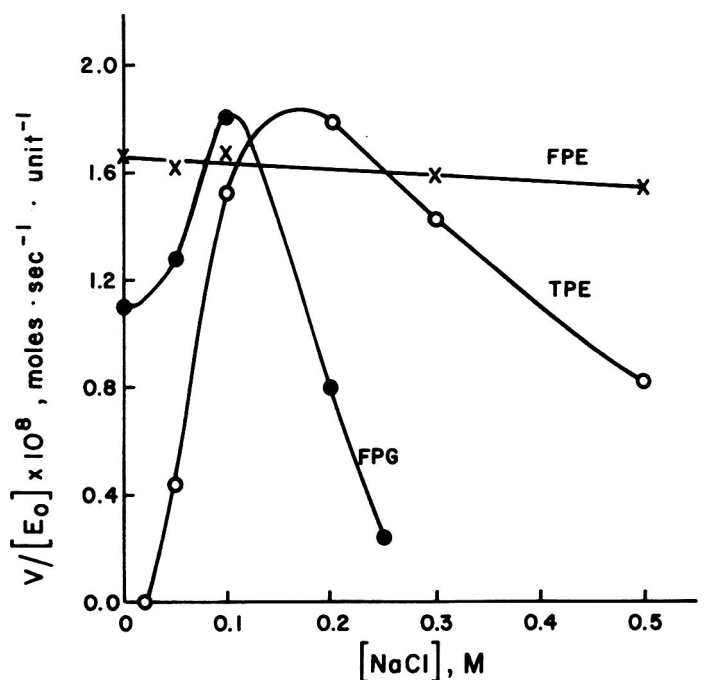
Fig. 3—Effect of NaCl concentration on the activity of pectin enzymes. Activities of TPE (o——o), FPE (x——x) and FPG (•——•) are represented on the same ordinate axis. The reaction conditions for each enzyme at pH 4.5 and 30°C were: TPE—28 units/liter enzyme and 0.25% pectin; FPE—27.5 units/liter enzyme and 0.5% pectin; FPG—633 units/liter enzyme, 1% PGA and 0.02M acetate buffer.

Effect of substrate concentration

Conventional double reciprocal plots of TPE and FPE are shown in Figure 4. Both enzymes show linear behavior. The apparent K_m values for TPE and FPE at pH 4.5 evaluated from Figure 4 are 0.055% and 0.20% wt/vol, respectively. The ordinate intercepts yield k_{cat} values for TPE and FPE of 0.0185 and 0.0227 μmoles $\cdot$ s^{-1} per unit enzyme, respectively.

Initial velocity data for pectinase kinetics are difficult to obtain as the method used for the determination of reducing groups requires discrete sampling and finite interval measurements. In addition, as the reaction progresses there is an increase in the effective molecular concentration of the substrate as depolymerization occurs. Integral analysis has not been used in pectic enzyme kinetics, although the procedure would appear to be ideally suited for endo-depolymerizing enzymes such as pectinases.

The data of Table 1 as shown in Figure 5 do not yield a linear plot for a simple integrated Michaelis-Menton kinetic mechanism. It was found that integral rate data could be linearized by using a functional turnover equation of the form

$$\frac{E_t}{V} = \theta_1/(S) + \theta_2(S) + \theta_3 \qquad (1)$$

The second term containing θ_2 arises from substrate inhibition and appears to be quite significant at our experimental conditions. The integrated form of equation 1 reduces to

$$\ln(S/S_o) = E_T \, t/\theta_1 - \theta_2(S^2 - S_o^2)/\theta_1 - \theta_3(S\text{-}S_o)/\theta_1 \qquad (2)$$

Figure 6 shows a plot of ln (S/S_o) versus S^2-S_o^2 for the data of Table 1. The linear behavior indicates that minor variations of the term in S-S_o are not significant at constant reaction time intervals.

Table 1—Relative magnitude of substrate terms in Eq (2)[a]

PGA conc S_o M X 10^3	(S-S_o) M X 10^3	S^2-S_o^2 M^2 X 10^6	S/S_o
0.77	4.93	31.9	7.4
1.025	6.09	49.7	6.9
1.54	7.25	75.0	5.7
2.31	8.14	103.7	4.52
3.86	8.74	144.0	3.27
7.70	8.65	208.0	2.12

[a] Typical turnover data obtained over finite reaction time intervals for FPG. The substrate dependence of EQ (2) is reflected by terms in S-S_o, S^2-S_o^2, and S/S_o where S_o and S represent the reducing group concentration at time equals 0 and 15 min, respectively. The reaction conditions were 633 units FPG/liter, 0.1 to 1.0% PGA, 0.1M NaCl and 0.02M acetate buffer at pH 4.5 and 30°.

Product inhibition of pectin esterase

PGA was used to investigate product inhibition of TPE and FPE. Figure 7 shows that while the tomato enzyme was inhibited by the presence of PGA, the fungal enzyme showed a small increase in activity. The inhibition observed in case of TPE appeared to be competitive and a K_i of 0.024% wt/vol PGA was obtained.

Effect of pectinase on pectin esterase activity

Pectin esterase initial hydrolysis rates were measured in the presence of varying concentrations of pectinase to determine

Fig. 4—*Conventional double reciprocal plots of TPE and FPE kinetics. The substrate dependence of TPE (o––o) and FPE (x––x) was linear over a tenfold substrate range. The reaction conditions are 0.1–1% pectin, 0.1M NaCl, pH 4.5 and 30°C. Both enzymes are at a relative concentration of 28 units per liter. pH stat kinetics at pH 4.5 were uncorrected for fractional dissociation of product.*

Fig. 5—*FPG integrated turnover kinetics: Nonlinearity of the integrated Michaelis-Menton equation. The data of Table 1 are plotted as the log (S/S_o) versus S-S_o where S_o and S represent reducing group concentrations at time 0 and 15 min, respectively. The reaction conditions are 633 units FPG/liter, 0.1–1.0% PGA, 0.1M NaCl and 0.02M acetate buffer at pH 4.5 and 30°C.*

the effect of pectinase upon the esterase activity. Since minor pectin esterase activity was present in the partially purified FPG, a suitable set of control experiments were performed in order to eliminate the esterase contribution endogenous to FPG. Of the two lines in Figure 8 the lower line represents the endogenous pectin esterase activity in FPG preparation. The upper line was obtained when FPG preparation was supplemented by an additional 15 units per liter of TPE. Both lines have the same slope indicating that there is no significant effect on TPE activity due to the presence of pectinase. Jansen et al. (1945) have reported some activation of pectin esterase by pectinase. Such activation may be due to an increased molecular concentration of polygalacturonide oligomers resulting from the depolymerizing action of pectinase on partially esterified PGA.

Effect of pectin esterase on pectin degradation by pectinase

Optimization in the ratio of pectin esterase to pectinase is advantageous in that a maximum rate of pectin degradation can be rapidly achieved with a minimum amount of methanol introduction into the fruit juice. A series of experiments were designed to investigate the sequential enzyme catalyzed depolymerization of pectin. The depolymerization of a 0.5% pectin solution at pH 4.5 in the presence of 0.1M NaCl was systematically investigated using fixed amounts of pectinase and varying TPE activity. Both the viscosity of the solution and the concentration of methanol in the reaction mixture are recorded as a function of time. Methanol was not directly measured but calculated from the stoichiometry of ester hydrolysis based upon pH stat measurements. Figure 9 shows the experimental profile for several enzyme ratios. Two experiments were performed using endogeneous pectin esterase ac-

tivity of FPG while three experiments contained 14.85, 32.3 and 61.4 units per liter of additional TPE. It can be seen that in first two experiments the viscosity decreases very slowly using the commercial preparation while upon addition of TPE a rapid decrease in viscosity, proportional to TPE concentra-
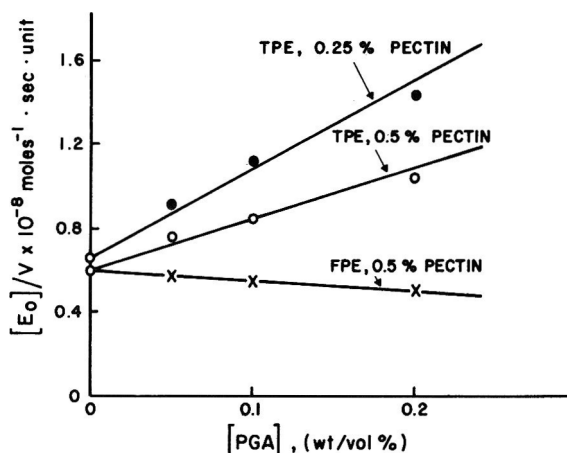
Fig. 7—Inhibition of TPE and FPE by PGA. TPE inhibition kinetics were carried out in presence of 0.5% (o——o) and 0.25% (●——●) pectin concentrations and FPE inhibition kinetics were performed at a pectin concentration of 0.5% (x——x). The reaction conditions were 28 units/ liter TPE or FPE, 0—0.2% PGA, 0.1M NaCl at pH 4.5 and 30°C.

Fig. 6—FPG integrated turnover kinetics: Incorporation of substrate inhibition into the turnover equation. The data of Table 1 are plotted as log S/S_O (ordinate) versus S^2-S_O^2 (abscissa) according to the integrated turnover Eq (2) where S_O is the initial reducing group concentration and S that after a reaction period of 15 min. The reaction conditions are 633 units FPG/liter, 0.1—1.0% PGA and 0.1M NaCl and 0.02M acetate buffer at pH 4.5 and 30°C.

Fig. 8—Effect of FPG on TPE activity. The lower line o——o represents endogeneous pectin esterase activity in the FPG preparation and represents the control experiment. The upper line x——x was obtained when FPG preparation was supplemented by an additional 15 units/liter of TPE. Parallel lines indicate that FPG does not influence TPE activity under these assay conditions. The reaction mixture contained 0.25% pectin and 0.1M NaCl at pH 4.5 and 30°C.

tion, is observed. In Figure 10 the data of Figure 9 are replotted in terms of the time needed to reach a viscosity ratio n/n_w equal to 1.05 and the amount of methanol subsequently accumulated versus the TPE concentration. The value of n/n_w chosen reflects a realistic value which would be useful in fruit juice processing. At low TPE activity considerable time is required to achieve the desired pectin degradation. However, the amount of methanol produced appears minor. As the TPE concentration is increased, the degree of pectin degradation correspondingly increases while methanol production appears maximal at an esterase concentration of 35 units per liter.

Temperature effect on pectic enzymes

The temperature profile of TPE, FPE, and FPG are shown in Figure 11. The initial velocity measurements were obtained immediately after the enzyme is introduced into the reaction mixture. In all cases thermal inactivation was ruled out as appropriate integral kinetic plots indicated an absolute conservation of E_T. For FPE the optimum occurs at 40°C. The temperature rate profile of TPE appears biphasic. From 20–35°C the rate rises rapidly, though not exponentially. When the temperature is increased further from 35°C, only a small rise in the activity is observed. FPG gives an exponential increase in the activity as the temperature is raised to 50°C and at 60°C the activity drops rapidly. In Figure 12, Arrhenius plots of TPE, FPE and FPG activities are shown. Only FPG

yields a linear relationship with an activation energy of 3.16 kcal. Apparent activation energies for TPE and FPE at 30°C and 6.0 and 2.04 kcal, respectively.

DISCUSSION

THE COMMERCIAL application of pectolytic enzymes to fruit juice clarification is usually an empirically optimized process. Significant differences with respect to soluble juice components and solution parameters such as pH, viscosity, ionic strength and physical heterogeneity are observed for different fruit preparations. Indeed, such differences may arise within a single type of fruit in which the fruit population being processed is highly heterogeneous with respect to state of maturation, species and growth conditions. As commercial pectolytic enzyme preparations are fixed blends of two or more enzymes, the possibility of obtaining a commercial enzyme preparation which is optimal for all applications is unlikely. Therefore it would be desirable to either modify commercial preparations to meet specific needs or conversely modify processing parameters to match the behavior of the commercial enzyme preparation. The characterization of critical chemical and physical properties of the enzymes in question are necessary to rationally choose between these two alternatives and optimize the overall process.

The pH rate profile of FPG shows optimal kinetic behavior
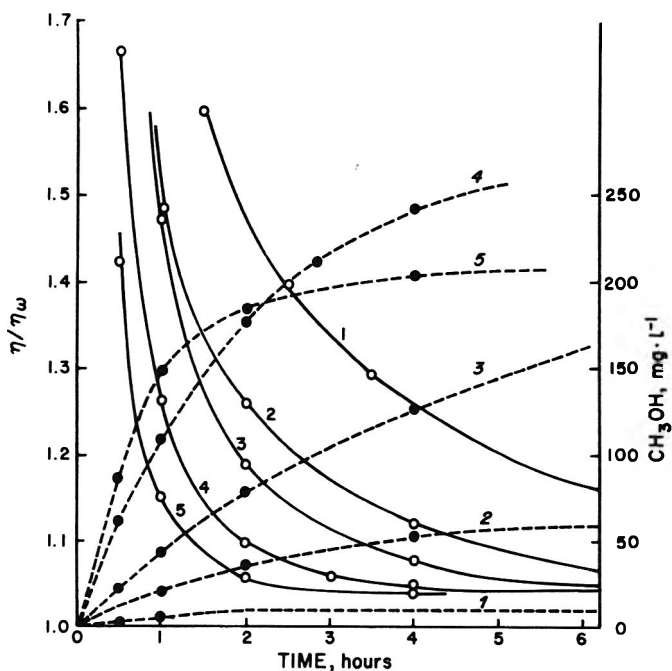
Fig. 9–Pectin degradation by concerted action of TPE and FPG. The left ordinate represents the viscosity ratio of the reaction mixture o——o while the right ordinate represents the methanol production o---o. The reactions were carried out in presence of 0.5% pectin and 0.1M NaCl at pH 4.5 and 30°C. Methanol concentrations are calculated from the stoichiometry of ester hydrolysis and are based upon pH-stat measurements which are corrected for partial dissociation of the resulting polygalacturonic acid. The following enzyme combinations were used.

(1) FPG–161.5 units/liter, FPE (endogeneous) 3.2 units/liter;

(2) FPG–323 units/liter, FPE (endogeneous) 6.4 units/liter;

(3) FPG–161.5 units/liter, total pectin esterase 14.85 units/liter;

(4) FPG–161.5 units/liter, total pectin esterase 32.3 units/liter;

(5) FPG–161.5 units/liter, total pectin esterase 61.4 units/liter.
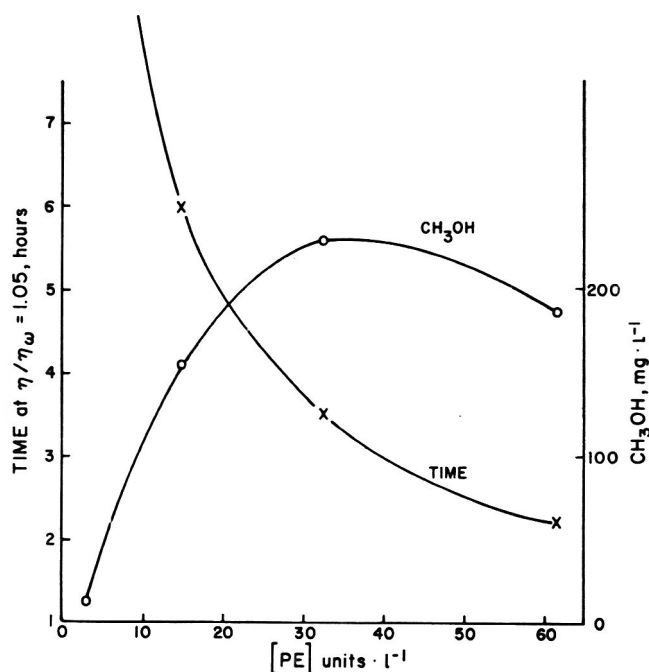
Fig. 10–The influence of coupled enzyme ratios upon accumulated methanol when a specific viscosity of the reaction solution is desired. The data in Fig. 9 are replotted as the time needed to reach a viscosity ratio n/n_w equal to 1.05 x——x (left hand ordinate) and the amount of methanol accumulated in the process o——o (right hand ordinate) as functions of the esterase activity present in solution with 161.5 units/liter of FPG.

at pH 4.4 in the presence of 0.1M NaCl. Using established analytical methods (Alberty and Massey, 1954), it can be shown that FPG displays a symmetrical bell shaped profile with approximate pK values for the left and right shoulder of 3.8 and 4.7, respectively. The decrease in activity displayed by the low pK shoulder probably reflects the gross status of substrate ionizations and the preference of the enzyme for anionic forms. The pK of galacturonic acid is 3.5 and would be expected to shift to slightly higher values due to the polyelectrolytic nature of the high molecular weight oligomer. The partially esterified pectin (65% esterified) used for the esterase activity measurements displayed an apparent pK of 3.9 with slight skewing of the titration profile at the high pH side. The high pH limb of the FPG profile probably reflects an ionization state of the enzyme which influences either substrate binding or first order processes on the catalytic pathway. Our data do not distinguish between these alternatives as the principal coefficient in the turnover equation at the optimal pH under our assay conditions contains a significant contribution from both processes. The apparent pK's for FPE and TPE are 5.0 and 4.3, respectively, in the presence of 0.1M NaCl. These pK's are also assigned to ionization states of the enzyme as they appear to be significantly higher than that assigned to the substrate. FPE possesses a plateau in the profile at pH values less than 3.5. The enzyme appears to be rapidly inactivated at pH values less than 2. Both TPE and FPE at pH 4.5 exhibit similar activity with respect to their pH optimum. Depending upon the operational pH of interest FPE at pH values less than 5 or TPE at pH values greater than 4 would be preferred.

At pH 4.5 both FPE and FPG display high sensitivity to NaCl. The optimum NaCl concentration with either esterase

and FPG would be in the vicinity of 0.1M. Provided the NaCl effect is purely an ionic strength effect, most fruit juices with an ionic strength equivalent of 0.05—0.15M salt can be readily processed with either combination. At low salt levels the combination of fungal enzymes appears more suitable.

Conventional methods of enzyme kinetic analysis yield apparent K_m values of 0.055% and 0.20% for TPE and FPE, respectively, at pH 4.5, 0.1M NaCl and 30°C. Product inhibition using PGA was observed only in the case of TPE and appeared to be competitive with a K_i of 0.024% (wt/vol). Values of k_{cat} cannot be directly compared for the two esterase enzymes as an arbitrary standard state based upon intrinsic catalytic activity is used for the assignment of enzyme concentrations.

As was demonstrated in the results section, the FPG preparation appeared to be predominantly an endo enzyme. The finite interval rate data could not be fitted to an integrated form of the Michaelis-Menton equation. Incorporation of a substrate inhibition term in the turnover equation results in a form of the integrated rate equation which yields appropriate linear plots for substrate dependence. Substrate inhibition of this functional form must arise from unproductive binding of at least two oligomers. In the case of lysozyme and amylase subsite affinity studies have documented numerous degrees of partial active site coverage in which one or more substrate oligomers may participate (Rupley and Gates, 1967; Hiromi, 1972).

As depolymerization proceeds, the molecular weight distribution of substrate progressively shifts to lower weight average values with a concomitant increase in substrate concentration. In the case of an endo depolymerizing enzyme this shift
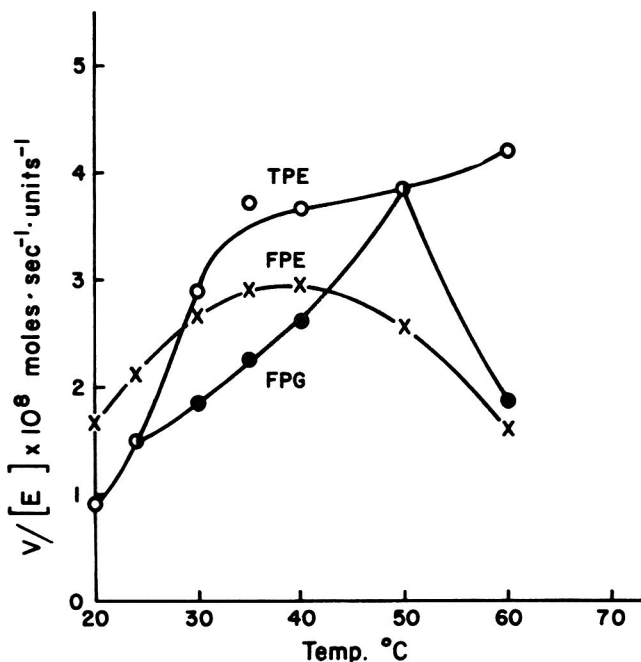
Fig. 11—Effect of temperature on the activity of pectic enzymes. Relative activities of TPE (o——o), FPE (x——x) and FPG (●——●) at pH 4.5. The assays for TPE and FPE at relative concentrations of 28 units/liter were performed with 0.5% pectin solutions in presence of 0.1M NaCl and pH 4.5. The pH-stat data is uncorrected for fractional dissociation of product. FPG activity assays were obtained as described in the text and reflect a mean velocity over a 15-min interval. The reaction conditions were 633 units/liter, 1% PGA, 0.1M NaCl, 0.02M acetate and pH 4.5.
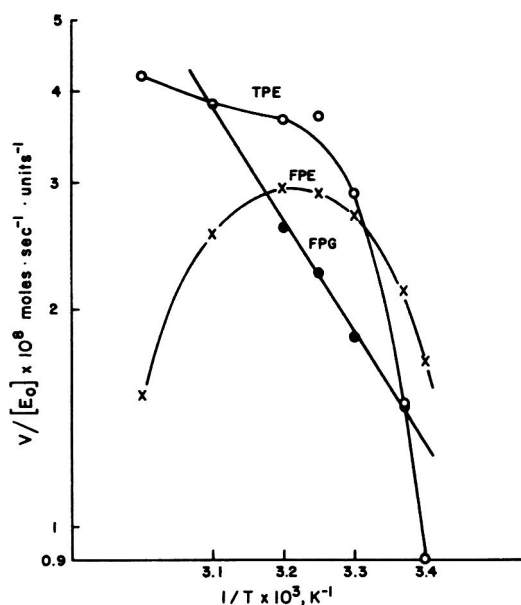
Fig. 12—Arrhenius plots for TPE (o——o), FPE (x——x) and FPG (●——●). The data were obtained from Fig. 11.

may produce significant concentrations of lower molecular weight oligomers such that a high incidence of partial active site coverage occurs from two or more substrate molecules. The consequence would lead to an unproductive enzyme substrate(s) complex when the composite free energy of binding two substrates is not sufficient to achieve the transition state configuration for hydrolysis. This state also could arise when the terminal ends of the two substrates are adjacent to the cleavage locus at the active site and possibly result in polymerization.

A minimal kinetic scheme for the enzyme under our experimental conditions would be

$$E + S_h \underset{\longrightarrow}{\overset{K_m}{\rightleftharpoons}} ES_h \overset{k_{cat}}{\longrightarrow} E + S_{l \text{ or } h}$$

$$E + S_{h \text{ or } l} \overset{K_1}{\rightleftharpoons} E'S$$

$$E'S + S_{h \text{ or } l} \overset{K_2}{\rightleftharpoons} E'S_2$$
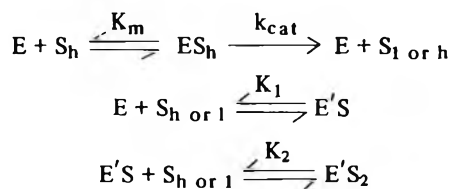
where S_l and S_h represent low and high molecular weight oligomers, respectively, and which are arbitrarily defined about a critical molecular weight. Assuming rapid equilibrium for all substrate encounters and defining all equilibrium constants as dissociation constants the turnover equation is

$$E_T/V = K_m/k_{cat}(S_h) + 1/k_{cat} + K_m(S_{h \text{ or } l})/K_1(S_h)k_{cat}$$

$$+ K_m(S_{h \text{ or } l})^2/K_1K_2(S_h)k_{cat} \qquad (3)$$

As the occurrence of a new reducing end group can produce both high and low molecular weight species we define a partitioning coefficient associated with S_h

$$S_h = S_h^o + \alpha\Delta(RS) \qquad (4)$$

where $\Delta(RS)$ represents the change in reducing sugar equivalents. The commercial polygalacturonic acid used in our studies contained a considerable low molecular weight contribution to the molecular weight average. Considering the manufacturer's average value specification of 40–200 galacturonic acid units per reducing end group, the native citrus pectin had obviously suffered considerable depolymerization. In all likelihood this depolymerization resulted from contaminating endo polygalacturonases. With this assumption we may then express the distribution of $S_{h \text{ or } l}$ and S_h in terms of the original reducing sugar equivalents and the distribution coefficient α. Substitution into Eq (4) yields

$$S_h = \alpha(RS^o) + \alpha\Delta(RS) = \alpha[(RS)^o + \Delta(RS)]$$

$$S_{h \text{ or } l} = (RS^o) + \Delta(RS) \qquad (5)$$

Substitution of Eq (5) into Eq (3), integration and rearrangement yields

$$\ln((RS^o)/(RS^o + \Delta RS)) = E_T \cdot t \cdot k_{cat}/K_m - (\alpha K_l + K_m)$$

$$\cdot \Delta RS/\alpha K_1 K_2 - [(RS^o + \Delta RS)^2$$

$$- (RS^o)^2]/2\alpha K_1 \cdot K_2 \qquad (6)$$

As shown in the result section a plot of the left hand side of Eq (6) versus- $[(RS^o + \Delta RS)^2 - (RS^o)^2]$ is linear indicating that the term in ΔRS, whose coefficient is $(\alpha K_l + K_m)/\alpha K_l K_m$, is not a significant contributor under our experimental conditions. The slope of Figure 6 yields a value for

$\alpha K_1 K_2$ of 69 mM^2. As α is less than unity, the lower limit of $K_1 K_2$ is 69mM^2. A realistic value of α for the polygalacturonase preparation used in our experiments would be of the order of 0.9–0.95 assuming 5 monomeric units comprise a minimal binding unit for depolymerization. Kimura et al. (1973) have shown that a minimum of 4 galacturonide units are necessary to initiate glycolytic cleavage for polygalacturonase from Aerocylindrium. Since competative and unproductive binding of multiple oligomer fragments would not be expected to produce a substantially more negative free energy than the productive binding of a single substrate oligomer, we estimate the upper limit of K_m to be 69 μM.

Commercial enzyme preparations for fruit juice clarification contain predominately endo- FPG as the major depolymerizing enzyme and it catalyzes hydrolysis of the pectin molecule where the galacturonide unit has been deesterified. Thus PE is essential for depolymerization. Extensive deesterification, however, is neither necessary nor desirable. The product, methanol, is a toxic substance. Therefore an optimization in the ratio of PE to PG is desirable in that the maximum amount of pectin degradation be achieved with a minimum amount of methanol accumulation. When processing requires that methanol be maintained less than 50 ppm a ratio of FPG/PE greater than 50 should be used with the maximum PE concentration less than 3.2 units per liter. If rapid processing on the order of 2 hr or less is required, an acceptable viscosity can be achieved with FPG/PE ratios near 2 and the FPG concentration in excess of 200 units per liter. Under these conditions methanol production levels out at approximately 200 ppm.

FPG appears quite stable at elevated temperatures and a threefold increase in rate is observed for the interval 20–50°C. Both esterase enzymes appear to show a change in the rate limiting step of catalytic turnover as demonstrated by the biphasic temperature profiles. FPE displays decreasing activity at temperatures in excess of 35°C. Such behavior may reflect a large negative entropic contribution to the binding of substrate, thus resulting in an apparent inversion of the temperature profile as substrate binding begins to dominate the kinetic mechanism. Provided flavor and other constituents of the juice can withstand incubation at 50°C it would appear that FPG supplemented with TPE provides the best combination.

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Ms received 1/26/74; revised 4/8/74; accepted 4/17/74.

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PREPARATION AND STABILITY OF TRYPSIN IMMOBILIZED ON POROUS GLASS

INTRODUCTION

TREATMENT of milk with trypsin has been shown to inhibit the development of oxidized flavor (Anderson, 1939; Doan and Miller, 1940; Forster and Sommer, 1951; Olson and Brown, 1944). It has been reported that the antioxygenic effect of trypsin in milk is a function of the degree of proteolysis (Lim and Shipe, 1972).

Trypsin has been immobilized on porous glass by covalent bonding (Weetall, 1969) and the bound enzyme used to retard oxidized flavor in milk (Shipe et al., 1972). The glass-bound trypsin can easily be removed from the milk, stored and reused at a later date. This study was undertaken to determine the optimum procedure for preparing the glass-bound trypsin and the stability of the bound enzyme under storage and operational conditions.

EXPERIMENTAL

Preparation of glass-bound trypsin

The general procedure described by Weetall (1969) was followed. Porous 96% silica glass particles (Pierce Chemical Co.) were silanized in 10% solution of γ-aminopropyltriethoxysilane (Union Carbide Corp.) overnight at 40°C when acetone was used as solvent or at 80°C when toluene or water was used. Two types of linkage between the carrier and the enzyme were prepared. Glutaraldehyde linkage was prepared as previously described with minor modifications (Shipe et al., 1972). Each gram of alkylamino glass was reacted with 10 ml of a glutaraldehyde-phosphate buffer mixture. The glass stood at room temperature in a vacuum desiccator evacuated with an aspirator for 1 hr and then at atmospheric pressure for 2 more hours. The excess glutaraldehyde was washed off with 4 aliquots of 100 ml of distilled water. Diazo linkage was prepared from alkylamino glass as described by Weetall (1969). Trypsin (2× crystallized, Nutritional Biochemical Corp.) was immobilized on glass through either type of the linkages by reacting each gram of the appropriate glass derivatives with 10 ml of a 0.5% trypsin solution in 0.1M phosphate buffer (pH 7.0) at 5°C overnight. The excess trypsin solution was removed by filtration.

Measurement of amino content of alkylamino glass

Alkylamino glass was exhaustively washed with distilled water to remove soluble amino compounds. The bound amino groups were then liberated from glass by washing 0.5g of the glass with 10 ml 0.1N NaOH in a 50 ml Erlenmeyer flask at room temperature for 2 hr. The bound amino groups were completely liberated in five washes. Therefore, after proper dilution the amino content of the five washings was determined by using the procedure of Satake et al. (1960).

Removal of loosely bound or entrapped trypsin from glass

Glass-trypsin derivatives were washed by either of the following two processes.

Batch process. An aliquot of 300 ml of distilled water, 0.1M phosphate buffer (pH 7.0) or 1M NaCl was added to 5g (wet weight) of glass-bound trypsin in a 1 liter Erlenmeyer flask. The flask was moderately shaken for 2 hr at room temperature. The washing was removed by decantation, filtered through Whatman No. 1 filter paper, and the trypsin activity was determined.

Continuous process. Glass-bound trypsin (5g, wet weight) was packed into a glass column (i.d. 2.2 cm). Phosphate buffer (0.1M, pH 7.0) was pumped through the column at a flow rate of 150 ml/hr at room temperature so that 300 ml of washing were collected every 2 hr.

Measurement of trypsin activity in washings

Trypsin activity in washings was assayed by the hydrolysis of N-benzoyl-L-arginine ethyl ester (BAEE) as described by Bergmeyer (1963). BAEE was prepared in 0.1M phosphate buffer (pH 7.0). 1.6 ml of washing was mixed with 1.6 ml 1 mM BAEE and the change in absorbance measured every 30 sec for 5 min. One BAEE unit increases absorbance 0.001/min at 254 nm, pH 7.0 and 23°C. The absorbance was measured in a Beckman Acta C11 spectrophotometer.

Determination of degree of leaching

The extent of leaching of enzyme from glass-trypsin derivatives was indirectly determined as follows. An aliquot of 50 ml of 0.5 mM BAEE was added to 0.5g (wet weight) glass-bound trypsin at 23°C. The enzyme-substrate mixture was moderately shaken and 4 ml aliquots were removed at 10 min intervals and filtered through Whatman No. 1 filter paper. Absorbance of the filtrates was read immediately at 254 nm and again after being held at 23°C for 60 min. The degree of leaching was expressed as the amount of soluble trypsin pres-

Table 1—Amino content of alkylamino glass derivatives[a] silanized in different solvents

Solvent	Toluene	Acetone	Water (pH 10.5)	Water (pH 3.5)
Amino content (μmoles/g)	65	110	110	75

[a] Particle size 80—120 mesh, pore diameter 550 Å

Table 2—Effect of washing on removal of soluble trypsin from glass support

Washing solution	Number of washes[a]					
	4	8	12	16	20	24
Direct assay—Active trypsin detected in washings						
(BAEE units per 1200 ml)						
Water	19300	0	0	0	0	0
0.1M PO₄ buffer	60200	1900	0	0	0	0
Indirect assay—Relative amount of soluble trypsin remaining[b]						
Water	13	6	5	6	3	0
0.1M PO₄ buffer	46	17	12	8	4	0

[a] 5g of sample were washed with 300 ml solution for 2 hr with shaking.
[b] Amount of soluble enzyme in BAEE units removed from 0.5g of sample in 50 ml of BAEE after the specified number of washings.

ent in the enzyme-substrate mixture in BAEE units.

Determination of enzymatic activity of glass-bound trypsin

A continuous process was employed when BAEE (0.5 mM) was used as the substrate. Glass-trypsin derivative (0.5g, wet weight) was packed in a glass column (i.d. 1 cm), water-jacketed at 30°C, and BAEE was pumped through at various flow rates from a 30°C reservoir. Results were expressed as percent BAEE hydrolyzed. A batch process was used when casein (1%) was the substrate. Glass-trypsin derivative (0.5g, wet weight) was added to 25 ml casein solution in a 60 ml test tube. The tube was shaken on a reciprocating shaker (Virtis Research Equipment) for 250 min at 23°C. After reaction the supernatant was filtered through Whatman No. 1 filter paper and nonprotein nitrogen (NPN) was determined in the filtrate. The method described by Bergmeyer (1963) was followed with minor modifications. A 1-ml aliquot of sample was added to 4 ml 10% trichloroacetic acid in a 10 ml centrifuge tube. The mixture was shaken, held for 30 min and centrifuged at 540 × G for 10 min. The supernatant was filtered through Whatman No. 1 filter paper. Absorbance of the filtrate was read at 280 nm against a water reference in a Beckman Acta C11 spectrophotometer.

Determination of storage stability of glass-bound trypsin

Glass-trypsin derivatives were stored at 5°C as wet cakes in closed glass containers. Their activity on BAEE was measured before and after storage.

Determination of operational stability of glass-bound trypsin

Casein solution (1%) was passed continuously through a glass column (i.d. 1 cm) containing 1g (wet weight) of azo-linked glass-bound trypsin at 5°C. A slow flow rate (3 ml/hr) was employed so that a measurable increase in nonprotein nitrogen could be obtained. The NPN of the effluents was measured as previously described.

RESULTS & DISCUSSION

ALKYLAMINO glass derivatives silanized in different solvents contained different amounts of amino group. Table 1 shows that alkylamino glass derivatives (80–120 mesh, pore diameter 550 Å) silanized in acetone or alkaline water (pH 10.5) had higher amino contents than those silanized in toluene or acidic water (pH 3.5). The 10% silane solution in toluene appeared to have damaged the physical structure of the glass beads resulting in the formation of clumps during the final immobilization steps. The degree of structural damage to the beads was decreased and the amino content increased by about 50% when a lower silane concentration (5%) was used. Because of the possibility of solubilization of glass beads in alkaline aqueous solution, acetone appeared to be the best silanization solvent among those tested.

After immobilization of trypsin through glutaraldehyde or azo linkage, glass-trypsin derivatives contained a substantial amount of loosely bound or en-

Table 3—Enzymatic activity of glass-bound trypsin washed with different solutions

| | Activity (% BAEE hydrolyzed) | |
| | Substrate flow rate (ml/hr)[a] | |
Wash solution	180	360
Water	31	16
0.1M phosphate buffer	42	23
1M NaCl solution	42	24

[a] BAEE (0.5 mM) was pumped through 0.5g (wet weight) of a glass-trypsin preparation.

trapped enzyme. In a preliminary study it was found that a total of 2.4 liters of aqueous solutions was required to adequately wash a gram (wet weight) of glass-bound trypsin. When the glass-trypsin derivative was washed more systematically in a flask with shaking and the washing removed by decantation, the amount of aqueous solution needed to properly wash unit weight of glass-bound trypsin was reduced by about 50%. In the top half of Table 2, the data indicate that

trypsin was detectable only in the first few washings. However, when a more sensitive technique was used for detecting soluble enzyme, it was observed that 24 washings of water or phosphate buffer were required to thoroughly remove loose enzyme from 5g (wet weight) of glass-bound trypsin. Phosphate buffer removed more active enzyme from the glass-trypsin derivative than water. Similar results were obtained when 1M NaCl was used as the washing solution. This presumably was due to the ionic strength of phosphate buffer and NaCl solution. The presence of charged ions probably facilitated the desorption of enzyme which had been ionically attached to the glass. However, the lower amount of active trypsin in the washings (Table 2) and the lower activity of the water washed preparation (Table 3) could also be explained by the possible adverse effect of water on trypsin activity.

When a large quantity of glass-enzyme preparation needs to be washed, a batch process is difficult and impractical. Therefore, the effectiveness of washing the glass-bound trypsin continuously in a

Table 4—Comparison of washing efficiency between batch and continuous processes

Method of washing	Total volume in ml of phosphate buffer used/g of sample[a]				
	240	480	720	960	1200
Direct assay—Active trypsin detected in washings (BAEE units per 1200 ml)					
Batch	23600	1100	0	0	0
Continuous	29200	3400	1300	0	0
Indirect assay—Relative amount of soluble trypsin remaining[b]					
Batch	69	51	27	9	0
Continuous	62	44	23	4	0

[a] 5g of glass-bound enzyme (wet weight) were washed with 0.1M phosphate buffer (pH 7.0).
[b] Amount of soluble enzyme in BAEE units removed from 0.5g of the washed sample in 50 ml of BAEE.

Table 5—Hydrolytic activity of azo- and glutaraldehyde-linked glass-trypsin derivatives on BAEE and casein

| | | Activity | |
| | | Substrate used | |
Type of linkage	Silanization solvent[a]	BAEE[b] % hydrolyzed	Casein[c] Δ NPN
Azo	toluene	97	0.16
	acetone	100	0.27
	water (pH 10.5)	100	0.24
	water (pH 3.5)	–	–
Glutaraldehyde	toluene	73	0.10
	acetone	95	0.22
	water (pH 10.5)	91	0.23
	water (pH 3.5)	93	0.16

[a] Porous glass (80–120 mesh, pore diameter 550 Å) was silanized in 10% γ-aminopropyltriethoxysilane. 5% silane was used in the case of toluene.
[b] BAEE (0.5 mM) was pumped through 0.5g of glass-trypsin preparation at 360 ml/hr and 30°C.
[c] 25 ml 1% casein were reacted with 0.5g of glass-trypsin preparation for 250 min at 23°C.

Table 6—Hydrolytic activity of replicates of glass-trypsin derivatives linked through glutaraldehyde.

Silanization solvent[a]	Replicate	Activity BAEE[b] % hydrolized	Casein[c] Δ NPN
Acetone	1	97	0.21
	2	95	0.22
	3	92	0.24
Water (pH 10.5)	1	93	0.22
	2	91	0.23

[a] Porous glass (80—120 mesh, pore diameter 550 Å) was silanized in 10% γ-aminopropyltriethoxysilane.
[b] BAEE (0.5 mM) was pumped through 0.5g of glass-trypsin preparation at 360 ml/hr and 30°C.
[c] 25 ml 1% casein were reacted with 0.5g of glass-trypsin preparation for 250 min at 23°C.

glass column was compared with that of the batch process. Table 4 shows that the continuous washing process was at least as effective as the batch process. A total of 1.2 liters of 0.1M phosphate buffer was required to wash a gram (wet weight) of the glass-trypsin preparation free of soluble enzyme in both cases.

To determine whether it is easier to wash glass-trypsin derivatives having a larger pore diameter, glass-trypsin derivatives were prepared from glass beads with pore diameter of 550 Å and 1350 Å (particle size 20—80 mesh) and washed with phosphate buffer. The results showed that equal amounts of phosphate buffer were needed to wash both preparations free of soluble trypsin.

The hydrolytic activity of glutaraldehyde-linked glass-trypsin derivatives on BAEE and casein is presented in Table 5. All preparations showed similar enzymatic activity except those silanized in toluene. The toluene-silanized preparations had lower activity presumably due to the damaging effect of high silane level in toluene on the structure of porous glass. The fact that these glass-trypsin derivatives showed similar activity despite the difference in their amino contents suggests that amino content at these levels is not a limiting factor for the immobilization of enzyme on porous glass. In fact, the amino content of their corresponding alkylamino glass preparations was in excess of the number of moles of trypsin they bound. When casein was used as the substrate, glass-trypsin preparations silanized in acetone and alkaline water (pH 10.5) showed better activity.

Glass-bound trypsin linked through glutaraldehyde is easy to prepare and very reproducible. Table 6 shows the activity of replicates of glutaraldehyde-linked glass-bound trypsin on BAEE and casein. The similar activities among respective replicates indicate excellent reproducibility.

More work and steps are involved in the preparation of azo-linked glass-trypsin derivatives than that of glutaraldehyde-linked glass-trypsin derivatives. This contributes a poorer reproducibility to the preparation of azo-linked glass-bound trypsin. However, good azo-linked glass-trypsin derivatives generally had better activity than their glutaraldehyde-linked counterparts as shown in Table 5. Among all glass-trypsin derivatives prepared, the ones with the best activity were prepared by silanizing porous glass (20—80 mesh, pore diameter 550 Å) in 10% acetone solution of γ-aminopropyltriethoxysilane, coupling the enzyme to the alkylamino glass through azo linkage, and washing the glass-trypsin derivative with 0.1M phosphate buffer (pH 7.0).

The protein content of glass-trypsin derivatives was determined by measuring the depletion of protein using the Lowry method (Lowry et al., 1951). The protein content of glutaraldehyde-linked glass-trypsin derivatives ranged from 25—33 mg per gram dry weight, whereas that of azo-linked glass-trypsin derivatives ranged from 10—18 mg per gram dry weight. The fact that glutaraldehyde-linked glass-trypsin preparations contained more enzyme but less activity than azo-linked ones indicates that a larger portion of the enzyme immobilized through glutaraldehyde linkage was inactive. That is, a higher percentage of the bound enzyme

Table 7—Storage stability of glutaraldehyde-linked and azo-linked glass-trypsin derivatives

Glass-trypsin derivative[a]	Length of storage (days)[b]	Initial activity retained
Azo-linked	114	100%
Glutaraldehyde-linked	156	94%

[a] Acetone-silanized and phosphate buffer washed
[b] Glass-trypsin derivatives were stored at 5°C as wet cake.

was active after immobilization in the case of azo linkage than in the case of glutaraldehyde linkage.

Both glutaraldehyde-linked and azo-linked glass-trypsin derivatives had excellent storage stability. Table 7 shows that one glutaraldehyde-linked preparation retained 94% of its initial activity after 156 days at 5°C and that one azo-linked preparation retained completely its activity after 114 days at 5°C. Such storage stability was considerably better than that reported by Weetall (1970).

Operational stability of glass-bound trypsin was determined by measuring the proteolytic activity of a glass-bound trypsin column at 5°C for 150 days. Casein solution (1%) was passed continuously through the column and increase in nonprotein nitrogen in the effluents was determined. During the first 90 days of operation the activity gradually declined to about one-half of its original activity. No preservative was used during this period to control microbial growth in the column, and the standard plate counts (APHA, 1967) of the effluents from the column were of the order of 10^8. At the end of this period 40 ppm of tetracycline (Upjohn Co.) were incorporated in the substrate to eliminate microbial growth. No additional loss of activity was observed during the following 60-day period. Presumably most of the initial loss of activity was due to microbial action.

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Ms received 4/2/74; revised 5/25/74; accepted 5/28/74.

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IMMUNOCHEMICAL STUDIES ON HUMAN AND BOVINE MILK LYSOZYMES

INTRODUCTION

IMMUNOLOGICAL TECHNIQUES, because of their high degree of specificity, have been used extensively to compare similar or related enzymes and proteins. Lysozymes (mucopeptide N-acetylmuramylhydrolase, EC 3.2.1.17) from various biological and food sources have been shown to possess similar antigenic properties (Fujio et al., 1962; Arnheim and Wilson, 1967; Maron et al., 1970; Arnheim et al., 1971; Arnon and Maron, 1971). The use of immunological techniques in conjunction with chemical modification allows not only the localization of the amino acid residues in the antigenic sites, but also yields valuable information about their spatial organization. The antigenic properties of egg white lysozyme (EWL), after chemical modification, have been studied extensively. Specifically, modifications of the lysine (Strosberg and Kanarek, 1968, 1970), histidine (Strosberg and Kanarek, 1970), tyrosine (Atassi and Habeeb, 1969), and methionine residues (Strosberg and Kanarek, 1970; Bonavida et al., 1969) have been reported.

Bovine milk lysozyme (BML) and human milk lysozyme (HML) have been isolated and characterized (Chandan et al., 1965; Parry et al., 1969; Eitenmiller, 1971). Recently, Jolles and Jolles (1972) established the complete primary sequence of HML. Faure and Jolles (1970) studied the antigenic relationship between several lysozymes and bovine α-lactalbumin and found no antigenic cross reactivity between any of the lysozymes and anti-bovine α-lactalbumin antiserum. Cross-reactivities were reported between hen, guinea-hen, duck II and duck III egg-white lysozymes, but not between hen and goose egg-white or human milk lysozymes. However, little is known about the antigenic relationship between the milk lysozymes and EWL, or the amino acid residues which may be involved in the antigenic sites of BML or HML. The present paper presents the results of investigations on the immunological properties of native and some chemically modified milk lysozymes.

MATERIALS & METHODS

BML AND HML used in this study were isolated in an electrophoretically and ultracentrifugally pure form as previously reported (Chandan et al., 1965; Parry et al., 1969). EWL, used as a control enzyme, was a three times recrystallized enzyme (lot No. 7812) obtained from Nutritional Biochemicals Corp., Cleveland, Ohio.

Preparation of antisera

28 mg of BML were dissolved in 2.8 ml of 0.85% saline. 2 ml of the antigen solution were emulsified with an equal volume of complete Freund's adjuvant and injected intramuscularly at three sites in each of two rabbits. Sample bleeding by heart puncture commenced 2 wk after injection, with the animals ultimately being bled out 6 wk after immunization.

20 mg of HML were dissolved in 2 ml of 0.85% saline and emulsified with 2 ml of complete Freund's adjuvant. 2 ml of the mixture, containing 10 mg of the antigen, were injected intramuscularly into each of two rabbits at two sites. 1 wk later, the rabbits were reinjected with an additional 10 mg of the antigen. Sample bleeding commenced at the third week and continued throughout the fifth week after the first injection.

Antiserum for EWL, used as a control enzyme, was purchased from Kallestad Labs., Inc., Minneapolis.

Immunodiffusion of native lysozymes

Immunodiffusion tests were conducted by double diffusion techniques (Crowle, 1961). The gel medium was a 1% solution of agarose (Kallestad Labs) in 0.01M phosphate buffer, pH 7.0, containing 1% NaCl and sodium-azide (1/2500 dilution) as a preservative. The antigens at various concentrations were prepared in 0.01M phosphate buffer, pH 7.0, containing 1% NaCl. Approximately 10 μl of antigen solution was added to the antigen wells and 10 μl of undiluted antiserum was added to each center well. After incubation at room temperature for 48 hr in a humidity cabinet, the slides were washed overnight with 0.85% NaCl and dried. The precipitin bands were then stained for 5 min with amido black in 1% acetic acid and destained using a water:methanol:glacial acetic acid (5:5:1 v/v) solution. The native lysozymes were also analyzed by intragel specific absorption (Crowle, 1961).

Inhibition studies

To 0.5 ml of 50 μg/ml solutions of BML and HML in M/15 phosphate buffer, pH 6.2, was added 0.1 ml and 0.2 ml of their corresponding antisera. The volume was made up to 1 ml with the phosphate buffer. To check for cross-reaction between the antisera, 0.1 ml of anti-HML and anti-EWL was added to 0.5 ml of the BML solution and 0.1 ml of anti-BML and anti-EWL was added to the HML solution. The volume was then made up to 1 ml with phosphate buffer. The enzyme and antisera mixtures were then incubated with shaking at 37° for 30 min before being assayed for activity according to the procedure of Parry et al. (1965). 0.1 ml of the HML and 0.2 ml of the BML and antiserum mixtures were assayed. For the control assays it was necessary to add amounts of normal serum corresponding to the antisera added in the inhibition tests, since the antisera inherently possessed lysozyme activity and lysozymes are activated by salts normally present in the antisera.

Chemical modification of the lysozymes

All modifications were performed on the milk lysozymes and EWL. At the completion of each reaction, the modified product was isolated by dialysis or gel filtration, lyophilized and retained for further analysis. The acetylation of the lysine and nitration of the tyrosine residues was completed by the methods of Frankel-Conrat (1957) and Atassi and Habeeb (1969), respectively. The histidine residues were alkylated with iodoacetic acid at pH 5.5 according to the method of Parsons et al. (1969). In addition, the conformation of the lysozymes was altered by reduction with 2-mercaptoethanol (Friend et al., 1972) or oxidation with performic acid (Hirs, 1956). The extent of modification of the lysine, tyrosine, and cystine residues in the lysozymes is shown in Table 1. Extent of acetylation of lysine residues was calculated from the ninhydrin reaction using the method of Moore and Stein (1968). Determination of the number of moles of 3-nitrotyrosine formed per mole of protein was calculated by the method of Sokolovsky et al. (1966). Reduction of cystine residues was followed by titration of the reduced proteins with p-mercuribenzoate (PMB) according to the procedure of Boyer (1954). Extent of modification of histidine residues was not determined, since Parsons et al. (1969) have shown that in lysozyme iodoacetate alkylated the histidine residues completely. Performic acid oxidation results in oxidation of methionine and tryptophan residues together with conversion of cystine to cysteic acid. This reaction was assumed to be essentially complete as reported by Hirs (1956).

Immunodiffusion of modified lysozymes

The modified proteins were cross-reacted

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with their homologous native antiserum as previously described for the native lysozymes.

RESULTS

Immunodiffusion of native lysozymes

Figure 1 presents the immunodiffusion pattern obtained for the milk lysozymes and EWL against their corresponding antisera. Slide A is a 48 hr diffusion pattern for BML. The BML concentrations in the antigen wells ranged from 0.05 mg/ml to 0.5 mg/ml. At these concentrations only a single precipitin band was observed, with the optimum precipitation occurring at 0.4 and 0.5 mg/ml BML concentrations. It was noted that at higher BML concentrations, a second weak precipitin band was formed that disappeared upon further incubation. The weak band was likely the result of antigen excess causing secondary precipitation in the gel. The weak precipitin band never appeared at the lower antigen concentrations which produced sharp precipitin bands at 0.4 and 0.5 mg/ml levels.

Slide B is a 48 hr diffusion pattern for HML diffused against undiluted anti-HML. The HML concentrations ranged from 0.25 to 2.5 mg/ml. A single precipitin band was obtained at all concentrations. The best balance between antigen and antibody appeared to be at 2.0 mg/ml HML, as the band was very sharp at this concentration. No precipitin bands were formed at 0.25 and 0.50 mg/ml HML (a and b in slide B).

Slide C is a 48 hr diffusion pattern of EWL. The EWL concentrations ranged from 0.025 to 0.15 mg/ml. Optimum precipitin band formation occurred at 0.05 mg/ml of EWL.

Inhibition studies

The results of the addition of the antisera to the lysozymes are shown in Table 2. BML was inhibited by the anti-BML. With the addition of 0.1 ml and 0.2 ml of anti-BML, the enzyme lost 33.4 and 60.0% of its activity, respectively. Anti-HML and anti-EWL did not inhibit the BML. Likewise, HML was inhibited by anti-HML but not by anti-BML or anti-EWL. The HML solution was inhibited by 23.5 and 32.4% with 0.1 and 0.2 ml of anti-HML, respectively. The failure of the milk lysozymes to be inhibited by antibodies of the other lysozymes indicates a lack of serological similarity between the milk lysozymes and EWL.

Intragel specific absorption

To further examine the possibility of serological relationships between the milk lysozymes and EWL, the technique of intragel specific absorption was used.

The results of the intragel specific absorption study in Figure 2 indicate BML to be serologically different from HML and EWL. In slide A, the absorbing antigen was BML. A 0.5 mg/ml BML sol-

Table 1—Extent of modification of amino acids by various treatments

Lysozyme	Amino acid residues modified	Amino acid residues observed in		% Modification
		Native lysozyme	Modified lysozyme	
BML				
Acetylated	Lysine	11[a]	1.8	84
Nitrated	Tyrosine	7	3.0	57
Reduced	Cystine	3[b]	0	100
HML				
Acetylated	Lysine	7	3.8	46
Nitrated	Tyrosine	5	2–3	40—60
Reduced	Cystine	3[b]	0	100
EWL				
Acetylated	Lysine	7	1.6	77
Nitrated	Tyrosine	3	1.0	67
Reduced	Cystine	4[c]	0	100

[a] In the case of lysine, the number of residues refers to the number of lysines plus the N-terminal amino group.
[b] Six half-cystines or —SH groups
[c] Eight half-cystines or —SH groups

ution in 0.01M phosphate buffer, pH 7.0, containing 1% NaCl was placed in the three center wells. After 24 hr, undiluted antisera of the milk lysozymes and EWL were placed in their respective center wells. The antigens at their optimum concentrations were placed in the surrounding wells. As would be expected, the anti-BML was absorbed by its homologous antigen and precipitated around the antibody well. However, the BML did not cross-react with the anti-HML or anti-
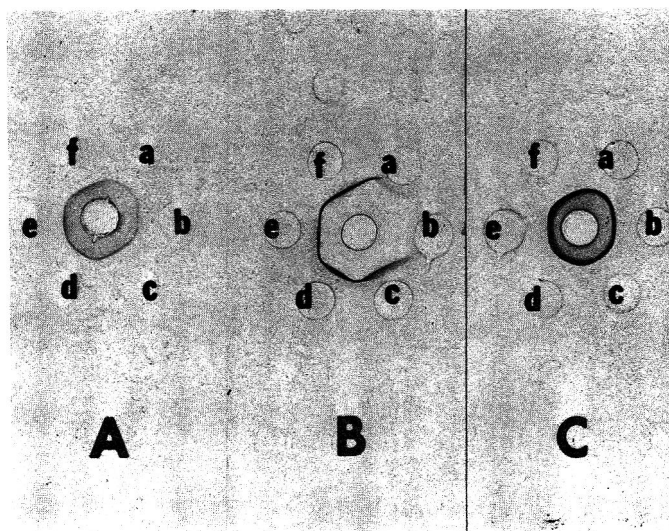
Fig. 1—Immunodiffusion patterns.

A. Central well filled with antiserum to BML. Well a = BML (0.05 mg/ml); b = BML (0.10 mg/ml); c = BML (0.2 mg/ml); d = BML (0.3 mg/ml); e = BML (0.4 mg/ml); f = BML (0.5 mg/ml).

B. Central well filled with antiserum to HML. Well a = HML (0.25 mg/ml); b = HML (0.5 mg/ml); c = HML (1.0 mg/ml); d = HML (1.5 mg/ml); e = HML (2.0 mg/ml); f = HML (2.5 mg/ml).

C. Central well filled with antiserum to EWL. Well a = EWL (0.025 mg/ml); b = EWL (0.05 mg/ml); c = EWL (0.075 mg/ml); d = EWL (0.10 mg/ml); e = EWL (0.125 mg/ml); f = EWL (0.15 mg/ml).

EWL, and the presence of BML antigen did not interfere with the normal precipitin bands being formed for the other two antigens.

In slide B, the absorbing antigen was HML at 2.0 mg/ml. Following the same procedure as for BML (slide A), no cross-reaction could be observed between HML and anti-BML and anti-EWL. The anti-HML was completely absorbed by its homologous antigen. In slide C, the absorbing antigen was EWL at the concentration of 0.05 mg/ml. The EWL did not cross-react with the HML and BML antisera.

Immunodiffusion of the modified lysozymes

Figure 3 presents the immunodiffusion patterns obtained for the modified milk lysozymes and EWL against their corresponding antisera. Slide A is a 24 hr diffusion pattern for BML. Concentration of native or modified BML in each well is given in Figure 3. There were no bands for either the oxidized or reduced BML at various concentrations. The concentrations of the native, carboxymethylated, and nitrated correspond to the optimal concentrations reported for the native BML. The concentration of acetylated BML, however, had to be increased almost twofold to obtain a precipitin band.

Moreover, the bands for the acetylated and nitrated BML were weaker and more diffused than those obtained for the native and carboxymethylated BML.

Slide B is a 24 hr diffusion pattern for HML. Concentration of native or modified HML in each well is given in Figure 3. As in the BML, there were no bands for either the reduced or oxidized HML. All concentrations of modified HML corresponded to the optimum concentrations for precipitin formation noted for native HML. The acetylated HML had a

sharp precipitin band while the nitrated HML, as with the BML, had a weaker, more diffuse band.

Slide C is a 24 hr diffusion pattern for EWL. Concentration of native or modified EWL in each well is described in Figure 3. There were no bands for either the reduced or oxidized EWL which is in accordance with the results obtained for the milk lysozymes and for the results obtained for EWL by Arnon and his co-workers (Arnon and Maron, 1971; Arnon and Sela, 1969) and Maron et al. (1970). The concentrations for the native and carboxymethylated EWL for the precipitin band formation correspond to the optimal concentration (0.05 mg/ml) observed in earlier studies. The concentration of the acetylated EWL had to be increased twofold and the concentration of the nitrated EWL had to be increased fourfold to obtain a precipitin band. Moreover, the bands for both the acetylated and nitrated EWL were weaker and more diffuse than those obtained for the native and carboxymethylated EWL.

DISCUSSION

Immunological comparison of native lysozymes

Immunological studies of the human and bovine milk lysozymes showed them

Table 2—Enzyme inhibition by antisera

Treatment	% Inhibition
BML Control	0
BML + 0.1 ml anti-BML	33.4
BML + 0.2 ml anti-BML	60.0
BML + 0.1 ml anti-HML	0
BML + 0.1 ml anti-EWL	0
HML Control	0
HML + 0.1 ml anti-HML	23.5
HML + 0.2 ml anti-HML	32.4
HML + 0.1 ml anti-BML	0
HML + 0.1 ml anti-EWL	0
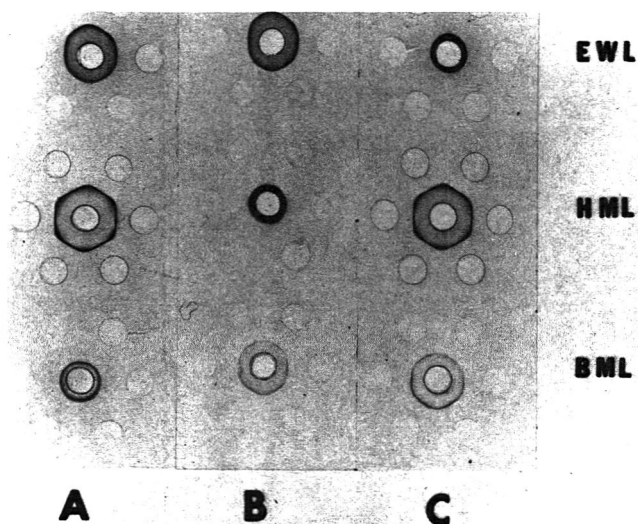
Fig. 2–Intragel specific absorption patterns.

A. Central wells filled with BML (0.5 mg/ml) and 24 hr later with antisera to BML, HML, and EWL. Surrounding wells were filled with antigen at equivalence concentration corresponding to the antiserum placed in the center well (BML = 0.5 mg/ml, HML = 2.0 mg/ml, EWL = 0.05 mg/ml.

B. Central wells filled with HML (2.0 mg/ml) and 24 hr later with antisera to BML, HML, and EWL. Surrounding wells contain same materials as in A.

C. Central wells filled with EWL (0.05 mg/ml) and 24 hr later with antisera to BML, HML, and EWL. Surrounding wells contain same materials as in A.
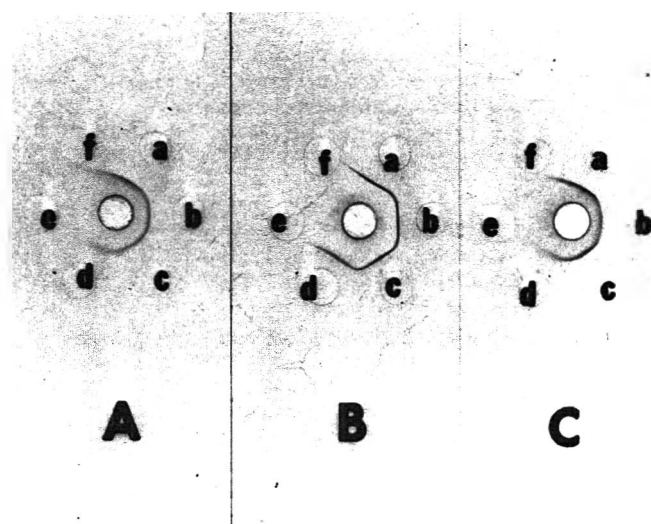
Fig. 3–Immunodiffusion patterns.

A. Central well filled with antiserum to BML. Well a = BML (0.5 mg/ml); b = Acetylated BML (0.75 mg/ml); c = Carboxymethylated BML (0.4 mg/ml); d = Nitrated BML (0.4 mg/ml); e = Reduced BML (0.4 mg/ml); f = Oxidized BML (0.4 mg/ml).

B. Central well filled with antiserum to HML. Well a = HML (1.5 mg/ml); b = Acetylated HML (2.0 mg/ml); c = Carboxymethylated HML (1.5 mg/ml); d = Nitrated HML (2.0 mg/ml); e = Reduced HML (1.5 mg/ml); f = Oxidized HML (1.5 mg/ml).

C. Central well filled with antiserum to EWL. Well a = EWL (0.05 mg/ml); b = Acetylated EWL (0.1 mg/ml); c = Carboxymethylated EWL (0.05 mg/ml); d = Nitrated EWL (0.2 mg/ml); e = Reduced EWL (0.05 mg/ml); f = Oxidized EWL (0.05 mg/ml).

to be antigenically different from each other and from EWL. Antisera against the milk lysozymes did not inhibit the activity of the other lysozymes under study. Also, lack of serological relationship was indicated in the specific intragel absorption studies as anti-HML did not cross-react with BML and EWL, and anti-BML did not cross-react with HML and EWL. Likewise, anti-EWL did not absorb HML and BML.

Immunodiffusion of the modified lysozymes

All three acetylated lysozymes gave a weak and diffuse precipitin band when reacted against the native antisera. In addition, the concentration of the acetylated BML and EWL had to be increased almost twofold over the native concentration to obtain a precipitin band while HML reacted at native concentration. The concentration of acetylated lysozyme necessary for precipitin formation is related to the extent of acetylation. BML and EWL were heavily acetylated and contained only 16% and 23% free amino groups, respectively. HML, however, was only slightly acetylated and contained 54% free amino groups. Heavily acetylated samples, therefore, required a higher protein concentration to provide the necessary biologically active molecules for the precipitin reaction. Moreover, the precipitin reaction was weak for all acetylated samples regardless of concentration. It seems, therefore, that certain amino groups have a definite role in the antigenic makeup of the lysozymes while others aid in the precipitin reaction, possibly by providing electrostatic attraction with the antibody. It has been proposed that at least one of the six lysine residues is part of the antigenic determinant in EWL (Strosberg and Kanarek, 1970).

Carboxymethylation of the histidine residues of the lysozymes did not seem to affect their precipitin reaction. At concentrations corresponding to the native, all three lysozymes gave sharp precipitin bands which were indistinguishable from those given in the native reaction. This is in agreement with the findings of Strosberg and Kanarek (1970) for carboxymethylated EWL. Histidine residues are, therefore, probably not involved in the antigenic site of the lysozymes.

As in the case of the acetylated samples, the nitrated samples gave weak and diffuse precipitin bands. In addition, the concentration of the nitrated EWL had to be increased almost fourfold to obtain a

precipitin band. The present study, however, does not eliminate the possibility that conformational changes may be responsible for the decrease in precipitin reaction. Atassi and Habeeb (1969) nitrated EWL and noted quantitative decrease in precipitin reaction. Moreover, reduction of the nitro group restored almost full antigenic activity although conformational changes were still present. These workers concluded that one or both of Tyr 20 and 23 take an active part in the binding of EWL with its antibodies. The present study seems to indicate a similar role for the tyrosine residues of the milk lysozymes.

Disruption of the conformation of the lysozymes by reduction of the cystine residues to cysteines, or oxidation of the cystine and methionine residues destroyed their antigenic activity. Several workers including Strosberg and Kanarek (1968 and 1970) and Bonavida et al. (1969) have suggested that the methionine side chains do not directly participate in the antigenic site but are important for maintaining the conformation of at least one of the antigenic sites of EWL. Our results suggest a similar role for the methionine as well as the cystine residues in the milk lysozymes. It has also been reported by Sela et al. (1967) that antibodies in the native lysozyme seem to be directed for the most part against conformation rather than sequential determinants. Methods of modification which disrupt conformation prevent the interaction of the antigen with its corresponding antibody. The milk lysozymes also seem to be dependent on conformation for their precipitin reaction and could possess conformational determinants.

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TREATMENT OF SOYBEAN SPENT SOLUBLES BY MEANS OF YEAST CULTIVATION

INTRODUCTION

SPENT SOLUBLES from the preparation of soybean protein isolates and heat-coagulable whey proteins account for about one-fourth of the original dry weight of defatted soybean flakes. Dry spent solubles contain about 60% carbohydrates and 7% organic acids. Crude protein content (Kjeldahl-N × 6.25), however, is only 18%. Moreover, one-third of Kjeldahl-N is composed of non-amino acid nitrogen. A large part of these carbohydrates are made up of oligosaccharides such as raffinose and stachyose, which may induce flatulence on usual liverstocks (Murphy, 1964; Rackis et al., 1967). About one-fifth of the dry solubles is crude ash of which a major part should be potassium salts. These factors lower the feed value of spent solubles.

These spent solubles usually have been disposed of as sewage. However, because of their high biological or chemical oxygen demand (BOD or COD), they present a serious waste disposal problem. The solution of this problem by means of usual biological treatments, such as an activated sludge method, requires a large area and considerable capital for the facilities. During an investigation on the biological treatment of these spent solubles, the author noted that some yeast strains, particularly *Candida quilliermondii* or *Debaryomyces kloeckeri (D. hansenii)* grew very well on the spent solubles without supplementation.

MATERIALS & METHODS

Yeast strains

Among the yeast species recorded in Lodder's work (1970), those assumed to grow on any of sucrose, melibiose, galactose, citric acid or L-glutamic acid as sole carbon source, were picked up, and 425 strains of yeasts were collected from several research institutions in Japan. Among them, 377 had been stored in our laboratories, and the others were supplied from Hokkaido Univ. (AHU), Osaka Univ. (OUT), Hiroshima Univ. (HUT), Institute of Fermentation (IFO) and Dr. H. Iizuka of the Institute of Applied Microbiology (IAM).

Preparation of soybean spent solubles

As shown in Figure 1, the freeze-dried soybean spent solubles were prepared from defatted soybean flakes (Fuji Seiyu Co., Ltd., Tokyo) according to a modification of Rackis'

procedure (Rackis et al., 1971). The yield was 25.09% on a dry base. The original soybean flakes had the following composition: moisture 10.61%, crude protein (Kjeldahl-N × 6.25) 50.78%, nitrogen solubility index (NSI: AOCS, 1965) 89.6% and crude lipid 0.5%.

Preliminary screening of yeast strains

6.7g of Bacto-yeast nitrogen base (Difco Labs., Detroit) and 17g of agar were dissolved into 1,000 ml of pure water and the mixture was autoclaved at 120°C for 10 min. Into this basal medium, sucrose (5g), or galactose (5g), or melibiose (10g), or monosodium-L-glutamate (10g) or sodium citrate (20g) was added aseptically through a MF-Millipore®, type GS (Millipore Corp., Bedford, Mass). Each 10 ml of medium, solidified in a Petri dish, was inoculated with yeast previously grown on malt extract agar slant (Van Der Walt, 1970), and incubated at 30°C for 120 hr. 42 yeast cultures showing good growth on any of these carbon

sources were selected for the second screening.

Second screening of yeast strains

50g of the freeze-dried soybean spent solubles were dissolved in pure water. The solution was adjusted to pH 4.0 with HCl and brought up to 1,000 ml. After sterilization by autoclaving, a 10 ml aliquot of the medium in a large test tube (2.0 × 20 cm) was inoculated with a loopful of yeast cells. Tubes were set on a reciprocal shaker operating at 330 strokes per min with a stroke distance of 20 mm. The cultivation was carried out at 30°C for 72 hr. The optical density (OD) of the culture broth was measured at 660 nm.

Cultivation of yeasts on soybean spent solubles

A 50-ml aliquot of the same medium as employed in the second screening was poured into a 500 ml shaker flask, sterilized by autoclaving and inoculated with 1-ml aliquot of the seed culture of yeast. The seed culture was obtained

Defatted soybean flakes

Extract with water:meal ratio, 10:1, at pH 7.2, 50°C for 1 hr

Reextract residue with 5:1 ratio, for 0.5 hr

Water-insoluble residue　　**Water extracts**

Acidity to pH 4.4 with 1 N-HCl

Whey solution　　**Precipitates**

Heat-treat at 80°C, at pH 5.0 for 15 min

Wash once with cold water, add washings to whey

Spent solution　　**Heat-coagulable proteins**　　**Protein isolates**

Lyophilize

Freeze-dried soybean spent solubles

Fig. 1—Procedure for preparation of soybean spent solubles.

Table 1—Chemical composition of soybean spent solubles

Item	% on a dry base
Nitrogenous substances:	
Kjeldahl-N	2.88
Non-amino acid-N	0.87
5% TCA soluble-N	2.45
Formol-N	0.40
Ammonium-N	0.03
Carbohydrates:	
Total carbohydrate[a]	58.10
80% EtOH solubles[a]	57.52
Reducing sugars[a]	8.18 (22.31)[e]
Ketohexoses[b]	20.20
Total organic acid[c]	6.56 (6.72)[e]
Crude lipid	0.75
Crude ash	21.51
Total phosphorus[d]	1.79
Inorganic phosphorus[d]	1.12

[a] As glucose
[b] As fructose
[c] Nonvolatile, as citric acid
[d] As phosphorus pentoxide
[e] After autoclaving 5% (w/v) solution, pH 4.0, at 120°C for 10 min

after 24 hr incubation in the same procedure as applied for the second screening. The cultivation was performed at 30°C for 72 hr on a reciprocal shaker operating at 140 strokes per min with a stroke distance of 75 mm.

Thin-layer chromatography (TLC)

Carbohydrates. 2 ml of the supernatant of the culture broth were mixed with 8 ml of pure water. The mixture was deproteinized (Somogyi, 1945), desalted (Sugimoto and Van Buren, 1970) and concentrated in vacuo to 1 ml of syrup. 10 μl of the concentrated syrup were applied to a microcrystalline cellulose plate (Funakoshi Pharmaceutical Co., Ltd., Tokyo), and developed by ascending partition chromatography using the solvent system, n-butanol-pyridine-water (6:4:3 by vol; Jeanes et al., 1951). Resolutions of carbohydrates were enhanced by employing a multiple development technique, with four successive developments. To locate and identify carbohydrate spots on the chromatograms, five detectors were used. Ketohexoses, substantially fructose, were detected with the improved α-naphthol reagent (Albon and Gross, 1950; Sugimoto and Van Buren, 1970). Alkaline silver nitrate (Trevelyan et al., 1950), benzidine-TCA (Bacon and Edelman, 1951) and alkaline triphenyltetrazolium chloride (Burton et al., 1951) reagents were applied to detect reducing sugars. Diphenyl-amine-aniline-phosphate was used to detect both reducing and nonreducing sugars (Buchan and Savage, 1952; Bailey and Bourne, 1960).

Nonvolatile organic acids. After adjusting to pH 2.0 with H_2SO_4, 20 ml of the supernatant of the culture broth were extracted with ethyl ether in a Soxlet-type apparatus for 96 hr. After dehydrating as usual with anhydrous Na_2SO_4, the solvent was distilled off in vacuo. The residual organic acids were redissolved in pure water and passed through an Amberlite CG-4B column (OH⁻ form; 0.9 × 35 cm). After washing the column with water, the absorbed

acids were eluted with 100 ml of 2% NaOH. An excess amount of alkali in the effluent was eliminated by passing it through an Amberlite IR-120B column (H⁺ form; 2.0 × 30 cm). The organic acid solution, thus purified, was concentrated in vacuo to 5 ml. 20 μl of the concentrated organic acids were applied to a microcrystalline cellulose plate and developed by ascending partition chromatography with the upper layer of the 1:1 mixture of 1-pentanol and 5M aqueous formic acid (Buch et al., 1952). For the detection of organic acid spots, ethanol solution of 0.04% brom phenol blue (BPB) was employed.

Other chemical analyses

All samples for carbohydrate analyses were first deproteinized and desalted as described above. Total carbohydrate was assayed by a phenol-sulfuric acid method (Dubois et al., 1956) and expressed as glucose. Reducing sugars and ketohexoses were determined by Somogyi-Nelson's method (Somogyi, 1945; 1952) and by the modified resorcinol reaction (Kulka, 1956). These sugars were expressed as glucose and fructose, respectively. The formol titration procedure followed was that described by Hawk et al. (1954). Ammonium nitrogen was estimated by a micro-diffusion analysis (Conway, 1933). The determinations of both total and inorganic phosphates were carried out according to the method of Allen (1940). Crude lipid was estimated in accordance with the AOAC method (1965). Crude ash content was determined gravimetrically by ignition at 500°C for 48 hr. The samples for the determination of total organic acid were prepared as for TLC. A 50-ml aliquot of purified aqueous organic acids was titrated with 0.05N NaOH to pH 8.2. Total organic acid was calculated as citric acid. Determination of BOD or COD was performed in conformity to Japanese Industrial Standards (1964).

RESULTS

Chemical composition of soybean spent solubles

The spent solubles prepared from defatted soybean flakes had the chemical composition shown in Table 1.

Since 99% of the total carbohydrate were soluble in 80% EtOH, almost all of them seemed to be made up of low molecular carbohydrates.

15% of the nitrogenous substances were insoluble in 5% TCA and therefore, might be composed of high molecular peptides. However, according to the calculation of total amino acid, the non-amino acid nitrogen occupied about 30% of Kjeldahl-N. The total amino acid composition was almost the same as that of nonheat coagulable protein in soybean whey analyzed by Rackis et al. (1971). The ratio of free amino acid to total amino acid was 0.208. This value decreased to 0.202 after autoclaving the solubles. In this case, however, no significant change in the total amino acid composition was observed. Since usual yeasts assimilate peptide nitrogen with difficulty, the surely assimilable nitrogen including ammonium nitrogen in the solubles was calculated to represent only about 15% of Kjeldahl-N.

The amount of total organic acid was equivalent to 6.6% of the dry matter of the solubles. Since defatted soybean contained almost no volatile acids (Moriguchi et al., 1961), the author did not take them into consideration.

One of the characteristics of the chemical composition was the ash content, which was over one-fifth of the dry matter of the solubles.

Preliminary screening of yeast strains

Through the preliminary screening, 42 yeast strains were selected which grew well on one of the media containing sucrose, melibiose, galactose, monosodium-L-glutamate, or sodium citrate as sole carbon source. These strains were composed of four strains of Candida guilliermondii, three of C. membranfaciens, one of C. parapsilosis var. hokkai, 13 of Debaryomyces hansenii, two of D. cantarellii, two of D. phaffii, one of D. vanrijii, two of Monilia sitophila, four of Pichia scolyti, three of P. polymorpha, two of P. pseudopolymorpha, one of P. farinosa, three of Torulopsis candida and one of Saccharomyces tikumaensis.

Table 2—Yeast strains demonstrating good growth on soybean spent solubles[a]

Strain		Growth (OD at 660 nm)	Final pH
Candida guilliermondii	IFO 0961	20.85	7.65
	OUT 6005	23.40	7.60
Debaryomyces hansenii	OUT 6030	22.47	5.80
Debaryomyces kloeckeri	AHU 3929	23.22	7.20
	AHU 3930	20.15	6.40
	AHU 3931	20.15	7.00
	AHU 3932	23.22	7.10
	AHU 3934	20.15	6.95
Torulopsis candida	OUT 6187	23.03	5.75
Pichia scolyti	IFO 1113	21.38	6.75

[a] The culture conditions are shown in the text.
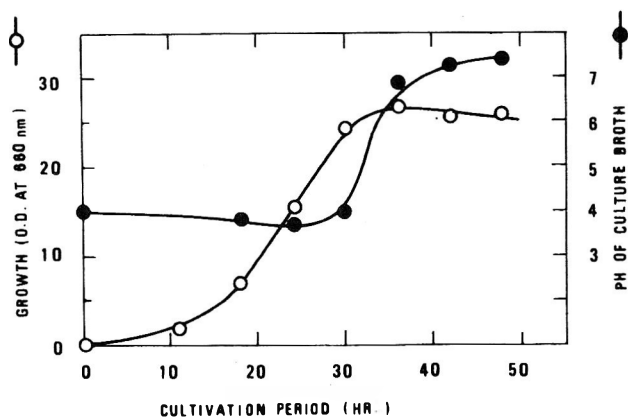
Fig. 2–Relationship between cultivation period and growth of Candida guilliermondii OUT 6005 on soybean spent solubles. (Experimental details are shown in the text.)
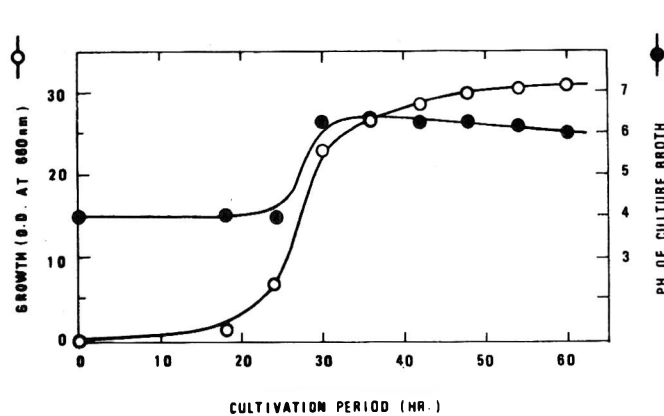
Fig. 3–Relationship between cultivation period and growth of Debaryomyces kloeckeri AHU 3932 on soybean spent solubles. (Experimental details are shown in the text.)

Second screening of yeast strains

Ten strains grew especially well on the solubles (see Table 2). Since, *D. kloeckeri* has been combined with *D. hansenii* (Kreger-Van Rij, 1970), and *T. candida* has also been recognized as an imperfect form of *D. hansenii* (Van Uden and Vidal-Leiria, 1970), these strains were arranged into three groups: *C. guilliermondii*, *D. hansenii* and *P. scolyti*.

Since the cells of *P. scolyti* IFO 1113 were very small and the culture broth smelled unpleasant, it was omitted from subsequent experiment. The following investigations were done mainly with *C. guilliermondii* OUT 6005 and *D. kloeckeri* AHU 3932 as shown in Figures 2 and 3, respectively.

The growth of *C. guilliermondii* OUT 6005 on the solubles was so rapid as to reach its maximum within 36 hr. On the contrary, that of *D. kloeckeri* AHU 3932 showed a longer lag phase. Although it had a relatively high growth rate between 18–30 hr it continued to grow slowly even after that time, at least until 60 hr.

As for both strains, a rapid pH shift of the culture broth was observed at the end of the logarithmic growth phase. The same phenomenon was observed by Takei et al. (1969) in the cultivation of *Torulopsis xylinus* OUT 6182 on a cooking soybean drain. Since this pH shift corresponded to the disappearance of organic acids from the culture broth, the cause was suspected to be due to the loss of buffer action through the consumption of acids by yeasts.

The dry cell yields of *C guilliermondii* OUT 6005 and *D. kloeckeri* AHU 3932 were 13.82 and 14.61g per 1,000 ml of the culture broth at 36 hr, or 60 hr, respectively. These yields coincided with 27.64% and 29.22% recoveries from the dry matter of the solubles. The crude protein contents of both strains were 39.2% and 34.4% on a dry base, respectively.

However, since there was a report (Rieth, 1970) suggesting that a strain belonging to *C. guilliermondii* was occasionally detected in human mycetoma, the subsequent experiment was carried on only with *D. kloeckeri* AHU 3932.

Consumption of carbohydrates by D. kloeckeri AHU 3932

The time course of the consumption of carbohydrates in the solubles during the cultivation of *D. kloeckeri* AHU 3932 is shown in Figure 4.

At the end of the 60-hr cultivation, 97.2% of total carbohydrate, 97.6% of reducing sugars and 99.8% of ketohexoses in the medium had disappeared. In conclusion, the carbohydrates in the solubles could be almost completely metabolized or assimilated by the yeast.

To investigate the consumption of the individual carbohydrate, TLC was performed, Figure 5. The medium contained much more reducing monosaccharides (fructose, glucose and galactose) than the original solubles, and the ratio of reducing sugars to total carbohydrate was 38.4%. In comparison with that on the original solubles, 14.1%, this value seemed to be significantly large. A great part of those monosaccharides was suspected to have been formed from the oligosaccharides by the heat-sterilization process of the medium.

Besides, those sugar monomers, oligosaccharides corresponding to stachyose, raffinose, manninotriose and melibiose, were detected on TLC. However, higher oligosaccharides including verbascose were not recognized. Among the constitutive
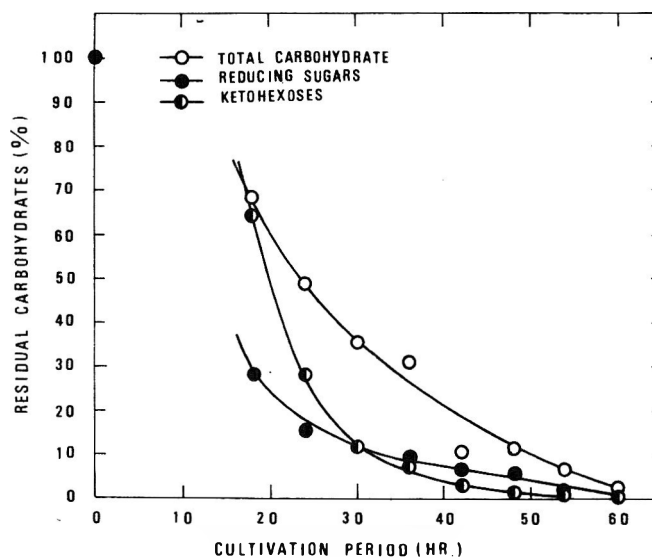
Fig. 4–Consumption of total carbohydrate, reducing sugars and ketohexoses in soybean spent solubles by Debaryomyces kloeckeri AHU 3932. (Experimental details are shown in the text.)
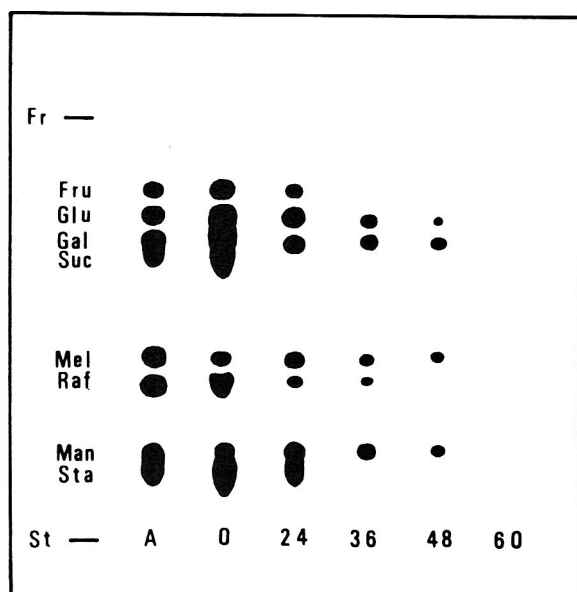
Fig. 5—Thin-layer chromatogram of carbohydrates in soybean spent solubles during cultivation with Debaryomyces kloeckeri AHU 3932. [Numerals indicate the cultivation period (hr). Abbreviations: Fr = front line of development; St = start line of development; A = authentic standards (Fru = fructose, Glu = glucose, Gal = galactose, Suc = sucrose, Mel = melibiose, Raf = raffinose, Man = manninotriose, Sta = stachyose). Detector: diphenylamine-aniline-phosphate reagent.]
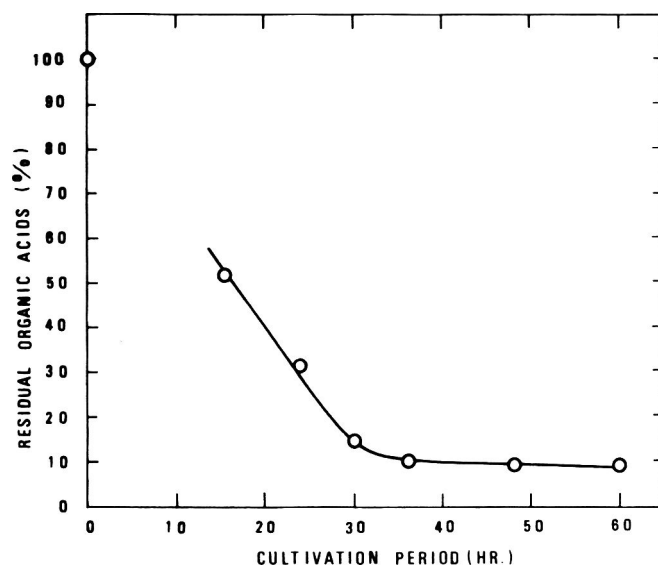
Fig. 6—Consumption of total organic acid in soybean spent solubles by Dabaryomyces kloeckeri AHU 3932. (Experimental details are shown in the text.)

sugars, the fructose moiety seemed to be the most easily liberated and utilized by the yeast.

Sucrose, stachyose and raffinose disappeared at 24, 36 and 48 hr, respectively. Instead of those oligosaccharides, their metabolic intermediates, manninotriose and melibiose increased temporarily during the cultivation. However, all of those carbohydrates completely disappeared by 60 hr. α-1,6-Galactobiose was not detected throughout the incubation period.

Consumption of nonvolatile organic acids by D. kloeckeri AHU 3932

The time course of the consumption of nonvolatile organic acids is shown in Figure 6.

The consumption of organic acids was more rapid than that of carbohydrates. Although the consumption slowed by 36 hr, it still continued till 60 hr, and removed 88.7% of the initial acids. The detailed investigation was carried out on TLC, Figure 7.

On the medium, the spots assumed to be citric, malic, lactic, α-ketoglutaric, fumaric, glycolic, succinic and tartaric acids were detected. The list is given according to the order of concentration. The first to disappear were both fumaric and glycolic acids. They were followed by lactic, malic and tartaric acids. Only the spot corresponding to α-ketoglutaric acid was still observed at 60 hr.

Consumption of nitrogenous substances by D. kloeckeri AHU 3932

The time course of the consumption of Kjeldahl-N by D. kloeckeri AHU 3932 is shown in Figure 8. The Kjeldahl-N in the supernatant of the culture broth reached to its minimum at 42 hr and almost no change was observed after that time. However, the reduction was only about 56% even at 60 hr. As measured by a biuret reaction (Gornall et al., 1949), with ovalbumin (3x cryst.) as a standard, over 89% of the residual Kjeldahl-N corresponded to peptides or proteins.

Chemical composition and BOD or COD of supernatant of culture broth

The dry matter in the broth supernatant was 30% of that in the original medium.

At the same time, BOD and COD values of the medium, 27,600 and 25,700 ppm, were decreased to 4,470 ppm and 5,040 ppm, respectively. In consequence, 83.8% of BOD or 80.4% of COD was removed through the yeast cultivation. A large part of residual BOD or COD might consist of high molecular nitrogenous substances.

The amount of crude ash in the broth supernatant was 62% of the dry matter. The ash was largely composed of potassium salts.

DISCUSSION

A FEW YEARS AGO, Takei and Mochizuki (1968) and Takei et al. (1969) attempted the cultivation of *Torulopsis xylinus* OUT 6182 on a medium composed mainly of soybean cooking drain, a spent sewage from a Japanese miso (a fermented soybean paste) manufacturing plant. They reported that nearly 90% of the BOD could be removed through their process. However, in the case of the spent solubles used in this work, the cultivation of *T. xylinus* OUT 6182 could eliminate only 61% of BOD and produce only 18.78g cells from 100g of the solubles on a dry base, in comparison with 84% and 29g with *D. kloeckeri* AHU 3932. The differences among those values might derive from the difference in chemical compositions between the waste materials.

In this paper, dealing with *C. guilliermondii* OUT 6005 and *D. kloeckeri* AHU 3932, the latter strain was studied in more detail because of a pathogenic possibility of the former. However, the former strain had some superiorities in comparison with the latter. For instance, it showed more rapid growth, higher protein content in the cells, and better assimilation of the nitrogenous substances in the spent solubles. If the pathogenic possibility of the former would be discounted by future investigations, a more efficient treatment of the soybean spent
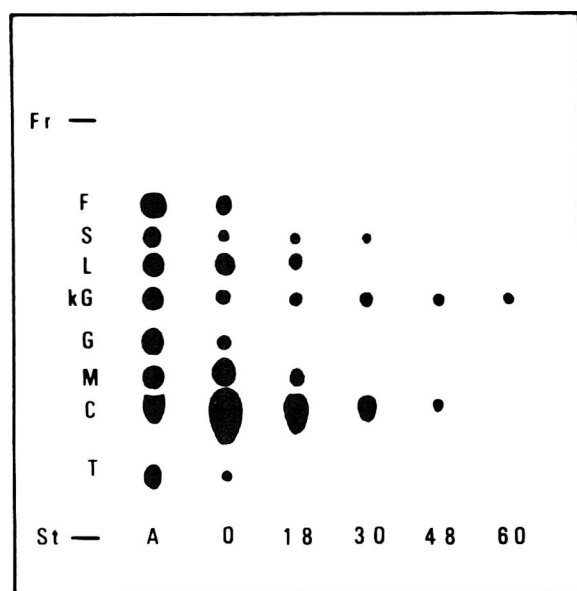
Fig. 7–Thin-layer chromatogram of nonvolatile organic acids in soybean spent solubles during cultivation with Debaryomyces kloeckeri AHU 3932. [Numerals indicate the cultivation period (hr). Abbreviations: Fr = front line of development; St = start line of development; A = authentic standards (F = fumaric, S = succinic, L = lactic, kG = α-ketoglutaric, G = glycolic, M = malic, C = citric, T = tartaric acid). Detector: 0.04% B.P.B. in ethanol.]
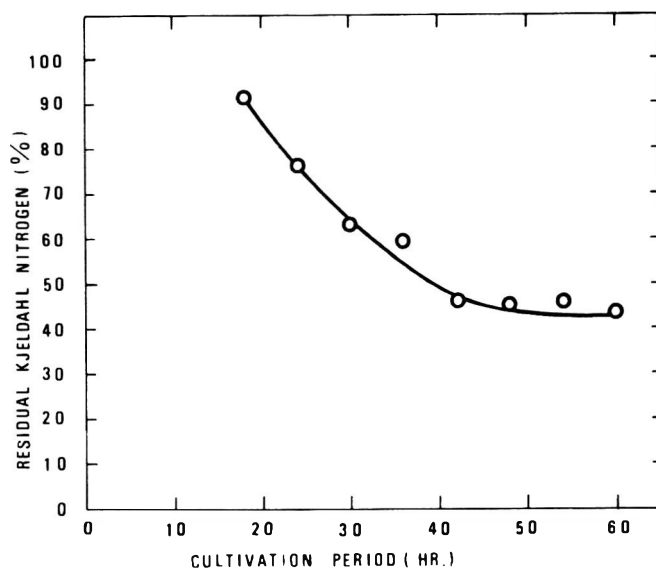

Fig. 8–Consumption of nitrogenous substances in soybean spent solubles by Debaryomyces kloeckeri AHU 3932.

solubles might be possible with *C. guilliermondii* OUT 6005 instead of *D. kloeckeri* AHU 3932.

On the other hand, the cells of *C. guilliermondii* were smaller than those of *D. kloeckeri*. Moreover, they smelled characteristically fruity. In contrast, the cells of *D. kloeckeri* could be more easily separated from the culture broth. The separated cells were almost white, and smelled like freshly compressed baker's yeast.

Anyway, the fact that it is possible to remove about 84% of BOD from the spent solubles with the use of *D. kloeckeri* AHU 3932, suggests the possibility of the reduction of facilities required for the conventional biological treatment, represented by activated sludge ponds, and of the simultaneous SCP production from the cost-free raw material.

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Ms received 12/20/73; revised 3/15/74; accepted 3/20/74.

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SYNERGISTIC EFFECT OF ETHANOL AND SODIUM CHLORIDE ON AUTOLYSIS OF BAKER'S YEAST FOR PREPARING FOOD-GRADE YEAST EXTRACTS

INTRODUCTION

AUTOLYZED yeast extracts are inexpensive flavor substitutes for animal meat extracts. The demand for them as food additives has increased (e.g., Albrecht and Deindoerfer, 1966; East et al., 1966).

Living yeast cells undergo autolysis if they stand at temperatures above 35–40°C. This process, however, requires an incubation period of 2 wk or more before autolysis starts (Tohoyama and Takakuwa, 1972). Therefore, this process has never been used industrially.

In practice, some plasmolysing agents are usually employed to shorten this preliminary incubation period (Peppler, 1970).

During an investigation on the effect of ethanol (EtOH) as a plasmolyser for baker's yeast, the author noted that yeast extracts of high quality could be obtained in a high yield by combined use of EtOH and NaCl as plasmolysers.

MATERIALS & METHODS

Yeast

Fresh compressed baker's yeast was obtained from Oriental Yeast Ind. Co., Ltd., Tokyo. This material had the following composition: moisture 69.22%, crude protein (Kjeldahl-N $\times$ 6.25) 16.56% and total carbohydrate 18.1% as glucose.

Enzyme substrates

Urea-denatured bovine hemoglobin solution was purchased from Pentex Biochemicals, Kankakee, Ill. DNP-casein was prepared according to Hata and Doi (1959).

Enzyme preparations

30g of compressed baker's yeast was suspended in phosphate buffer, pH 5.50, μ = 0.02, brought up to 100 ml, and a homogeneous slurry was prepared with gentle stirring at 30°C for 30 min. The slurry was then cooled with ice and treated for 6 min in a Braun cell homogenizer using glass beads with a diameter of 0.25–0.30 mm. From the homogenate the supernatant was obtained by centrifugation, 26,000 $\times$ G for 60 min. The supernatant was used as the enzyme preparation from the raw material, compressed baker's yeast.

Enzyme preparations from the autolysates were prepared as follows: Into the preparation of the yeast slurry as mentioned above, EtOH (0–5%, v/v) and NaCl (0–5%, w/v) were added. After incubating the mixture at 40°C for 1–8

days, the supernatant was collected by centrifugation, 12,000 $\times$ G for 20 min. The enzyme activities in the supernatant were assayed as those of extracellular enzymes. The precipitate was washed twice by centrifugation with the phosphate buffer, resuspended in the same buffer and brought up to 100 ml. The suspension was disintegrated under the same condition. The supernatant separated from the insoluble cell debris was regarded as the intracellular enzyme preparation from the autolysates.

Assay of enzyme activities

Proteinase activities were assayed at three different pH's, 3.0, 5.5 and 9.0. For the assay at pH 3.0, hemoglobin solution previously diluted twice and acetate buffer, μ = 0.1 were used. For the assay at pH 5.5 or 9.0, 3% aqueous DNP-casein and phosphate or ammonium buffer, μ = 0.1 were employed, respectively. The procedure for the assay of those proteinases was as follows: 1 ml of enzyme solution, 2 ml of buffer solution and 1 ml of substrate were mixed. The mixture was incubated at 37°C for 1 hr. To stop the reaction, 4 ml of 5% aqueous solution of trichloroacetic acid (TCA) were added. After diluting the filtrate 10 times with water, the optical density (OD) was measured at 275 nm (for hemoglobin) or 360 nm (for DNP-casein). The proteinase activities thus measured were expressed in increased OD per g of yeast cells on a wet base. In the experiments performed with the presence of EtOH and NaCl in the reaction mixture, these plasmolysers were preliminary dissolved in the buffer solution. When the reaction mixture contained over 5% (w/v) NaCl, the assay with hemoglobin was impossible because of marked coagulation of the substrate.

Preparations of yeast plasmolysates and autolysates

To 100g of compressed baker's yeast, 0–15 ml of EtOH and 0–7.5g of NaCl were added. After mixing well, the mixture was brought up to 150 ml with water. The homogeneous slurry (plasmolysates) was prepared by agitating the mixture at 30°C for 30 min. The apparent viscosity of the whole plasmolysate was measured in Viscosimeter model BM (Tokyo Keiki Seisakusho, Tokyo) under a fixed shear rate at 30°C. The volume of the supernatant separated by centrifugation, 20,000 $\times$ G for 20 min, the contents of total carbohydrate and Kjeldahl-N in the supernatant were estimated.

The yeast autolysates were prepared as follows: 0–66 ml of EtOH and 0–54g of NaCl were mixed with 500g of the compressed yeast. The mixture was then brought up to 600 ml volume with water. In the experiments without an addition of NaCl, the mixture was brought up to 700 ml. The plasmolysates thus prepared

were incubated for the autolysis at 40°C for 3–7 days. After being brought up to 1,000 ml with water, the autolysates were autoclaved at 100°C for 30 min. The supernatant was obtained from the heat-processed autolysates by centrifugation, 12,000 $\times$ G for 20 min and the contents of Kjeldahl-N, formol-N and DM were determined.

Preparation of yeast extracts

To 500g of the compressed yeast, 30 ml of absolute EtOH and 30g of NaCl were added. The plasmolysates, thus obtained brought up to 600 ml. The incubation period and temperature for the autolysis were set at 7 days and 40°C, respectively. After collecting the supernatant from the autolysates, the residue was heat-treated with 500 ml water in an autoclave, and the washings obtained by centrifugation. The washing operation was repeated twice. The supernatant was mixed with the washings and concentrated in a rotary vacuum evaporator. The substances precipitating during the concentration were eliminated by two filtrations through a celite-coated Buchner funnel.

Counts of viable bacteria

During the autolysis for the preparation of yeast extracts, the number of viable bacteria in the autolytic mixture was counted at appropriate intervals. The counts of total viable bacteria were carried out after 24 hr incubation at 37°C on an eurocidin (50 μg/ml)-supplemented glucose-bouillon agar (Sakaguchi, 1964). For the counts of lactic acid bacteria and *E. coli*, the methods described by Yamashita and Suda (1967) were employed. Staphylococcus medium 110 (Chapman, 1946; 1952) was applied for the detection of pathogenic Staphylococci. Also in these cases, eurocidin was added to the medium to inhibit growth of viable yeast.

Chemical analyses

The formol titration procedure followed was that described by Hawk et al. (1954). Moisture content was determined gravimetrically after drying a sample with purified sea-sand at 105°C for 6 hr. Mohr's procedure (AOAC, 1955) was applied for the determination of NaCl. Dry matter (DM) was expressed as that excluding NaCl. Total carbohydrate was determined by means of phenol-sulfuric acid method (Dubois et al., 1956).

RESULTS & DISCUSSION

Plasmolytic effect of EtOH on yeast cells

In the simultaneous employments of EtOH and NaCl, the contribution of EtOH toward the reduction of viscosity
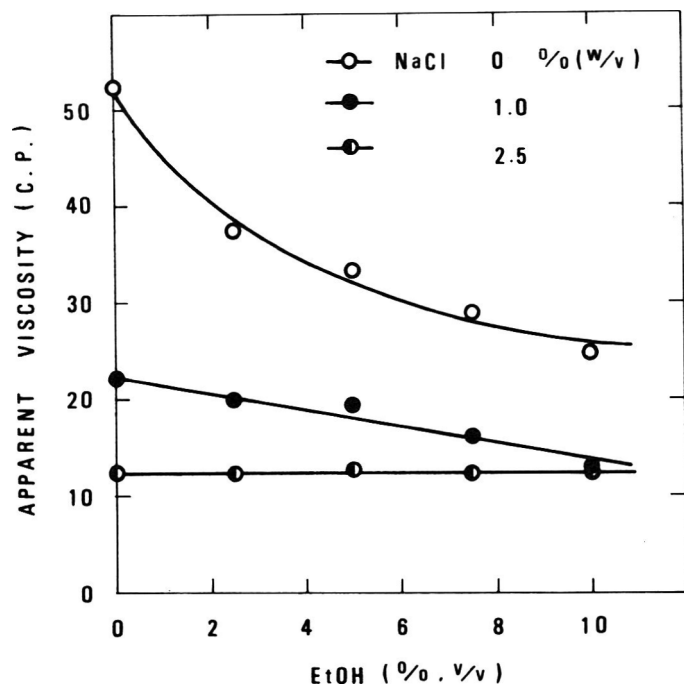
Fig. 1—*Relationship between concentration of EtOH and apparent viscosity of plasmolysates of baker's yeast under co-existence of NaCl of three different concentrations.*

Fig. 2—*Effect of concentrations of EtOH and NaCl in reaction mixture on intracellular proteinase activities of baker's yeast. (pH 3.0 = A; pH 9.0 = B; and pH 5.5 = C.)*

was apparently zero at least when NaCl was above 2.5% (w/v) (Fig. 1).

Recoveries of the supernatant volumes from the plasmolysates prepared with EtOH and NaCl of several different concentrations, the contents of total carbohydrate and Kjeldahl-N in the supernatants, and those recoveries from the yeast cells are summarized in Table 1.

The addition of NaCl led to a rapid excretion of the intracellular fluid of the yeast; nevertheless, the liberations of both total carbohydrate and Kjeldahl-N were very slight, below 0.3% and 1.3%, respectively. This fact indicates that the enzyme reactions following plasmolysis are very important for the effective preparation of yeast extracts.

All Kjeldahl-N liberated during the plasmolysis was soluble in 5% TCA. No proteinase activity was recognized in the supernatant.

Effects of EtOH and NaCl on the enzymatic reactions by proteinases from baker's yeast

The fact that the presence of EtOH and NaCl promotes the autolysis leads one to the first simple question; whether EtOH and NaCl directly enhance the proteolytic reactions.

To investigate the propriety of this hypothesis, the proteinase activities in the compressed yeast were assayed under conditions with different EtOH and NaCl concentrations (Fig. 2). This showed that the activity at pH 3.0 (proteinase A) was considerably inhibited by EtOH of above

Table 1—Effects of EtOH and NaCl concentrations in plasmolysates on yield of supernatant, and on recoveries of total carbohydrate and Kjeldahl-N liberated from baker's yeast

EtOH (%, v/v)		0	5	0	5
NaCl (%, w/v)		0	0	5	5
Yield of supernatant	(%, v/v)	19.0	25.0	53.0	55.0
Recovery of total carbohydrate	(%, w/w)	0.21	0.24	0.24	0.28
Recovery of Kjeldahl-N	(%, w/w)	0.30	0.32	1.05	1.26

5% (v/v) concentration. The activity at pH 9.0 (B) was not significantly affected by NaCl, but was slightly inhibitied by EtOH. On the other hand, the activity at pH 5.5 (C) was inhibited much more by NaCl than by EtOH. At all events, the proteinases were never activated by EtOH or NaCl.

In conclusion, the primary mechanism of the promotion of the autolysis did not include a direct acceleration of any proteinase reactions by those plasmolysers.

Changes in intra- and extracellular proteinase activities of baker's yeast after plasmolysis

Intra- and extracellular enzyme solutions were prepared from the autolysates after incubation at 40°C for 24 hr. The residual proteinase activities in them were assayed (Fig. 3).

With an exception of the activity A, a large part of the activity remained within the yeast cells after 24 hr. In comparison with the control values (at 0 hr) shown in

Figure 2, considerable decrease was observed in a majority of those proteinase activities. However, in the cases when the autolytic mixture contained 5% (v/v) EtOH, the activity C still remained at a high level.

Recently, Hayashi et al. (1972) isolated a precursor of proteinase C from a chloroform-autolysate of baker's yeast They reported that this pro-proteinase was activated through a treatment with 30% EtOH to give proteinase C. The fundamental mechanism of the proteinase activation observed here may be similar to that reported by them.

However, the fact that this activation by EtOH was enhanced by the presence of NaCl, synergistic effect may be interesting from a view point of practical application.

Taking special notice to the proteinase C, its activity was assayed during the autolysis of 8 days incubation (Fig. 4). This activity reached its maximum at 2
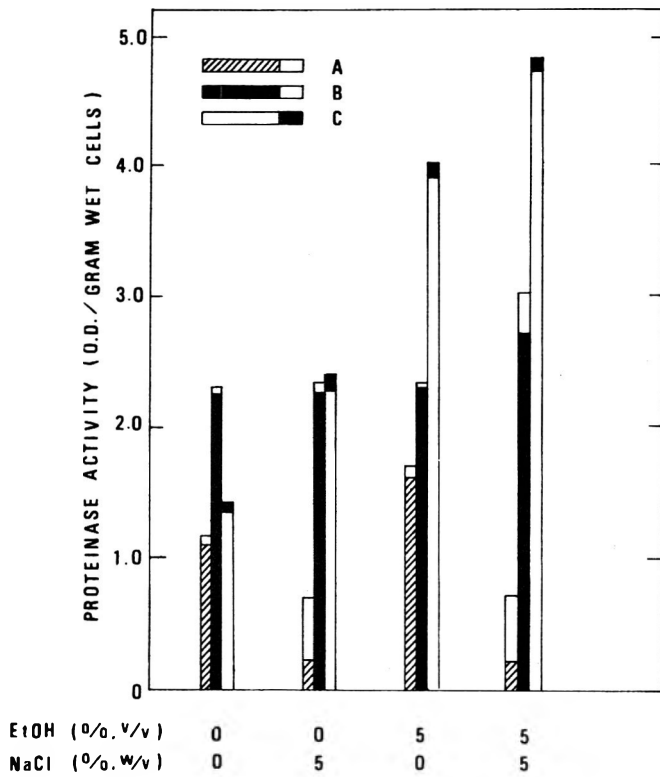
Fig. 3—Intra- and extra-cellular proteinase activities of baker's yeast assayed after 24 hr incubation at 40°C. (Upper black or white part of each column shows the extracellular enzyme activity. Lower part corresponds to the intracellular activity. Compare with the control value illustrated in Fig. 2. pH 3.0 = A, pH 9.0 = B and pH 5.5 = C.)
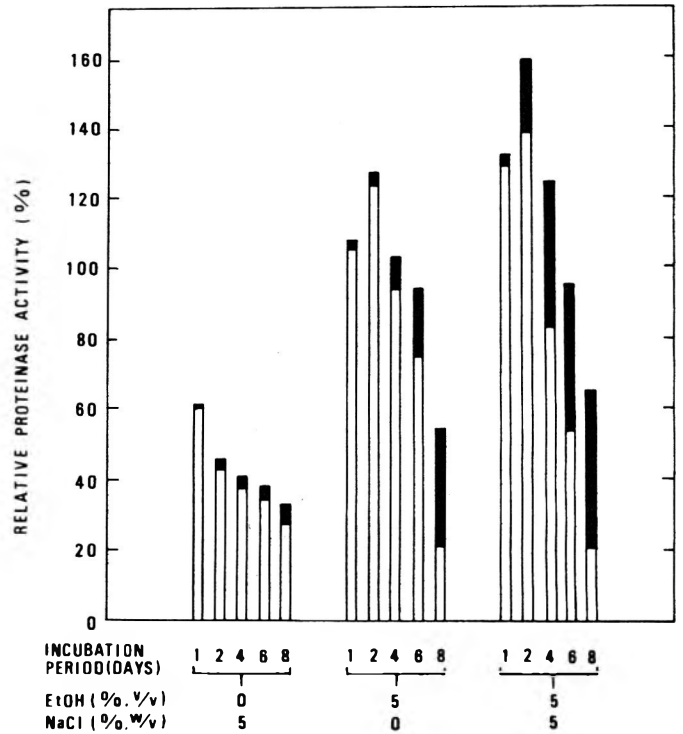
Fig. 4—Effect of incubation period (at 40°C) on intra- and extra-cellular proteinase activities of baker's yeast assayed at pH 5.5 (proteinase C). (Upper black part of each column shows the extracellular proteinase activity. Lower white part corresponds to the intracellular activity. Activity at zero time is expressed as 100%.)

days after the start of incubation. In comparison with the initial activity, the maximum activity obtained with 5% (v/v) EtOH and that with 5% (v/v) EtOH plus 5% (w/v) NaCl reached 128% and 157%, respectively.

These activities decreased gradually after 2nd day. However, the integral activity demonstrated with EtOH plus NaCl should become much greater than the others. This fact may represent a part of the synergism of EtOH and NaCl.

Concentrations of EtOH and NaCl in autolytic reaction mixture

The relationship between the concentrations of EtOH in the autolytic system, and the contents of Kjeldalh-N and DM in the supernatant of the autolysates, was investigated after 4 days incubation. Figure 5 suggests that the presence of EtOH in the autolytic reaction mixture contributes to the solubilizations of both Kjeldahl-N and DM. As shown in Figure 5, the effect of EtOH reached its optimum at the concentration of around 5% (v/v) at 5% (w/v) NaCl concentration. Higher EtOH concentration than 5% gave little advantage. Within the 0−9% (w/v) range of NaCl concentration, appreciable differences in the recoveries of both Kjeldahl-N and DM were not seen. A very low

NaCl concentration resulted in the development of a strongly bitter taste in the autolysates, which might have been due to some peptides in them. The development of bitterness was enhanced by a

higher concentration of EtOH. From the technological point of view, the following investigations were carried out with the concentrations of EtOH and NaCl set at 5% (v/v) and 5% (w/v), respectively.
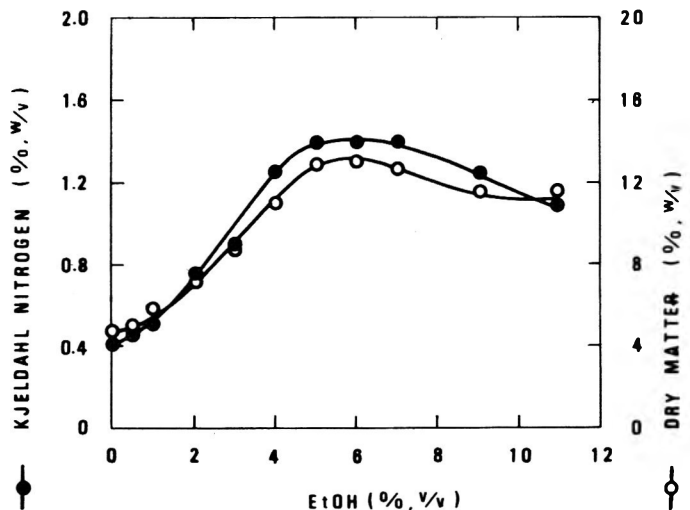
Fig. 5—Effect of EtOH concentration on liberations of Kjeldahl-N and DM from baker's yeast through 4 days autolysis. In this experiment, NaCl concentration was fixed at 5% (w/v). (DM does not include NaCl.)

Effects of incubation temperature and pH on autolysis

Three different preparations of the autolysates were prepared at three different incubation temperatures, 40°, 45° and 50°C. The effects of those temperatures on the recoveries of Kjeldahl-N, formol-N and DM in the supernatants were investigated.

3 days after the start of incubation the best recoveries were demonstrated at 45°C. At 5 days, however, the incubation at 40°C always led to the best recoveries on three different items.

While holding the temperature at 40°C, the effect of pH was investigated within the range of pH 4.0–6.0.

The results showed that the effect of pH was very slight. Without any adjustment, the autolytic reaction mixture was pH 5.3 at the start of incubation, then gradually increased by 2 days to pH 5.6 (Fig. 6).

Effect of incubation period on autolysis

The effect of incubation period on the liberations of Kjeldahl-N, formol-N and DM from the yeast cells, was investigated.

Although the liberations of both Kjeldahl-N and DM were rapid till 3 days and slowed after that time; that of formol-N was relatively slow and kept at an almost constant rate during the autolysis of 7 days. It seemed to require a longer incubation period than 5 days for preparing yeast extracts of better quality, usually marked with a higher ratio of formol-N per Kjeldahl-N.

Counts of viable bacteria during autolysis

The change of viable bacteria counts in the autolysates was investigated at appropriate intervals (Fig. 6).

The number of total viable bacteria was 1.5×10^4/ml at the start of incubation, decreasing rapidly to 3.1×10^2/ml until 2nd day. After that time, it held at almost the same level. Thus, the prevention of putrefaction of the autolytic reaction mixture during the incubation was achieved, at least up to 8 days.

Among the bacteria detected, 10–20% were designated as lactic acid formers. Neither *E. coli* nor *S. aureus* was detected throughout the incubation period.

Preparation of food-grade yeast extracts

A trial production of food-grade yeast extracts was performed. This product had the following composition: moisture 20.7%, NaCl 18.1%, Kjeldahl-N 6.81%, formol-N 2.94%, ammonium-N 0.13%, total carbohydrate 7.20% as glucose, crude lipid 0.40% and crude ash (excluding NaCl) 2.69%. No organoleptic bitterness was present. A high recovery (62.1% as DM or 86.9% as Kjeldahl-N) was obtained.
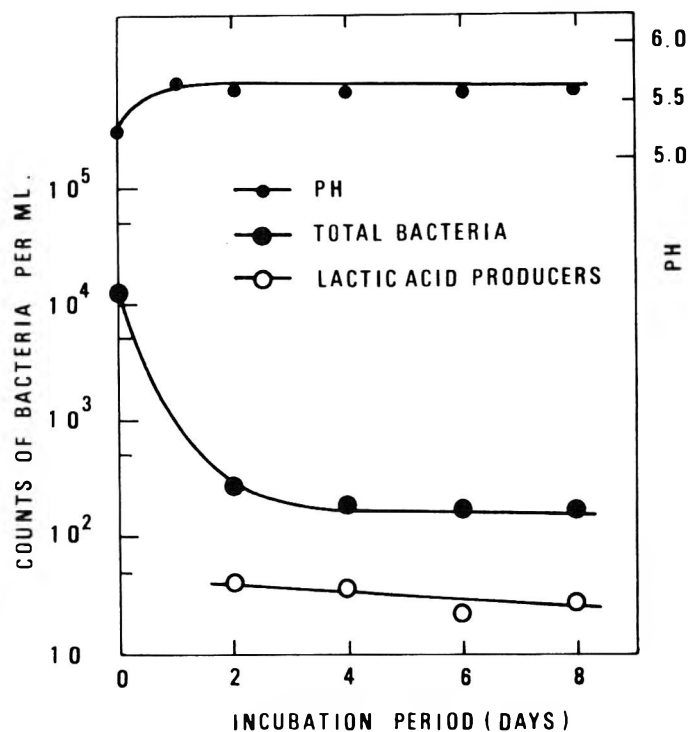
Fig. 6—Counts of viable bacteria in autolysate of baker's yeast and pH change during incubation for autolysis.

CONCLUSION

AUTOLYZED yeast extracts are sometimes manufactured employing nonpolar organic solvents such as toluol or chloroform as plasmolysers (Peppler, 1970). However, the use of these solvents prohibited by law as food additives should be avoided. NaCl is also known to be an excellent plasmolyser. However, NaCl acts as an inhibitor of the autolytic enzyme reaction, thus, it is hard to avoid a lower yield of yeast extracts.

The process described in this paper, in which both EtOH and NaCl were simultaneously employed as plasmolysers, indicates a possibility to overcome almost all of these defects. It promises the preparation of yeast extracts of high organoleptic quality with a high recovery. Also the product is safe as food. Therefore, the process may be able to be put into industrial practice, immediately.

Data pertaining to the organoleptic quality and detailed composition of the yeast extracts through this process, will be presented soon in another short publication.

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USE OF RESPONSE SURFACE METHODOLOGY IN THE DEVELOPMENT OF ACCEPTABLE HIGH PROTEIN BREAD

INTRODUCTION

MUCH LITERATURE has been published in recent years on the formulation and production of high protein breads. Articles concerned with the incorporation of soy flour (Tsen and Tang, 1971; Turro and Sipos, 1968) have appeared most frequently. The use of fish protein concentrate (FPC) has also been reported but to a lesser extent (Sidwell and Hammerle, 1970). Tsen and co-workers (Tsen et al., 1971) have summarized the use of various protein-rich foodstuffs such as FPC, cottonseed flour, nonfat dry milk and chickpea flour at a protein level equivalent to 12% soy flour. The main emphasis of these articles has been the study of dough properties and baking behavior which has led to the development of new and improved breadmaking processes for protein enriched breads.

Conspicuous, however, has been the lack of published data indicating the acceptance of these fortified breads by consumers. The primary purpose therefore, of the present research was not just to produce a high protein bread, but to produce one that was acceptable to consumers. The key factor appears to be the interaction of protein function and flavor attributes. Three protein sources were selected as variables for a Response Surface computer program to be integrated with flavor responses. Optimum levels of protein combinations were then selected to produce a consumer acceptable bread with a protein level of 20% (a 12% protein addition).

MATERIALS & METHODS

Experimental design and data evaluation

A Response Surface Computer Program was used to optimize both sensory responses and physical properties of the bread. This system using fractional factorial designs, response surface methodology and a computer program to make calculations and plot contour maps has recently been described (Henika, 1972).

The fractional factorial design chosen was a three-variable, three-level pattern investigating the three protein sources, each at protein level additions of 0, 4 and 8%. Table 1 lists these three independent variables, the ranges explored and the 15 combinations specified by the 3 x 3 fractional factorial design used as part of the Response Surface System. Responses measured include loaf volume, weight, specific volume, grain quality (visual examination of size, shape and number of air cells) and flavor.

Table 1—Independent variables, experimental pattern, response results, regression coefficients and analysis of variance

Independent variables	−1	0	+1
Milk protein — % protein from	0	4	8
Soy protein — % protein from	0	4	8
Fish protein — % protein from	0	4	8

Experimental pattern and results fed to computer

	Level of					
Run	Milk protein (%)	Soy protein (%)	Fish protein (%)	Loaf vol (cc/500g)	Grain quality (units)	Overall impression (units)
1	0	0	4	3542	9.05	6.0
2	8	0	4	2900	8.85	6.0
3	0	8	4	2733	8.05	4.0
4	8	8	4	2292	7.08	4.5
5	0	4	0	3295	9.25	6.5
6	8	4	0	2990	8.70	6.0
7	0	4	8	2917	7.73	4.0
8	8	4	8	2508	8.58	4.0
9	4	0	0	3092	9.30	7.0
10	4	8	0	2558	7.85	5.5
11	4	0	8	2883	8.00	4.5
12	4	8	8	2292	7.40	3.5
13	4	4	4	2751	8.40	5.0
14	4	4	4	2778	8.55	5.0
15	4	4	4	2725	8.35	5.1

Regression coefficients

Coefficient	Loaf vol	Grain quality	Overall impression
B_0	2754	8.43	5.033
B_1	−224.6	−0.109	0.0
B_2	−317.7	−0.603	−0.75
B_3	−166.9	−0.424	−1.13
B_{11}	+168.2	0.123	0.046
B_{22}	−53.01	−0.302	0.046
B_{33}	+7.819	0.00583	0.0458
B_{12}	+50.00	−0.193	0.125
B_{13}	−25.83	0.350	0.125
B_{23}	−14.55	0.213	0.125

Analysis of variance and explained variance

	Mean squares (F values) for		
	Loaf vol	Grain quality	Overall impression
First order	0.478×10^6 (673*)	1.48 (137*)	4.88 (1462*)
Second order	0.402×10^5 (56*)	0.140 (13)	0.0067 (2)
Interaction	0.451×10^4 (6)	0.273 (25*)	0.062 (19)
Lack of fit	0.155×10^5 (22*)	0.113 (11)	0.083 (25*)
Experimental error	0.710×10^3	0.011	0.0033
Variability % explained	96	80	97

* Significant

Table 3—Formula and procedure

%	Dough Ingredient	%	Brew Ingredient
100.0	Flour	1.0	Sugar
0.5	Emplex (SSL)[a]	0.25	Yeast food
5.0	Sugar	3.0	Yeast
2.0	Salt	10.0	Basic water
3.0	Reddi-sponge[b]		
3.0	Shortening		
0.002	Potassium bromate		
35.0	Basic water		
Variable	Additional water		

Procedure

Set brew @ 32–38°C and ferment for 30 min. Add brew to dough ingredients; add additional water as necessary and mix to optimum development. Give 30 min floor time, scale dough pieces (560g), round, rest 20 min and mould. Proof 70 min; bake 20 min @ 205°C; cool 60 min. Measure weights and volumes. Store samples for 24 hr, score for grain quality and submit for Descriptive Flavor Analysis.

[a] C.J. Patterson Co.
[b] Foremost Foods Co.
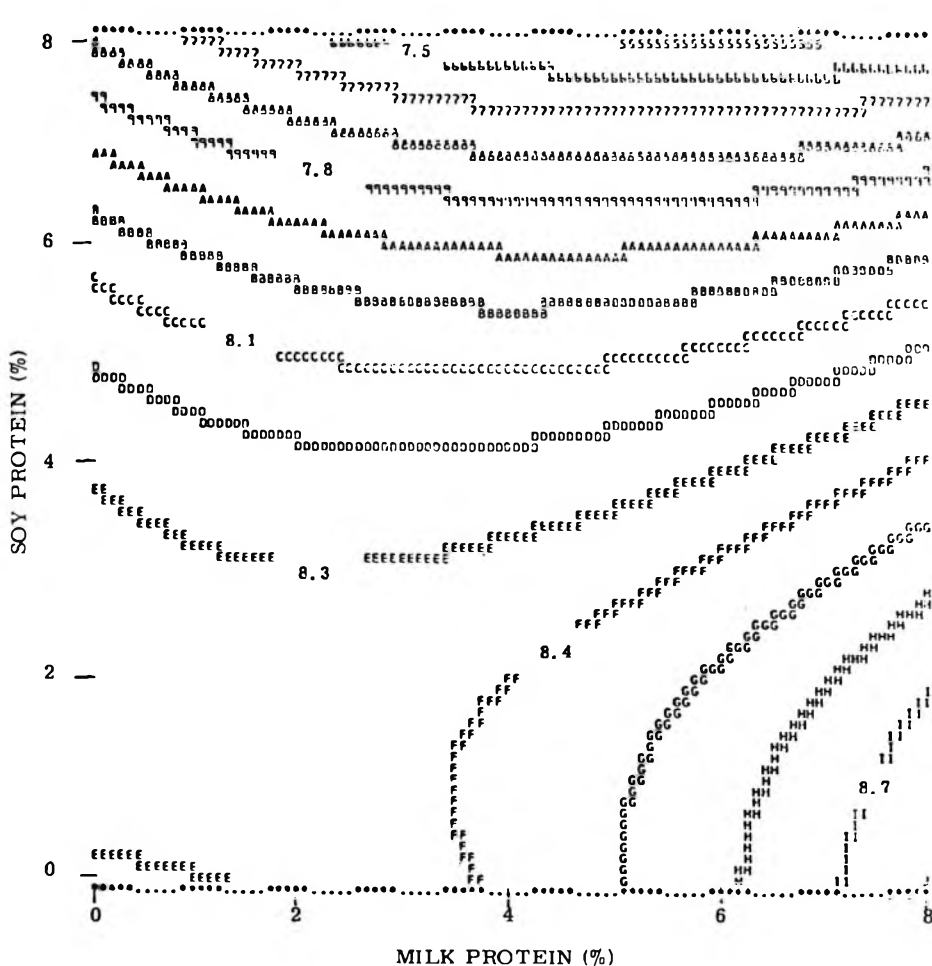
Table 2—Protein sources

Source	% Protein	Percent of product to give added protein level		
		0%	4%	8%
Milk protein concentrate[a]	35	0	11.4	22.8
Soy flour[b]	60	0	6.7	13.4
Fish protein concentrate[c]	90	0	4.45	8.9

[a] Whey, sodium/calcium caseinate, Foremost Foods Co.
[b] Textrol®, Central Soya Co.
[c] Astra Nutrition, Möludal, Sweden

The Descriptive Analysis Flavor Panel was used to identify and quantify those flavor characteristics considered most important to the consumer.

These responses became input data to the computer and regression coefficients were calculated for a second order Taylor Expansion Equation with three variables. The coefficients were then used to make the statistical calculations and generate computer-calculated and printed contour maps covering the entire range investigated.

Formula and procedure

The proteins selected for this study and identified in Table 2 were milk protein concentrate (MPC), soy flour, and fish protein concentrate (FPC). The basic bread formula and baking procedure appear in Table 3. The amount of water used in the various runs was established by setting the "basic water" at 45% and calculating the additional water required. As determined on the farinograph, MPC and FPC required 100% absorption and soy flour 200% absorption. Mixing was done in a Hobart A-200 dough mixer equipped with a McDuffee bowl. In trials where the dough appeared too dry during initial mixing, additional water was added in 20 ml increments to the desired consistency.

These breads were made with a commercially available short-time dough system (Henika and Rodgers, 1965; Henika and Zenner, 1960). In addition, sodium stearoyl-2 lactylate was used throughout to add strength to the high protein doughs and broaden their tolerance (Tsen et al., 1971; Tenney and Schmidt, 1968).

Sensory evaluation methods

The breads, as described in Table 1, were characterized 24 hr after baking by a Descriptive Flavor Analysis Panel trained according to the method of Arthur D. Little and described by Caul (1957). This five-member panel is trained to objectively analyze food products for

Fig. 1—Loaf volume (cc) as a function of soy and milk protein. Basic formula with 6% FPC.

Table 4—Selected protein combinations: Computer predicted vs. observed results

		Protein combinations					
Milk protein (%)		8	8	8	7	2	0
Soy protein (%)		4	2	0	5	4	6
Fish protein (%)		0	2	4	0	6	6
Responses	**Limits**	**Computer predicted results**					
Mix time	max 10 min	9.0	9.5	9.0	8.5	8.8	9.0
% Water	none	85.0	85.0	82.5	86.0	82.5	81.0
Volume	min 2600 cc	2900	2900	2900	2800	2850	2850
Spec vol	min 5.5	5.8	5.9	5.9	5.7	5.8	5.9
Grain qual	min 8.0	8.6	8.9	8.9	8.3	8.2	8.1
Responses	**Limits**	**Observed results**					
Mix time	max 10 min	9.0	9.5	9.0	8.5	8.8	8.5
% Water	none	85.0	83.0	82.5	86.0	82.5	84.0
Volume	min 2600 cc	2800	2800	2750	2700	2700	2850
Spec vol	min 5.5	5.7	5.7	5.6	5.5	5.5	5.8
Grain qual	min 8.0	9.2	9.1	8.5	8.3	7.9	8.7

Fig. 2—Grain quality as a function of soy and milk protein. Basic formula with 6% FPC.

aroma and taste components and give an intensity score to each specific character note. The panel evaluated the 15 bread samples for milk, soy and fish notes. In addition each sample received an overall impression rating. This rating takes into account all sensory characteristics including texture, color, aroma and flavor. Numerical values were given to each intensity score and the data were entered into the computer as responses.

RESULTS & DISCUSSION

TABLE 1 summarizes the response data for loaf volume, grain quality and the Descriptive Flavor Panel's judgment of "overall impression." These three were considered the most critical.

Although breads were made containing an added 4–20% protein, analysis of the data suggested that addition of 12% would (1) add substantial amounts of protein and (2) still give an acceptable loaf of bread.

Figure 1 shows the effect of MPC and soy when added to bread containing 6% protein from FPC. While both MPC and soy flour depress loaf volume independently, combinations can be found which minimize this undesirable effect.

Figure 2 shows the influence of MPC and soy on grain quality in bread containing 6% FPC protein. MPC from 0–8% added protein did not change grain quality at all, provided there was at least 4% soy protein present. These representative maps illustrate that combinations of protein should be carefully selected to maximize benefits and minimize undesirable effects.

Response values compatible with commercial breadmaking operations were used as guidelines in selecting bread for sensory evaluation. These values are shown in Table 4 along with actual baking data for six protein combinations. Table 4 also shows responses predicted for those combinations using the Taylor equation coefficients described in Table 1. Analysis of descriptive flavor profiles of these six breads narrowed the selection to two combinations: 7/5% or 8/4% MPC protein/soy protein, respectively. These bread combinations had a minimal soy (beany) character with a good balance between the flour, yeast and milk notes. Bread containing FPC was eliminated from further evaluation due to inappropriate fish aroma and flavor.

Given an equal sensory rating, the 7% milk protein and 5% soy protein combination was selected over the 8/4% blend for use in the consumer study because of the economic advantage. The flavor acceptance of this bread was compared to: (a) a standard straight dough bread with no added protein and (b) a high protein bread made with 6% added soy protein following the method described by Tsen and Tang (1971).

Judges (79) from the surrounding community, ranging in age from 8 to 65,
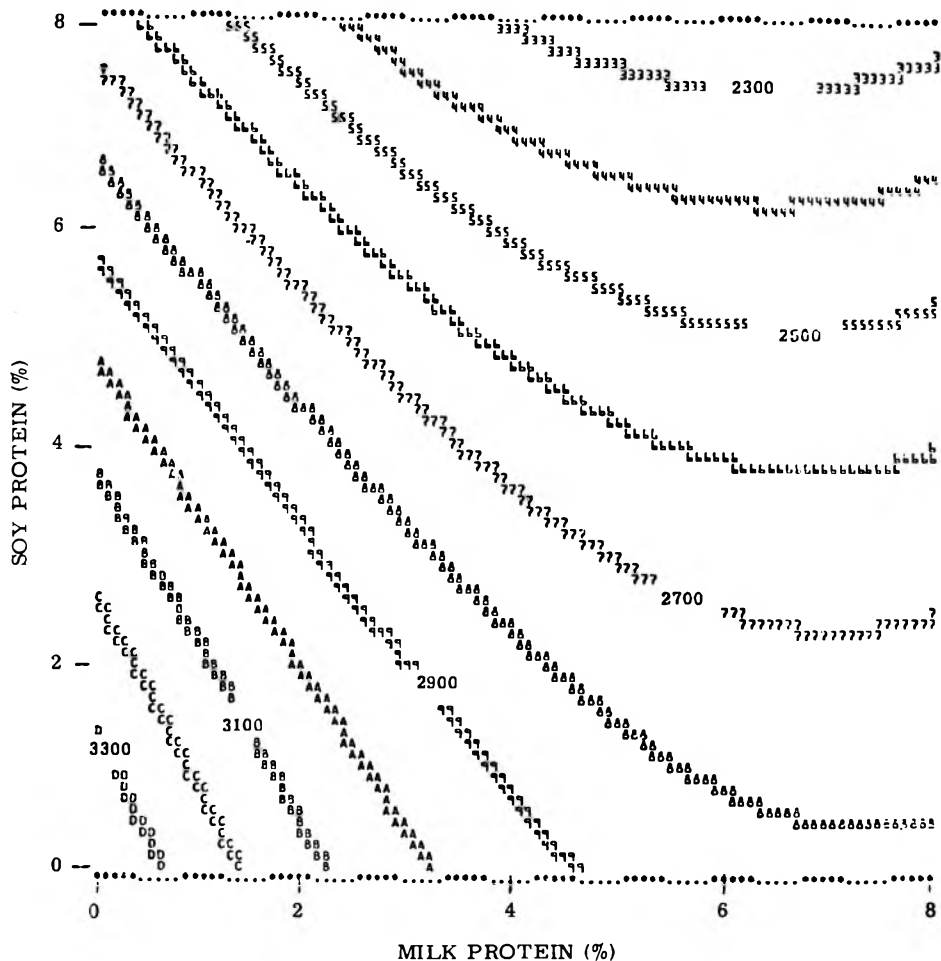
Table 5—Analysis of consumer test data

	Consumer taste test results		
Type of bread	MPC/soy flour	Straight dough	Soy flour
Ranked first	37	24	18
Ranked second	26	28	25
Ranked third	16	27	36

To analyze the results, the ranks were transformed into scores according to Fisher and Yates (1942). Numerical scores given were:

Ranked first	0.85
Ranked second	0.0
Ranked third	−0.85

	Analysis of variance			
Variables	df	SS	MS	F
Samples	2	7.08	3.54	7.70***
Panelists		0		
Error	234	107.08	0.46	
Total	236	114.16		

Standard Error = 0.076

	Duncan's multiple range test			
Number of means	2		3	
Probability (P)	5%	1%	5%	1%
Factor	2.77	3.64	2.93	3.80
Shortest sig. diff.	0.21	0.28	0.22	0.29

Samples	MPC/soy flour	Straight dough	Soy flour
	A	B	C
Means	0.23	−0.03	−0.19

A–C	0.23 −	(−0.19) = 0.42 < 0.22 and	0.29**
A–B	0.23 −	(−0.03) = 0.26 < 0.21	> 0.28*
B–C	−0.03 −	(−0.19) = 0.16 > 0.21	NSD

* Significantly different at 0.05% level
** Significantly different at 0.01% level
*** Significantly different at 0.001% level
NSD = not significantly different

evaluated the three breads in one session. Samples were served in randomized order in individual air-conditioned booths under white lights. Judges were instructed to taste each sample from left to right rinsing with water between samples. They were then asked to rank the three samples in order of preference from "like most" to "like least." Additionally, they scored each bread using a seven point hedonic scale, with seven equaling "like very much" and one equaling "dislike very much."

The data from the consumer test was analyzed in accordance with Larmond's (1970) method for ranked difference analysis (Table 5). Results of the analysis showed no significant differences in preference between the straight dough bread and the 6% soy protein bread. The MPC/soy bread, however, was significantly preferred at the 99% level over the 6% soy protein bread and at the 95% level over the straight dough bread. Hedonic scores on the three breads were in agreement with the ranking scores. They were:

MPC/soy, 6.0; straight dough, 5.5; and 6% added soy protein, 5.1 on a seven point hedonic scale.

These results provide evidence that unfamiliar or inappropriate flavor is the limiting factor in using high levels of soy as the sole protein source for fortification. The milk protein in the form of MPC served to complement the natural aroma and flavor of the high protein bread. Thus significant levels of protein can be added to bread without compromising flavor attributes provided care is exercised in choosing from among the many available protein sources.

This work demonstrates the compatibility of the breadmaking process and sensory evaluation with response surface methodology in developing a "consumer acceptable" protein-rich loaf of bread. These techniques are not limited to bread. Proteins as well as other ingredients can be selected as variables for use in the Response Surface Program. By utilizing the format of this system those combinations whose responses meet the preset limits can be identified. Confirming tests are then made and products evaluated by consumer panels for acceptance.

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STRUCTURAL FUNCTIONS OF TASTE IN THE SUGAR SERIES: SENSORY PROPERTIES OF DEOXY SUGARS

INTRODUCTION

THE MOST DIRECT method for testing and assessing Shallenberger's sweetness hypothesis in the sugars (Shallenberger et al., 1969; Shallenberger, 1966; Shallenberger and Acree, 1967) is to selectively eliminate oxygen atoms at single positions around a sugar ring and to then evaluate the sensory properties of the products. In this way it might be anticipated that superfluous oxygen atoms could be identified, and Shallenberger's AH,B system located, at least in certain sugar structures.

We have therefore tested a number of mono- and dideoxy derivatives of the analogous and conformationally stable and well-defined models, α,α-trehalose and methyl α-D-glucopyranoside. Their sensory properties are described in this communication and compared with those of some previously known monodeoxy sugars. As many of these deoxy derivatives were novel compounds, prepared by sophisticated techniques (Lee, 1973), their chemistry and synthesis will be described elsewhere. However, their conformations, as determined by nuclear magnetic resonance spectroscopy, are in each case the expected $^4C_1$ favored form.

EXPERIMENTAL

ALL DEOXY DERIVATIVES of methyl α-D-glucopyranoside and trehalose were prepared by classical or novel carbohydrate techniques (Lee, 1973). Known compounds synthesised in this way agreed in melting point and optical rotation with literature reports and the structure of all compounds was in any case confirmed by nuclear magnetic resonance spectroscopy. (1-Deoxy derivatives were a kind gift from Dr. R. Barker of Iowa State University; free reducing deoxy sugars were obtained from British Drug Houses (Chemicals) Ltd., Poole, Dorset.)

Panellists were selected and trained from college personnel according to a previous publication (Birch et al., 1972). Each was asked to place a few mg of each substance (powdered crystals) on the tongue and to comment whether it was trace sweet (tr S), sweet (S), intensely sweet (SS), trace bitter (tr B), bitter (B) or intensely bitter (BB). The decisions listed in the tables are those obtained in at least 70% of total judgements, each panellist carrying out duplicate tasting sessions. Ten subjects were asked to taste all substances listed in Table 1 once each at each session, rinsing with distilled water between substances, and pausing 1 min before passing on to the following substances.

Table 1—Taste properties of deoxy derivatives of α,α-trehalose and methyl α-D-glucopyranoside[a]

Substance	Sweetness	Bitterness
Methyl α-D-2-deoxy arabino-hexopyranoside	S	tr
Methyl α-D-2-deoxy ribo-hexopyranoside	S	tr
Methyl α-D-3-deoxy arabino-hexopyranoside	tr	0[b]
Methyl α-D-3-deoxy ribo-hexopyranoside	tr	0
Methyl α-D-4-deoxy xylo-hexopyranoside	S	0
Methyl α-D-6-deoxy xylo-hexopyranoside	S	tr
Methyl α-D-2,6-dideoxy arabinohexopyranoside	0	BB
Methyl α-D-2,6-dideoxy ribohexopyranoside	0	BB
Methyl α-D-3,6-dideoxy arabinohexopyranoside	0	B
Methyl α-D-4,6-dideoxy xylohexopyranoside	0	B
Methyl α-D-2,3-dideoxy glucopyranoside	—	—
O-α-D-2-deoxy arabinohexopyranosyl — (1→1)-α-D-2-deoxy arabinohexopyranoside	—	—
O-α-D-2-deoxy ribohexopyranosyl — (1→1)-α-D-2-deoxy ribohexopyranoside	S	tr
O-α-D-3-deoxy arabinohexopyranosyl (1→1)-α-D-3-deoxy arabinohexopyranoside	tr	0
O-α-D-3-deoxy ribohexopyranosyl (1→1)-α-D-3-deoxy ribohexopyranoside	tr	0
O-α-D-4-deoxy xylohexopyranosyl (1→1)-α-D-4-deoxy xylohexopyranoside	S	0
O-α-D-6-deoxy xylohexopyranosyl (1→1)-α-D-6-deoxy xylohexopyranoside	S	tr
O-α-D-2,6-dideoxy arabinohexopyranosyl (1→1)-α-D-2,6-dideoxy arabinohexopyranoside	—	—
O-α-D-2,6-dideoxy ribohexopyranosyl (1→1)-α-D-2,6-dideoxy ribohexopyranoside	0	BB
O-α-D-3,6-dideoxy arabinohexopyranosyl (1→1)-α-D-3,6-dideoxy arabinohexopyranoside	0	B
O-α-D-4,6-dideoxy xylohexopyranosyl (1→1)-α-D-4,6-dideoxy xylohexopyranoside	0	B
O-α-D-2,3-dideoxy-glucopyranosyl (1→1)-α-D-2,3-dideoxy-glucopyranoside	0	B

[a] S = Sweet; B = Bitter; BB = Intensely bitter; tr = trace; 0 = Zero response either way.
[b] Some panelists claimed this substance was perceptibly trace bitter.
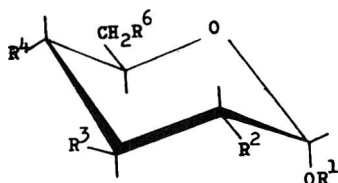
R^1 = CH$_3$ or repeated deoxy sugar residue
R^2 = H; R^3 = R^4 = R^6 = OH   S (Tr B)
R^3 = H; R^2 = R^4 = R^6 = OH   tr S
R^4 = H; R^2 = R^3 = R^6 = OH   S
R^6 = H; R^2 = R^3 = R^4 = OH   S (Tr B)
R^2 = R^6 = H; R^3 = R^4 = OH   not S (Very B)
R^3 = R^6 = H; R^2 = R^4 = OH   not S (B)
R^4 = R^6 = H; R^2 = R^3 = OH   not S (B)
R^2 = R^3 = H; R^4 = R^6 = OH   not S (B)

Fig. 1—Taste effects of deoxy substituents in deoxy glucopyranoside molecules.

RESULTS

THE RESULTS in all three tables of this communication depend to some extent on the crystalline properties of the sugars tasted. If the sugars had been tasted as solutions rather than crystals we would anticipate (as in previous studies) no qualitative differences, due to the intrinsic stability of glycoside structures. This would also apply, of course, to the 1-deoxy sugars but not to the free reducing monodeoxy sugars listed in Table 3.

These latter would undoubtedly anomerize and give rise to complex mixtures of isomers with different sensory properties. Some differences in intensity of response might occur with any of the substances listed in all three tables, because of the inter- and intramolecular hydrogen bonding systems: hydrogen bonding, which occurs in the crystal lattice is either altered or absent in solution.

The results listed in Table 1 confirm our initial belief that the two model sugars, α,α-trehalose and methyl α-D-glucopyranoside are completely analogous in their sensory properties. The results are indeed identical except for a marginal difference in the 4-deoxy xylo configuration. This adds support to a previous suggestion (Birch et al., 1970) that only one half of a disaccharide molecule binds to the taste bud protein, and in the case of glucopyranoside molecules the sensory effects of deoxy substituents are directly attributable to the position of the deoxy substituent in the sugar ring (Fig. 1).

The results in Table 1 also show that monodeoxy sugars are *always* either sweet or trace sweet and in a few cases trace bitterness also occurs. No marked degree of bitterness arises in these compounds. Dideoxy sugars, on the other hand, are *never* sweet nor even trace sweet and are either markedly bitter or intensely bitter in each case. If Shallenberger's AH,B system can be identified with a stereospecific moiety within these favored conformations of hexopyranose molecules, then at least some of the dideoxy sugars listed in Table 1 should have been sweet, unless some marked difference in solubility characteristics exists between the deoxy sugars and parent sugars. For example since methyl α-D-2-deoxy arabinohexopyranoside, methyl α-D-4-deoxy xylohexopyranoside and methyl α-D-6-deoxy xylohexopyranoside were all markedly sweet we can logically argue that both methyl α-D-2,6-dideoxy arabinohexopyranoside and methyl α-D-4,6-dideoxy xylohexopyranoside should have been sweet. No configurational change has occurred in the dideoxy derivatives in which the superfluous 2- and 6-oxygen atoms are simultaneously absent. The same reasoning applies to the corresponding trehalose compounds. The complete lack of sweetness in all these substances could be explained by assuming that the dideoxy derivative aligns itself differently from the parent sugar because the freshly available lipophilic sites (CH$_2$ groups) are bound to the protein, thus eliciting the bitter response. We have recently (Birch and Lindley, 1973a, b) tested a number of inositols and deoxy inositols containing four to six hydroxyl substituents, none of which elicited any response other than sweetness. Those with fewer than four hydroxyl substituents were not sweet and frequently bitter. Likewise Shallenberger (1973) has pointed out that the menthols (deoxy alkyl inositols) are extremely stereospecific in their olfactory response and hence the lipophilic sites must react with the olfactory epithelium in the odorous enantiomer. The 1-deoxy sugars which have previously been reported by us (Table 2) are purely sweet and we wish to modify our original result in erithritan (Birch and Lee, 1971) to accord with a recent finding of Hodge

Table 2—Taste properties of 1-deoxy derivatives[a]

Substance	Sweetness[b]	Bitterness[c]
1-Deoxy erythrofuranose (erythritan)	tr	0
1-Deoxy ribofuranose	S	0
1-Deoxy xylofuranose	S	0
1-Deoxy mannofuranose	S	0
1-Deoxy glucofuranose	S	0
1-Deoxy mannopyranose	S	0
1-Deoxy galactopyranose	S	0
1-Deoxy glucopyranose	S	0

[a] Birch and Lee (1971)
[b] S = Sweet; tr = trace.
[c] 0 = Zero response either way.

Table 3—Taste properties of free mono deoxy sugars[a]

Substance	Sweetness	Bitterness
6-Deoxy-D-glucopyranose (Quinovose)	S	tr
2-Deoxy-D-glucopyranose	S	tr
2-Deoxy-D-ribofuranose	S	tr
6-Deoxy-D-mannopyranose (Rhamnose)	S	0
6-Deoxy-L-galactopyranose (Fucose)	S	0

[a] S = Sweet; tr = tr bitter; 0 = Zero response either way.

(1973), from tasteless to trace sweet. The complete absence of bitterness in the 1-deoxy sugars substantiates our previous deduction (Birch and Lindley, 1973a, b) that the anomeric center, ring oxygen atom, primary alcohol group and second carbon atom are associated with the bitter response. The trace bitterness reported for the 2-deoxy derivatives listed in Table 1 accords with this idea. The deoxy derivatives of free reducing sugars are less agreeable models for this type of study than either the stable glycoside structures or 1-deoxy sugars listed in Tables 1 and 2. However, for comparison, five of these are listed in Table 3 and all exhibit sweetness and/or bitterness depending on the position of the deoxy group.

We have previously suggested that the fourth hydroxyl group of glucopyranosyl structures is of unique importance in eliciting the sweet response (Birch et al., 1971), possibly acting as the binding site to the receptor protein as it does in the hesperidin glycosides (Horowitz and Gentili, 1971) or again in fly taste receptors (Evans, 1963). The results in Table 1, however, indicate that it is the third hydroxyl group in the substances examined which is of greatest importance because, of the monodeoxy methyl glucosides and corresponding trehalose analogues, only the two 3-deoxy derivatives are trace sweet. All others listed in this group are markedly sweet. These results agree with those of Hodge et al. (1972) and parallel those obtained by Barnett (1973), for the binding of sugars to carrier protein during intestinal transport. Barnett's specificity studies have progressed so far that he has ascribed the unique importance of the third hydroxyl group to a covalent bond between it and the protein, the remaining sugar hydroxyl groups exhibiting only hydrogen bonding. Our original deduction may have been due to the complex and varied nature of the derivatives under examination at the time, such as N-acetyl amino derivatives possessing strong positive centers, or due to the difficulty of distinguishing AH from B in the saporific α-glycol moiety.

Deoxy sugars offer the simplest insight into the functions of hydroxyl substituents in their parent sugars and the results reported in this communication clearly indicate that certain hydroxyl groups are more important than others in eliciting the sweet response. If the third and fourth hydroxyl substituents really constitute Shallenberger's AH,B system for sweetness, the molecule may be "polarized" on the taste bud surface with the first, second, fifth and sixth carbon substituents, and the ring oxygen providing the lipophilic centers which may give rise to the bitter response, and having little or no effect on the sweetness properties (cf. glucose, mannose, xylose and the cyclitols) (Birch et al., 1972).

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GIBBERELLIC ACID IN MALTING OF OATS

INTRODUCTION

CHEMICAL modifications in germinated or malted oats have been the subject of few investigations. Naylor and Dawson (1936) studied the effect of different seed treatments on the development of amylolytic enzymes during germination of oats. Their preparation from carbon disulfide-treated oats, germinated for 3 or 8 days, showed a negligible saccharogenic (diastatic) power and a very high dextrinogenic (alpha-amylase) power. Kneen (1944) reviewed the literature on development of amylases in germinating cereals and concluded that ungerminated barley, wheat, or rye had much higher saccharifying activities than ungerminated oats. Germination increased both starch saccharifying and liquefying (or dextrinizing) activities of barley, wheat and rye; in oat malts, the ratio of saccharifying to liquefying activities was low. Investigations of Kneen (1944) indicated that the small quantity of beta-amylase in oats largely disappeared during germination. Consequently, oat malts could be used as sources of preparations rich in alpha-amylase containing little, if any, beta-amylase. Davidson (1945) found that wheat showed the highest diastatic power; barley was considerably lower; and oats, corn and rice showed very little activity. Preece (1948) reported that kilned oat malt, though of low diastatic power, may show appreciable alpha-amylase activity. For barley malts, beta-amylase activity as measured by production of reducing sugars, under the conditions of the standard diastatic power determination, was three to four times that of alpha-amylase activity. For oat malts, the two activities were essentially equal.

The response of germinating barley to gibberellic acid (GA₃) stimulated research at many locations (Dahlstrom and Sfat, 1961; MacLeod and Millar, 1962; Melcher and Varner, 1971; Prentice et al., 1963; Sparrow, 1964). The research included fundamental investigations on the mode of action of the applied GA₃ in the germinating barley grain and small-scale and commercial malting. Russell (1972) established that GA₃ enhances the production of at least six hydrolytic enzymes in the aleurone cells of barley and that the synthesis of two of these is *de novo*. Originally, 15 mg GA₃ per kg of dry barley was recommended. Applications of 1 mg/kg barley are considered completely adequate, and as little as 0.1 mg per kg may give excellent results without undesirable side effects (excessive proteolysis) (Sparrow, 1964).

Most studies on the effects of GA₃ on germination of oats concerned the mechanism of dormancy in wild oats (*Avena fatua*, L.). Dormancy and inhibition of germination involve restrictions in two metabolic pathways. Both restrictions can be overcome by GA₃ (Naylor and Simpson, 1961). The first restriction occurs in the endosperm and was postulated as lack of sugar production in the absence of active maltase. The second restriction occurs in the germ and results in a block in sugar utilization (Simpson and Naylor, 1962). Simpson (1965) concluded that exogenous GA₃ promotes the synthesis of enzymes or activates preformed enzymes necessary for the utilization of endosperm constituents as substrate for germination and growth.

Palmer (1970) found that aleurone cells of oats produce significantly less alpha-amylase than the aleurone cells of rye, wheat, or barley after treatment with GA₃. Excised embryos from oats, rye, wheat, or barley were more efficient than exogenous GA₃ in catalyzing the production of alpha-amylase in the oat endosperm. Enzymatic modification of the endosperm of oats, as distinct from other cereal grains, was associated with rapid elongation of the scutellar apex under the aleurone cells. Palmer's observation regarding the scutellum corroborates the findings of Shands (1960).

Cereal grains contain in addition to alpha- and beta-amylases, limit-dextrinase which can break 1.6 linkages of starch. According to Manners (as reported by Anon., 1971), GA₃ enhances activity of limit-dextrinase in barley malt. Corn, rye and wheat have little limit-dextrinase activity in the ungerminated grain but after 2–3 days of germination a considerable increase takes place. Oats are unusual in having before germination a high limit-dextrinase activity which does not increase with germination.

The authors know of no published data on the effects of gibberellic acid in malting of oats, in general, and on malt modification and solubilization of proteins, in particular. Such effects were the subject of this study.

Table 1—Effects of gibberellic acid (GA₃) on barley and oat malt parameters[a] (1971 crop)

Grain and treatment[b]	Kernel weight mg	Malt extract[c] %	Extract fine-coarse grind[d] %	Wort color[e]	Grain N %	Ratio wort N malt N %	Diastatic power deg.	Alpha-amylase 20° units
Larker barley								
H₂O	31.4	77.0	1.5	1.9	2.05	33.4	189	27.0
5.0 ppm GA₃	–	78.9	2.1	2.5	–	50.6	202	60.1
Dal oats								
H₂O	35.1	53.5	1.7	1.8	2.70	21.7	23	26.6
5.0 ppm GA₃	–	53.8	1.8	1.6	–	25.1	39	36.0

[a] Data expressed on a moisture-free basis
[b] Final moisture content of steeped grain was about 45%
[c] % solids extracted from finely ground malt
[d] Difference between solids extracted from finely and coarsely ground malt
[e] Lovibond Tintometer method

Table 2—Effects of N fertilization and gibberellic acid on oat malt parameters[a] (1972 crop)

N-fertilizer kg/ha	Gibberellic acid ppm	Oat kernel weight mg	Plump oat %	Malt extract[b] %	Extract fine-coarse grind %[c]	Wort color[d]	Oat N %	Ratio wort N malt N %	Dia-static power deg.	Alpha-amylase 20° units
Dal										
0 N	0	25.2	9.2	51.2	1.9	3.2	2.62	24.1	29	32.8
112 N	0	24.5	10.5	50.4	2.6	3.1	2.77	25.1	28	32.8
0 N	0.5	25.2	9.2	51.2	1.5	2.6	2.62	25.6	42	39.3
112 N	0.5	24.5	10.5	50.5	1.6	2.7	2.77	24.8	42	39.3
0 N	2.5	25.2	9.2	49.7	0.7	2.7	2.62	28.0	46	40.8
112 N	2.5	24.5	10.5	51.3	2.4	3.0	2.77	27.8	32	43.6
X1196-1										
0 N	0	32.2	53.7	54.9	3.0	2.8	2.07	27.0	20	25.6
112 N	0	32.4	54.3	53.3	1.1	3.3	2.22	26.6	21	26.0
0 N	0.5	32.2	53.7	55.9	0.8	2.7	2.07	27.5	26	27.8
112 N	0.5	32.4	54.3	54.1	1.0	2.9	2.22	27.3	28	30.3
0 N	2.5	32.2	53.7	55.8	0.6	3.0	2.07	31.3	31	31.3
112 N	2.5	32.4	54.3	54.0	1.8	3.0	2.22	32.3	35	32.9
X1656-1										
0 N	0	26.9	7.2	49.5	0.9	2.5	2.80	18.5	18	18.9
112 N	0	26.3	8.2	49.5	1.5	3.1	2.84	21.7	21	20.0
0 N	0.5	26.9	7.2	50.6	1.0	2.8	2.80	23.3	30	25.3
112 N	0.5	26.3	8.2	50.5	1.5	2.7	2.84	23.4	29	26.4
0 N	2.5	26.9	7.2	51.3	1.2	2.7	2.80	25.1	34	30.4
112 N	2.5	26.3	8.2	50.7	2.3	3.0	2.84	24.9	40	31.3

[a] Data expressed on a moisture-free basis
[b] % solids extracted from finely ground malt
[c] Difference between solids extracted from finely and coarsely ground malt
[d] Lovibond Tintometer method

EXPERIMENTAL

Materials and methods

The three oat cultivars ('Dal,' CI 9159; Wis. X1196-1; and Wis. X1656-1) were grown at Madison, Wis. in 1972 with 0 or 112 kg/hectare (0–100 lb per acre) of N fertilizer. A solution of KNO$_3$ was applied as top dressing immediately prior to heading on June 17 when plants were 45 cm tall. Protein content (Kjeldahl-N × 6.25) of the oats was 12.9–17.8%. In addition, single samples of the following cereal grains from the 1971 crop were used: barley ('Larker,' CI 10648) and a plump kerneled sample of Dal oats. Grain of three replications produced in randomized blocks was composited for the samples to be malted.

Malting

Lots of 170g cleaned grain were malted, essentially as described by Dickson et al. (1968). Preliminary tests were run to determine steeping times required to attain a moisture of 43%. The 1971 samples were steeped at 16°C, sprayed with water or GA$_3$ solution to increase moisture content from 43–45%, and germinated at 16°C for 5 days. Final kiln temperature was 65°C. The malting conditions were the same for the 1972 lots except that the concentrations of GA$_3$, on a dry grain basis, varied; exogeneous GA$_3$ was 5 ppm in the 1971 samples and 0.5 and 2.5 ppm in the 1972 samples. Levels of 5 ppm GA$_3$ are routinely used in our laboratory in preliminary investigations. Excellent responses are generally obtained from much lower levels of added GA$_3$.

Analytical determinations

The cereal grains, malts, and worts were analyzed according to the methods of analysis of the American Society of Brewing Chemists (1958).

RESULTS & DISCUSSION

THE EFFECTS of spraying barley and oats with gibberellic acid (5 ppm GA$_3$ on dry grain basis) are summarized in Table 1. Practically all important malt data values were much higher in barley than in oats. Adding GA$_3$ increased malt extract in barley 1.9% and only 0.3% in oats, but markedly increased alpha-amylase activity and diastatic power of barley and oat malts. There was a large increase in protein solubility in Larker barley malts.

The more comprehensive results of the effects of various levels of GA$_3$ on malting of three oat cultivars grown with or without N fertilizer are given in Table 2. The three cultivars varied widely in behavior during malting and in response to GA$_3$. Malt extract and ratio of wort N:malt N were highest in X1196-1; this was presumably associated with the lowest oat-N level (among the three cultivars). Malting had little effect on Kjeldahl-N content of oats. To simplify presentation of results, Kjeldahl-N con-

tent of oats, only, is given. The high extract of X1196-1 can be attributed, in part, also to the relatively high kernel weight.

The protein content of X1656-1 oats was the highest, and was followed closely by Dal, both of which were much greater than for X1196-1. The Kjeldahl-N content of Dal was little less than that in X1656-1; yet, the alpha-amylase activity of Dal malt was highest among the three cultivars. N fertilization had little effect on oat kernel weight or on grain color as measured by the Agtron instrument (the data on oat color are not included); plumpness increased about 1%. The KNO$_3$ application increased oat N; generally increased fine-coarse grind extract and wort N; and slightly increased alpha-amylase activity in malts of X1196-1 and X1656-1; but usually decreased malt extract a small amount.

Treatment with GA$_3$ increased solubility of proteins (ratio of wort N:malt N), diastatic power and alpha-amylase activity. Unfortunately, good methods are not available for determination of beta-amylase. The increases in diastatic activity were somewhat similar to the increases in alpha-amylase activity. The increase in alpha-amylase activity due to the addition of GA$_3$ was 18.5% for X1196-1, 24.2% for Dal and 45.5% for X1656-1 which also had the highest Kjeldahl-N content. In agreement with the report of Preece (1948) malted oats contained substantial amounts of alpha-amylase approaching that of barley, but diastatic power in oat malt even after GA$_3$ treatments was much below the amounts in malted barley. Consequently, it may be possible to produce from GA$_3$-treated oat malts concentrated preparations of alpha-amylase, essentially free of beta-amylase.

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METAL PROTEIN COMPLEXES IN ETHANOL MEDIA

INTRODUCTION

ONE OF THE prime indicators of beer quality is its colloidal stability (Gorinstein, 1968; Fertman and Gorinstein, 1968, 1970). This stability depends on the amounts of minerals in proteins in an aqueous medium (Gorinstein, 1971; Raible, 1967). The main task facing us was to discover the nature of the bonds between the mineral and protein components of beer and their effect on the colloidal stability of the product.

EXPERIMENTAL

THE INVESTIGATION was carried out on "Zhuguli" nonfiltered beer (a type of light beer of 2.8% mas. alcohol), produced at Lvov by Kolos Breweries by use of the double-decoction method, from 60% light malt and 40% nonmalted adjuncts. Standards of comparison for beer were the brews clarified by cotton filtering masses "Kineshma" (control) and "Evlakh" (test). ["Kineshma" and "Evlakh" are the Russian names of samples of cotton fibers. "Kineshma" mass is of 34 nephelos units and the "Evlakh" of 55 nephelos units; the two are distinguished by their filtering ability. For the manufacture of these filtering masses, raw materials such as cotton and cellulose were utilized.] "Kineshma" (control) was processed according to a well-known procedure while "Evlakh" (test) was treated in a different manner (Gorinstein, 1970). Because of this, "Kineshma" was selected as the control.

It is known that four main groups of proteins in beer are distinguishable according to their solubility in different solvents: (a) proteins soluble in water and in dilute salt solutions, as represented by albumin; (b) proteins soluble in dilute salt solutions but dissoluble in water, as represented by globulin; (c) proteins soluble in 60–75% ethyl alcohol, as represented by prolamine and hordein; and (d) proteins soluble in weak alkali solutions, as represented by glutelin.

The solubility of proteins in these solutions was the basis for selecting and utilizing the procedure of fractionation. In this procedure, the proteins were extracted directly from the beer samples with 5% potassium sulfate, 0.2% sodium hydroxide and 70% ethyl alcohol. The extracts were filtered and the proteins precipitated out by 10% trichloroacetic acid, in filtrates of every fraction. The protein sediments were then washed with 1% trichloroacetic acid. The filtrates remaining after precipitation were also investigated.

Iron, copper, zinc, cobalt and manganese in the sediments and the filtrates were determined spectroscopically. Calcium was investigated titrimetrically according to Frey (1968) and by flame photometry; oxalates were examined both gravimetrically and titrimetrically (Koch

and Strong, 1965). The stability of metal-protein complexes was determined thermochemically using the Paulik-Paulik-Erdey derivatograph (Paulik et al., 1958). To perform a thermochemical analysis, it is not necessary to first fractionate the proteins. Instead of this, the proteins were precipitated by tannin-caffeine and by ammonium sulfate. The tannin-

caffeine method is based on the protein complex-forming property of tannin. These complexes were then dissolved in caffeine (Fertman and Gorinstein, 1968, 1970). The sediments were dried at 30°C.

Four curves were recorded simultaneously on the derivatograph and are presented in Figure 1, a–d: (1a) Curve of differential thermo-
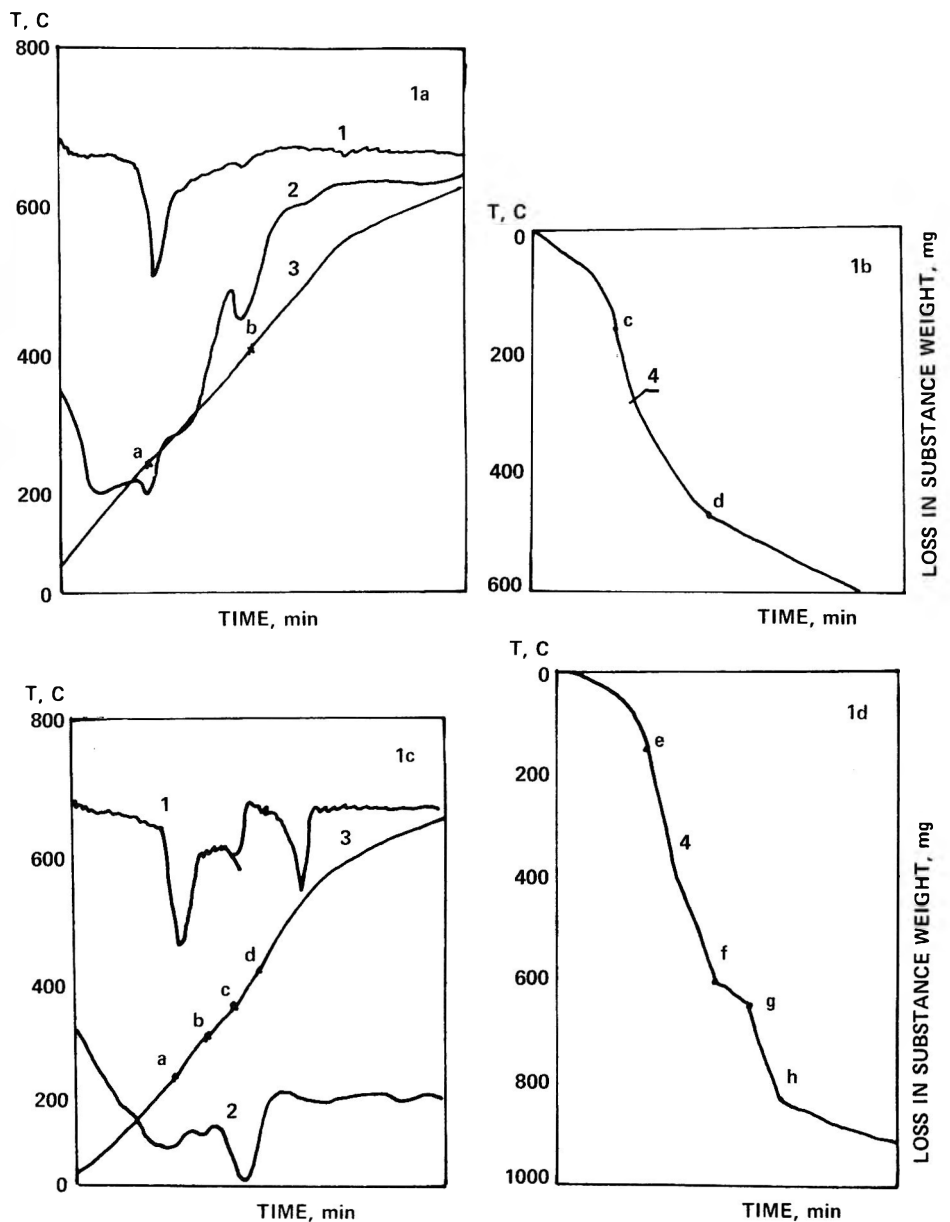
Fig. 1—Derivatograms of metal-protein complexes of samples of control beer (a, b) without added Fe^{3+} and (c, d) with added Fe^{3+}. The curves of heating: (1) DTG; (2) DTA; (3) T; and (4) TG.

gravimetric analysis, DTG; (1b) Curve of differential thermal analysis, ETA; (1c) Curve of temperature, T; and (1d) Curve of integral thermogravimetric analysis, TG. Points a, b, c, d, e, f and h are the sites of the respective endothermic effects of loss in substance weight at varying temperatures. The conditions of the experiment are shown in Table 1.

The colloidal stability of beer was determined using the limit of precipitation of ammonium sulfate (Fertman and Gorinstein, 1968; Gorinstein, 1971, 1973). As the limit of precipitation decreased, the colloidal stability was found to increase.

In the series of experiments, standard solutions of cations of heavy metals were introduced into the samples of control beer. The results of the experiments express the dependence of the elements on colloidal stability.

RESULTS & DISCUSSION

THE BOND STRENGTHS between the trace elements and proteins were determined by the ratio of their quantities in protein fractions to the total amount in beer. The data obtained show that in nonfiltered beer, iron and copper are found in complexes with proteins of all fractions. The various protein fractions can be classified according to their iron content in descending order as follows: alkali soluble (glutelin), alcohol soluble (prolamine-hordeine), salt soluble (globulin) and water soluble (albumin).

Zinc, nickel, manganese and cobalt are found mainly in the filtrates after protein precipitation, suggesting that those metals form unstable bonds with proteins. These data are found in Tables 2 and 3. The complexing abilities of the investigated metals, determined by taking a sum total of metal percentages in the protein fractions (aqueous + salt + alkaline + alcoholic), are also shown in Table 3.

The most common complexing agents are, as seen in the Tables, the transition elements copper and iron (Davies et al., 1969; Ritsma et al., 1969; Bagger, 1969; McKenzie, 1969). The considerable complexing ability of copper results from its being contained in beer mainly in the oxidized form (Cu^{2+}) (Suchov and Mitsuya, 1967; Michailidis and Martin, 1969; Nancollas and Poulton, 1969). The same property of iron is explained by the prevalence of the oxidized form over the reduced one.

The complexing ability of the oxalate-ion results from its being a bidentate ligand, which together with amino groups of beer, forms mixed ligands (Fig. 2).

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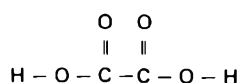
As stated above, the majority of trace elements, especially copper and iron, combine with proteins of all fractions, their amino acids being their ligands (see Tables 2 and 3). Thus, complexes of metals with proteins are formed (Makinen

et al., 1969; Nakao et al., 1967; Hamada et al., 1969). It is clear that in these complexes, a metal is bonded not only with a carboxyl group, but with nitrogen as well, by secondary covalent bonds (Fig. 3) when the metal ion (Me^{2+}) is Co^{3+}, Co^{2+}, Fe^{3+}, Fe^{2+}, Ni^{2+}, Cu^{2+}, Cu^{+}, Mn^{2+} or Zn^{2+}. Copper (III) and Fe (III) cause another carboxyl group to be picked up.

Thermochemical investigations were carried out to determine the position of the metals in the metal-protein complexes and their bond strengths with the ligand.
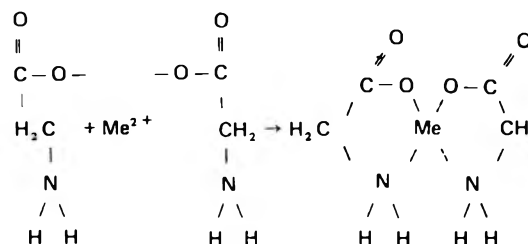
Fig. 3–

Table 1—Experiment conditions

Weight of substance	100 mg
Thermopair	Pt-Pt/RH
Resistance of electric circuit	DTA, 1/10; DTG, 1/10 megohms
Rate of heating	10 deg/min
Range of error of temperature	±5°C

Table 2—Mineral components in filtrate fractions of proteins in nonfiltered beer

Trace elements			Filtrate fractions			
			Aqueous	Salt	Alkaline	Alcoholic
Iron	Total amt ($\times 10^{-3}$ mg/ℓ)	16.0	1.04	4.37	1.16	1.18
	% of total	–	0.66	2.63	0.74	0.75
Copper	Total amt ($\times 10^{-3}$ mg/ℓ)	39.21	0.77	2.04	1.15	–
	% of total	–	1.97	5.19	2.97	–
Zinc	Total amt ($\times 10^{-3}$ mg/ℓ)	24.83	11.34	–	12.74	–
	% of total	–	45.62	–	51.38	–
Nickel	Total amt ($\times 10^{-3}$ mg/ℓ)	15.94	12.88	–	1.91	–
	% of total	–	80.66	–	12.09	–
Cobalt	Total amt ($\times 10^{-3}$ mg/ℓ)	9.92	7.39	–	2.13	–
	% of total	–	74.36	–	21.49	–
Manganese	Total amt ($\times 10^{-3}$ mg/ℓ)	8.47	1.50	–	6.78	–
	% of total	–	17.71	–	80.05	–
Calcium	Total amt (mg/ℓ)	26.00	13.83	–	11.58	–
	% of total	–	53.18	–	44.56	–
Oxalate-ion	Total amt (mg/ℓ)	24.91	–	19.78	–	3.88
	% of total	–	–	79.45	–	15.55

Metal-protein complexes of nonfiltered, test and control beers were studied. The same samples were treated with the ferric ion in the amount of 3.5×10^{-3} mg/ℓ, causing some protein precipitation. In samples both with and without Fe^{3+}, proteins were precipitated by tannin-caffeine and ammonium sulfate. Thermogram data were obtained under the conditions described in Tables 4 and 5 (see also Fig. 1).

Investigations of metal-protein complexes by the thermogravimetric method have shown that in the temperature interval of $180-240°C$, one or two endothermic effects of dehydration take place on the DTG and TG curves (see Fig. 1). The nature of the complex as well as that of the external sphere of the heavy metals (Fe, Cu, etc.) depends on the temperature of dehydration. The first effect exists in each of the derivatograms presented.

The loss of mass of the complex in the TG curve is attributed to the removal of water. In the beginning, loss of mass ranging from $9.7-17.9\%$ occurs in the different samples, corresponding to the removal of molecules of water. Following this, there occurs a sharp decrease in mass $(27.6-45.1\%)$ and then a smaller one indicating insufficient decomposition of the complex. The loss of mass in complexes of mixed composition (with added Fe^{3+}) begins only under conditions of higher than usual temperature. Complexes are more stable when a smaller loss of mass occurs. The decomposition of anhydrous complexes is accompanied by an endoeffect in the temperature interval $300-700°C$. The nature of this effect is somewhat complicated. It may be explained by the decomposition of anhydrous complexes, the oxidation of volatile products produced as a result of the composition of the complex, or by the property of the precipitate of protein substances. Where the addition of Fe^{3+} has been made, the complex decomposes following dehydration due to oxidation of the organic ligand. By examining the TG curve (see Fig. 1d) it can be seen that heating is accompanied by a gradual loss of mass in the sample. In some cases, no change in mass occurs at all, which can be explained by internal rearrangement and reconstruction of the complex. Data on the derivatograms of nonfiltered, control and test beers reveal a sharp distinction between their qualitative characteristics and their endoeffects (Tables 4, 5).

The introduction of Fe^{3+} ions into the nonfiltered, control and test beer samples alters the character of the derivatograms. A comparison of positions 1b and 1d in the figure reveals the difference in behavior of these systems. The second endoeffect takes place at a higher temperature than the first. With the introduction of Fe^{3+}, the number of endoeffects increases to 3 or 4.

According to Gorinstein (1973), beer stability depends mainly on the content of the cation forms of iron, copper and other microelements capable of forming compounds with different protein fractions. Increasing the concentration of these microelements negatively affects beer stability (Gorinstein, 1973). In a

Table 3—Mineral components in protein fractions of nonfiltered beer

Trace elements			Protein fractions				Complexing ability (%)
			Aqueous	Salt	Alkaline	Alcoholic	
Iron	Total amt ($\times 10^{-3}$ mg/ℓ)	16.0	4.16	15.46	105.49	28.14	—
	% of total	—	2.53	9.62	65.68	17.49	95.22
Copper	Total amt ($\times 10^{-3}$ mg/ℓ)	39.21	7.08	1.05	27.16	—	—
	% of total	—	18.04	2.54	69.29	—	89.87
Zinc	Total amt ($\times 10^{-3}$ mg/ℓ)	24.83	0.75	—	—	—	—
	% of total	—	3.00	—	—	—	3.0
Nickel	Total amt ($\times 10^{-3}$ mg/ℓ)	15.94	1.15	—	—	—	—
	% of total	—	7.25	—	—	—	7.25
Cobalt	Total amt ($\times 10^{-3}$ mg/ℓ)	9.92	0.40	—	—	—	—
	% of total	—	4.15	—	—	—	4.15
Manganese	Total amt ($\times 10^{-3}$ mg/ℓ)	8.47	0.19	—	—	—	—
	% of total	—	2.24	—	—	—	2.24
Calcium	Total amt (mg/ℓ)	26.00	0.59	—	—	—	—
	% of total	—	2.26	—	—	—	2.26
Oxalate-ion	Total amt (mg/ℓ)	24.91	—	0.75	—	0.50	—
	% of total	—	—	3.00	—	2.00	5.0

Table 4—Result of the differential thermal analysis

Indices	Samples of beer					
	Non-filtered	Test	Control	Non-filtered with Fe^{3+}	Control with Fe^{3+}	Test with Fe^{3+}
Endoeffects (°C)	180	230	240	210	230	240
	320	440	410	340	290	290
				380	350	360
					410	410

Table 5—Loss in substance weight of metal-protein complexes by thermogravimetric analysis

Indices	Samples of beer					
	Non-filtered	Test	Control	Non-filtered with Fe^{3+}	Control with Fe^{3+}	Test with Fe^{3+}
Loss in substance weight (%)	13.3	9.7	17.9	11.9	12.3	12.9
	45.1	43.3	38.3	27.6	36.0	36.0
				24.9	4.0	4.9
					14.7	14.7

series of experiments, standard solutions of cations of bivalent copper, iron, zinc, nickel, cobalt, manganese, trivalent iron, calcium and oxalate-ion, with $C = 10^{-3}$ mg/100 ml, were introduced into samples of control beer. The dependence of the colloidal stability of this beer on some of the heavy metals contained in it was investigated. Upon introduction of copper ions in the amount of $(0.5-1.0) \times 10^{-3}$ beer. It reflects the dehydration process mg/100 ml, the deposition limit of beer increased. This is because copper cations in small amounts are stabilizers. A further increase of copper concentration from $(1.0-7.0) \times 10^{-3}$ mg/100 ml sharply decreased the deposition limit, because of the breakdown in stability of the colloidal systems. The addition of bivalent iron, cobalt, nickel, zinc and manganese did not greatly affect the colloidal stability of beer, while trivalent iron in concentrations of $(3.5-7.0) \times 10^{-3}$ mg/100 ml sharply decreased it. This confirms the supposition that free Fe^{3+} ions are less active than those of Fe^{2+}. When calcium and the oxalate-ion were added in concentrations of 1.5×10^{-3} and 10.0×10^{-3} mg/100 ml respectively, the colloidal stability decreased.

CONCLUSION

THE PRESENT investigation of clarified samples of beer has shown that the minor inorganic components of beer (iron, copper, zinc, nickel, cobalt and manganese) are bonded with the same protein fractions as are its major inorganic components (calcium and oxalate-ion), even after filtration. It was also found that in-creasing the concentrations of oxidized iron, copper, oxalate-ion and calcium, decreases the colloidal stability of beer.

The first endoeffect noted in the derivatograms from the test beer occurs at higher temperatures than with nonfiltered taking place in the isolated metal-protein complexes. The second endoeffect takes place at an even higher temperature. With the introduction of Fe^{3+}, the number of endoeffects increases to 3 or 4.

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Ms received 12/9/73; revised 3/9/74; accepted 3/13/74.

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INFLUENCE OF WINE INTAKE ON MOUSE GROWTH, REPRODUCTION AND CHANGES IN TRIGLYCERIDE AND CHOLESTEROL METABOLISM OF OFFSPRING

INTRODUCTION

WINE has been extolled as a therapeutic agent that has probably been in use for a longer period than any other medicine or food (Lucia, 1954; 1963). In the past few years, however, wine made from certain cultivars of hybrid grapes, e.g., *Vitis vinifera* x *Vitis riparia* or *Vitis labrusca*, has been reported to contain natural toxicants (Breider et al., 1965; Breider and Wolf, 1967). Although these alleged toxicants have not been chemically identified, Breider (1973) has proposed that they are responsible for the grapes natural resistance to plant pathogens and insects. We have shown that hybrid grapes and wines are not toxic (Stoewsand et al., 1969; Stoewsand and Robinson, 1970); indeed, it appears that the diet used in the experimental toxicity studies of Breider and Wolf (1967) was nutritionally inadequate (Stoewsand and Robinson, 1972). Schürch et al. (1968; 1972) also observed no physiological abnormalities in experimental animals fed hybrid grape juices for four generations.

Wine is generally indicated as a mild tranquilizing agent or sedative, primarily because of its largest single constituent, ethyl alcohol. However, due to the multitude of other wine components, i.e., aldehydes, organic acids, polyphenols, etc., in addition to ehtyl alcohol, the use of wine has been attributed to a variety of beneficial physiologic responses (Wine Advisory Board, 1965).

The use of wine and brandy in control of cardiovascular diseases has been advocated as early as the 13th century (Amatuzio and Hay, 1958). Although ethyl alcohol intake in animals and man was reported to increase serum cholesterol (Grande et al., 1960), Masquelier (1961) reported that polyphenols in wine reduce serum cholesterol. Morgan et al. (1957) showed liver cholesterol in hamsters increases with either ethyl alcohol or wine ingestion, but the increase is much less with wine.

The objectives of this wine feeding study were to observe two generations of laboratory mice for 26 wk regarding (a) growth or body weight changes and reproduction; and (b) blood and liver cholesterol and triglyceride levels in the offspring or second generation. These parameters were compared with controls fed either 12% ethyl alcohol in aqueous solution or distilled water. The mouse was used as our animal model, since it grows and breeds easily and rapidly in relatively small quarters.

EXPERIMENTAL

Laboratory animals and management

Weanling mice, BDF_1 hybrid strain purchased from ARS/Sprague-Dawley, Madison, Wisc., were placed in suspended cages, one male and one female per cage, within a 24°C temperature-controlled room. Lights were on 12 hr per day. All liquids i.e., wine, 12% ehtanol (EtOH), or distilled water, were fed from graduated glass feeding tubes together with a complete pelleted diet (Charles River Mouse Formula, Agway, Inc., Syracuse, N.Y.) ad libitum. Ten pairs were gradually introduced to wine, starting after weaning (wine-young treatment), or after sexual maturity (wine-mature treatment) at 60 to 70 days of age. Control groups of 10 pairs per group were fed EtOH starting at either age, as in the wine treatments, or water. After 10 wk of gradually increased intake, the experimental mice were receiving exclusively wine or EtOH as their sole dietary liquid. Births were allowed to go to term. Ten pairs of offspring (F_1), produced from the wine-young or water-treated parents (F_0) during the 26th wk of treatment, were weaned and fed either wine, EtOH, or water for another 26-wk period. After gradually increasing the wine and EtOH concentrations, the F_1 mice attained 100% wine or aqueous alcohol intake at 6 wk post-weaning.

Wine

The red wine used in the experiment was made from Seibel 10878 (Chelois) grapes after crushing and stemming. 100 ppm of SO_2 was added to suppress wild yeast growth. The crushed grapes were inoculated with Montrachet strain 522 yeast and allowed to ferment for 3 days. The mixture was then pressed, ameliorated with a sugar solution in order to standardize the final alcohol content at 12%, and allowed to ferment at 10°C until the fermentable sugars were less than 0.25%.

Analytical

The F_1 mice were fasted overnight at the end of the 26th wk. The following morning the animals were weighed and blood was obtained via decaptiation after brief, light, ether anesthesia. Livers were immediately excised, weighed and frozen. Cholesterol and triglycerides were obtained on the same blood plasma sample or on a 0.3-g sample of macerated liver. This meth-
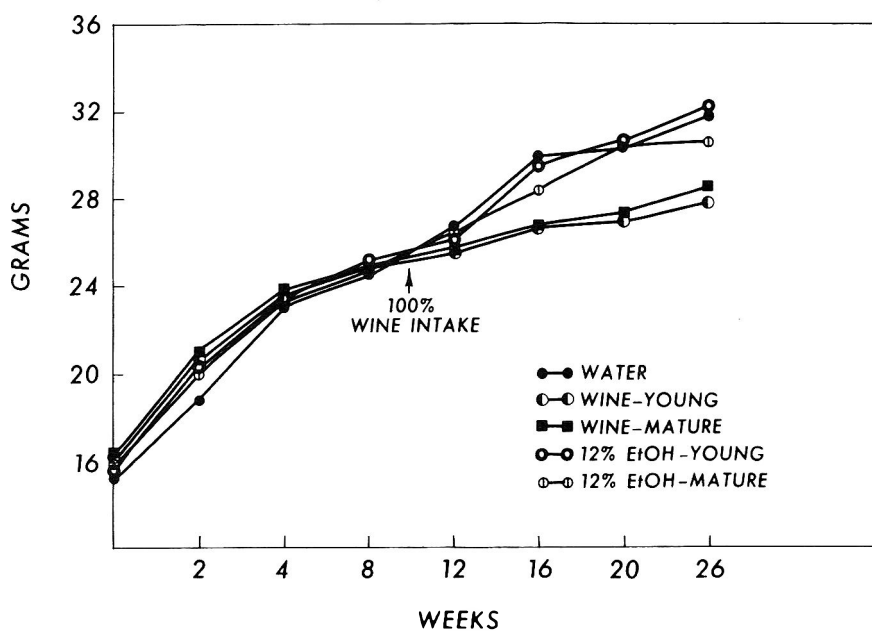
Fig. 1—Mean body weights of male mice (F_o) fed wine, starting at a young (weanling) or mature (60–70 days old) age, as compared to EtOH or water-treated controls.

Table 1—Reproductive index[a] of F_0 mice[b] fed wine starting at a young or mature age[c]

Months	Water %	Wine-young %	Wine-mature %	Ethanol-young %	Ethanol-mature %
1	120	54	100	124	128
2	150	130	125	154	110
3	123	128	118	100	140
4	144	138	118	166	140
5	122	118	145	110	130

[a] No. litters born/No. pairs X 100
[b] F_0 = Parent or first generation of mice
[c] Young indicates weanlings (20–23 days old); mature indicates sexually mature (60–70 days old).

od, developed by the Oxford Laboratories, San Mateo, Calif., and supplied as "Tri-Chol" reagent set, is a clinical technique for blood analysis. The sample is extracted with isopropyl alcohol and powdered activated alumina. From one aliquot of this lipid extract the triglycerides are saponified; the glycerol is oxidized by sodium metaperiodate to formaldehyde; combination with acetylacetone produces a color, directly related to the amount of total triglycerides present, measured at 405 nm. Total cholesterol is determined on another aliquot of the lipid extract by a reagent containing ferric ion in sulfuric and acetic acid (Zak, 1957). This resulting color is measured at 560 nm.

Statistical analyses of the data were performed according to Steel and Torrie (1960) by analysis of variance and comparisons of mean values.

RESULTS & DISCUSSION

Parents (F_0)

Mice fed wine as their exclusive dietary liquid, after gradual introduction to 100% wine intake at 10 wk, had significantly (P < 0.05) lowered body weights as reflected in the male mice body weight curves of Figure 1. Weight changes of females did not reflect growth because of pregnancies. Although wastage of food made accurate intake measurement difficult, both the estimated food and liquid intake of the wine drinkers were less than the mice drinking either EtOH or water. Aschkenasy-Lelu (1957) reported male rats receiving wine showed a decreased weight gain in 2–6 months when compared to control animals. Evidence (Tamir and Alumot, 1970; Glick and Joslyn, 1970) indicates that dietary plant tannins produce lowered energy conversion of food together with binding of protein, thus depressing growth. Phenolic compounds present in the red wine, i.e., tannins, anthocyanidins, etc., may be partially responsible for the observed growth depression. Liver weights of all male mice from each treatment, as a % of body weight, were similar.

The average number of live pups produced per litter (Fig. 2) generally increased with age of parents in all treated groups. The mice drinking water had an average of 8.14 live pups per litter through the entire 5-month period. The wine-young treated group had the lowest number–6.15 live pups per litter for this period. Comparison of the values of all treatments indicated only the wine-young treated group had a significantly (P < 0.05) lowered number of offspring when considered over the entire 5 months. However, the numbers of live pups per litter at the end of 5 months are about equal in all treated groups, and approach the litter size of the water-consuming controls.

The "reproductive index," defined as number of litters born/number of mouse pairs × 100 is presented in Table 1. Since mice have a gestation period of 17–21 days, one or more litters per pair, when calculated on a monthly basis, can be expected. However, during the initial month only 54% of the young mice drinking wine had litters, while the mice on the other treatments showed the expected 100% or higher index. Subsequent months showed this normal increased reproductive index in the wine-young treatment.

It is apparent from the weight gain and reproduction data of the parents, that the wine-drinking mice, particularly mice starting to drink wine at the young age, gained weight slower and generally lagged in maturation rate. Incidence of fetal death was low, but occasionally was encountered within all treated groups. No malformed offspring were noted; however, by allowing the dams to go to term, possible fetal resorption or consumption
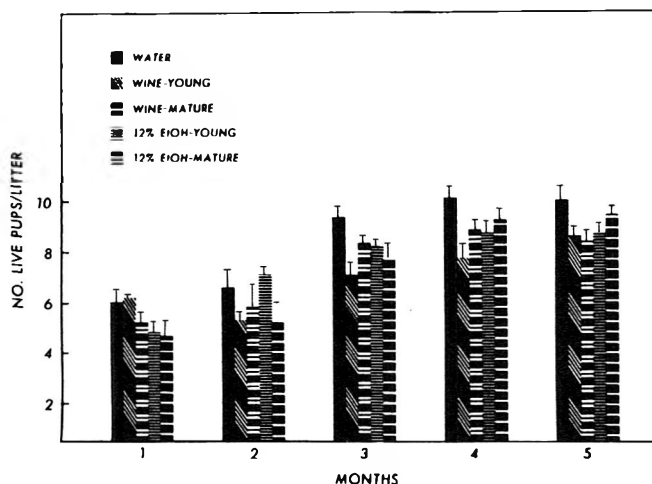
Fig. 2—Mean number of live pups born per litter per month from mice fed wine, EtOH or water. Wine or EtOH was started either at a young (weanling) or sexually mature (60–70 days) age.
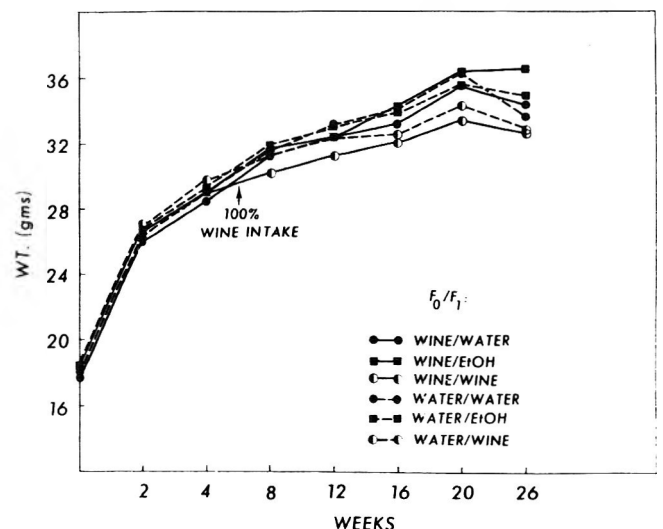
Fig. 3—Mean body weights of male mice (F_1) fed wine, EtOH or water. The parents (F_0) were from either the wine-young treatment or water-fed control group.

Table 2—Mean weight of newborn pups from F_1 mice and analysis of variance summary

	Treatments: F_0/F_1					
Months	Water/water (g)	Water/EtOH (g)	Water/wine (g)	Wine/water (g)	Wine/EtOH (g)	Wine/wine (g)
1	1.33 ± 0.03	1.51 ± 0.06	1.25 ± 0.03	1.34 ± 0.03	1.29 ± 0.03	1.29 ± 0.02
2	1.43 ± 0.03	1.38 ± 0.03	1.38 ± 0.05	1.40 ± 0.05	1.44 ± 0.06	1.41 ± 0.04
3	1.49 ± 0.05	1.38 ± 0.03	1.34 ± 0.04	1.44 ± 0.04	1.49 ± 0.04	1.41 ± 0.03
4	1.44 ± 0.02	1.38 ± 0.03	1.41 ± 0.05	1.44 ± 0.03	1.48 ± 0.04	1.48 ± 0.07
5	1.46 ± 0.03	1.46 ± 0.03	1.39 ± 0.03	1.44 ± 0.04	1.44 ± 0.04	1.42 ± 0.03

Summary of analysis of variance

Source	df	f Value
F_1 treatments	2	4.21*
F_0 treatments	1	0.53 ns
Months	4	6.58**
$F_1 \times F_0$	2	1.84 ns
$F_1 \times$ Months	8	1.32 ns
$F_0 \times$ Months	4	1.95 ns
$F_1 \times F_0 \times$ Months	8	2.00*

** Significant ($P < 0.01$)
* Significant ($P < 0.05$)
ns Nonsignificant ($P > 0.05$)

of the pup could conceal any possible teratogenicity (Fitzhugh, 1968).

Offspring (F_1)

Male and female pups, weaned from dams on either the "wine-young" treatment or water controls, were gradually introduced to wine or EtOH until 100% intake of these liquids was attained at the 6th wk. As observed in the mean body weight changes (Fig. 3), the male offspring gained weight faster and matured to a larger size than their sires (Fig. 1). The F_1 wine groups, i.e., animals born from water- or wine-fed parents, however, again exhibited a slower body weight in-

crease as compared to water or EtOH treated groups. The slight depression of body weights of all animals at the 26th wk probably reflects the overnight fast necessary for blood analysis.

The mean weight of the newborn pups (F_2 generation) were tabulated including a summary of the analysis of variance (Table 2). It is evident that the pups from all treatments became heavier as the dam matured. The F_1 wine treated dams, particularly during the first month of reproduction had smaller offspring. This again reflects a slower maturation rate in wine-fed mice; again these animals had an apparent lowered food intake. As in the

F_1 generation, the F_2 pups showed no abnormal development. There was not a depressed "birth index" as seen in the first month of reproduction of the F_0 generation (Table 1). The number of pups produced per litter from the F_1 generation increased with time. However, unlike the F_0 generation (Fig. 2), the differences seen between treatments were not significant ($P > 0.05$). These last two parameters reflect that, although the wine-fed F_1 mice were maturing slower as compared to the other F_1 treated groups, they matured at a faster rate as compared to their wine-fed parents (F_0).

The levels of cholesterol and triglyceride in the blood plasma and livers of the male and female F_1 mice are graphically presented in Figures 4, 5, 6 and 7, respectively. Analysis of variance of the blood cholesterol data shows no significant differences ($P > 0.05$) due to treatments or sex. Evidence with dogs and to a lesser extent with man (Grande et al., 1960) indicate elevated levels of blood cholesterol with ethanol ingestion. In the case of blood triglyceride (Fig. 5), there is a highly significant difference ($P < 0.01$) due to F_1 treatments and sex and a significant difference ($P < 0.05$) due to the F_0 treatments. Although there are variations within each mean blood-triglyceride value, as seen by the wide spread of the standard error of the mean, the EtOH-fed female mice born from wine-fed parents had almost a threefold increase of blood triglyceride compared to the controls. This increase is significantly higher ($P < 0.05$) than all other treated animals. Wine-fed female mice had elevated blood triglyceride values, but this increase was less than in female mice fed the EtOH solution. Blood triglyceride has been
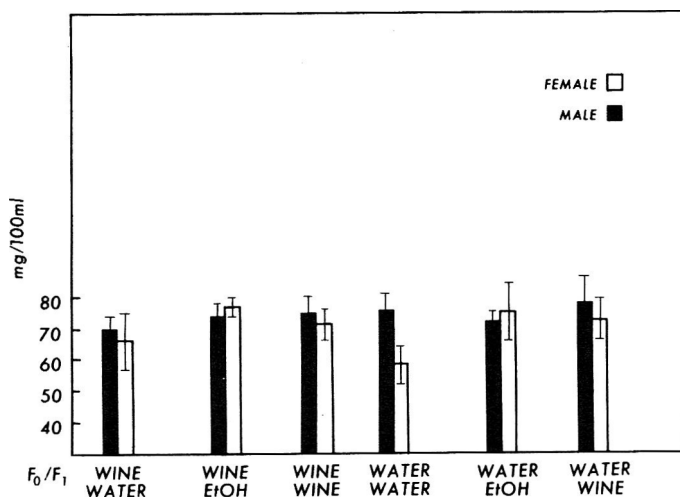
Fig. 4—Mean ± standard error of blood plasma cholesterol of male and female offspring (F_1) fed wine, EtOH or water born from wine- or water-fed parents (F_0).
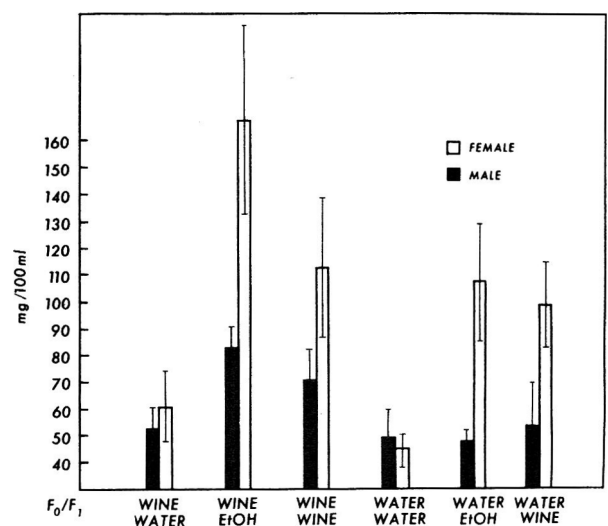
Fig. 5—Mean ± standard error of blood plasma triglyceride of male and female offspring (F_1) fed wine, EtOH or water born from wine- or water-fed parents (F_0).

reported by Barboriak and Meade (1971) to increase with ethanol ingestion in rats. Our data, in addition to confirming this observation, show that relatively long term, exclusive intake of wine by parental mice can enhance the elevated blood triglyceride values of offspring, especially females.

Liver cholesterol of the offspring from wine-fed parents were significantly (P < 0.05) higher than liver cholesterol of the offspring from water drinking parents (Fig. 6). However, it is interesting to observe that the wine consuming male F_1 mice born from water-fed parents had significantly (P < 0.05) lower values of both

liver triglyceride (Fig. 7) and cholesterol than did wine drinking male F_1 mice born from wine treated parents. This difference did not occur with the male F_1 mice fed EtOH. Studies by Bode et al. (1971) indicate that ethanol ingestion increases liver triglyceride through enhanced lipid lipolysis in adipose tissue. Our data indicate that wine intake, especially in male mice born from water-fed parents, depresses the levels of liver triglyceride and cholesterol, as compared to animals fed a solution of the equivalent amount of ethanol.

Recently, Jones et al. (1973) have associated aberrant morphogenesis in the offspring of chronic alcoholic human mothers. Although we did not encounter this phenomenon in our animal study, evidence seems to indicate that prolonged intake of alcoholic beverages can affect offspring by causing a variety of metabolic and perhaps anatomic changes.

This study on wine intake in an animal model system indicates a rather complex interaction of the influence of parental treatment and sex on cholesterol and triglyceride metabolism in a normal, but slower maturing animal. Much further study is indeed warranted, but it is obvious that the variety of physiologic effects of wine are related to both the ethanol and the nonethanol wine components.
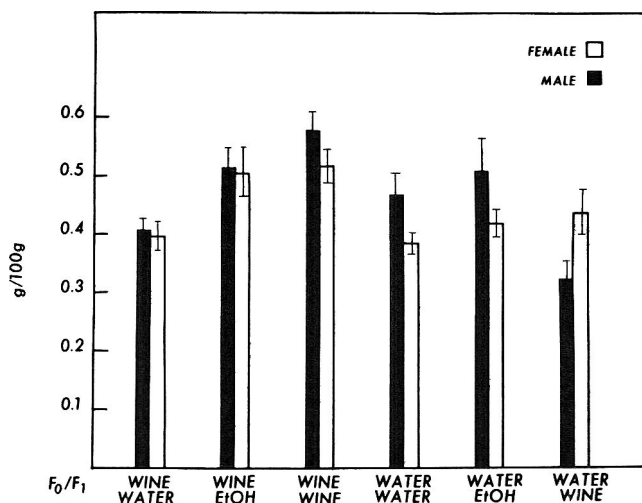
Fig. 6—Mean ± standard error of liver cholesterol of male and female offspring (F_1) fed wine, EtOH or water born from wine- or water-fed parents (F_0).
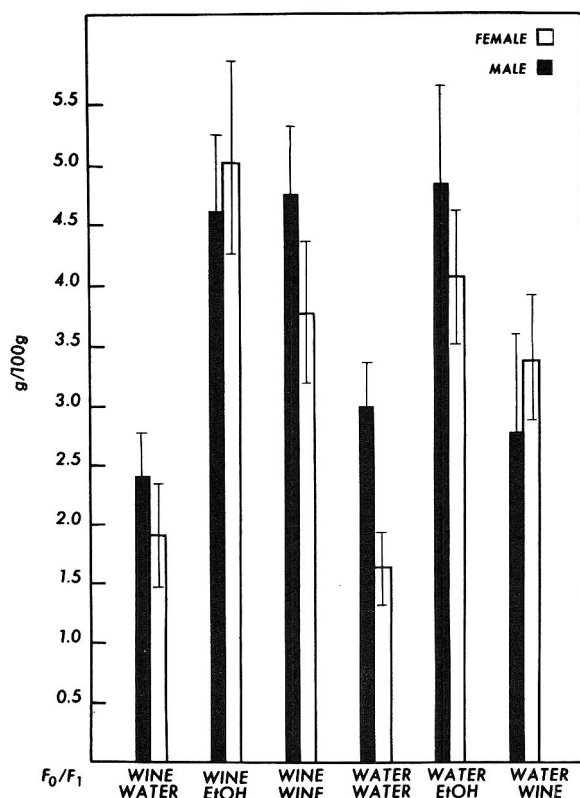
Fig. 7—Mean ± standard error of liver triglyceride of male and female offspring (F_1) fed wine, EtOH or water born from wine- or water-fed parents (F_0).

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FACTORS INFLUENCING AIR-BORNE
CONTAMINATION OF FOODS. A Review

INTRODUCTION

THE AIR is just one of many environments which may contact a food product during the many stages of handling, storage, processing and packaging. The influence of any environment on the product quality is dependent on quality of the environment and length of time that the product is exposed to the environment. Air-borne contamination of foods depends on microbial population of the air in contact with the product and length of exposure of the product to air.

The importance of air-borne contamination is twofold. The first concern is public health related due to the possibility of product contamination by air-borne pathogens. The second consideration is economic and deals with the reduction of product shelf life resulting from air-borne contamination. Both factors are of sufficient importance to deserve the continued interest of various researchers.

Air quality can be defined in many ways, depending on the application. In general, air quality must include an evaluation of particle population, humidity, odor, microbial population and temperature. During the handling of foods, microbial particles represent the quality attribute of primary concern in air, although contamination by other air-borne particles will occur in the same manner.

The mechanisms involved in air-borne contamination of a food product are illustrated schematically in Figure 1. The microbial particles would be generated at a source, either within the same room or space as the product or in an adjoining room or space. The generated contaminant is dispersed throughout the room or space by air flow patterns or mixing. Microbial particles generated in the room
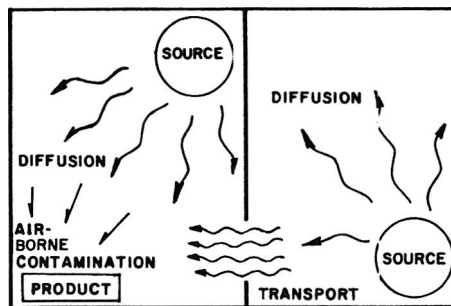
Fig. 1—Mechanisms leading to air-borne contamination of a food product.

next to the exposed product will be transported through an opening or openings between the two rooms. Actual contamination of the product occurs when microbial particles are brought sufficiently close to the exposed surface to result in deposition. Opportunities for preventing contamination are numerous, but the complexity of each mechanism dictates need for careful analysis in order to establish the most efficient control technique.

The objectives of this manuscript include: (a) to review published literature dealing with populations and sources of air-borne microorganisms in food process-
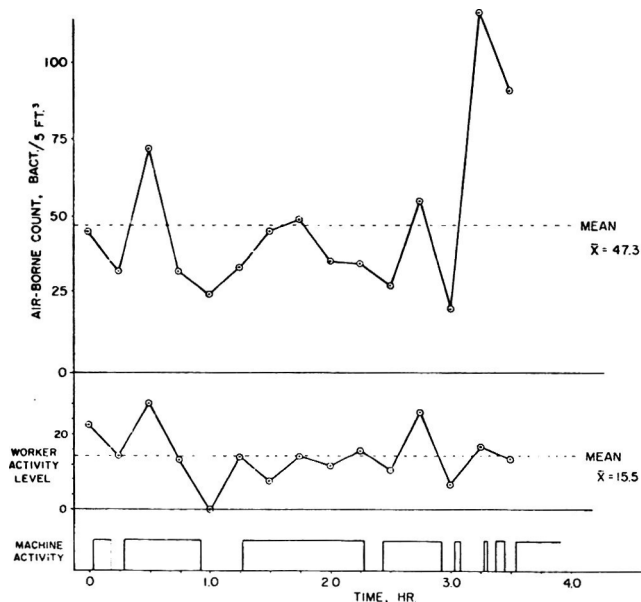
Fig. 2—Variation in air-borne bacteria count in milk packaging area. (From Heldman et al., 1964)

Table 1—Microorganism shedding rates from arms and hands of human subjects on different days[a]

Subject	Day #1		Day #2		Day #3		Day #4		Day #5	
	UT	T	UT	T	UT	T	UT	T	UT	T
A	5–60	5–93	0–11	1–19	1–25	2–40	0–5	2–9	2–30	1–15
B	5–95	6–57	0–7	0–10	2–11	2–24	1–14	0–11	3–16	1–22
C	2–27	3–11	1–14	2–29	3–13	1–7	7–37	1–26	–	–
D	1–13	1–10	0–9	2–8	1–9	0–25	0–4	0–4	0–7	1–25

[a] UT = untreated arm; T = treated arm. (From Sunga, 1968)

ing plants; (b) to discuss factors influencing diffusion and transport of microbial particles within enclosed spaces; and (c) to evaluate the effectiveness of techniques used to prevent air-borne contamination of foods.

POPULATIONS AND SOURCES OF AIR-BORNE MICROORGANISMS

THE MEASUREMENT of microbial populations in food processing plants dates back to 1934, when Olson and Hammer (1934) reported the numbers of bacteria, yeast and mold which settled on exposed agar surfaces in open petri dishes in dairy plants. Since 1958, there have been numerous reports (Cannon, 1966; Cerna, 1961; Cjetanovic, 1958; Hedrick and Heldman, 1969; Heldman et al., 1964; Kotula and Kinner, 1964; Labots, 1961; Perry et al., 1958; Sunga et al., 1966) providing quantitative information on populations of bacteria, yeast and mold in various types of processing plants. Typical results obtained by Heldman et al. (1964) are presented in Figure 2. These results illustrate the significant variations in population which occur in short time intervals. In addition to variations with time, published results indicate (a) considerable similarity in populations in different types of processing plants; (b) large differences in population between different areas of a given plant; (c) relationships between quality of atmosphere around plant and microbial populations within plant; and (d) a definite influence of worker activity in the plant on populations of air-borne microorganisms.

An obvious conclusion based on attempts to quantitatively evaluate populations in any enclosed space is the definite relationship of the population to the many sources of air-borne microorganisms which are present in the enclosed space. Several of the sources which exist in most food processing facilities have been identified and approximate rates of microbial particle generation have been established.

A source which is present in almost every room of a food processing plant is the ventilation system. Figure 3 provides an indication of the microbial particle contribution from a ventilation system which has been started after being idle for a 15-hr period. The population of microbial particles in air from the system increases rather significantly immediately after the system was started before decreasing to the normal population. The microbial particle population in any enclosed space cannot be lower than the population in the atmosphere from which ventilation air is drawn. Above-normal contributions after the system is idle are attributed to microbial populations associated with dust which collects in the duct during the idle period.

Floor drains are a rather unique and possibly unexpected source of air-borne microorganisms. The results in Figure 4 illustrate the significant increase in population which occurred in the isolated space above the drain during flooding with rinse water after the drain was idle for a 12–15-hr period. The results indicate that microbial populations which grow on product solids collected on the interior surfaces of the drain become air-borne when air is displaced from the drain during flooding. It was demonstrated that the number of particles generated reduced with the number of times the drain was used.

Results reported by Riemensnider
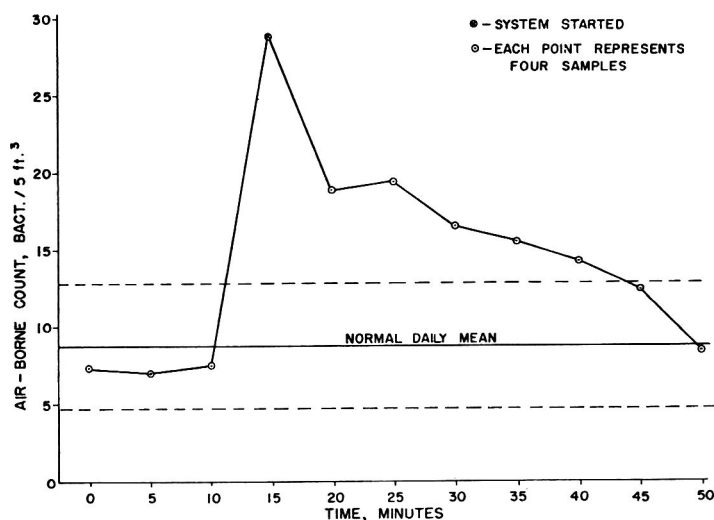
Fig. 3—Contribution of ventilation system to air-borne microorganism populations. (From Heldman et al., 1966)
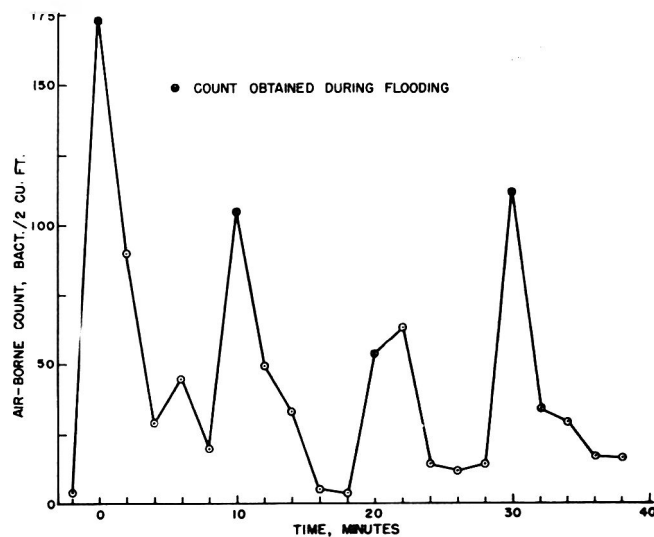
Fig. 4—Effect of flooding at 10-min intervals on air-borne bacteria counts in isolated space above drains in four food plant areas. (From Heldman et al., 1965)
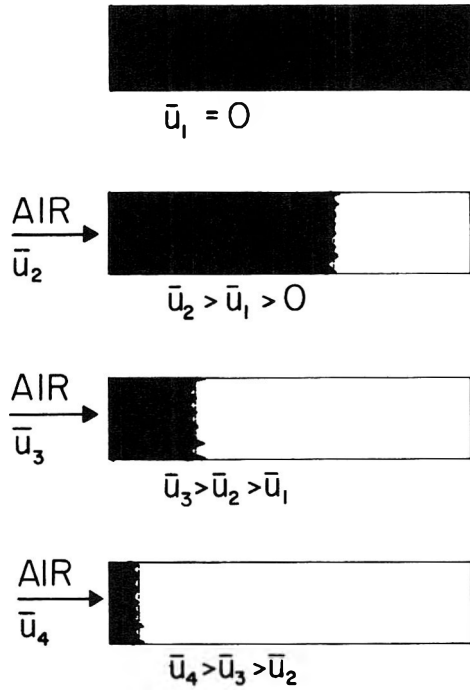
Fig. 5—Influence of air velocity on re-entrainment of small particles from a flat surface.
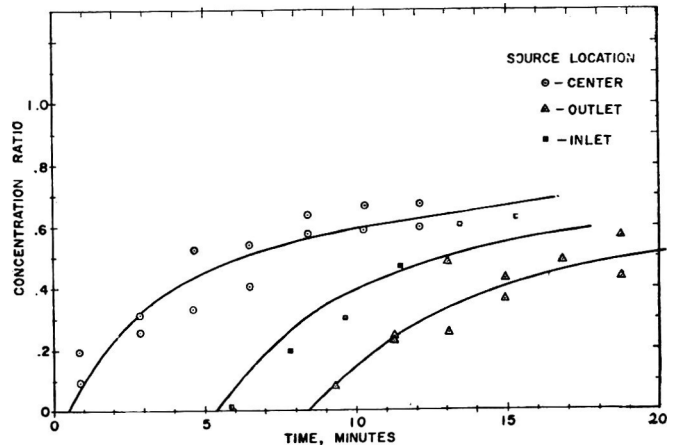
Fig. 7—Aerosol diffusion response at one sampling location and 0.75 air changes per hour. (From Heldman, 1970)

(1966; 1967) indicated that human subjects may generate from 3300–72,000 viable particles per minute. The results in Table 1 were reported by Sunga (1968) and represent the number of viable particles shed from the arm and hand of four different dairy plant workers. In addition to considerable variability for each subject, numbers generated vary with date and with subject. Treatment of the exposed arm and hand by washing and sanitizing did not seem to significantly decrease generation. Identification of the microorganisms shed by human subjects indicated that the flora was closely related to the working environment of the subjects (Sunga, 1968).

The potential for any exposed surface to collect microbial particles by sedimentation seems to be very high. The re-entrainment of these particles into an air stream would make any surface a potential source of air-borne microorganisms. This re-entrainment mechanism which occurs on a flat surface in a manner illustrated in Figure 5 was demonstrated by Heldman and Punjrath (1970). These results reveal that re-entrainment of small particles will occur at the downstream edge of a flat surface at air velocities normally existing in a food processing facility. Additional results (Punjrath and Heldman, 1972a, b) have indicated that the extent of particle re-entrainment is increased by increased air turbulence and increased surface roughness. The key consideration is that the surface must have sufficient length in the direction of air flow for re-entrainment to occur.

DIFFUSION OF MICROBIAL PARTICLES WITHIN A ROOM

THE FIRST STEP occurring in the movement of microbial particles from source to exposed product is diffusion into space around the source. The manner in which this diffusion of particles occurs is influenced by many factors.

The rate of ventilation in a dilution-type system (most typical in food processing plants) has a significant influence on microbial particle diffusion. The influence of ventilation rate is illustrated in
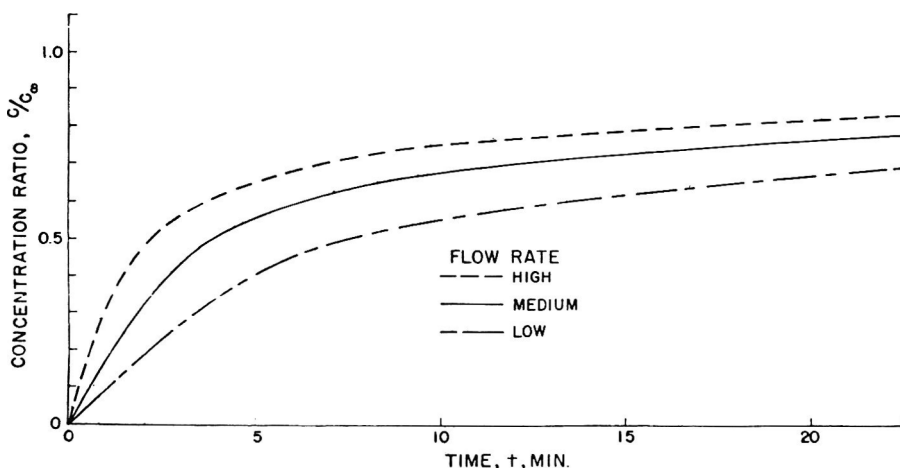
Fig. 6—Influence of ventilation rate on concentration-time history during aerosol concentration increase due to a single contamination source. (From Heldman et al., 1967b)
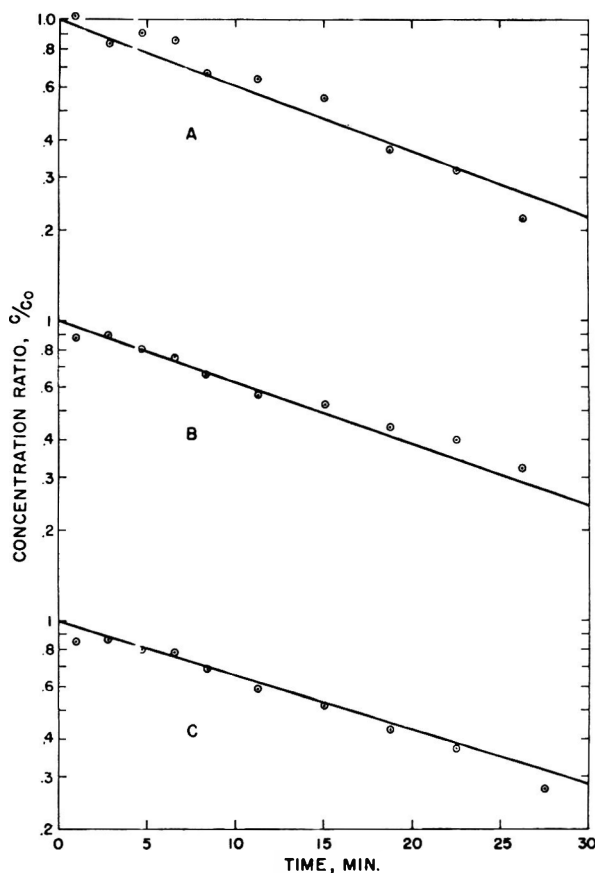
Fig. 8—Bacterial aerosol removal curves at various conditions: (A) middle sampling location—high ventilation rate; (B) back sampling location—medium ventilation rate; (C) back sampling location—low ventilation rate. (From Heldman et al., 1967b)

Figure 6 where the increase in viable particle population at a specific location in a room has been measured as a function of a source at another location in the room. These results indicate that the population increases more rapidly at higher ventilation rates. The observed relationship can be attributed to both bulk transport of particles from source to sampling location and increased dispersion due to more air turbulence at the higher ventilation rate.

Diffusion within an air space is a definite function of microbial particle source location and location of this source with respect to the ventilation system inlet and outlet locations (Figure 7). When the source is near the sampling location (center), the microbial population at the sampling location increases rapidly to some uniform concentration. If the source is located near the inlet, the population increase at the same sampling location is delayed but increases at nearly the same rate. The population increase is delayed even more when the source is located near the ventilation system outlet. These results emphasize that the potential of product contamination is dependent on distance between source and exposed product as well as location of the source with respect to ventilation system inlets or outlets.

One of the unique characteristics of microbial aerosols is the loss of viability which occurs when a microorganism is air-borne. The decay or decrease in microbial population which occurs in a room with no sources of viable particles is illustrated in Figure 8. The rate of population decrease at three different locations could be predicted by accounting for: (a) removal due to ventilation; (b) loss by sedimentation of particles to floor; and (c) decrease in viable population due to bacterial death rate. All factors contributing to aerosol decay must be accounted for in any analysis of viable particle diffusion in a room.

The direct influence of diffusion on viable particle deposition and the associated contamination was measured by Cown and Kethley (1967) and is illustrated in Figure 9. These results indicate that viable particles generated near a ventilation system inlet will be dispersed in a definite pattern as illustrated by the deposition populations. Using these results, Heldman (1971) derived a mathematical model to describe the diffusion and deposition. Using the model, the influence of viable particle generation rate and source height on extent of deposition or contamination can be analyzed in detail as presented in Figure 10.

TRANSPORT OF MICROBIAL PARTICLES FROM ROOM TO ROOM

IN MANY SITUATIONS, air-borne contamination of a food product may occur due to microbial particles which have been transported from an adjoining room. This transport of microbial particles will occur through various openings between the two rooms and is a function of concentration gradient as well as several other factors. A mathematical expression which describes the microbial particle transport would be:

$$N_A = \bar{k}_c \, A \, (C_H - C_L)$$

where N_A = number of particles transported through opening per unit time; A = area of opening; $C_H - C_L$ = microbial particle population gradient; and $\bar{k}_c$ =

Fig. 9—Experiment deposition from a bacterial aerosol. (From Cown and Kethley, 1967)
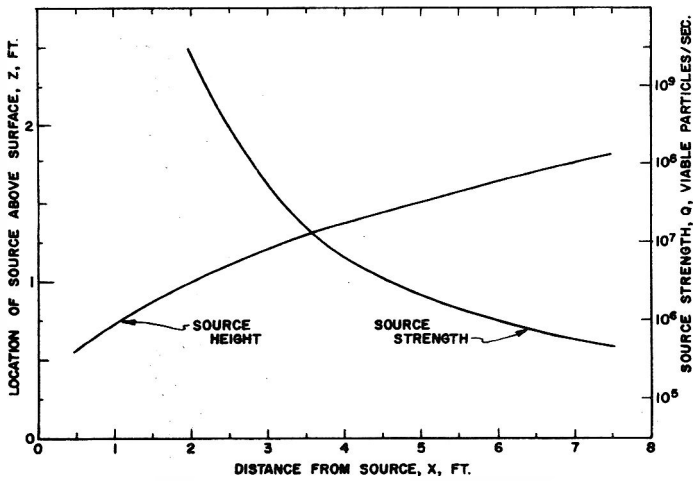
Fig. 10—Influence of source height and source strength on the distance downstream at which deposition begins (From Heldman, 1971)
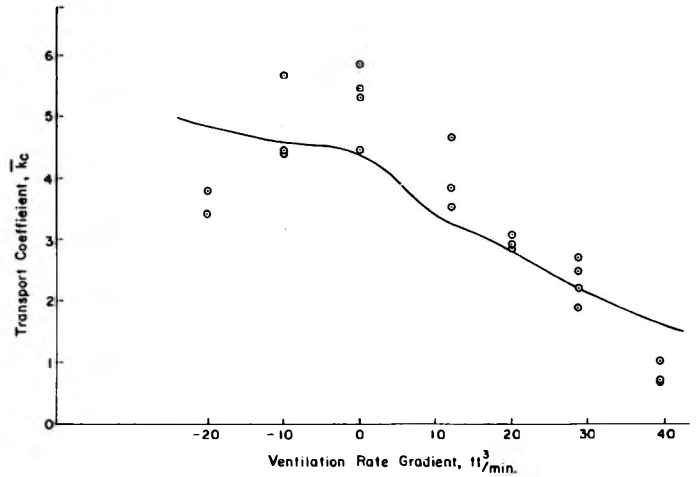
Fig. 11—Influence of a ventilation rate gradient on bacterial aerosol transport. (From Heldman, 1967)

transport coefficient. The transport coefficient ($\bar{k}_c$) was measured experimentally for a variety conditions by Heldman (1965) with no bulk transport of air through the opening (pressure gradient = 0).

One factor which influences microbial particle transport is ventilation rate as illustrated in Figure 11. When rates in both rooms are equal and are increased, the transport coefficient ($\bar{k}_c$) increases rather significantly. If the ventilation rates of the two rooms are not equal, it is desirable to have the lower rate in the room with the high air-borne population. A higher ventilation rate in the high population room can result in higher transport than occurs with equal ventilation rates.

The influence of opening geometry appears to be most dependent on the dimension of the opening in the direction of air flow past the opening. The influence of this dimension on the transport coefficient ($\bar{k}_c$) is illustrated in Figure 12. Although the values of the coefficient decrease with increasing size, the total number of microbial particles being transported would not decrease due to a corresponding increase in opening area (A).

A rather unexpected and interesting influence on microbial particle transport is caused by a temperature gradient as illustrated in Figure 13. The temperature gradient is measured with respect to the microbial population gradient across the opening. The results presented indicate that a temperature gradient in the same direction as the population gradient increased particle transport while a temperature gradient in the opposite direction decreased particle transport. The increase or decrease in transport measured has been explained by the influence of thermal forces on small particles and

the influence of changes in temperature and relative humidity on microbial particle viability (Heldman, 1968).

Experimental evidence indicates that transport of air-borne microbial particles through an opening between rooms is increased by air turbulence at the opening. This influence is illustrated by the increase in transport coefficient created by an increase in turbulent energy at the opening (Fig. 14). These results indicate the significant influence of air mixing on diffusion and transport of a microbial aerosol. The extent of mixing is directly related to the type of ventilation system and the flow characteristics around the opening.

EFFECTIVENESS OF AIR-BORNE CONTAMINATION CONTROL TECHNIQUES

AN ANALYSIS of the various factors which contribute to air-borne contamination of any exposed surface provides considerable insight into potential control techniques. Probably the most positive approach is to remove the source of air-borne microorganisms from the room or space in which the product is exposed. In addition, transport of microbial particles into the space must be eliminated. This approach cannot be justified for many situations due to costs of system components required. As analysis of each source
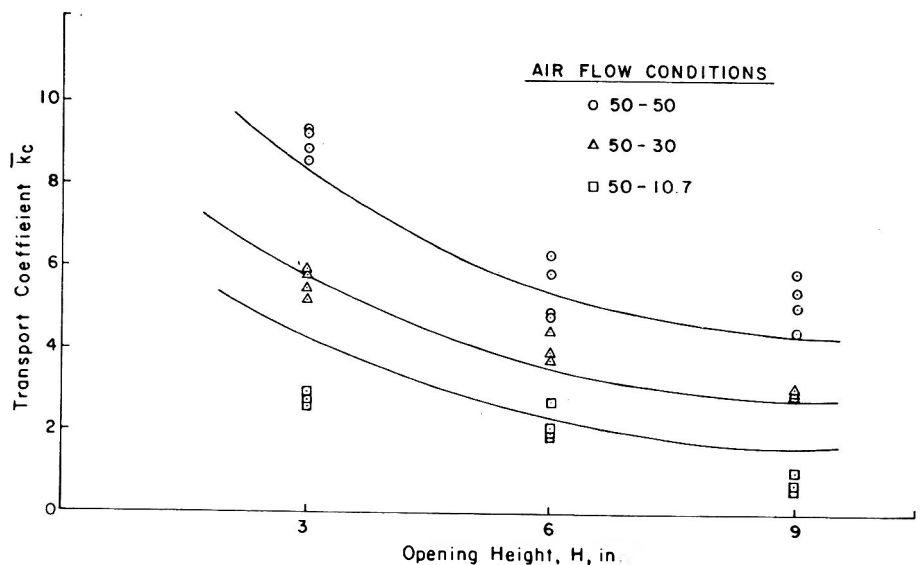
Fig. 12—Influence of opening height on bacterial aerosol transport. (From Heldman 1967)

of air-borne microbial particles as well as diffusion and transport mechanisms indicates that considerable control is possible through individualized control techniques for each situation.

Control or elimination of ventilation systems as a source of microbial particles can be accomplished through the use of air filters. The effectiveness of control is as good as the efficiency of the filter, as long as the filter is placed at the ventilation inlet to the room. Recent results by Burmester and Witter (1972) indicate that available filters are highly effective in removal of virus particles.

The control of air-borne microorganisms generated from a floor drain can be achieved through the use of a sanitizer of appropriate strength as indicated in Figure 15. By applying the sanitizing solution to the interior surfaces of the drain before flooding occurs, a significant reduction in the number of viable particles generated results. The results (Fig. 15) illustrate that the reduction is directly proportional to the strength of the solution applied. These results as well as experiments conducted without sanitizer indicate that drains have a self-cleaning action in that the number of microbial particles generated reduces with the number of times the drain is flooded.

The employees or observers in a room may be the most difficult source of airborne microorganisms to control. Probably the most successful attempts to control microorganism shedding by humans have involved the use of special clothing. These techniques and clothing have not been developed for use in food processing plants and probably would require considerable modification. Certainly a direct approach to reducing the contribution of workers to air-borne microorganism populations in a room would be to reduce the number of employees in the space of concern and the length of time they are in the room.

Techniques for control of surfaces as a source of air-borne microbial particles are not apparent. Based on available evidence, several recommendations can be made. Any contamination control measures which reduce the air-borne microorganism population in the room will reduce the number of viable particles which may deposit on surfaces and reduce the potential for re-entrainment. A treatment of the surface to increase the forces required for particle re-entrainment will contribute to the effectiveness of control. In addition, any modifications in the ventilation system design which will reduce air turbulence will also reduce extent of microbial particle re-entrainment.

Results on diffusion of microbial particles within a room or enclosed space indicate that two approaches to control might be used. The first approach which might satisfy many situations would involve factors which would promote rapid and uniform dispersion of the generated particles throughout the room or space. This will result in the minimum air-borne population for a given rate of microbial particle generation and therefore in the minimum probability of contamination of the exposed product. The second approach would involve a control technique which would prevent the generated particles from being diffused from the source into the surrounding space. Application of this approach requires knowledge of the source and its characteristics as well as unique control techniques which have not been developed.

The techniques for control of microbial particle transport through openings between rooms or spaces are established by experimental results. Ventilation rates, opening geometry, air turbulence and
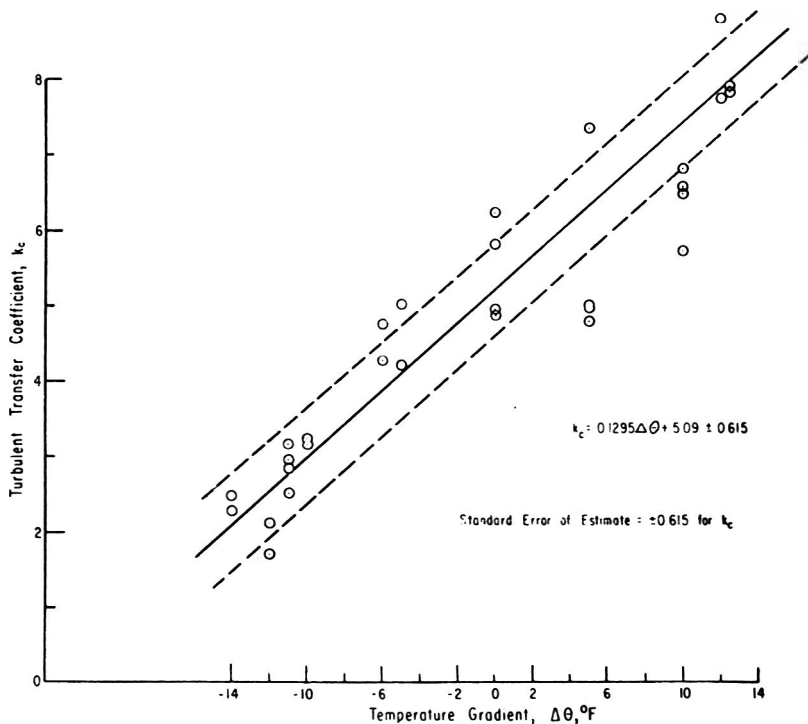
Fig. 13—Influence of temperature gradient on turbulent transfer coefficient. (From Heldman et al., 1967c)

$$k_c = 0.1295 \Delta\theta + 5.09 \pm 0.615$$

Standard Error of Estimate = ±0.615 for k_c
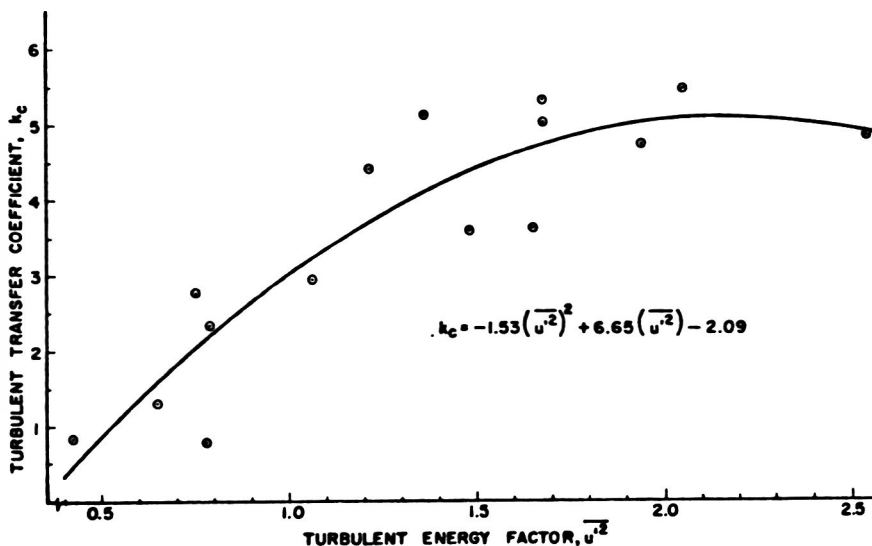
Fig. 14—Influence of turbulent energy factor on transfer coefficient. (From Heldman et al., 1967a)

$$k_c = -1.53\left(\overline{u'^2}\right)^2 + 6.65\left(\overline{u'^2}\right) - 2.09$$
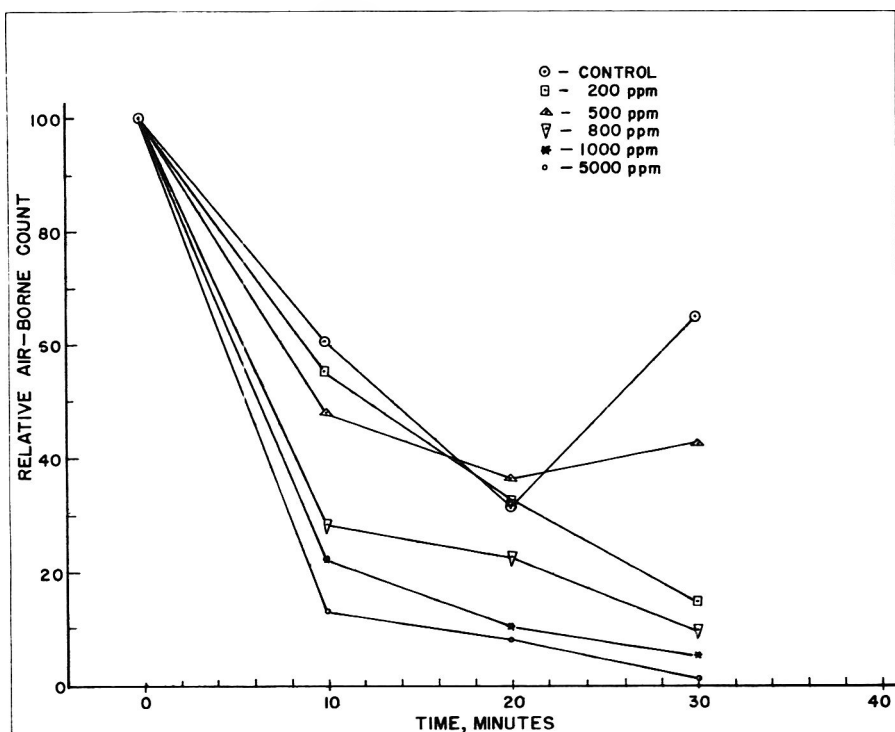
Fig. 15—Effect of chlorine sanitizer concentration on air-borne bacteria populations caused by flooding drains. (From Heldman et al., 1965)

temperature gradients might be used to reduce particle transport. In addition, a pressure gradient in the direction opposite the population gradient can be effective in reducing viable particle transport.

If all other control measures fail to reduce air-borne contamination, localized control at the point of product exposure can be used. The "laminar air flow" technique illustrated in Figure 16 appears to be the most effective localized control measure. This technique uses a low velocity stream of filtered air to continuously "wash" the space of concern. The success of the technique for controlling air-borne microorganism population was illustrated by Heldman et al. (1968) and McDade et al. (1968).
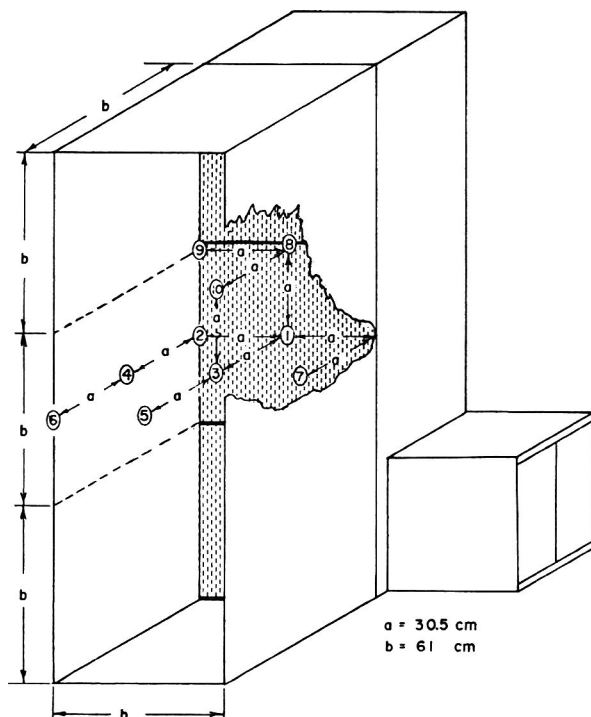
SUMMARY

AN ANALYSIS of available research directed toward preventing air-borne contamination of food products indicates that there is considerable information available to be used in reducing air-borne contamination in food processing plants. Published information includes:

(1) Typical populations in various areas within various types of food processing plants have been measured.

(2) Most sources of air-borne microorganisms in food processing plants have been identified and quantitatively evaluated.

(3) The diffusion of microbial particles with an enclosed air space has been described in considerable detail.

(4) The transport of air-borne microbial particles through an opening between two adjoining spaces has been described.

(5) Localized control of air-borne contamination can be effective under most circumstances.

The ultimate control of air-borne contamination has not been developed as yet. This control technique would prevent the air-borne viable particles from leaving the source. The use of air filters to eliminate the ventilation system as a source is the best example of the ultimate approach. This example illustrates, however, that the best control techniques are those which are source-oriented and must be designed especially for the source being considered. Several other areas deserve research attention:

(a) Sources of air-borne contamination in food processing which could generate pathogenic microorganisms must be identified and probabilities of contamination by a pathogen.

(b) Identify economic considerations related to reduced shelf life caused by air-borne contamination. The relationships between air-borne populations to which the product is exposed and reductions in shelf life which results must be established.

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a = 30.5 cm
b = 61 cm

Fig. 16—Experimental laminar air flow chamber. (From Heldman et al., 1968)

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AN APPARATUS FOR MEASURING PLUCKING FORCE OF PIG HAIRS

INTRODUCTION

THE SUCCESS of the dehairing process (during slaughter of pigs) varies throughout the year, but extreme difficulty is often encountered during the late fall and early winter. Since the hair is difficult or hard to remove it is referred to as "hard hair." 4 min in the scalding bath at 60°C as recommended by Wang et al. (1954) will not loosen the so-called hard hairs, and the packer is then forced to use auxiliary practices such as singeing, excessive shaving, or dipping in hot resin to remove the hair from the carcass. Such procedures result not only in extra labor costs, and a slow down of the processing line, but also will destroy the possibility of using such skin in the manufacture of leather.

An apparatus was developed to meas-ure objectively the degree of attachment of a given hair to the skin layers. The apparatus measures the force in grams required to pluck a single hair and it was tested on a group of pigs from an experiment designed to study the effect of climatic conditon (season) on characteristics of the hair follicle.

EXPERIMENTAL

THE APPARATUS (Fig. 1) was designed and manufactured with the co-operaticn of the Dept. of Mechanical Engineering. A commercially available force gauge, commonly used for mechanical testing, was used to record force of plucking. It has a magnetic hold feature which registers maximum force. A beaded chain was used to connect the force gauge to the hair clamp thereby eliminating differences due to the angle between the gauge and the animal. A modified electric test clip with increased spring force was used as the hair clamp.

Repeatability of the apparatus was determined by testing it with standard weights (100, 200, 300, 400, 500g). Table 1 shows an intraclass correlation. Less than 1% variation was observed within a given weight class indicating a very high repeatability.

Two rooms at the University of Wisconsin Biotron were programmed so that the one simulated summer (25–30°C and 15 daylight hr) and the other simulated early winter (2–4°C and 10 daylight hr). 18 Hampshire barrows weighing from 80–100 kg were equally divided into two groups designated as summer and winter; they were exposed to the above described environment for a continuous period of 4–5 wk. This time interval was chosen on the basis of preliminary observations on the hair growth cycle of the pig. 100 hairs were plucked randomly from two locations: the frontal plane of the cranial skin, and an area lateral to the middle back line and approximately over the 10th rib.

The hairs were classified into five categories according to the force that was required to pluck them. The categories were: (1) 5–100g; (2) 105–200g; (3) 205–300g; (4) 305–400g; (5) over 400g. Plucked hair follicles were classified by microscopic investigation as growing or resting (Adachi, 1969). The animals were slaughtered, scalded for 2 min at 60°C, de-haired for 30 sec and observed for effectiveness of the hair removal process. Data were statistically treated as described by Steel and Torrie (1969).

RESULTS & DISCUSSION

COMPARISON of results between the two climatic groups and the different body locations within each group are shown in Tables 2 and 3. For the purpose of this discussion hard hairs were arbitrarily defined as hairs requiring 300g or more plucking force. The data revealed that the summer group had approximately 32% hard hairs while the winter group had about 55%. While 13.7% of the hairs from the back region in the winter group required a plucking force of over 400g, no hairs in the summer group were found to be that difficult to pluck. Approximately 26% of the hairs from the back region of the summer group were found to require a low plucking force of 100–200g while only 6.1% of the hairs from the back region of the winter group were in this category. Such findings suggest that keeping the pigs for 4–5 wk at 2–4°C and 10 hr daylight per day caused a strengthening of the attachment of the hair to the skin which resulted in a great-

Table 1—Intra-class correlation

Source of variation	Degrees of freedom	Mean square	r_1
Standard wt	4	25000	0.52
Individual readings[a]	20	130	

[a] Five readings were taken for each standard weight

Table 2—Average plucking force[a] of summer and winter pigs as measured in two different body locations

	No.	Mean	S.E.[b]
Back			
Summer	550	253.09 ± 10.89	
Winter		278.18 + 25.68	
Head			
Summer	550	270.18 ± 12.72	
Winter		290.82 ± 19.21	

[a] Force is measured in grams
[b] Standard error

Fig. 1—The apparatus used for measuring the plucking force: (1) meter; (2) reset button; (3) 500g maximum load force gauge; (4) chain connecting spring to (5) the clamp that grasps the hair being tested.

Table 3—Hairs found in the different categories expressed as percentages of total hairs plucked ± S.E.

	0—100g	105—200g	205—300g	305—400g	> 400g	Total hard hairs (> 300)
Back						
1-Summer	15.7% ± 1.14	26.2% ± 0.73	26.0% ± 0.78	26.2% ± 1.28	6.0% ± 0.44	32.2% ± 1.
Probability level[a]	0.01	0.01	0.01	0.01	0.01	0.01
2-Winter	0.05% ± 0.24	6.12% ± 0.28	38.5% ± 0.38	41.0% ± 0.90	13.7% ± 0.60	54.7% ± 0.7
Head						
1-Summer	5.9% ± 0.48	18.5% ± 0.89	29.5% ± 0.84	28.7% ± 1.11	17.6% ± 0.88	46.3% ± 1.6
Probability level[a]	0.05	0.01	0.01	N.S.	N.S.	N.S.
2-Winter	1.3% ± 0.42	7.2% ± 0.63	43.7% ± 1.49	31.2% ± 1.23	16.3% ± 1.23	47.5% ± 1.1

[a] Significance of the differences between summer and winter pigs is expressed as being highly significant (0.01), significant (0.05) or not significant (N.S.).
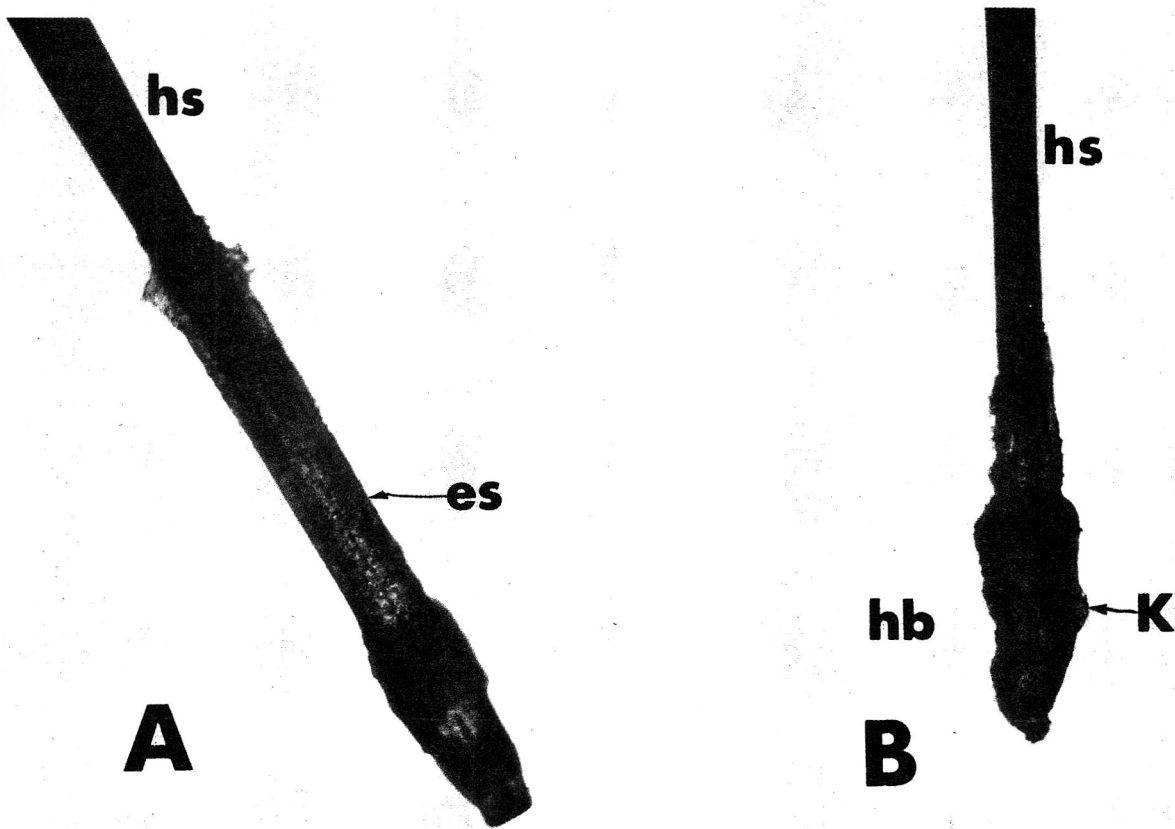
Fig. 2—Plucked hairs which illustrate the growing (A) and resting (B) states. The epithelial sac (es) is prominent in the growing follicle but keratinized (K) in the resting follicle. The hair bulb (hb) and hair shaft (hs) are larger in the growing follicle.

er plucking force. The effect of the winter climatic condition was manifested not only in greater plucking force but also in more hairs remaining on the carcass after routine dehairing.

Microscopic study showed that 80% of the hairs with a plucking force over 300g (hard hairs) were in the growing or anagen phase of the hair growth cycle. Resting hairs did not require high plucking force, probably because their attachment to the surrounding sheaths is much less than that of growing follicles. The depth of the resting hairs in the corium is also less than that of the growing follicles. A

comparison between growing and resting follicles is shown in Figure 2.

No significant differences in the plucking force were found between the two climatic groups or the two body locations. However, when the absolute numbers of hairs in each category were expressed as percentages of the total hairs plucked (Table 3), the differences between the summer and winter pigs were significant in all categories.

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CURED HAM PROPERTIES AS AFFECTED BY NITRATE AND NITRITE AND FRESH PORK QUALITY

INTRODUCTION

NITRATE AND NITRITE salts have been used for many years as curing ingredients for pork. Haldane (1901) reported that color of cured pork was caused by a reduction of nitrate to nitrite which, in turn, reacted with the meat pigments. Kerr et al. (1926) reported the beneficial effects of sodium nitrite as a curing ingredient and as a result, the U.S. Department of Agriculture permitted its use in meat curing. Typical dry-cure procedures contain nitrate and/or nitrite (Kemp et al., 1968). Meat was dry cured, however, long before this method was approved by the meat inspection division of the U.S. Department of Agriculture. In the book, *Secrets of Meat Curing and Sausage Making* by B. Heller & Co. (1918), no mention is made of either nitrate or nitrite in any of the curing recommendations. Although the color probably was not as good as when nitrate or nitrite was used, the meat was preserved. Nitrite, as a curing ingredient, improved the flavor of pork when cured by the injection of pickle (Cho and Bratzler, 1970). Little work has been reported, however, on the effects of nitrate or nitrite on the color or flavor of dry-cured hams.

Fresh ham quality affects the weight loss, tenderness and other organoleptic properties of dry-cured hams (Kemp et al., 1968, 1971; Fox et al., 1970). These workers, however, did not report the effects of using nitrate or nitrite on hams varying in quality as measured subjectively or varying in myoglobin levels or pH values.

This project was devised, therefore, to compare hams cured with the use of nitrate or nitrite with hams cured with salt and sugar only and to study the relationships of these treatments to ham quality.

MATERIALS & METHODS

24 NORMAL and 24 PSE hams (Wisc. Standards, 1963) were selected from the 1973 Louisville Barrow Show. Although initial selections were based on subjective criteria, pH values and myoglobin (Rickansrud and Henrickson, 1967) contents also were recorded for the semimembranosus (SM) and gluteus medius (GM) muscles (Table 1). Each quality group was subdivided into three groups of eight hams each for curing. Group 1 was cured with salt and sugar only (control). Group 2 was cured with salt, sugar and potassium nitrate (2 oz per 100 lb meat). Group 3 was cured with salt, sugar and

Table 1—Mean pH and myoglobin (MYO)[a] values of fresh ham muscles

Quality group	Salt sugar only (Control) SM[b] pH	Salt sugar only (Control) SM[b] MYO	Salt sugar only (Control) GM[c] pH	Salt sugar only (Control) GM[c] MYO	Nitrate SM[b] pH	Nitrate SM[b] MYO	Nitrate GM[c] pH	Nitrate GM[c] MYO	Nitrite SM[b] pH	Nitrite SM[b] MYO	Nitrite GM[c] pH	Nitrite GM[c] MYO	Average by quality SM[b] pH	Average by quality SM[b] MYO	Average by quality GM[c] pH	Average by quality GM[c] MYO
Normal	5.82	0.84	5.77	1.04	6.02	1.05	5.83	1.04	6.11	0.92	6.02	1.23	5.99[d]	0.94	5.88[d]	1.11[e]
PSE	5.61	0.62	5.54	0.64	5.62	1.03	5.62	0.80	5.81	0.96	5.69	0.71	5.68[d]	0.87	5.62[d]	0.72[e]

[a] MYO expressed as mg total pigment per g tissue
[b] Semimembranosus
[c] Gluteus medius
[d] pH higher (P < 0.01) in normal hams in both muscles for all cure groups
[e] MYO higher (P < 0.01) in normal hams in gluteus medius

Table 2—Mean percent weight loss by periods

Quality group	Salt-sugar only (Control) Cured	Salt-sugar only (Control) S.E.[a]	Salt-sugar only (Control) Smoked	Salt-sugar only (Control) 1 Mo	Salt-sugar only (Control) 2 Mo	Salt-sugar only (Control) 3 Mo	Nitrate Cure	Nitrate S.E.	Nitrate Smoked	Nitrate 1 Mo	Nitrate 2 Mo	Nitrate 3 Mo
Normal	4.6	14.7	16.0	19.6	22.9	25.0	3.8	14.6	15.3	18.8	22.1	24.0
PSE	6.6	16.9	18.1	22.2	25.7	28.0	5.0	16.6	17.4	21.8	25.6	28.1
Avg by cure[c]	5.6	15.8	17.1	20.9	24.3	26.5	4.4	15.6	16.4	20.3	23.9	26.0

Quality group	Nitrite Cure	Nitrite S.E.	Nitrite Smoked	Nitrite 1 Mo	Nitrite 2 Mo	Nitrite 3 Mo	Avg by quality[b] Cured	Avg by quality[b] S.E.	Avg by quality[b] Smoked	Avg by quality[b] 1 Mo	Avg by quality[b] 2 Mo	Avg by quality[b] 3 Mo
Normal	4.2	14.4	15.1	18.7	21.9	23.7	4.2	14.5	15.4	19.1	22.3	24.2
PSE	6.1	16.6	17.5	21.6	25.3	27.7	6.1[c]	16.5	17.5	21.6	25.3	27.7
Avg by cure[c]	5.5	15.2	16.0	19.8	23.2	25.3						

[a] S.E. = Salt equalization
[b] Weight loss greater (P < 0.01) for PSE hams at all periods
[c] Difference significant (P < 0.01) for cure treatment at cured period only

sodium nitrite (1 oz per 100 lb meat). The cure had a ratio of eight parts salt to two parts sugar and was applied in three applications at 3- to 5-day intervals until an amount equal to 8% of fresh weight was used. Hams were cured 2 days per lb at 2°C held 4 wk at 13°C for salt equalization, smoked at approximately 38°C for 24 hr and aged at 24°C for 3 months. Weight was recorded fresh and after curing, salt equalization and smoking, and after 1, 2 and 3 months aging. The hams were then cut, evaluated subjectively for color, aroma and general appearance. Center slices of 1-in. thickness were broiled soon after slicing and tested for tenderness with a Warner-Bratzler type shear attached to an Instron testing device. Other slices of 1/2-in. thickness were broiled and evaluated by a palatability panel for tenderness, flavor, saltiness and over-all satisfaction. An additional slice of one-half inch thickness was trimmed of spearable fat and analyzed

Table 3—Subjective evaluation scores of cured ham slices

Quality group	Salt-sugar only (Control)			Nitrate			Nitrite			Avg by quality		
	Color[a]	Aroma[b]	Gen app[c]	Color	Aroma	Gen app	Color	Aroma	Gen app	Color	Aroma	Gen app
Normal	2.6	2.9	2.8	2.9	3.0	3.0	2.8	3.0	2.9	2.8	3.0	2.9[f]
PSE	2.1	3.0	1.5	2.9	3.0	2.2	3.0	3.0	2.6	2.7	3.0	2.1[f]
Avg by cure	2.4[d]	2.9	2.1[e]	2.9[d]	3.0	2.6[e]	3.0[d]	3.0	2.8[e]			

[a] Scores: 3 = Red; 2 = Light red; 1 = Dark pink
[b] Scores: 3 = Typical aroma; 2 = Slightly off odor
[c] Scores: 3 = Excellent; 2 = Good; 1 = Fair
[d] Color scores higher (P < 0.01) when either nitrate group or nitrite group is compared with controls with no difference between nitrate and nitrite groups.
[e] General appearance scores higher (P < 0.01) when either nitrate or nitrite group is compared with controls with no difference between nitrate and nitrite groups.
[f] General appearance scores higher (P < 0.01) in normal than in PSE hams.

Table 4—Tenderness (shear value) scores[a]

Quality group	Salt-sugar only (Control)			Nitrate			Nitrite			Avg by quality		
	SM[b]	ST[c]	BF[d]	SM	ST	BF	SM	ST	BF	SM	ST	BF
Normal	8.8	9.4	10.6	8.4	9.6	9.6	9.4	9.1	10.2	8.8[e]	9.4	10.2
PSE	6.3	8.6	9.3	8.1	9.0	9.7	7.5	8.0	11.3	7.3[e]	8.6	10.1
Avg by cure	7.5	9.0	9.9	8.2	9.3	9.7[f]	8.4	8.6	10.7[f]			

[a] Kg force to shear 1 inch cores.
[b] Semimembranosus
[c] Semitendinosus
[d] Biceps femoris
[e] SM more tender (P < 0.05) for PSE group
[f] BF more tender (P < 0.05) for nitrate than for nitrite group

Table 5—Organoleptic panel scores

Quality group	Salt-sugar only (Control)				Nitrate				Nitrite				Avg by quality			
	Tend	Flavor	Salt	O.S.[a]	Tend	Flavor	Salt	O.S.	Tend	Flavor	Salt	O.S.	Tend	Flavor	Salt	O.S.
Normal	6.3	6.9	6.1	6.7	6.2	7.4	6.2	7.2[b]	6.1	7.1	6.0	6.9	6.2	7.1[b]	6.2	7.0[d]
PSE	6.4	6.6	6.2	6.6	6.3	6.6	6.1	6.6[e]	6.2	7.2	6.1	7.0	6.4	6.8[b]	6.2	6.8[d]
Avg by cure	6.4	6.8[c,f]	6.2	6.7[e]	6.3	7.0[f]	6.2	7.0[e]	6.2	7.2[c,f]	6.1	7.0[e]				

[a] Over-all satisfaction: All scores based on 9-point hedonic scale
[b] Flavor was higher (P < 0.01) for normal hams.
[c] Flavor was higher (P < 0.01) for nitrite group than for controls.
[d] O.S. was higher (P < 0.05) for normal than for PSE hams.
[e] O.S. was higher (P < 0.05) for both nitrate and nitrite as compared to control hams.
[f] There was a significant (P < 0.01) interaction between quality and cure for flavor.

Table 6—Nitrite, salt and water content of cured pork

Quality group	Salt-sugar only (Control)			Nitrate			Nitrite			Avg by quality		
	NO₂ (ppm)	NaCl (%)	H₂O (%)	NO₂ (ppm)	NaCl (%)	H₂O (%)	NO₂ (ppm)	NaCl (%)	H₂O (%)	NO₂ (ppm)	NaCl (%)	H₂O (%)
Normal	4.96	6.76	56.2	8.21	7.02	57.0	14.54	6.74	56.8	9.24	6.74	56.8
PSE	3.94	7.12	56.0	5.02	7.62	56.1	13.81	6.46	58.2	7.59	7.07	56.8
Avg by cure	4.45[a]	6.94[b]	56.1	6.61[a]	7.32[c]	56.6	14.18[a]	6.44[b,c]	57.6			

[a] Nitrite group contained more NO_2 (P < 0.01) than control or nitrate group
[b] Control group contained more salt (P < 0.05) than nitrite group
[c] Nitrate group contained more salt (P < 0.01) than nitrite group

Table 7—Correlations among variables studied

Variable	pH GM	MYO SM	MYO GM	Wt loss cured	Wt loss S.E.[b]	Wt loss smoked	Wt loss 1 mo	Wt loss 2 mo	Wt loss 3 mo	Color
pH SM	0.89**	0.36*	0.55**	−0.42**	−0.55**	−0.55**	−0.57**	−0.56**	−0.58**	0.34*
pH GM		0.31*	0.58**	−0.52**	−0.63**	−0.63**	−0.63**	−0.63**	−0.64**	0.40**
MYO SM			0.05	−0.28*	0.21	−0.30*	−0.28	−0.25	−0.25	0.16
MYO GM				−0.32*	−0.17	−0.32*	−0.33*	−0.32*	−0.35*	0.24
Wt loss cured					0.74**	0.83**	0.84**	0.79**	0.80**	−0.33*
Wt loss S.E.						0.87**	0.87**	0.86**	0.87**	0.29*
Wt loss smoked							0.99**	0.97**	0.96**	0.35*
Wt loss 1 mo								0.99**	0.99**	−0.34*
Wt loss 2 mo									0.99**	−0.35*
Wt loss 3 mo										−0.32*
Color										
Aroma										
Gen app										
Shear SM										
Shear ST										
Shear BF										
Tend										
Flavor										
Saltiness										
O.S.										
Nitrite										
NaCl										

[a] Over-all satisfaction
[b] Salt equalization
* Significant $P < 0.05$
** Significant $P < 0.01$

for nitrite, sodium chloride and moisture. Nitrite and moisture were determined according to the methods of AOAC (1970). Sodium chloride was extracted by blending 10g ground ham in 90 ml water for 2½ min, filtering through Whatman #1 filter paper and determining as the chloride on a Buchler-Cotlove chloridameter. Data were analyzed by the method of Barr and Goodnight (1972) with additional "t" tests as described by Snedecor (1955).

RESULTS & DISCUSSION

HAMS were selected subjectively on the basis of muscle color and firmness and then assigned to normal or PSE groups. Table 1 shows the pH and myoglobin values for the SM and GM muscles and verifies that pH values were higher (P < 0.01) in normal hams for both muscles. Myoglobin content was greater for both muscles in normal hams, with the difference being significant (P < 0.01) for the GM muscle. This creates the possibility that different salt and nitrite values might occur in cured hams if pH or myoglobin are related to cure penetration and cure-pigment reactions.

Weight loss was greater (Table 2) for the PSE hams at all periods (P < 0.01). This agrees with the work of Kemp et al. (1968, 1971). There were no significant differences in weight loss owing to curing treatment except immediately after cure where the control hams lost more weight (P < 0.01) than the nitrate group hams. Since the effects did not carry through to

succeeding periods this difference probably has little meaning.

Subjective evaluation of cured ham slices (Table 3) revealed differences (P < 0.01) in color and general appearance in favor of either nitrate or nitrite hams since the slices were redder. General appearance scores also were higher in the nitrate and nitrite groups indicating that, from a color producing standpoint, either nitrate or nitrite is beneficial, with scores of each being equal to the other.

Aroma was similar for all groups as all were rated excellent. General appearance, which included uniformity of color and firmness as well as color per se, was significantly (P < 0.01) more desirable in the normal than in the PSE hams. This indicates the desirability of having firm, normal-colored hams for dry curing, especially if hams are to be sliced before merchandising.

Shear values were lower (P < 0.05) for PSE than for normal hams for the SM only (Table 4). This agrees with previous work by Kemp et al. (1968, 1971). In a few cases, however, in the previous work, the ST and BF muscles were also more tender in the PSE hams. The only differences in shear tenderness attributable to curing procedures occurred in the BF muscle where the nitrate group was more tender (P < 0.05). Even though this occurred, little importance is attached to it as all values are within normal range for

hams of this type (Kemp et al., 1968, 1971).

There were no significant differences in panel tenderness scores (Table 5) owing to quality or cure. Several differences in flavor or over-all satisfaction scores were noted, however. Flavor scores were higher (P < 0.01) for normal than for PSE hams indicating that there is reason to select for fresh quality. Flavor scores also were higher for hams cured with nitrate or nitrite than for controls, with the difference between nitrite and controls being significant (P < 0.01) and the differences between nitrate and nitrite and between nitrate and controls approaching significance at the 0.05 level. There also was significant (P < 0.01) interaction between quality and cure. Over-all satisfaction scores followed a similar pattern. Scores for normal hams were higher (P < 0.05) than for PSE hams. Scores were higher (P < 0.05) for nitrate or nitrite hams than for control hams, with a significant quality-cure interaction. Saltiness scores were not affected by either quality or cure. Flavor and over-all satisfaction scores indicate that there was a beneficial effect due to either nitrate or nitrite. Most of the scores for hams where neither was used, however, were within an acceptable range, indicating that it is possible to have good tasting hams of this type without using nitrate or nitrite.

Values for residual nitrite, sodium

Table 7—Continued.

Aroma	Gen App	Shear SM	Shear ST	Shear BF	Tender-ness	Flavor	Salt	O.S.[a]	NO$_2$	NaCl	H$_2$O
-0.21	0.38**	0.37**	-0.11	0.20	0.04	0.38**	-0.09	0.32*	0.44**	-0.30*	0.18
-0.26	0.45**	0.42**	-0.03	0.21	0.08	0.30*	-0.26	0.21	0.40**	-0.34	0.15
-0.01	0.36*	0.33*	-0.02	0.09	0.15	0.19	-0.07	0.21	0.21	-0.03	0.15
0.11	0.46**	0.25	0.10	0.03	-0.05	0.09	-0.10	0.06	0.06	-0.28	0.13
0.11	-0.35*	-0.44**	-0.42**	0.07	0.18	-0.25	-0.02	-0.24	-0.14	-0.06	0.18
0.41**	-0.32*	-0.25	-0.13	-0.14	-0.04	-0.28	0.14	-0.24	-0.31	0.20	-0.07
0.04	-0.41**	-0.32*	-0.23	-0.08	0.01	-0.37**	0.18	-0.34*	-0.37**	0.24	-0.13
0.06	-0.44**	-0.29*	-0.19	-0.09	-0.01	-0.37**	0.18	-0.34*	-0.35*	0.27	-0.14
0.06	-0.43**	-0.29*	-0.20	-0.11	0.01	-0.36*	0.18	-0.33*	-0.34*	0.28	-0.13
0.07	-0.43**	-0.30*	-0.21	-0.09	0.03	-0.38**	0.14	-0.33*	-0.35*	0.25	-0.11
-0.07	0.38**	0.18	0.15	0.27	-0.21	0.13	-0.11	0.08	0.38**	-0.11	0.22
	-0.10	0.02	0.16	-0.07	-0.15	0.03	0.18	0.07	0.10	0.10	-0.02
		0.20	0.04	0.09	-0.10	0.37**	-0.25	0.33*	0.25	-0.36*	0.23
			0.41**	0.27	-0.41**	0.15	0.14	0.14	0.20	0.18	-0.27
				0.15	-0.40**	-0.06	0.28	-0.05	-0.09	0.22	-0.22
					-0.29*	0.01	0.10	-0.01	0.13	-0.11	0.19
						-0.06	-0.36*	0.12	-0.25	-0.29*	0.32*
							0.12	0.89**	0.41**	-0.03	-0.02
								0.11	0.05	0.71**	-0.49**
									0.20	0.01	0.05
										-0.16	0.17
											-0.54**

chloride and water are given in Table 6. Hams cured with nitrite contained more residual nitrite (P < 0.01) than either controls or those cured with nitrate. Differences in control and nitrite groups approached significance at the 0.05 level. The pattern was similar to the color score patterns of Table 3. Even the hams cured without nitrate or nitrite had a small amount (4.45 ppm) of residual nitrite. It is possible that this was due to contamination as all hams were cured in the same room and handled by the same workers. There is a possibility that nitrite might have been absorbed from the smoke. Other workers (Christiansen et al., 1973; Hustad et al., 1973; Simon et al., 1973) also have shown low levels of nitrite in products where none was added. The acceptable flavor of some of the controls may have been due to the low levels of nitrite. However, many hams with satisfactory flavor are produced annually by noncommercial producers who cure slowly and age under natural conditions.

Normal hams, which contained more myoglobin, had a brighter color (Table 3), possibly indicating that more nitrosomyoglobin was formed. If this were true it might be expected that more residual nitrite would be found in the PSE group. Such was not the case. In fact, normal hams contained slightly more (P < 0.09) nitrite.

Sodium chloride content was slightly

higher (P < 0.09) in PSE than in normal hams. The softer tissue and more unbound water could cause greater absorption in the PSE group. The only significant difference in salt content owing to cure was between the nitrate group with 7.32% salt and the nitrite group with 6.44%. The reason for this is unknown.

Several significant correlations among various traits are noted in Table 7. Significant correlations (P < 0.01) were noted between pH and myoglobin. Firmer darker muscles had higher pH values which, in turn, had higher myoglobin contents. pH was negatively (P < 0.01) correlated with weight loss at all periods. This is logical as the PSE group, with the lower pH values, lost more weight (P < 0.01) (Table 2) than normal hams. Other significant correlations with pH were cured color (P < 0.01), general appearance (P < 0.01), shear value of SM (P < 0.01), flavor (P < 0.01), over-all satisfaction (P < 0.05), nitrite (P < 0.01) and NaCl (P < 0.05). In general, the higher pH hams were more desirable.

Myoglobin was negatively related (P < 0.05) to weight loss at most weigh periods. This can be explained partially by the higher myoglobin content of normal, firmer pork which, in turn, had less weight loss. Myoglobin was also correlated with cured color (P < 0.05). The high (P < 0.01) correlations among weight loss at various periods were part-

whole relationships and have little meaning. There were several significant negative relationships (P < 0.01) between weight loss and color scores and weight loss and general appearance. Again weight loss, low myoglobin scores and low pH all are associated with lower color and general appearance scores. There also were significant negative correlations between weight loss and flavor and between weight loss and over-all satisfaction scores showing that hams with more weight loss were less desirable in these respects. Weight loss also was negatively related to residual nitrite levels. This indicates a relationship between pH and nitrite levels as low pH values, lower nitrite values are, in turn, related to higher weight loss values. Color, as expected, was related (P < 0.01) to higher general appearance scores and nitrite values. Shear scores were negatively (P < 0.01) related to panel tenderness scores indicating that the two types of tenderness ratings show the same trends. Flavor scores were highly related to over-all satisfaction scores and nitrite values. Saltiness was correlated (P < 0.01) with chemical salt and negatively correlated (P < 0.01) with moisture content. Chemical salt also was negatively (P < 0.01) related to water content.

SUMMARY & CONCLUSIONS

NORMAL and PSE hams were dry cured with salt and sugar only, salt, sugar and

potassium nitrate or salt, sugar and sodium nitrite. PSE hams lost more weight during curing and aging but were generally more tender than normal hams although normal hams were more flavorful. Hams cured with nitrate or nitrite had more desirable color and general appearance scores than controls. Aroma was similar for all groups. Flavor was more desirable in either the nitrate or nitrite groups than in controls, with no difference between nitrate and nitrite groups. Saltiness was not affected by either quality or cure.

In general, dry-cured hams cured with a curing mixture containing either nitrate or nitrite were superior to those cured with salt and sugar only although many of the latter were highly acceptable.

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CHARACTERISTICS OF CURED HAM AS INFLUENCED BY LEVELS
OF SODIUM NITRITE AND SODIUM ASCORBATE

INTRODUCTION

THE USE OF sodium nitrite for curing meat was investigated by Kerr et al. (1926) who found that 156 ppm was sufficient for color fixation. Following this investigation, the United States Bureau of Animal Industries authorized the use of sodium nitrite for curing meat. More recent investigations have been conducted concerning the chemistry of cured meat color (Fox, 1966), color stability (Draudt and Deatherage, 1956; Bailey et al., 1964) and color uniformity of cured meat. Watts and Lehmann (1952a, b) reported that cured meat color formation could be accelerated and color uniformity increased by using reducing agents such as ascorbate and processing conditions conducive to reducing conditions.

There has been some concern during the past several years that nitrite present in cured meat can combine with secondary amines in meat or in the gastrointestinal tract to form nitrosamines. The investigations of Fiddler et al. (1971) and Fazio et al. (1971) showed that nitrosamine levels rarely exceed 10 ppb in various cured meat products and Fiddler et al. (1972) found no correlation between the concentrations of added nitrite and dimethylnitrosamine in meat. Lane and Bailey (1973) recently demonstrated that the optimal pH for formation of dimethylnitrosamine in human gastric juice was 2.5 and discussed the responsible reaction mechanisms.

Although the use of nitrite in cured meats may be questioned, its presence in cured meats has been shown to inhibit development of toxin production by *C. botulinum* (Emodi and Lechowich, 1969; Johnston et al., 1969; Greenberg, 1972) and to prevent the growth of other putrefactive organisms (Bulman and Ayers, 1952).

Sodium nitrite has been reported to serve in the development of a characteristic cured meat flavor in various meat products such as bacon and ham (Brooks et al., 1940), pork loins (Cho and Bratzler, 1970), canned comminuted pork (Ockerman et al., 1973), frankfurters (Wasserman and Talley, 1972; Simon et al., 1972) and pork middles (Mottram and Rhodes, 1973). Nitrite when used in excess may cause meat to become hard

and thus adversely affect textural characteristics (Thornton, 1962).

The present investigation was conducted to determine if nitrite has an effect on the flavor and texture of cured ham; if a lower level of nitrite could be used in cured ham than currently used and still achieve comparable qualitative characteristics; and to determine the effect of sodium tripolyphosphate and sodium ascorbate on nitrite retention during refrigerated storage.

EXPERIMENTAL

TWO SEPARATE experiments were conducted. The number of hams used in each experiment is given in Table 1. The hams ranged in weight from 8.7 to 9.1 kg, had color and firmness scores that averaged 2.5 and ranged from 2 to 3 relative to the Wisconsin standards of 1 to 5. Hams in Experiment 1 were blast-frozen and stored at $-18°C$ while those in Experiment 2 were processed without freezing. The frozen hams were removed from storage and thawed prior to being cured. In each experiment hams were stitch pumped to 110% of their fresh weight with the brines designated in Table 1.

In Experiment 1, brines were formulated with three levels of nitrite (0, 1.0 and 2.0 g/l.). Hams pumped to 110% of fresh weight theoretically had nitrite levels of 0, 91 and 182 ppm following pumping (assuming 1/11 dilution). In Experiment 2, brines were formulated with 2.0g/liter sodium nitrite, four levels of ascorbate (0, 2.5, 5.0 or 7.5g/liter) and 50.0g/liter sodium tripolyphosphate. Sodium chloride (198g/liter) and sucrose (35.9g/liter) were the same for all curing brines. Hams were cured for 3 days after being stitch pumped and then fully

cooked in a smoke house without smoke where the temperature was increased from 57°C to 88°C in five degree increments during processing for 20 hr. The internal temperature was 69°C and the humidity was maintained at approximately 80%.

Sample preparation and nitrite analysis

In Experiment 1, three slices (2.5 cm thick) were removed from each ham and residual nitrite analysis, sensory panel evaluation and Warner-Bratzler shear determinations were made on the semimembranosus muscle. The remaining shank portion of the ham was packaged and stored at 2°C. After 14 days storage a slice (2.5 cm thick) was removed for sensory panel evaluation. The slice was kept at room temperature for approximately 30 min then cut into sample size portions and served to the panel.

In Experiment 2, two slices (2.5 cm thick) were removed from each ham and nitrite analyses and Warner-Bratzler shear determinations made on the semimembranosus muscle. 25-g samples were stored in glass jars for 0, 2, 5, 8 and 16 days at 2°C prior to analysis for residual nitrite. Nitrite was determined by the AOAC (1965) method.

Sensory panel evaluation

A six member trained sensory panel evaluated samples for flavor and texture compared to reference samples from hams cured with a brine containing 91 ppm nitrite. Texture was evaluated using two criteria, softness to tooth and ease of fragmentation, and was defined as the textural impression of the sample from initial bite through complete mastication. Flavor and texture were scored separately on a 7 point difference-rating test. A score of 4 indicated flavor or texture identical to the refer-

Table 1—Composition of curing solutions and number of hams used in Experiments 1 and 2

Ingredient (g/liter)	Curing solution						
	Level of nitrite (Exp 1)[a]			Level of ascorbate (Exp 2)[a]			
	0 ppm	91 ppm	182 ppm	0 ppm	227 ppm	455 ppm	568 ppm
Sodium chloride	198.0	198.0	198.0	198.0	198.0	198.0	198.0
Sucrose	35.9	35.9	35.9	35.9	35.9	35.9	35.9
Sodium nitrite	0.0	1.00	2.00	2.00	2.00	2.00	2.00
Sodium tripoly- phosphate	—	—	—	50.0	50.0	50.0	50.0
Sodium ascorbate	—	—	—	0.00	2.50	5.00	7.50
Number of hams	8	8	8	3	3	3	3

[a] Theoretical level following pumping assuming 1/11 dilution

Table 2—Sensory characteristics of the semimembranosus muscle of cured ham as influenced by nitrite level in the curing solution and period of storage

Nitrite level ppm	Flavor[a]		Softness to tooth[a]		Ease of fragmentation[a]	
	0 day	14 day	0 day	14 day	0 day	14 day
0	2.48[b]	2.77[b]	4.21[d]	3.58[e]	4.14[f]	3.58[f]
91	3.47[c]	3.77[c]	3.94[f]	4.02[f]	3.75[f]	3.09[f]
182	3.79[c]	4.08[c]	4.19[f]	3.73[f]	3.85[f]	3.83[f]

[a] Scale from 1—7 where 4 was equivalent to reference standard sample containing 91 ppm nitrite. Samples rated higher than 4 were more desirable and samples less than 4 were less desirable than standard.

[b,c] Means within the same column bearing different superscripts are different (P < 0.05). There were no significant differences in flavor due to storage.

[d,e] Means on the same line and within the same variable bearing different superscripts are different (P < 0.05).

[f] Means on the same line and within the same variable bearing the same superscript are not different (P > 0.05).

ence ham sample. Scores of 1, 2 or 3 indicated less intense cured meat flavor or less desirable texture than the reference cured ham sample while scores of 5, 6 or 7 indicated more intense cured meat flavor or a more desirable texture than the reference sample. The sensory panel evaluations were performed in individual booths located in a dark room. The individual booths were lighted by shaded red fluorescent light to eliminate color differences.

Following removal from the smokehouse, hams were cooled from 69°C to 21°C, cut and served immediately to the panel.

Shear evaluation

Warner-Bratzler shear values were obtained from three 1.3 cm cores removed from the semimembranosus muscle. Each core was sheared three times and the average calculated for the nine determinations.

Statistical analysis

Treatment differences were detected using analysis of variance, and Duncans New Multiple Range test was utilized to separate means (Snedecor and Cochran, 1967).

RESULTS & DISCUSSION

HAMS CURED without nitrite in Experiment 1 were scored lower in cured meat flavor intensity by the sensory panel (P < 0.05) than hams cured with either 91 ppm or 182 ppm nitrite. No significant differences (P > 0.05) were observed in cured meat flavor between samples from the 91 ppm and the 182 ppm cured hams. These results indicate that a desirable cured meat flavor was produced with 91 ppm nitrite and no additional improvement in flavor was attained by using 182 ppm nitrite. Storage did not significantly (P > 0.05) affect the flavor of hams stored for 14 days compared to hams stored 0 days (Table 2). The panel scored all samples stored 14 days as having slightly more intense cured meat flavor.

Panel scores for softness to tooth and ease of fragmentation were similar for samples from all treatment groups (Table 2). Level of nitrite in the curing solution

had no apparent influence on these textural properties. After 14 days storage, panel scores for softness to tooth and ease of fragmentation were lower. These observations indicate that textural properties were adversely affected by storage. The changes in texture were probably associated with loss of moisture during storage.

Residual nitrite levels were higher (P < 0.05) in hams cured with nitrite than in hams cured without nitrite (Table 3). Also, more nitrite was present in hams cured with 182 ppm nitrite than those cured with 91 ppm nitrite.

Hams cured with and without nitrite had similar Warner-Bratzler shear values. Color intensity differences were observed between the 91 ppm and 182 ppm nitrite cured hams. Hams cured with brines containing 182 ppm nitrite had darker more typical cured meat color than those cured with 91 ppm nitrite.

In Experiment 2, residual nitrite was influenced by ascorbate and period of storage (Table 4). Nitrite increased during the first 5 days of storage in nonascorbate-treated hams, then declined steadily throughout the remaining 16 days of storage. Nitrite level was lower in ascorbate-treated than in nonascorbate-treated hams. Also, the residual nitrite was lower in the 455 and 568 ppm ascorbate-treated hams than in the 227 ppm ascorbate-treated hams.

Results of visual observations indicated that color stability and color uniformity were superior in ascorbate-treated hams during the 16 day storage period. Hams cured without ascorbate had significantly (P < 0.05) higher mean Warner-Bratzler shears (14.0) than those cured with 2.5, 5.0 and 7.5 grams ascorbate per liter. The ascorbate-treated samples had mean shear values of 5.9, 7.6 and 8.5, respectively. Hams in Experiment 2 cured with ascorbate and phosphate were generally more tender than those in Ex-

Table 3—Residual nitrite content and Warner-Bratzler shear values of the semimembranosus muscle of cured ham

Treatment	Variable[a]	
	Residual nitrite (ppm)	Warner-Bratzler shear value (1.3 cm) (kg.)
0 ppm nitrite	1.25[b]	8.69
91 ppm nitrite	8.39[c]	9.30
182 ppm nitrite	24.18[d]	8.96

[a] Residual nitrite level and Warner-Bratzler shear values were determined at 0 days storage.

[b,c,d] Means within the same column bearing different superscripts are different (P < 0.05).

Table 4—Residual nitrite levels in the semimembranosus muscle of cured ham as influenced by ascorbate and days refrigerated storage

Days storage	Residual nitrite (ppm) for ascorbate levels (Hams pumped to 182 ppm nitrite)			
	0 ppm ascorbate	227 ppm ascorbate	455 ppm ascorbate	568 ppm ascorbate
0	49.7[b]	43.0[d]	18.0[h]	14.0[i,j]
2	49.7[b]	45.6[c]	19.7[g,h]	18.0[h]
5	58.7[a]	30.0[e]	21.0[g]	19.0[h]
8	32.0[e]	24.7[f]	15.7[i]	8.0[k]
16	12.3[j]	12.0[j]	8.3[k]	8.7[k]

[a,b,c,d,e,f,g,h,i,j,k] Means within the same row or column bearing different superscripts are different (P < 0.05).

periment 1, presumably because of greater moisture content of the phosphate-treated hams.

CONCLUSIONS

HAMS cured with nitrite had more intense cured meat flavor than hams cured without nitrite. Hams cured with 91 ppm nitrite were not significantly different in flavor from those cured with 182 ppm nitrite. Level of nitrite in the curing brine did not significantly affect tenderness of cured hams. The concentration of nitrite injected into the hams was directly related to residual nitrite in the cured hams. Hams treated with ascorbate had lower residual nitrite than nonascorbate-treated hams. Higher levels of ascorbate resulted in increased nitrite depletion.

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FACTORS INFLUENCING COOLING
OF POULTRY CARCASSES

INTRODUCTION

COOLING of eviscerated broilers is a common process in the poultry industry. Microorganisms growth is reduced by lowering the temperature on the inside and outside surface of the product. As a result the storage stability of the product is improved. In practice a number of different processes are employed for removing energy from the carcasses. The most commonly used coolants are water and air. If air is used, special slaughtering procedures are needed to prevent discoloration during cooling. In some cases the chilling process seems to be primarily a method of cleaning rather than cooling. Most research is done on the microbiological aspects of the process. The earlier literature was reviewed by Brant (1963). Recent papers describe the washing effect (Peric et al., 1971b; Mulder, 1972; Veerkamp et al., 1972; Ranken, 1973; Barnes, 1973); contamination (Grossklaus and Levetzow, 1967; Surkiewicz et al., 1969; Knoop et al., 1971; Peric et al., 1971a); crosscontamination (Peric et al., 1971a; Schothorst, 1973); or water uptake (Kotula et al., 1960; Katz and Dawson, 1964; Marion et al., 1968; Heath et al., 1968; Sanders, 1969; Woltersdorf, 1971; Veerkamp et al., 1973). Some of these investigators have shown that contamination conditions may be produced in chilling, but that no harm will result provided the bacterial load of the carcasses can be greatly reduced prior to the cooling process. Immersion cooling with water is the most applied process.

The cooling procedure for broilers may be defined as "the removal of energy in such a way that the temperature of the product at every point and time does not fall below the value at which ice can form." To avoid freezing any part of the product, the temperature — especially at the surface — must be measured or calculated. Temperature measurements of the thermal center of the breast are often used to describe cooling rates (Connolly et al., 1954; Brodine and Carlin, 1968; Thomson et al., 1966; Gisske and Glees, 1966; Scholtyssek and Gühne, 1973). Usually no further information is given about the geometry, weight or dimensions of the product. However, calculations with these figures lead to wrong conclusions about cooling time or capacity of the equipment. The energy removal can only be calculated if the average temperature is known as a function of the cooling time. It can also be determined directly by means of calorimetric measurements.

If the energy removal, which is dependent on the cooling time, is known then the surface temperature can be calculated in relation to cooling time. Hitherto cooling time has been measured experimentally after the design and construction of the equipment for all cooling processes. There is no simple method for calculating cooling time as a function of the weight of the broilers for several processes.

The object of this study was to make it possible to calculate the most important factors defining the cooling process of broiler carcasses. This paper presents the results of extensive experiments used to elaborate simple equations for calculating total energy removal during cooling.

MATERIALS & METHODS

POULTRY CARCASSES without giblets and without neck were obtained from the Institute's pilot plant slaughterhouse. Most chickens were of Hypeco strain and aged from 6 to 12 wk. The broilers were processed with the simultaneous scalding and plucking equipment as described elsewhere (Veerkamp and Hofmans, 1973). The broilers for immersion chilling were processed at 65°C and those for air chilling at 52°C. Several weight classes of carcasses were used, viz., 0.600, 0.800, 0.950, 1.150 and 1.400 kg.

Immersion cooling

The carcasses were hung by their wings in a water bath (two to five in each experiment) (Fig. 1-a) and slush ice was added to the water. A screen was used to separate the ice from the broiler carcasses.

Experiments I — Immersion cooling with high heat-transfer coefficient. Intense water mixing was obtained by two propeller-type stirrers (1380 rpm), which results in a high heat-transfer coefficient.

Experiments II — Immersion cooling with low heat-transfer coefficient. With a low speed propeller-type stirrer (200–300 rpm) a low degree of water mixing was obtained.

Spray cooling

A full cone spray nozzle (Schlick, bore diam 6.5 × 10^{-3}m, water flow 5 × 10^{-4} m³/s and spray angle 140°) was placed 1.5m above the carcasses (Fig. 1-b). Assuming a useful spray area of 15m² and 50 carcasses hung in 1m², gives a water consumption of 0.04 liter/carcass/minute.

In all these experiments the carcasses were hung by their wings and a maximum of eight broilers per experiment were used.

Air cooling

An experimental air cooler was installed in an air conditioned room in which the temperature was kept at 0.5 ± 0.5°C and the relative humidity close to 100% (Fig. 1-c). The air stream flowed from the top to the floor at an air speed of 5.5m/s. The broilers were hung by their wings and eight carcasses were cooled at the same time.
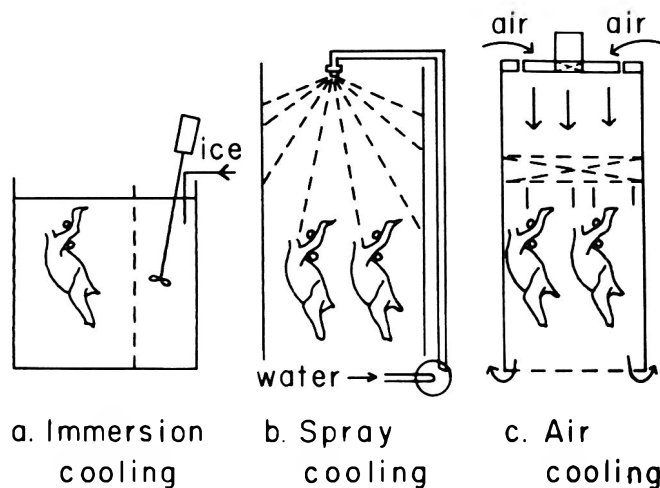
Fig. 1—Experimental cooling processes.

Theory

The enthalphy H_t of the broilers at any time is equal to

$$H_t = \sum_{k=0}^{k=n} G_k \times cp \times T_k = G \times cp \times T_t \qquad (1)$$

where G = total wt of the broilers; G_k = wt of vol element k; cp = specific heat; T_t = avg temp at time t; T_k = temp of vol element k; and n = number of elements.

Two to five broilers were used for each experiment in order to obtain the same total weight in each. The relative energy removal is

$$\frac{\Delta H}{\Delta H_i} = \frac{H_o - H_t}{H_o - H_\infty} \qquad (2)$$

where H_o = enthalphy at time = 0; H_t = enthalphy at time = t; and H_∞ = enthalphy at time = ∞.

H_o and H_∞ are readily calculated from Eq (1) provided the temperature of the broilers before cooling is uniform and the coolant temperature during the experiment is constant. ΔH_i is the maximum removable energy of the carcasses after an infinitely long cooling time. ΔH is the real energy that is removed during t seconds.

A uniform initial temperature of the broilers was obtained by hanging them at least 300 sec in a water bath with a temperature of 40 ± 0.1°C. The coolant temperature was:

I and II immersion cooling	0.3 ± 0.2°C
III spray cooling	0.5 ± 0.3°C
IV air cooling	0.5 ± 0.5°C

The enthalphy after cooling for t sec was measured by means of four well insulated calorimeters.

Calorimetric measurements procedure

First 10 kg water is added to the calorimeter (Fig. 2) and the temperature recorded by a Leeds and Northrup recorder with copper – constantan thermocouples (accuracy ± 0.1°C). Cooling with at least two carcasses simultaneously was executed during a defined cooling time.

The chilled carcasses are then placed in the box where they are able to equalize the temperature profile for 1 hr. The box is shaken every 20 min. The temperature of the calorimeter is recorded during the process of equalization. The following temperatures are used for the calculation:

T_o = initial temperature of the broiler carcasses
T_1 = temperature of the water in the calorimeter prior to equalization
T_2 = temperature in the calorimeter after equalization
T_g = coolant temperature

Since the carcass weight changes during immersion in water prior to cooling to obtain a uniform initial temperature, and also during cooling, they are weighed at different stages during the experiments:

Fig. 3—Immersion cooling with high heat transfer coefficient.

g_1 = wt after evisceration (kg)
g_2 = wt prior to cooling directly after 300 sec in a water bath of 40°C (kg)
g_3 = wt after cooling, before equalization in the calorimeter (kg)

The mean temperature inside the calorimeter is held approximately equal to laboratory temperature; in this case the heat leakage to the surroundings can be ignored.

The average specific heat of the calorimeters is determined separately and was found to be 1265 J/K.

The specific heat for broilers is 3350 J/kg K (Walters and May, 1963) and the specific heat for water 4186 J/kg K. The relative energy removal can be calculated with the following equations:

$$H_o = \left\{ 3350\, g_1 + 4186\, (g_2 - g_1) \right\} T_o$$

$$H_t = H_2 - H_1 - H_3$$

$$= \left\{ 3350\, g_1 + 4186\, (g_3 - g_1) \right\} T_2 - (10 \times 4186 + 1265)(T_1 - T_2)$$

$$- 4186\, (g_3 - g_2) T_g$$

where H_1 = enthalphy calorimeter before measurement; H_2 = enthalphy calorimeter and carcasses after equalization; and H_3 = enthalphy of water taken up during cooling. $H_\infty = 3350 g_1 + 4186 (g_2 - g_1) T_g$.

insulating material
x thermocouples

Fig. 2—Calorimeter.
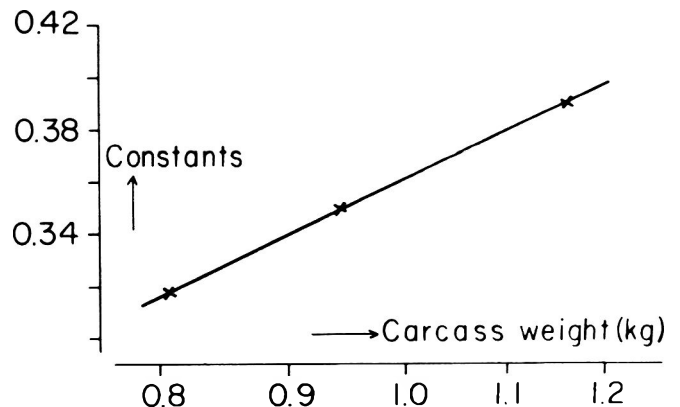
Fig. 4—The intersection of the $\Delta H/\Delta H_i$-axis of Eq 4a, 5a and 6a, as a function of carcass weight.
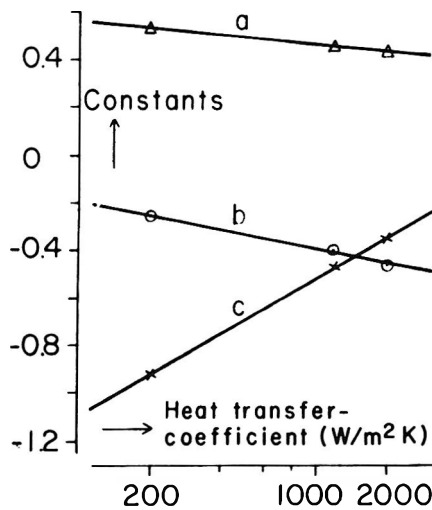
Fig. 5—Regression lines for the constants of Eq 8, 9 and 10.

Introducing these equations in Eq (2) gives the following result:

$$\frac{\Delta H}{\Delta H_i} = \frac{\{3350g_1 + 4186\,(g_2 - g_1)\}\,T_o - \left[\{3350g_1 + 4186\,(g_3 - g_1)\}\,T_2\right.}{\{3350g_1 + 4186\,(g_2 - g_1)\}\,(T_o - T_g)}$$

$$\left. - 43125\,(T_1 - T_2) - 4186\,(g_3 - g_2)\,T_g\right] \qquad (3)$$

The energy of the water taken up during cooling $4186\,(g_3 - g_2)\,T_g$ is subtracted from the estimated amount of energy.

The accuracy of the calorimetric method was determined separately. For an average value of $\Delta H/\Delta H_i = 0.712$, the standard deviation was 0.018 (n=8).

The cooling time was varied as a function of the weight and the cooling process, so that the relative energy removal varied from 0.40 to 0.95.

RESULTS & DISCUSSION

Immersion cooling

Data from experiments I (high heat-transfer immersion cooling) are presented in Figure 3. The mean values of three weight classes are plotted in this graph. It is possible to fit the results into three linear regression lines:

$$\frac{\Delta H}{\Delta H_i} = 0.432 \log t - 0.318 \text{ when } G = 0.807 \text{ kg} \qquad (4)$$

$$\frac{\Delta H}{\Delta H_i} = 0.451 \log t - 0.398 \text{ when } G = 0.942 \text{ kg} \qquad (5)$$

$$\frac{\Delta H}{\Delta H_i} = 0.412 \log t - 0.399 \text{ when } G = 1.158 \text{ kg} \qquad (6)$$

The slope of these lines can be averaged, giving 0.4317. Now, three new lines with this slope are produced by the average values of all measured data of $\Delta H/\Delta H_i$ and log t. Another approach is to take the average value of the intersections of the y-axis which gives finally a similar equation. The lines can be defined by the following formulae:

$$\frac{\Delta H}{\Delta H_i} = 0.432 \log t - 0.318 \qquad (4a)$$

$$\frac{\Delta H}{\Delta H_i} = 0.432 \log t - 0.350 \qquad (5a)$$

$$\frac{\Delta H}{\Delta H_i} = 0.432 \log t - 0.391 \qquad (6a)$$

The intersections of the y-axis (log t=0) for the three weight classes are plotted as a function of log G in Figure 4. These points can be fitted into the following linear regression line:

$$C = 0.464 \log G + 0.360 \qquad (7)$$

Finally it becomes clear that the relative energy removal in the immersion cooling process with a high degree of water mixing can be defined by the following equation:

$$\frac{\Delta H}{\Delta H_i} = 0.432 \log t - 0.464 \log G - 0.360 \qquad (8)$$

Results of experiments II (immersion cooling with a low degree of water mixing) are treated equally as those of experiments I. It was possible to arrange the figures in one formula which is valid for this process:

$$\frac{\Delta H}{\Delta H_i} = 0.456 \log t - 0.400 \log G - 0.478 \qquad (9)$$

Spray cooling

The spray cooling process can also be defined by one equation:

$$\frac{\Delta H}{\Delta H_i} = 0.523 \log t - 0.263 \log G - 0.922 \qquad (10)$$

Eq 8, 9 and 10 are all of the same pattern:

$$\frac{\Delta H}{\Delta H_i} = a \log t + b \log G + c \qquad (11)$$

The only difference among these three processes is the heat-transfer coefficient. Therefore, an attempt was made to find a correlation between the above calculated constants and an apparent heat transfer coefficient. The apparent heat-transfer coefficients are estimated by cooling experiments with a stainless steel sphere. The well-known nonsteady state heat-flow equations are used for calculating the heat transfer coefficient. In Figure 5 the constants a, b and c are plotted against the logarithm of the apparent heat transfer coefficient.

All the points of this graph can be fitted into three linear regression lines. These regression equations are substituted in Eq (11) which, after rearrangement, gives the following result:

$$\frac{\Delta H}{\Delta H_i} = (-0.09\log\alpha' + 0.73)\log t - (0.194\log\alpha' - 0.187)\log G$$

$$+ 0.564 \log\alpha' - 2.219 \qquad (12)$$

This equation makes it possible to calculate the relative energy removal for the cooling processes described in which the coolant used is water. The relative energy removal is only a function of the cooling time, the weight of the broiler carcasses and the apparent heat transfer coefficient.

Rearrangement of Eq (12) gives formulae for the cooling time and the apparent heat transfer coefficient:

$$t = 10^{\left[\dfrac{\frac{\Delta H}{\Delta H_i} + (0.194\,\log\alpha' - 0.187)\log G + 0.564\,\log\alpha' - 2.219}{-0.09\,\log\alpha' + 0.73}\right]} \qquad (13)$$

$$\alpha' = 10^{\left[\dfrac{\frac{\Delta H}{\Delta H_i} - 0.73\,\log t - 0.187\,\log G + 2.219}{-0.09\,\log t - 0.194\,\log G + 0.564}\right]} \qquad (14)$$

An experiment with a few broiler carcasses of the same weight is needed to determine the apparent heat transfer coefficient
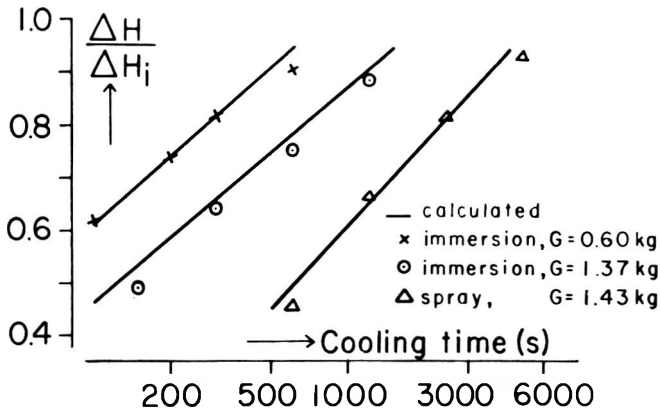
Fig. 6—Experimental and calculated cooling times.
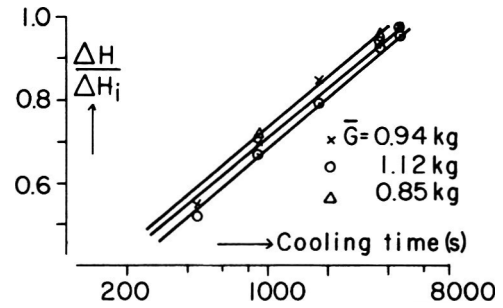
Fig. 7—Experimental results of air cooling.

of the cooling equipment. The relative energy removal is therefore measured after cooling for t sec. Eq (14) makes it possible to calculate α'. All other calculations can be made after changing the weight of the carcasses, the cooling time or the relative energy removal. The results of calculations with equation (12) are compared with the results of experiments using immersion cooling with a high heat transfer coefficient and spray cooling. The weight classes for these experiments are carcasses of about 0.600 and 1.400 kg. The experimental data and the lines calculated for the energy removal are presented in Figure 6. They show a very good agreement.

In Table 1 the calculated apparent heat transfer coefficients of cooling turkey fryers are given. The deviation of the average value is smaller than 17% so it seems reasonable to describe this cooling process with the given equations. The conclusion so far is that it is a simple matter to calculate the cooling times when water is used as coolant.

Air cooling

The experimental data for cooling in a high velocity air stream are given in Figure 7. As in the case of water cooling processes, it is possible to fit these data into a formula defining this cooling process:

$$\frac{\Delta H}{\Delta H_i} = 0.417 \log t - 0.402 \log G - 0.549 \qquad (15)$$

The constants in this equation do not agree with those given in Figure 5 using one value for the apparent heat transfer coefficient. Comparing the air-cooling process with the other experimental processes, calculations with Eq (14) are executed.

Table 2 presents the apparent heat transfer coefficients cal-

culated with Eq (14) for the air chilling experiments. These values are not equal and even very high. The value of the apparent transfer coefficient that agrees with the velocity of the air (5.5 m/sec) is 40 W/m²K, which is much smaller than the calculated value. The high values of the heat transfer coefficients calculated can be explained by evaporation of water, especially at the start of the cooling process. The evaporation during this process was measured and is shown by equation:

$$X_t = 8.1 \log t - 11.5 \text{ g/carcass} \qquad (16)$$

where X_t is the amount of water evaporated in t sec.

The high evaporation rate is due to immersion in 40°C water prior to the cooling process, and the high air speed. As a result, the evaporation and hence the cooling rate is very high compared with normal air chilling processes.

In Figure 8 the cooling time calculated with the empirical Eq (15) is compared with values calculated according to Eq (13) for one weight class of broiler carcasses of 1 kg. The experimental values are not equal for all values of the relative energy removal either for an apparent heat transfer coefficient of 200 J/m² s K or one of 325.

The conclusion of the last experiment is that the air chilling process cannot be calculated using Eq (12), (13) and (14). More experimental work is needed to extend the model Eq (12) to air cooling.

CONCLUSIONS

THE EXPERIMENTAL cooling processes for broiler carcasses, using water as coolant, can be all described with a single equa-

Table 1—Calculations of the apparent heat transfer coefficient with use of Eq (14) for cooling turkey fryers

Cooling time (S)	Weight (kg)	$\frac{\Delta H}{\Delta H_i}$	Calculated apparent heat transfer coefficient (W/m² K)	Relative deviation of mean value %
100	1486	0.450	2188	−10.0
200	1476	0.577	2133	−12.4
300	1506	0.669	2480	+1.8
600	1520	0.795	2483	+2.0
800	1518	0.840	2313	−1.9
1000	1493	0.898	2598	+6.7
1200	1490	0.942	2847	+16.9

Table 2— Apparent heat transfer coefficient (J/m² s K) for air chilling according to Eq (14)

Cooling time (s)	450	900	1800	3600	5400
Carcass wt (kg)					
0.848		332		208	
0.940	319	312	309	188	110
1.120	318	311	241	247	107

Fig. 8—Cooling lines calculated for carcasses in air.

tion, Eq (12). Changing the weight of carcasses, Eq (12) is verified for other conditions than those from which it was derived. A good agreement between theory and experimental results was found.

The energy removal and hence the average temperature and the surface temperature during the cooling processes of broiler carcasses can be calculated. Only a few experiments are needed for estimating the apparent heat transfer coefficient of the cooling process. The process using air as the cooling medium cannot be calculated with Eq (12) as the evaporation of water makes it more complicated. More experimental work is needed to adapt Eq (12) to this process.

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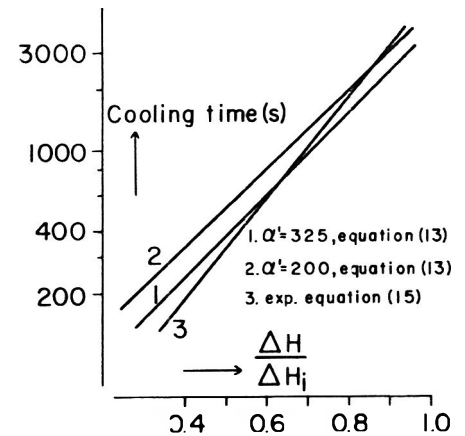
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EVALUATION OF SUCCINIC ACID AND HEAT TO IMPROVE THE MICROBIOLOGICAL QUALITY OF POULTRY MEAT

INTRODUCTION

THE USE OF heat to pasteurize raw poultry surfaces was explored by Dawson et al. (1963). They reported that water at temperatures higher than 60°C effectively reduced numbers of bacteria on skin but caused discoloration due to partial cooking. Klose et al. (1971) using steam at subatmospheric pressures, attained reduction by a factor of 1000–5000 times in surface bacterial loads on whole ready-to-cook carcasses with temperatures not exceeding 75°C for 4 min. Cox et al. (1974) concluded that batch immersion of whole broiler carcasses in hot water (up to 71.1°C) for 1–5 min reduced initial count but did not greatly increase shelf life. They also reported that the appearance of the carcasses immersed at 71.1°C was greatly impaired.

Decreased heat resistance of certain bacteria, particularly salmonellae, at low pH has been reported and successfully demonstrated with liquid eggs (Lategan and Vaughn, 1964). Reducing pH with organic acids, has been shown to inhibit bacteria on poultry surfaces and to extend shelf life (Perry et al., 1964; Murphy and Murphy, 1962). Mountney and O'Malley (1965) demonstrated that the degree of bacterial inhibition with acids does not depend solely upon low pH but also on the particular acid used. These investigators determined the effect of immersing cut-up poultry parts for 2 hr in 2°C solutions of various organic acids adjusted to pH 2.5. Of the 10 acids tested, they concluded that the most promising, in terms of bacterial inhibition and flavor acceptability, were adipic and succinic. Of these, they suggested that succinic would be the more suitable for possible commercial application because of its lower cost per pound and the smaller amount needed to obtain a pH of 2.5.

Consequently, the present study was undertaken to determine the effect of succinic acid and heat treatments of raw poultry parts on initial bacterial count, shelf life, appearance and spoilage organisms. As of this writing succinic acid is classified as a GRAS substance, but is presently undergoing re-evaluation (Federal Register, 1973).

EXPERIMENTAL

Initial count reduction

For each treatment, freshly processed whole eviscerated broiler carcasses were purchased from a commercial poultry processor. Left and right legs of the same bird were determined to have no significant difference in bacteria count before treatment. Therefore, to establish bacterial concentrations, right legs were used before treatment, and left legs were treated then used after treatment. The left leg (i.e., thigh and drumstick) of each of four broiler carcasses was removed and placed on aluminum foil with the inner surface of the thigh downward. This piece was obtained by a cut through the junction of the thigh muscles with the pelvic girdle to the hip joints disjointing the femur. The loin or "oyster" muscle was left on the back.

Using a sterile template, a 12.3 cm² area of skin on the right leg remaining on the carcass was swabbed for 30 sec using a calcium alginate swab. A 1% sodium citrate solution was used for making serial dilutions. A total aerobic plate count was made on these right legs to establish the initial bacterial load on the skin surface prior to treatment. The medium was standard methods agar (BBL) and the plates were incubated for 72 hr at 20°C.

The left legs which had been previously excised were then immersed, four at a time, into an unheated (24°C) or heated (60°C), 0, 1, 3 or 5% succinic acid solution for either 1 or 3 min. The pH's of the 1, 3 and 5% acid solutions were 2.7, 2.4 and 2.3, respectively. (Stock solutions of these concentrations maintained their respective pH's for a minimum of 6 wk). Immediately after immersion, the four legs were dipped into a second container of either 24°C or 60°C tap water for 1 min. A 12.3 cm² area of each of these legs was then swabbed to determine bacterial density on the skin after treatment. The ratio of the weight of the legs to liquid volume in both steps of the treatment was approximately 700g to 3500 ml.

The two-step procedure of (a) immersing four legs into the solution in the first beaker, immediately followed by (b) immersion in water in the second beaker, constituted a treatment. Each of the 32 treatments (resulting essentially from a 4 × 2 × 2 × 2 factorial design) was evaluated twice. For each treatment, the difference between the mean log of the number of bacteria per cm² of the treated leg and that of the corresponding untreated leg was determined. These differences (log reductions) were then subjected to analysis of variance to ascertain treatment effects.

Appearance

A second group of legs from freshly proc-

essed broilers were subjected to all the treatments previously described. Immediately after each treatment the appearance of each of the legs was evaluated by two experienced judges. Appearance was described as "good," "fair" or "poor." Specific descriptive comments regarding appearance were also made.

Shelf life determination

A third group of legs from freshly processed broilers were subjected to all the acid treatments previously described. After treatment, each leg was placed individually in a polyethylene bag and stored at 1.1°C for the first 24 hr, then transferred to a 4.4°C room.

Eight legs were used for each succinic acid treatment and for each control. Controls were handled exactly the same as the acid treatments, the only difference being that water (of the same temperature) was used instead of succinic acid solution in the first beaker.

On the fifth day of storage, and every day thereafter, three experienced judges made independent determinations as to whether or not each leg exhibited spoilage odor. A leg was judged to be spoiled only when at least two of the three judges agreed that spoilage odor was evident. The experiment was terminated when all samples spoiled.

Spoilage organisms

Legs of freshly processed birds were immersed four at a time, for 3 min into 3500 ml of 3% succinic acid at 60°C, then immersed in 24°C tap water for 1 min. Then individual legs were aseptically placed into polyethylene bags (18 oz) and stored at 4.4°C. For control samples, water at 60°C was used instead of succinic acid. A total of 16 legs were used: eight acid treated and eight were controls.

For bacteriological examination after spoilage odors developed, 100 ml of sterile phosphate-buffered diluent (0.3 mM PO⁴; pH 7.2) (Butterfield, 1932) was poured into the polyethylene bag and the contents shaken thoroughly for 1 min. Then 0.1-ml aliquots of this suspension, which had been serially diluted to 10⁻⁶, were spread on plates of brain heart infusion agar (Difco). The plates were incubated at 4.4°C for 14 days. All colonies (up to 10) from the highest dilution showing growth were isolated (Barnes and Thornley, 1962). A statistical randomizing procedure employing a numbered grid was used for selecting colonies. Table 1 shows some of the tests that were used to differentiate the cultures that were isolated from the spoiled legs. In addition to those shown in the table, gram stain and motility (hanging drop), were also done on all cultures. The identification procedure was similar to that prescribed by Thornley and Barnes, 1962.

Table 1—Microbiological tests used to differentiate gram-negative bacteria isolated from spoiled poultry

Test	Reference
Mode of attack on glucose	Hugh and Leifson (1953)
Hydrolysis of arginine	Thornley (1960)
Oxidase activity	Kovacs (1956)
Sensitivity to penicillin and oxytetracycline	Shewan et al. (1954)
Fluorescence	King et al. (1954)
Flagella stain	Rhodes (1958)

Table 3—Reduction in microbial counts on broiler thigh skin surfaces by succinic acid treatments (60°C)

Succinic acid (1st step)		Water (2nd step)		
% Succinic acid	Duration of dip (min)	24°C 1 min	60°C 1 min	Mean
0	1	0.14[a]	0.60	
	3	0.54	0.14	0.36
1	1	0.46	0.52	
	3	0.52	1.04	0.64
3	1	0.87	0.85	
	3	1.61	1.13	1.12
5	1	0.74	1.18	
	3	0.93	1.98	1.21

[a] Each value is the mean log reduction of bacteria/cm^2 of skin surface of eight legs (4 X 2 reps) after treatment as compared to mean log bacterial count of legs prior to treatment. A difference of 0.43 or greater between log reduction values is significant at the 5% level.

Table 2—Reductions in microbial counts on broiler thigh skin surfaces by succinic acid treatments (24°C)

Succinic acid (1st step)		Water (2nd step)		
% Succinic acid	Duration of dip (min)	24°C 1 min	60°C 1 min	Mean
0	1	0.07[a]	0.01	
	3	0.03	0.33	0.11
1	1	0.34	0.69	
	3	0.40	0.68	0.53
3	1	0.32	0.35	
	3	0.49	0.50	0.42
5	1	0.65	0.60	
	3	0.71	1.16	0.78

[a] Each value is the mean log reduction of bacteria/cm^2 of skin surface of eight legs (4 X 2 reps) after treatment as compared to mean log bacterial count of legs prior to treatment. A difference of 0.43 or greater between log reduction values is significant at the 5% level.

Table 4—Storage life at 4°C of raw chicken legs treated with succinic acid (24°C)

1st Step	2nd Step	Extension of storage life (days)
1% Succinic acid (3 min)	24°C Water (1 min)	1.5[a]
1% Succinic acid (3 min)	60°C Water (1 min)	1.0
3% Succinic acid (3 min)	24°C Water (1 min)	2.0
3% Succinic acid (3 min)	60°C Water (1 min)	3.5
5% Succinic acid (3 min)	24°C Water (1 min)	4.5
5% Succinic acid (3 min)	60°C Water (1 min)	5.0

[a] Number of additional days when compared with water treatments (control). Controls here were 24°C water for 3 min followed by either 24° or 60°C water for 1 min.

RESULTS & DISCUSSION

Initial count reduction

The mean log reductions in numbers of bacteria per cm^2 skin surface as a result of each of the treatments are presented in Tables 2 and 3. The standard error of the difference between any two log reduction means was 0.17, so that a difference between any two treatment means of 0.43 log was considered significant.

Exposure of the legs to unheated succinic acid, followed by a 1-min dip in 24°C water, was only slightly more effective than water in reducing initial surface bacterial counts. Significant reductions of greater than 0.5 log were attained only when legs were exposed for 1 or 3 min in the unheated 5% succinic acid solution. The degree of bacterial destruction increased slightly when exposure to the unheated succinic was followed by the 60°C water dip, but the differences were not significant.

Immersion of legs in the heated (60°C) succinic acid without subsequent 60°C hot water treatment reduced bacterial counts more than the corresponding 24°C succinic acid treatments, but the difference was statistically significant only with the 3% succinic acid treatments.

Analysis of variance of the log reductions indicated that the effectiveness of succinic acid treatment in destroying bacteria on the skin was greater (a) with heated than with unheated succinic, (b) after exposure for 3 rather than exposure for 1 min, (c) when the acid treatment was followed by a 60°C rather than a 24°C water dip, and (d) as the concentration of the succinic acid was increased. When unheated succinic acid was used, the mean log reduction for the 1, 3 and 5% solutions were 0.53, 0.42 and 0.78, respectively, whereas when heated succinic acid was used the mean log reductions were 0.64, 1.12 and 1.21, respectively.

Effects of the acid depended on its concentration, temperature, duration of the dip and the temperature of the second dip.

Appearance

Legs receiving any of the heated acid treatments received scores of "poor," while those receiving any of the other treatments (unheated acid and water controls) were scored "good" or "fair."

After the 3% and 5% unheated acid treatments, there was a slight whitening (greyish appearance) of the cut edges of muscle and a slight loss of yellow skin color.

Heated acid treatments caused a cooked appearance and loss of yellow skin color. Deterioration of appearance increased with acid concentration.

Shelf life determination

Unheated 1% succinic acid treatments increased shelf life of chicken legs 1–2 days over that of unheated water control treatments (Table 4). The 3% succinic acid gave 2–4 days extra shelf life and the 5% treatments, 4–5 days. With unheated acid treatments, the shelf life increased with the concentration of succinic acid.

With heated succinic acid treatments,

Table 5—Storage life at 4°C of raw chicken legs treated with succinic acid (60°C)

1st Step	2nd Step	Extension of storage life (days)
1% Succinic acid (3 min)	24°C Water (1 min)	0.0[a]
1% Succinic acid (3 min)	60°C Water (1 min)	0.0
3% Succinic acid (3 min)	24°C Water (1 min)	3.0
3% Succinic acid (3 min)	60°C Water (1 min)	6.5
5% Succinic acid (3 min)	24°C Water (1 min)	4.5
5% Succinic acid (3 min)	60°C Water (1 min)	4.5

[a] Number of additional days when compared with water treatments (control). Controls here were 60°C water for 3 min followed by either 24° or 60°C water for 1 min.

Table 6—Percentages of total isolates at time of spoilage

Organism	Untreated	Acid treated
Pigmented pseudomonas	44	69
Nonpigmented pseudomonas	34	15
Aeromonas	6	9
Achromobacter	5	1
Enterobacteriaceae	1	1
Unclassified	10	5

the 1% samples spoiled at about the same time as the heated water control treatments (Table 5). The 3% samples had from 3–6 days extra shelf life and the 5% samples about 4–5 days. The 5% heated acid samples underwent gross appearance and odor changes.

Spoilage organisms

In shelf life studies the character of spoilage odors of acid-treated samples was occasionally quite different from that of normal spoilage. We thought, therefore, that the normally predominant spoilage organisms might be more sensitive to the hot succinic acid treatment than other organisms present. However, the percentages of the different genera found on both the untreated and acid-treated legs at the time of spoilage were very similar (Table 6). Data suggested, however that nonpigmented pseudomonads may be more susceptible to the succinic acid than pigmented strains.

Pseudomonas was the predominant genus at the time of spoilage on both the untreated (78%) and acid-treated (84%) samples. These findings are very similar to those of Barnes and Thornley (1962) who reported that 71% of the spoilage flora of eviscerated chickens consisted of pigmented and nonpigmented strains of *Pseudomonas*. Ayres et al. (1950), Barnes and Shrimpton (1958) and Nagel et al.

(1960) also found that strains of pigmented and nonpigmented *Pseudomonas* were the main spoilage organisms of eviscerated poultry.

Results of these studies substantiate the findings of Mountney and O'Malley (1965) that succinic acid effectively destroyed bacteria on poultry skin surfaces and extended shelf life. Our findings further indicate that such benefits may be derived by an exposure time much shorter than the 2 hr used by the above investigators by increasing the concentration and temperature of the acid solution. To obtain a meaningful reduction in initial bacterial count and extension of shelf life, exposure to a hot 3% succinic acid solution for 3 min may be practical. Although appearance of legs receiving this treatment was judged to be "poor," the basis of the judgment was only some slight whitening or bleaching of the skin, which was not evident in the controls, but which was substantially less pronounced than that of parts exposed to the hot 5% acid solution. Although no evaluations were made of the flavor of the cooked-treated products, Mountney and O'Malley had indicated that washing prior to cooking of broilers immersed for 2 hr in 2°C acid solutions removed most of the acid taste. We assumed that this would also apply to these treated legs,

but recognize that this would need to be confirmed by appropriate sensory tests before such a treatment would be commercially acceptable. We conclude that treatment with a 3% or 5% succinic acid solution at 60°C is effective for reducing microbial contamination of raw poultry carcasses, but appearance is impaired.

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SIMPLIFIED METHODOLOGY FOR MEASURING MEAT COLOR

INTRODUCTION

THE COLOR of fresh meat is determined mainly by the relative proportions of three meat pigments: purple reduced myoglobin (Mb), red oxymyoglobin (MbO_2) and brown metmyoglobin (MetMb). Under normal marketing conditions reduced myoglobin in the presence of O_2 is completely changed to MbO_2 within a few hours (Van den Oord and Wesdorp, 1971a). The bright red of the MbO_2 is the preferred color of fresh beef packaged for retail sales. The change of MbO_2 to MetMb can be effected by bacterial growth or oxygen tension levels, resulting in slow oxidation of the heme iron to its ferric state. As this change occurs the meat becomes less acceptable to consumers. The relative proportions of MbO_2 and MetMb are a prime factor in the acceptability of fresh beef color to consumers. At approximately 50% conversion to MetMb the meat is unacceptable to most consumers and therefore unsuitable for retail sale (Van den Oord and Wesdorp, 1971a).

The preferred method for measuring consumer acceptability is color evaluation by a panel of trained observers This method has several serious disadvantages for continuous evaluation of meat color changes. Panel measurements are time consuming, prone to subjective errors and limited in the number of evaluations which can be made at one time.

Reflectance measurement is the instrumental technique of choice because it measures the color on the surface of the meat as observed by the consumer and it is nondestructive. Two types of instruments can be used in reflectance studies: colorimeters and spectrophotometers with reflectance attachments. Previous investigators have employed a variety of techniques for meat studies. Haas and Bratzler (1965) used Munsell disk colorimetry and a Gardner color difference meter to follow oxygenation rates in beef, pork and lamb. Snyder (1965) used the Gardner R_d, a, b, and a/b values to indicate changes taking place in intact beef samples. The "R_d" value is a measure of the total light reflected while "+a" is a measure of redness, "−a" is a measure of greenness, "+b" is a measure of yellowness and "−b" is a measure of blueness in the reflected light. He found that a high a/b value indicated a high concentration on the surface of the meat of either Mb or MbO_2, while a low a/b value indicated a high concentration of MetMb. Romans et al. (1965) attempted correlation of Munsell disk colorimetry values with myoglobin concentration but found low correlation coefficients (r = −0.5). Jeremiah et al. (1972) related color difference values to consumer acceptability of beef color. Their study was designed to measure the preferred intensity of color from MbO_2. They correlated the values from the Macbeth-Munsell disk colorimeter, the Gardner color difference meter, and the Photovolt-610 reflectance meter. Dean and Ball (1960) used the Gardner a_L value as a measure of redness or bloom in beef.

Other reflectance methods which could be used for estimating consumer acceptability of beef color measure the concentration of the myoglobin pigments with a reflectance attachment to a spectrophotometer. Dean and Ball (1960) calculated % Mb, % MbO_2 and % MetMb by using ratios of K/S values, the ratio of absorption coefficient over the scattering coefficient (Judd, 1952) of % reflectances at 507 and 573 and at 473 and 597 nm. The wavelengths of 507 and 573 nm were chosen, since Broumand et al. (1958) found these to be absorp-

tion isobestic points for MbO_2 and MetMb. Snyder (1965) used R_a values (reflectance value on a log scale corresponding to absorbance, used in transmittance data) from the reflectance spectra after R_a had been adjusted to 1.0 at 525 nm (an isobestic point for the three forms of myoglobin). He measured concentration of the pigments by plotting R_a at 473 nm for MetMb and MbO_2 and at 571 nm for Mb and MbO_2 and from these plots he calculated the concentration of the individual pigments. Standard curves for Mb, MbO_2 and MetMb were made from suspensions of the myoglobin derivatives in nonfat milk. Snyder and Armstrong (1967) found that with suspensions of MetMb or MbO_2 in nonfat milk, the K/S ratio at a single wavelength (571 nm) was sufficient for accurate measurement of MetMb. However, the authors stressed that for intact meat, ratios of K/S values such as proposed by Snyder (1965) were preferable. Stewart et al. (1965) used the K/S ratio at 525 nm to give total pigment concentration. Franke and Solberg (1971) found that % MetMb could be measured using: K/S 572 nm/K/S 525 nm, R_a 572 nm/R_a 525 nm, or a value called ΔR_a 632 nm. The ΔR_a 632 nm was found by setting the instrument at a constant value for 750 nm and subtracting the R_a 632 nm from the constant R_a 750 nm. Van den Oord and Wesdorp (1971b) stated that the myoglobin concentration and the relative proportions of oxy- and metmyoglobin are determined by using the difference R_a 630 nm − R_a 580 nm. These two wavelengths correspond to the minimums of met- and oxymyoglobin respectively. The above papers have established a linear relationship between reflectance measurements and concentration of the various myoglobin pigments. Van den Oord and Wesdorp (1971a) established a relationship between consumer acceptability of color, as related to aging of beef, and R_a 630 nm − R_a 580 nm.

We have investigated the relationships between beef color quality as judged by a trained panel and various types of reflectance measurements. The instruments used to make the measurements were color difference meters and reflectance spectrophotometers. Linear correlation coefficients were obtained for the various parameters measured versus the panel ratings (hedonic scale) as well as the correlation coefficients between the different parameters. The results from this study enable one to select a quick and accurate method for replacing a hedonic evaluation of color in storage studies on beef.

EXPERIMENTAL

Procedure

Eye-round roasts (USDA Choice semitendinosus muscles) obtained from commercial sources were used in the experiments. All equipment was scrubbed with a 3% hexachlorophene solution and rinsed with 70% ethanol to minimize bacterially produced discoloration of the meat samples. The eye-rounds were carefully trimmed to remove all fat and to expose fresh surfaces. Samples, approximately 1 × 1 × 2 in., were sliced across the grain from the trimmed roasts. One sample from each run (oxidized control) was sprayed with 1 ml of 1/2% $K_3Fe(CN)_6$ solution. All samples were wrapped in an oxygen permeable commercial wrap (PVC stretch film MC-FMC Corp.). The samples were packaged to approximate commercial supermarket methods, i.e., close contact with the meat surface. The samples were allowed to bloom at 4°C in the dark for 3 hr before initial hedonic evaluation. The samples were displayed on a white background over an ice bed. Lighting for hedonic appraisal was G.E. Cool White and illumination was an average of 80

ft-c at the meat surface. The color difference meter readings and the reflectance spectrophotometer readings were made immediately after the panel evaluations. A 10-member panel was selected to judge these samples on the basis of color quality. A scale of 0 to 50 was used with 50 being extremely acceptable, 30 being marginally acceptable and 0 being totally unacceptable. Each sample was rated by the panel and these ratings (H values) were compared with values obtained with a color difference meter and a reflectance spectrophotometer. Such procedures were carried out each day for a period of from 5 to 8 days. After this length of time most samples had unacceptable color.

Apparatus

A Beckman D.U. Model 2400 spectrophotometer with an integrating sphere reflectance attachment was used to make reflectance measurements at 520, 540, 560, 580, 600, 620, 630, 640, 650 and 700 nm. A magnesium carbonate block wrapped with the same packaging material as the samples was the reference standard. Color difference measurements were made by a Gardner color difference meter which was balanced using a standard tomato red ceramic plate: R_d 6.1, "a" 32.4, "b" 14.8. A clear glass optical flat was placed between the wrapped meat sample and the source (over the sample port) to provide a flat meat surface for the measurements.

Calculations

Method 1. The linear correlation coefficient for the Gardner "a" value versus the panel or hedonic (H) evaluation was calculated. Since linear correlation coefficients are not normalized functions, their confidence limits cannot be calculated in the normal manner. Confidence limits on the linear correlation coefficient were established by the following type of calculation (Steel and Torrie, 1960):

r = linear correlation coefficient, an estimate of ρ the population correlation coefficient;

Z = transformed r, Z is approximately normal with mean 0.5 ln [(1 + r)/(1 − r)] and standard deviation $1/\sqrt{N-3}$;

N = number of data points; and

$t_{.05}$ = 1.96 for a normally distributed function:

$$Z = 1/2 \ln [(1+r)/(1-r)]$$

$$Z^- = Z - \frac{1.96}{\sqrt{N-3}}$$

$$Z^+ = Z + \frac{1.96}{\sqrt{N-3}}$$

The 95% confidence limits for ρ are:

$$\frac{e^{2Z^-} - 1}{e^{2Z^-} + 1} \leqslant \rho \leqslant \frac{e^{2Z^+} - 1}{e^{2Z^+} + 1}$$

Linear combinations of "a," "R_d" and "b" versus H were also calculated but no significant improvement in fit was noted over the fit with "a" alone. Higher order polynomial fits of "a" versus H were calculated but the advantage of the slightly better fit obtained with a fifth degree polynomial in "a" over a simple correlation was outweighed by the complexity of the calculation necessary.

Method 2. Linear correlation coefficients for %R values versus H or "a" at selected wavelengths and the linear correlation coefficients' 95% confidence limits were calculated as described in Method 1. The wavelengths selected were 580 nm, which is a maximum for MbO_2; 630 nm, which is a maximum for MetMb; and 700 nm, which is independent of H. Linear correlation coefficients and their 95% confidence limits were also calculated for combinations (differences) of the selected wavelengths. The difference in two population values of r was tested by the following variation of a "large sample normal test" (Steel and Torrie, 1960). The example used to illustrate this test is r_1 for "a" versus H and r_2 for H versus %R 630 − %R 580.

N = number of data points;
r = linear correlation coefficient;
Z = transformed r:

	N	r	Z	$\frac{1}{N-3}$
H vs. "a"	629	.910	1.53	.0016
H vs. %R 630 − %R 580	277	.862	130	.0036

$$z = \frac{|Z_1 - Z_2|}{\sqrt{\frac{1}{N_1 - 3} + \frac{1}{N_2 - 3}}}$$

where z is normally distributed with a mean of zero and a standard deviation of 1. P, the probability that $\rho_1 = \rho_2$, is calculated by

$$P = 2 \int_z^\infty \frac{1}{\sqrt{2\pi}} e^{-X^2/2} dX$$

For this example P = 0.001, and it is safe to assume $\rho_1 > \rho_2$.

Method 3. Linear correlation coefficients for the logarithmic transformations of the %R at the selected wavelengths versus H or "a" and their 95% confidence limits were calculated as described in Method 1. Combinations (differences) of the selected wavelengths are expressed as logarithms of the ratios of the wavelengths. Comparisons were made among the linear correlation coefficients found using the different methods described in the manner shown in Method 2.

RESULTS & DISCUSSION

FIGURE 1 represents the reflectance spectra in terms of %R of three forms of myoglobin. Myoglobin itself is unstable in air and readily oxygenated to the oxymyoglobin form. The reflectance spectrum of oxymyoglobin has minimums between 540 nm and 580 nm and high reflectance in the 600−700 nm region, while the reflectance spectrum of metmyoglobin has increased reflectance in the 540−580 nm or yellow region and decreased reflectance in the red region, particularly at 630 nm. Meat surfaces which contain a high percentage of the oxymyoglobin form of the pigment are bright red in color while those surfaces which contain most of their pigment in the metmyoglobin form are brown or dark yellow in color.

Since the proportion of red color present on the surface of the meat was the criterion used for evaluating the acceptability of the meat color to consumers, the Gardner color difference
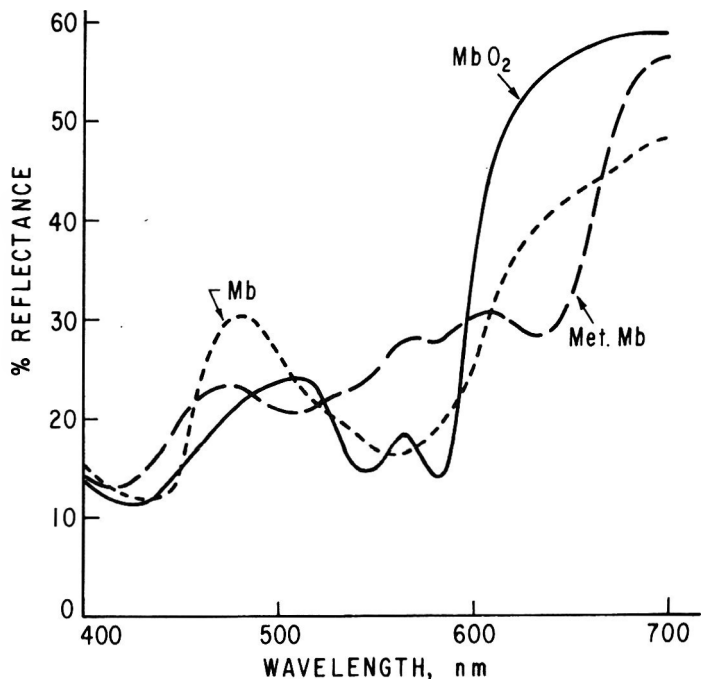
Fig. 1—Reflectance spectra of Mb, MbO_2 and MetMb in terms of % reflectance.

Table 1—Correlation of H[a] with reflectance at selected wavelengths

Source of reflectance values	Linear correlation coefficient	95% Confidence limits	
%R 580 nm	−0.66	−0.59,	−0.72
%R 630 nm	0.78	0.72,	0.82
%R 700 nm	0.21[b]	0.10,	0.32
%R 700 nm − %R 580 nm	0.57	0.48,	0.64
%R 700 nm − %R 630 nm	−0.64	−0.57,	−0.71
%R 630 nm − %R 580 nm	0.86	0.83,	0.89
2-1n %R 580 nm	0.70	0.63,	0.75
2-1n %R 630 nm	−0.76	−0.71	−0.81
2-1n %R 700 nm	−0.14[b]	−0.02,	−0.25
1n (%R 580 nm/%R 700 nm)	−0.69	−0.62,	−0.74
1n (%R 630 nm/%R 700 nm)	0.64	0.57,	0.71
1n (%R 580 nm/%R 630 nm)	−0.86	−0.83,	−0.89

[a] Consumer acceptability of color
[b] Not significant at 99% confidence level. N for all samples is 277.

Table 2—Correlation of "a"[a] with reflectance at selected wavelengths

Source of reflectance values	Linear correlation coefficient	95% Confidence limits	
%R 580 nm	−0.71	−0.65,	−0.76
%R 630 nm	0.78	0.73,	0.83
%R 700 nm	0.24[b]	0.12,	0.34
%R 700 nm − %R 630 nm	−0.64	−0.56,	−0.70
%R 630 nm − %R 580 nm	0.89	0.86,	0.91
2-1n %R 580 nm	0.73	0.67,	0.78
2-1n %R 630 nm	−0.78	−0.73,	−0.82
2-1n %R 700 nm	−0.14[b]	−0.02,	−0.26
1n (%R 580 nm/%R 700 nm)	−0.72	−0.66,	−0.77
1n (%R 630 nm/%R 700 nm)	0.66	0.59,	0.72
1n (%R 580 nm/%R 630 nm)	−0.90	−0.88,	−0.92

[a] From the Gardner Color Difference Meter
[b] Not significant at the 99% level. N for all samples is 277.

meter value "a," which is a mathematical representation involving a weighted integration of the reflectance primarily from the red region of the visible spectrum, was correlated with the hedonic (H) or panel score. The linear correlation coefficient (r) found for "a" versus H (hedonic score) is 0.91 with 95% confidence limits of (0.90, 0.92).

The reflectance spectra were determined using an integrating sphere reflectance instrument. The reflectance at three wavelengths 580 nm (a minimum for MbO_2), 630 nm (a minimum for MetMb) and 700 nm (highest reflectance found for the meat samples in the visible region of the spectrum) were selected for correlation with H.

Table 1 is a compilation of the linear correlation coefficients and their 95% confidence limits for the selected wavelengths versus the hedonic (H). Linear correlation coefficients were evaluated for both %R and 2−1n %R at the selected wavelengths versus H. The 2−1n %R values were included in the analysis of data because instrumentation in spectrophotometers often is designed to permit greater precision in reading absorbance or 2 − log %R values. Logarithmic transformations permit small variations in straight line fits to be eliminated. Although the use of natural instead of common logarithms, as employed here, will change the slope of the line, the linear correlation coefficients are not affected.

The extremely low linear correlation coefficients for H versus reflectance at 700 nm indicates that the reflectance at 700 nm is independent of both MbO_2 and MetMb concentrations. With a plot of H versus reflectance at 580 nm, the decrease in MbO_2 concentration is apparent. Similarly a plot of H versus reflectance at 630 nm shows an increase in MetMb concentration. However, linear correlation coefficients for both %R and for 2−1n %R at both 580 and 630 nm versus the hedonic are significantly lower than the linear correlation coefficient found for "a" versus H. The low values for these correlation coefficients indicate that a combination of wavelengths or other correction is needed.

Comparison of the linear correlation coefficients for the reflectance, either % or 1n%, at 630 and 580 nm versus H with the linear correlation coefficients for the differences in reflectance for either the %R or 1n %R, of 700 nm − 580 nm and

Fig. 2—Gardner Color Difference meter "a" vs. hedonic H.
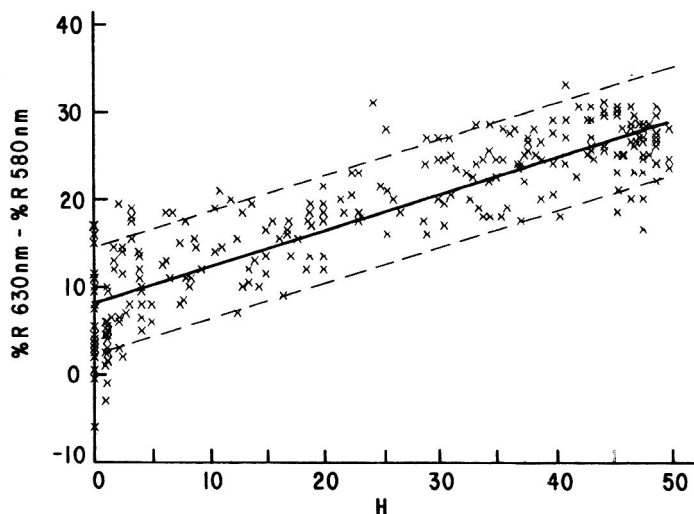
Fig. 3—Hedonic H vs. %R 630 nm − %R 580 nm.
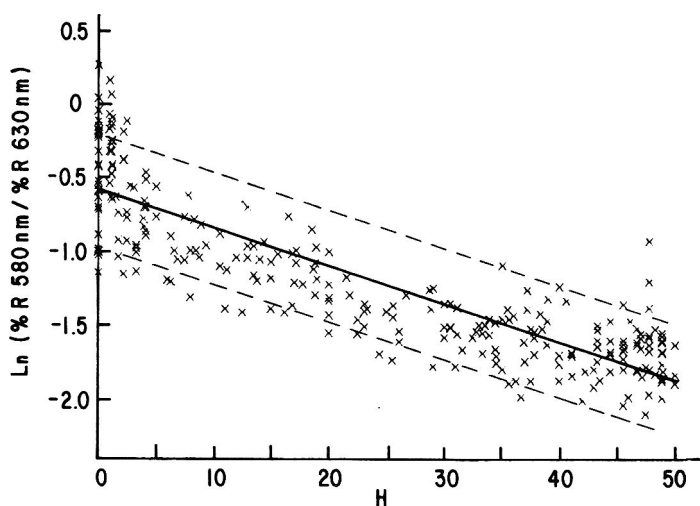
Fig. 4—Hedonic H vs. 1n (%R 580 nm/%R 630 nm).

700 nm − 630 nm, shows no improvement in a straight line fit with H. However, the linear correlation coefficients for the differences involving the wavelengths 630 nm and 580 nm show improvement in straight line fits with the hedonic score. This type of combination takes into account both the appearance of MetMb and the disappearance of MbO_2.

Figures 2, 3 and 4, respectively, show the graphs of "a" versus H, H versus %R 630 nm − %R 580 nm, and H versus 1n (%R 580 nm/%R 630 nm). Visual analysis of the graph of "a" versus H shows clustering of points about the values of 0 and 50 on the hedonic scale. This clustering is caused by boundary effects. The H value is not as elastic as the "a" value in describing either extreme in meat quality. This effect can be noted in all the graphs where the H value is one of the coordinates. A 5th degree polynomial fit of "a" with H was found. The curve given by this polynomial was sigmoid in shape with flattening about 0 and 50 of H. Since we have assumed that this flattening is caused by boundary effects in H, a straight line fit should give a more accurate measure of the color of the meat.
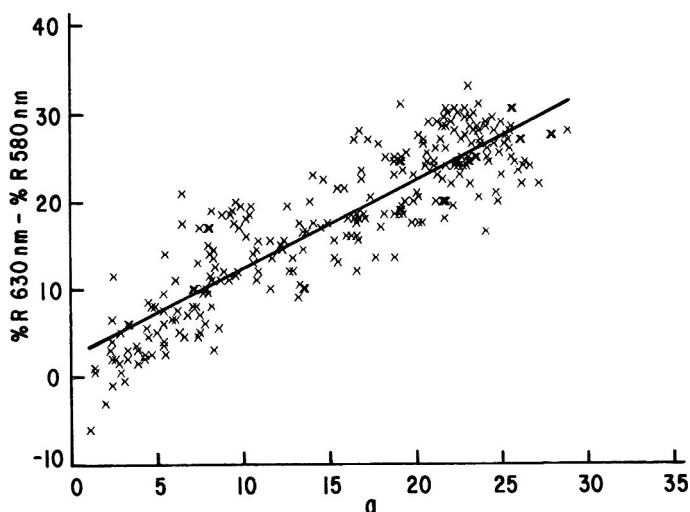
To check this hypothesis, comparisons of the two instrumental methods were made. The linear correlation coefficients found are listed in Table 2. The P value for the comparison of H versus %R 630 nm − %R 580 nm with "a" versus %R 630 nm − %R 580 nm is 0.156 and the P value for the comparison H versus 1n (%R 580 nm/%R 630 nm) with "a" versus 1n (%R 580 nm/%R 630 nm) is .036. These P values demonstrate the improvement in straight line fits, as shown by higher (in absolute value) linear correlation coefficients caused by eliminating the H value and substituting the "a" value from the Gardner color difference meter. The higher linear correlation coefficients are probably due to the objectivity of the instruments versus the subjectivity of the panel and support the boundary effect hypothesis. Visual inspection of the graphs for "a" versus %R 630 nm − %R 580 nm (Fig. 5) and for "a" versus 1n (%R 580 nm/%R 630 nm) (Fig. 6) shows a decrease in the clustering of points at both ends of the lines.

All data reported here were obtained on two instruments, a Gardner color difference meter and a Beckman D.U. spectrophotometer with an integrating sphere reflectance attachment. Similar instruments were evaluated for comparableness. A Neotec color difference meter and a Color Master color difference meter were checked. The absolute value of "a" or redness for these instruments differed from the Gardner "a" value but analysis of the data obtained from these instruments indicated that either instrument could be used to replace a hedonic evaluation of meat samples if a standard line of "a" or red value versus H was first obtained. Data from two spectrophotometers, a Beckman DBG and a B&L Spectronic 20, both equipped with integrating sphere reflectance attachments, gave extremely close agreement with the data obtained using the Beckman D.U.

From the linear correlation coefficients the three most effective methods for replacing the H are the Gardner "a" value, %R 630 nm − %R 580 nm and 1n (%R 580 nm/%R 630 nm). The P value for the comparison of "a" versus H with H versus %R 630 nm − %R 580 nm is .001 and the P value for the comparison of "a" versus H with H versus 1n (%R 580 nm/%R 630 nm) is .002. These P values indicate that the "a" value from the Gardner color difference meter is the most accurate replacement for the hedonic. The colorimeter requires less than 30 sec per measurement and can therefore be employed for large numbers of samples. Its main disadvantage is that a standard curve must be found for each different type of color difference meter used.

Fig. 5—Gardner Color Difference meter "a" vs. %R 630 nm − %R 580 nm).
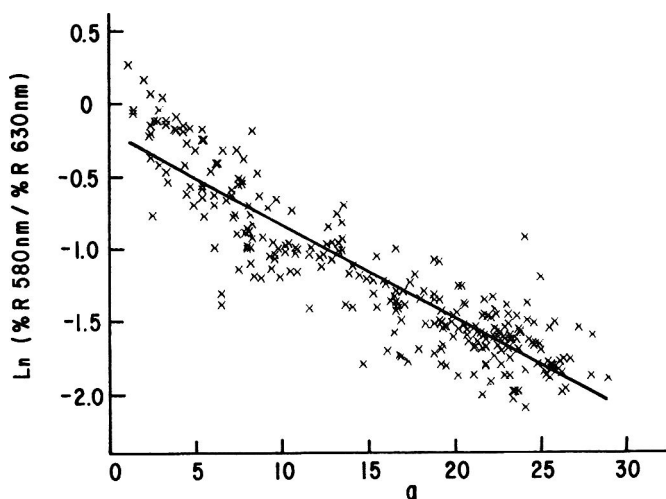
Fig. 6—Gardner Color Difference meter "a" vs. 1n (%R 580 nm/%R 630 nm).

The spectrophotometric methods outlined are slower, do not give quite as good agreement with the panel evaluation and entail expensive equipment. The main advantages of a reflectance spectrophotometric method are that the results from one instrument are comparable to results from another instrument, provided that the same type reflectance attachment is used, and that spectrophotometers are commonly available in laboratories. Analysis of the linear correlation coefficients of H versus %R 630 nm − %R 580 nm and H versus ln (%R 580 nm/%R 630 nm) gives a P value of .90. This P value indicates that there is no reason to prefer one method of calculation over the other.

The use of either colorimeters or reflectance spectrophotometers provides a quick, accurate replacement for panel or hedonic evaluations of meat color. Both methods require little training on the part of the operator for either procedure or interpretation.

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BOLOGNA PRODUCT CHARACTERISTICS AS INFLUENCED BY VARIOUS SOURCES AND LEVELS OF COTTAGE CHEESE WHEY

INTRODUCTION

THE U.S. FOOD industry, facing increased costs and diminishing profits, has had a growing interest in developing uses for their processing by-products. In addition, some of these processing wastes have presented waste disposal problems of some magnitude. In a world that is concerned about protein availability, the need for finding acceptable ways to use discarded sources of protein becomes apparent.

Due to the physical make-up and unique characteristics of sausage products, this facet of the meat industry has proved to be an acceptable outlet for such products when incorporated in small amounts as restricted by federal meat inspection regulations. It is also conceivable that a low cost product might be developed using higher levels of certain processing by-products and thus become a potential nutritional source for developing countries of the world.

Cottage cheese whey, a by-product of cheese manufacturing, is a protein source not utilized efficiently at the present time. Due to some of its emulsification, whipping and stabilizing properties, as reported by Hansen and Craver (1971), whey has proven worthy of more extensive study. With this in mind, the objectives of this work were to evaluate the composition and emulsifying properties of four different sources of whey protein in phase one, and to objectively and subjectively evaluate bologna product characteristics in phase two, which contained the different sources of whey at 0.0, 3.5, 7.0 and 10.5% of the meat block. The second objective was to be conducted with particular emphasis on the effects of lactose as a whey constituent and carboxymethylcellulose (CMC) as a protein precipitant.

EXPERIMENTAL

Phase 1

In phase 1, composition, emulsifying capacity (ml fat/g of sample), emulsifying efficiency (ml fat/mg of protein) and emulsion

stability (% fat lost) were determined for beef (boneless, USDA choice #2 chucks) and for four sources of whey protein. The sources of whey protein in the study included: (A) spray-dried whole whey, which was a direct by-product of cheese processing containing 50% lactose and no CMC; (D) spray-dried whey protein concentrate (26% CMC and 0% lactose); (C) a product obtained by the protein precipitation procedure of Hansen et al. (1971), spray-dried whey protein concentrate (26% CMC and 10% lactose), obtained by eliminating the final H_2O_2 refining step in their procedure; and (B) spray-dried whey protein concentrate (26% CMC and 50% lactose), the same product as above except that lactose was added to 50% so as to accomplish the objectives.

Phase 2

In phase 2, bologna products were manufactured with each source of whey protein added at 0.0, 3.5, 7.0 and 10.5% of the meat block containing boneless beef. In all cases, all-beef bologna (0.0% incorporated) was manufactured and evaluated as a control. This design was replicated three times.

A taste panel was used to evaluate each product for color, texture, juiciness, bologna flavor, off flavor, off aroma and general acceptability (a combination of all characteristics). The products were evaluated at the time of production, and again after 2 wk storage. In addition to the subjective evaluation, each product was objectively measured for yield, reflectance (Ockerman, 1971) and proximate analysis. This design permitted the evaluation of the whey sources in bologna and permitted an evaluation of the effects of lactose and CMC as they affected taste and general acceptability of each product.

Analytical methods

AOAC (1965) methods were used as the procedural reference for the moisture, fat, protein and lactose determination. The ash content

was determined using the method stated by Ockerman (1971).

A method similar to that used by Swift et al. (1961) was used to determine the emulsifying capacity of the protein sources. 25g of meat or 6.25g of the dried samples were added to 100 ml of cold (3.0°C) 1.0 molar salt solution and 25.0 ml of cottonseed oil and placed in a Waring Blendor jar. This mixture was blended at 15,000 rpm while adding additional oil at a rate of 1 drop/sec. When the emulsion collapsed, the total volume of oil used was determined and used in calculating the emulsifying capacity and efficiency.

The stability of the emulsions was determined with the process used by Inklaar and Fortuin (1969) and the percentage of CMC was determined using the methods prescribed by Hansen and Chang (1968).

RESULTS & DISCUSSION

COMPOSITION findings are shown in Table 1. Commercial spray-dried whole whey (A) had 35.1% protein and 51.2% lactose. The highest percentage of protein was found in product D. This is as one would expect since this source was a concentrate made from precipitated protein with lactose removed (Hansen et al., 1971). The differences observed in percents of protein among the whey protein sources B, C, and D were due primarily to levels of lactose and/or CMC. As the percentage of lactose and CMC increased, a proportional percentage decrease in water, fat and ash was observed.

It should be noted that the proximate analysis for beef has been presented in Table 1 in two ways. In the "fresh" column the analysis represents a fresh sample of beef just as it was used in the

Table 1—Percentage composition of beef and whey protein sources[a]

	Beef control		Commercial whey "A"	Whey protein (50% lactose, 26% CMC) "B"	Whey protein (10% lactose, 26% CMC) "C"	Whey protein (0% lactose, 26% CMC) "D"
	Fresh	Dry				
Protien, %	16.7	53.5	35.1	20.5	51.3	60.0
Fat, %	12.0	38.7	3.0	1.9	4.9	5.2
Water, %	70.5	5.5	6.5	2.2	5.5	7.0
Ash, %	1.0	3.1	2.2	1.0	1.7	1.5
Lactose, %	0.0	0.0	51.2	50.0	10.7	0.0
CMC, %	0.0	0.0	0.0	25.0	25.8	26.7

[a]Whey sources are spray dried

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Table 2—Emulsifying capacity, efficiency and stability of the protein sources

	Capacity[a] ml fat/g of sample	Efficiency ml fat/mg of protein	Stability % fat lost
Beef control	59.9	0.36	3.0
Commercial whey (50% lactose, 0% CMC) "A"	28.8	0.08	28.6
Whey protein (50% lactose, 26% CMC) "B"	54.6	0.27	29.5
Whey protein (10% lactose, 26% CMC) "C"	64.0	0.12	28.5
Whey protein (0% lactose, 26% CMC) "D"	127.0	0.21	30.0

[a] Tests were run on emulsions containing 30% fat.

Table 4—Least squares means for beef bologna yield and product acceptability as influenced by whey protein levels

	Whey protein sources as a % of the beef block[a]				
Characteristic[b]	0.0%	3.5%	7.0%	10.5%	Std error
Yield[c]	94.4	92.9	92.6	93.2	0.09
Internal color[d]	6.3	6.3	5.6	5.6	0.13
Texture[d]	6.3	6.6	5.4	5.2	0.14
Juiciness[d]	6.8	6.6	5.4	4.8	0.13
Bologna flavor[d]	5.8	6.3	5.2	4.7	0.14
Off-flavor[d]	7.6	8.0	7.1	6.7	0.13
General acceptability[d]	6.0	6.2	5.0	4.4	0.10

[a] Effects of whey source removed
[b] Yields are in %; remaining scores are based on a hedonic scale of 1–10 with 10 being the most desirable.
[c] Linear effects significant (P < 0.01)
[d] Quadratic effects significant (P < 0.01)

study. Data in the "dry" column are mathematical determinations of the compostion of beef as it would appear when dried to a moisture content of 5.5%. This portion of the table allows for a quick comparison of all the protein sources at a similar moisture content.

The effects of CMC, as a hydrocolloid stabilizer, and the potential value of the various protein sources in bologna-type products are elucidated by the emulsifying capacity, emulsifying efficiency and emulsion stability data presented in Table 2. Data for the beef control agreed with that reported by Potter (1971) and Neer et al. (1973). The emulsifying capacity of the commercial source of whey (A) is less than half that of beef; however, as the ratio of CMC to lactose increases, an increase in emulsifying capacity is noted. The highest capacity is associated with product D and quantitatively agrees with previous reports of Hansen and Craver (1971) and Neer (1972).

Two possible reasons for the previously mentioned findings involve the presence of CMC, as a stabilizer, and the level of protein available for fat encapsulation. Emulsions containing CMC had higher emulsifying capacities than did those containing no CMC. This is probably due to CMC's ability to encapsulate fat (Hansen, 1973). Also, of significant importance is the fact that as the amount of protein increased in samples without CMC the emulsifying capacity increased. This is in keeping with the basic premise of the nature of a meat emulsion.

Not only the amount of protein in a sample, but the type of protein alters the emulsion qualities of a sample. Beef protein is primarily composed of actin and myosin, both of which have excellent emulsifying capabilities (Hansen, 1960). The whey protein sources are deficient in actin and myosin, but rather, contain α and β lactoglobulin proteins (Wingerd, 1971).

Following exposure to heat stress, beef emulsions gave up 3.0% of the fat emulsified but whey protein emulsions demonstrated a high degree of instability. Vari-

ous protein types, such as represented in this study, coat fat in varying degrees of thickness, and the thickness of the protein coating affects its emulsifying stability (Potter, 1971). A thin coating of protein around a fat globule results in an unstable emulsion whereas a thick coating is associated with a stable emulsion (Potter, 1971). Lactoglobulin proteins have been observed (Wingerd, 1971) in forming thin protein coatings, thus accounting for the unstable emulsions associated with the whey protein sources.

Data from the bologna product evaluation phase of this project were subjected to the least square analysis of variance involving whey protein source, level of incorporation, and the two-way interactions (Snedecor and Cochran, 1971).

Proximate composition data for the finished emulsion products are presented in Table 3. Significant (P < 0.01) differences in protein content of the finished products resulted directly from the protein content of the whey protein sources as added to the beef block.

Products made with beef plus whey sources A, C and D had significantly (P < 0.01) higher protein content than did those made with beef alone. This is what would be expected since the whey sources contained a higher percentage of protein than beef (wet basis), thus slightly increasing the protein content of products containing whey.

The least squares means for bologna product characteristics as influenced by levels of whey protein in the formulation are presented in Table 4. In general, whey products incorporated in the beef block at levels of 7.0 and 10.5% resulted in a small but significant (P < 0.01) decline in smokehouse yield and in poorer product acceptability. At the 3.5% level whey products improved flavor probably due to

Table 3—Effect of protein source on beef bologna composition (%)[a,b]

	Beef control	Commercial whey "A"	Whey protein (50% lactose, 26% CMC) "B"	Whey protein (10% lactose, 26% CMC) "C"	Whey protein (0% lactose, 26% CMC) "D"
Protein, %	17.2a	18.1d	17.4a	19.0c	19.3b
Water, %	53.5a	51.2d	50.8c	51.0b	50.8c
Fat, %	27.6a	26.3c	26.2b	26.4c	26.4c
Ash, %	1.5a	1.6c	1.5a	1.6c	1.9b
Lactose, %	0.0a	2.6b	2.6b	0.5c	0.0a
CMC, %	0.0a	0.0a	1.3b	1.3b	0.7c

[a] Effects of level and storage time removed
[b] Means followed by like letters do not differ significantly (P < 0.01).

the presence of lactose in three of the whey protein sources.

The internal slice color was affected by the level of whey incorporation. Table 4 contains a quadratic relationship between level and internal color. Both the control (0.0%) and products at the 3.5% level have equal internal slice color means. Products at the 7.0 and 10.5% levels, however, were found to have less desirable color.

Table 4 also shows that the level of whey incorporated into a product affects texture, juiciness, bologna flavor, off flavor and general acceptability. As above, similar scores were seen in the 0.0% and 3.5% levels and in the 7.0% and 10.5% levels, thus, illustrating a quadratic effect.

Table 5 shows the effects of protein source on bologna product characteristics. The yield of the all beef bologna was significantly higher (P < 0.01) than that of bologna containing beef plus the various whey proteins. Since CMC acts as an emulsifier (Hansen and Craver, 1971), one might assume that it would help increase yield. Comparisons of the commercial whey (A) with products containing CMC (B, C and D) indicate that there was no such beneficial effect. The correlation between yield and CMC content was −0.22* (P < 0.05).

No significant differences were noted in internal color; however, a significant (P < 0.01) negative correlation between CMC and internal slice color persisted (r = −0.32**). It should be noted that CMC was also negatively correlated with moisture and fat (r = −0.70** and r = −0.63**, respectively) which have been positively correlated with slice color (r = 0.46** and r = 0.41**, respectively).

Taste panel evaluations for product texture indicated that product D was the least desirable. The fact that yield was correlated with texture (r = −0.45**) offers a possible reason for the undesirable texture evaluation of product D. The effect of lactose on texture (r = 0.28*) is also evident when comparing whey sources.

Bologna products B, C and D made with whey protein containing 26% CMC as a percent of the beef protein block, had significantly poorer bologna flavor development than product A containing lactose, but no CMC. Communications with Dr. P.M.T. Hansen (1973) support the theory that off-flavors can be associated with CMC that has been heat treated, such as we have in the cooking of bologna. The adverse effects of CMC on a bologna product are further substantiated by the highly significant correlations of CMC to flavor (r = −0.47**), and off-flavor (r = −0.29*).

As the effects of lactose are evaluated, a general increase in product flavor acceptability can be noted as the percent of lactose increases in the product. Appar-

ently the sweetness serves to mask some of the undesired CMC effects on flavor. Correlations between lactose content and flavor were influenced by CMC. However, bologna A, made with the commercial whey source was found to equal in bologna flavor to the beef bologna control. The increase in product flavor value is probably due to the carmalization of lactose. Deatherage (1973) called this carmalization of lactose a "candying effect" and further states that "as lactose is cooked it enhances the taste of the product in which it is incorporated."

Since the commercial whey (product A) was shown to be superior to the other whey protein sources, a look at the influence of level on the characteristics of bologna containing this product is most meaningful (Table 6). Levels as high as 7.0 and 10.5% present serious flavor and texture problems. The low level of lactose represented at the 3.5% level, however, actually enhanced flavor development.

From this project it can be recommended that spray-dried commercial whey could be added to the meat block at a level of 3.5%. Coincidentally, 3.5% is the allowed level of nonmeat material such as dried milk solids, vegetable starch, etc. (Bailey, 1970). Another federal regulation which might affect the use of commercial whey involves its lactose content. Lactose reduced dried whey is permitted in imitation sausage or nonspecific loaves but is not to exceed 2.5% of the finished product weight.

Obviously, some questions concerning the use of cottage cheese whey remain unanswered. However, the reclamation and use of an otherwise discarded protein source has distinct merit, especially if its utilization contributes to the solving of world food problems.

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Table 5—Least squares means for beef bologna product acceptability as influenced by protein source[a]

	Bologna products made with beef block plus whey[b]				
Characteristic[c]	Beef control	Commercial whey "A"	Whey protein (50% lactose, 26% CMC) "B"	Whey protein (10% lactose, 26% CMC) "C"	Whey protein (0% lactose, 26% CMC) "D"
Yield, %	94.4a	93.3b	93.4b	93.7c	92.5d
Internal color	6.3a	6.1a	5.8a	6.0a	6.0a
Texture	6.3a	6.6a	6.3a	6.2a	4.4b
Bologna flavor	5.8abc	6.0a	5.6bc	5.4c	4.9c
Off-flavor	7.6a	7.6a	7.4a	7.6a	6.8b
General acceptability	6.0a	6.1a	5.6b	5.4b	4.4c

[a] Means followed by like letters do not differ significantly (P < 0.01).
[b] Storage and level effects removed
[c] Yields are in %; remaining scores are based on a hedonic scale of 1–10 with 10 being the most desirable.

Table 6—Least squares means for beef bologna product acceptability as influenced by levels of commercial whey

	Commercial whey added as a % of the beef block				
Characteristic[a]	0.0%	3.5%	7.0%	10.5%	Std error
Yield, %	94.3	92.7	92.7	93.4	0.18
Internal color	6.3	6.3	5.6	5.6	0.24
Texture	6.3	6.6	5.4	5.2	0.26
Bologna flavor	5.8	6.3	5.2	4.7	0.26
Off-flavor	7.6	8.2	7.5	6.9	0.24
General acceptability	6.0	6.2	5.0	4.5	0.19

[a] Yields are in %; remaining scores are based on a hedonic scale of 1–10 with 10 being the most desirable.

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FATTY ACID COMPOSITION OF MUSCLE PHOSPHOLIPIDS FROM CALVES, AND GROWING AND MATURE STEERS FED PROTECTED SAFFLOWER OIL

INTRODUCTION

THE RELATION between fats and heart disease is controversial; nevertheless many consumers believe a strong correlation exists between high cholesterol (the saturated fat content of ruminant products) and coronary heart disease, and that polyunsaturated fats should be substituted for saturated fats in the diet whenever possible. However, the polyunsaturated fatty acid content in older ruminants seldom exceeds 4% of the fat fraction because the polyunsaturated fatty acids present in plant materials in the diet are hydrogenated in the rumen by microorganisms to form saturated and monounsaturated fatty acids (Reiser, 1951; Shorland et al., 1957).

Recently Australian investigators (Scott et al., 1970; 1971) developed a process of feeding ruminants oil supplements that were protected from hydrogenation in the rumen; animals showed marked increases in the linoleic acid content of the depot fats and phospholipids (Scott et al., 1971; Faichney et al., 1972; Cook et al., 1972). With this process, the Animal Physiology and Genetics Institute, and the Nutrition Institute, ARS, USDA, Beltsville, Maryland, produced calves, and growing and mature steers with increased linoleic acid in the depot fats. Meat samples from these animals were made available to us for chemical analysis. The history of the calves (Wrenn et al., 1973) and steers (Dinius et al., 1974) has been reported.

Stability properties of these meat samples are our primary interest. The triglycerides from the adipose tissues are the primary cause of deterioration during freezer storage (Sulzbacher and Gaddis, 1968), whereas the muscle phospholipids are the major contributors to the oxidative deterioration of cooked (Younathan and Watts, 1960) and freeze-dried meats (El-Gharbawi and Dugan, 1965; Chipault and Hawkins, 1971).

The stability of rendered subcutaneous fats from calves (Ellis et al., 1974) and from growing and mature steers (Kimoto et al., 1974), as well as the palatability data on freezer-stored roasts from these animals will be reported elsewhere.

We report herein the 2-thiobarbituric acid (TBA) values, tocopherol content, and fatty acid composition of the phospholipids from the rump roasts of these animals.

EXPERIMENTAL

ALL MEAT SAMPLES were obtained from the Animal Physiology and Genetics Institute, and the Nutrition Institute.

Calves

Two groups of 4-day-old bull calves, four in each group, were fed normal milk (NM) or polyunsaturated milk (PUM) from cows fed safflower oil-casein-formaldehyde (Plowman et al., 1972), both supplemented with 486 mg α-tocopheryl acetate per calf per day, for 10 wk. The groups were then further divided into smaller groups of two animals and fed either safflower oil-casein-formaldehyde (protected, P) or safflower oil-casein (unprotected, U) as supplements to the basal diet for 8 wk (Wrenn et al., 1972; 1973). The dietary groups thus formed were: NM-U, NM-P, PUM-U and PUM-P.

Growing and mature steers

Two sets of six growing steers each were fed for 6.5 wk on a regular basal diet supplemented with 10 or 20% concentrations of either safflower oil-casein-formaldehyde (P), or safflower oil-casein (U). Three animals were assigned to each treatment. The 8-month-old growing steers were fed a restrictive diet (2% of body weight per day) and ate all the feed offered. The average weight at slaughter was approximately 230 kg and 240 kg for the steers on the 10% and 20% treatments, respectively. Two mature steers were fed for 7 wk on the basal diet supplemented with 11% safflower oil-casein-formaldehyde, a third animal was fed safflower oil-casein, and a fourth mature steer was fed 6% sodium caseinate (C). The average weight of the 18-month-old mature steers was 500 kg.

Every animal received 20 mg of d-α-tocopheryl acetate per day (Dinius et al., 1974).

Fatty acid analysis

A portion from the round (rump roast) from each animal was excised 1 day after slaughter and stored at −18°C until needed. About 1 lb of the frozen rump roast was cut. Since the rump roast consists primarily of semimembranosus and semitendinosus muscles, with lesser amounts of biceps femoris and adductor, and minor amounts of gracilis muscles (Tucker et al., 1952), a representative sample of these muscles is obtained only by cutting against the grain of the roast. The partially thawed sample was cubed and, after removal of all visible fat tissues, was ground twice through a 1/8-in. plate, and hand mixed thoroughly. Samples of 30g and 10g were taken, in duplicate, for phospholipid and tocopherol determinations, respectively. The lipids were extracted in a blender with 300 ml and then 200 ml MeOH-CHCl₃ (1:2) according to the procedure of Folch et al. (1957). The combined extract was placed in a separatory funnel and mixed with 0.2 of its volume with water. The mixture was refrigerated for 1−2 hr, then allowed to stand at room temperature for 1 hr; two clear layers separated with an interfacial fluff. The lower layer was placed in a 1-liter round-bottom flask with 0.2 ml of 0.1% butylated hydroxytoluene (BHT) in CHCl₃ (v/v). The flask was placed in a 30−40°C water bath and the solvents were removed with a rotary evaporator under house vacuum. Lipids were separated on a silicic acid column with MeOH-CHCl₃ (1:20), MeOH-CHCl₃ (1:1), and MeOH by the procedure of Hornstein et al. (1961). The first fraction contains the neutral lipids (primarily triglycerides) and the last two fractions contain the phospholipids.

For calf samples, the phospholipid fractions, containing about 20 mg lipids, were placed in 50 ml test tubes and solvents removed with a jet of N_2. The residues were saponified with EtOH-KOH at 65°C and the fatty acids converted to their methyl esters with 5% $HClO_4$ in MeOH at 65°C (Schmitt and Wynn, undated).

For the growing and mature steer samples, the MeOH-CHCl₃ (1:1) and MeOH fractions from the separation of the lipids on silicic acid were combined and the fatty acids of the phospholipids were converted into their methyl esters by a modification of the transesterification procedure of Luddy et al. (1968), consisting of the addition of 2−3 drops of anhydrous benzene to the reaction mixture (Luddy, 1972). Conversion of the MeOH-CHCl₃ (1:20) or neutral lipid fraction into methyl esters was by the procedure of Luddy et al. (1968). The carbon disulfide extract of the methyl esters was transferred to a 5-ml vial and the solvent removed with a jet of N_2. Methyl heptadecanoate, 0.2 ml of a 1% solution in CHCl₃ (w/v), was added to the residue as an internal standard. The methyl esters were analyzed by gas-liquid chromatography as previously described (Ellis et al., 1974); in addition the programmed runs were held at the upper limit (210°C) for 10−13 min.

All of the methyl esters had retention times identical to those of standard compounds except 20:3, for which no standard was available. A semi-log plot of the carbon number against the ratio of the elution temperature of standard methyl esters to the elution temperature of the internal standard, 17:0, indicated that the compound was 20:3 (Schmitt and Wynn, undated).

This compound has been reported as a constituent of phospholipids from beef muscle (Hornstein et al., 1961; O'Keefe et al., 1967) and assignment was made on this basis. The multiplying factor, which was the ratio of peak height of the standard (17:0) to peak height of the standard methyl esters, was used to normalize the peak heights in the extracts, and the factor calculated for 20:4 was also used for 20:3. Numerous minor peaks before the 20:4 peak and three peaks appearing after the 20:4 peak represented 2% and 6% or less, respectively, of the total peak heights for the phospholipids. These peaks represented unidentified compounds and were not included in the present analysis.

Fatty acid compositions from $MeOH-CHCl_3$ (1:1), and MeOH fractions were combined for the calf phospholipid results.

Tocopherols

Tocopherols were isolated from the muscle tissues by a modification of the procedure of Erickson and Dunkley (1964). A mixture of 10g of ground lean tissues and 15 ml of absolute EtOH was placed in a 50 ml centrifuge tube and mixed thoroughly. Stirring was continued after the addition of 1 ml of 1N HCl and the mixture placed in a water bath at 60°C for 5 min with occasional stirring. While still warm the mixture was transferred into a 250 ml flat-bottom flask with 15 ml of hexane, shaken mechanically for 20 min, returned to the 50 ml centrifuge tube and centrifuged for 5–10 min. The solvents were decanted, and the extraction procedure was repeated on the residue two more times, with the mixture being shaken for 10 instead of 20 min. The extracts were placed in a separatory funnel, diluted with 45 ml of distilled or deionized water and 5 ml of 1N HCl and extracted with three 35 ml portions of hexane. The hexane extracts were combined and the volume reduced to 30 ml with a jet of N_2. After drying over Na_2SO_4 the hexane extracts were reduced to 20 ml with an N_2 jet; 10

ml aliquot was used for the tocopherol determination.

TBA numbers on ground meat stored at 3°C

About 1 lb of rump roast frozen at –18°C for 6 months for the growing steers and 1.5 months for the mature steers, was ground twice through a 1/8-in. plate and hand mixed thoroughly. Twelve 30-g aliquots of meat were taken from each sample and rolled into balls. Three balls were placed in each of four aluminum foil flat-bottom boats. These were placed in pyrex trays, covered with aluminum foil and stored at 3°C. After 0, 1, 2 and 3 days the three balls of ground meat in each boat were combined, thoroughly hand mixed and the 2-thiobarbituric acid (TBA) number was determined essentially by the method of Tarladgis et al. (1960). A simple still was used instead of the Kjeldahl apparatus, and the Kjeldahl flask was heated with a Meker burner. Absorbance of the TBA pigment was recorded at 538 nm on a Cary model 14 spectrophotometer. Absorbance was multiplied by K to give the TBA number in mg of malonaldehyde per 1,000g of sample. Our K value was 8.1, somewhat higher than the 7.8 reported by Tarladgis et al. (1960).

Data for the fatty acid composition and the TBA numbers from the growing steers were treated by analysis of variance (Snedecor, 1956).

RESULTS & DISCUSSIONS

Calves

Table 1 shows average fatty acid composition of the phospholipids from the rump roasts of four pairs of experimental and one pair of commercial calves. Calves fed polyunsaturated milk or protected safflower oil showed a trend toward greater incorporation of 18:2 into the phospholipid of meat than control calves

fed normal milk and unprotected safflower oil. The total polyunsaturated fatty acid content and 18:2 levels of the phospholipids were dependent on the diet in the order PUM-P = NM-P > PUM-U > NM-U. Commercial calf samples of unknown history were obtained fresh from the killing floor of a local slaughter plant. These samples were included for purposes of comparison.

During analysis of the fatty acid methyl esters by gas-liquid chromatography two extraneous compounds were observed which represented about 10% of the total peak heights. One peak eluted before methyl palmitate (16:0) and the other between methyl heptadecanoate (17:0) and methyl stearate (18:0). Extraction of these compounds with hexane after saponification of the phospholipids with alcoholic KOH, indicated that they were not fatty acids. They formed 2,4-dinitrophenylhydrazone derivatives with a maximum of 345 nm in CCl_4, which was in the region for the absorption maxima of alkanals (Gaddis et al., 1959), indicating they were saturated or unconjugated unsaturated carbonyl compounds. The retention time for the peak eluting between 17:0 and 18:0 was identical to that of stearic aldehyde and the peak eluting before 16:0 was believed to be palmitic aldehyde. The source of the aldehydes may be plasmalogens. The transesterification procedure of Luddy et al. (1968) and Luddy (1972) does not liberate aldehydes from phospholipids. With Luddy's procedure, the results of the analysis of the fatty acid composition of the phospholipids from the left rump

Table 1—Average tocopherol content and fatty acid composition (wt %) of phospholipids from rump roasts of calves[a]

	PUM-P[b]	PUM-U	NM-P	NM-U	Commercial (#1, #2)
16:0	14.9	14.6	16.8	15.7 ± 1.8	15.4
16:1	0.7	1.3 ± 0.3	0.7	1.6 ± 0.7	0.6 ± 0.1
18:0	16.8 ± 1.2	16.5	16.3	15.9 ± 2.1	16.5
18:1	7.2 ± 0.4	11.0	7.0	14.2 ± 1.1	22.5 ± 1.6
18:2	38.8	34.5	38.3	29.0	26.6 ± 1.8
18:3	0.4 ± 0.1	1.0	0.4	1.5 ± 0.4	1.4 ± 0.1
20:3	2.3	3.0	2.6 ± 0.5	3.9	2.9 ± 0.3
20:4	18.9 ± 1.6	18.1	17.9 ± 1.1	18.2	14.1
% Phospholipids, wet wt	0.71 ± 0.02	0.67 ± 0.01	0.71 ± 0.05	0.78 ± 0.01	0.86 ± 0.02
Tocopherols, µg/g	6.6 ± 1.0	5.2 ± 0.3	4.4 ± 0.3	4.7 ± 0.1	2.5 ± 0.1
% Saturated fatty acids	31.7	31.1	33.1	31.6	31.9
% Monounsaturated fatty acids	7.9	12.3	7.7	15.8	23.1
% Polyunsaturated fatty acids	60.4	56.6	59.2	52.6	45.0

[a] Standard deviation is shown if more than ±5% of the mean from two animals per treatment.
[b] Diet of normal milk (NM) or polyunsaturated milk (PUM), followed by diet supplemented with protected (P) or unprotected (U) safflower oil

roast of one sample from each of the four dietary groups was similar to the results from the saponification-esterification of the right rump roasts. The liberation of aldehydes, therefore, did not affect the analysis of the fatty acid composition of the phospholipids.

For the experimental animals tocopherol content varied from 4.2–7.3 $\mu g/g$ which was much higher than the 2.4–2.6 $\mu g/g$ level for the commercial calves. This difference was attributed to the supplemental vitamin E fed the calves the first 10 wk.

Growing and mature steers

Table 2 summarizes the average fatty acid composition of the phospholipids from growing and mature steers. Statistical analysis of the data shows significant differences (P < 0.01) in concentrations of 16:1, 18:1, 18:2, 18:3 and 20:3 in the phospholipids from animals receiving protected or unprotected safflower oil in the diet; the protected treatment produced

higher levels only for 18:2. With either 10% or 20% P in the diet the 18:2 level was about 40% of the fatty acid contents, nearly twice as much as in the phospholipids of animals fed the unprotected diet. The increase in 18:2 was counterbalanced by decreases in the other unsaturated fatty acids except 20:4, which was not significantly different. Similar results were reported by Cook et al. (1972). Differences were similar for mature steers fed protected safflower oil in comparison to those fed unprotected material or sodium caseinate.

Incorporation of tocopherols in the muscle tissues of growing and mature steers varied from 2.8–4.9 and from 2.7–3.1 $\mu g/g$, respectively, and were lower than the values for the experimental calves. Although the deposition of dietary tocopherol in animal tissue is rather inefficient (Machlin, 1962), tissue levels can be increased by increasing tocopherols in the diet over a period of time. Differences in the tocopherol level

in the diet were reflected in the muscle tissues of ewes (Buchanan-Smith et al., 1969) and calves (Eaton et al., 1958; Decker and Hill, 1957).

Table 3 summarizes the 18:2 composition of the neutral lipids (primarily triglycerides) from the rump roasts of growing and mature steers. Comparison between Tables 2 and 3 shows significant differences for the 18:2 content between the P treatments for the triglycerides (Table 3) but not for the phospholipids (Table 2). There was also a significant difference in 18:2 composition between the U treatments for the phospholipids but not for the triglycerides, although the triglyceride mean value for the 20% U was slightly higher than that for the 10% U treatment. The 18:2 composition of the phospholipids (38.8%) for the mature steers on the P treatment was similar to that for the growing steers (40.3%), although the corresponding composition of 18:2 for triglycerides was markedly different. These results suggest a more rapid

Table 2—Average tocopherol content and fatty acid composition (wt %) of phospholipids from rump roasts of growing and mature steers

	Growing steers[b]				Mature steers[c]	
	10% P[a]	20% P	10% U	20% U	P	U, C
16:0	15.9	15.0	16.4	15.5	15.8	16.2 ± 1.1
16:1	0.9b	0.8b	2.4c	1.3b	0.7	1.3 ± 0.3
18:0	17.5b,c	17.0b,c	16.5b	17.7c	14.1	14.1 ± 1.5
18:1	7.3b	6.4b	23.5c	18.4c	8.7	21.0
18:2	40.3b	40.2b	19.8c	26.1d	38.8	22.1
18:3	0.6b	0.5b	2.3c	1.6d	0.5	1.3 ± 0.3
20:3	3.0b,d	2.3b	3.9c,d	4.0c	2.9 ± 0.5	4.2
20:4	14.5	16.5	15.2	15.4	18.5 ± 1.1	19.8
% Phospholipids, wet wt	0.64 ± 0.03	0.65 ± 0.03	0.66 ± 0.02	0.65 ± 0.02	0.64 ± 0.04	0.60 ± 0.03
Tocopherols, $\mu g/g$	3.0 ± 0.3	3.3 ± 0.2	3.8 ± 1.1	3.5 ± 0.1	3.0 ± 0.2	2.7 ± 0.1
% Saturated fatty acids	33.4	32.0	32.9	33.2	29.9	30.3
% Monounsaturated fatty acids	8.2	7.2	25.9	19.7	9.4	22.3
% Polyunsaturated fatty acids	58.4	60.8	41.2	47.1	60.7	47.4

[a] Diet supplemented with protected (P) or unprotected (U) safflower oil, or sodium caseinate (C)
[b] Horizontal values on the same line not bearing the same letter differ significantly (P < 0.05).
[c] Standard deviation is shown if more than ±5% of the mean from two animals per treatment.

Table 3—18:2 composition (wt %) of triglycerides from rump roasts of growing and mature steers[a]

	Growing steers[b]				Mature steers	
	10% P[c]	20% P	10% U	20% U	P	U, C
18:2	8.6d ± 1.7	13.8e ± 1.6	2.9f ± 0.4	4.0f ± 1.3	4.4 ± 0.2	2.6 ± 0.4
% Lipids, wet wt	1.3 ± 0.4	1.3 ± 0.5	1.5 ± 0.6	1.4 ± 0.5	6.9 ± 0.3	5.9 ± 1.8

[a] Mean values and standard deviation of the mean. Fatty acid composition based on: 14:0, 16:0, 16:1, 18:0, 18:1, 18:2 and 18:3
[b] Horizontal values on the same line not bearing the same letter for the growing steers differ significantly (P < 0.01).
[c] Diet supplemented with protected (P) or unprotected (U) safflower oil, or sodium caseinate (C)

Table 4—TBA numbers for ground rump roasts stored at 3°C[a]

Days after storage	Growing steers[b]				Mature steers	
	10% P[c]	20% P	10% U	20% U	P	U, C
0	1.3 ± 0.3	1.0 ± 0.1	1.2 ± 0.3	1.5 ± 0.4	1.0 ± 0.1	1.3 ± 0.1
1	4.1	3.7 ± 0.1	3.1 ± 0.3	3.8 ± 0.6	4.1 ± 0.3	4.1 ± 0.3
2	6.3 ± 0.2	5.9 ± 0.2	5.1 ± 0.3	6.0 ± 0.8	6.1 ± 0.1	6.4 ± 0.3
3	7.5 ± 0.2	7.6 ± 0.3	6.6 ± 0.8	7.8 ± 1.0	7.6 ± 0.1	8.1 ± 0.5

[a] TBA number = mg malonaldehyde per 1,000g of sample. Mean values and standard deviation of the mean

[b] Analysis of variance showed differences were not significant (P < 0.05) among the four treatments or between protected and unprotected diet supplements.

[c] Diet supplemented with protected (P) or unprotected (U) safflower oil or sodium caseinate (C)

incorporation of 18:2 into the phospholipids than into the triglycerides of growing and mature steers.

TBA determination on ground meat stored at 3°C

Growing steers fed unprotected safflower oil had greater variations and somewhat lower TBA numbers than steers fed protected safflower oil, but these differences were not significant (P < 0.05). Mature steers fed unprotected safflower oil or sodium caseinate also had greater variations but slightly higher average TBA numbers than the steers fed protected safflower oil (Table 3).

TBA determinations by Keskinel et al. (1964) on fresh ground lean tissues of beef stored at 5°C indicated that increase in TBA numbers was greatest after storage for 2 days. On this basis, oxidation of ground lean tissues from growing and mature steers should have progressed far enough after 3 days at 3°C to indicate degrees of difference without significant bacterial growth. Oxidation of lipids in ground meat depends primarily on the degree of unsaturation and on amounts of lipid, antioxidants and heme catalysts (Tappel, 1962). The last should be comparable for the meat samples used, with some differences in tocohperol levels, and marked differences in degree of unsaturation of the lipids.

During the early stages of oxidation the TBA value correlates linearly with peroxide value, diene conjugation and oxygen uptake (Kenaston et al., 1955; Tarladgis and Watts, 1960; Dahle et al., 1962); its use for quantitative determination of lipid oxidation may be meaningful only during this period (Kwon and Olcott, 1966). Up to peroxide levels of 2,000 meg/kg, methylene-interrupted polyunsaturated fatty acids at the same peroxide values had TBA values that were approximately in the ratio 1:2:3:4, for the triene, tetraene, pentaene and hexaene, respectively, with none for linoleic acid (Dahle et al., 1962). These data show that TBA value depends on the profile of polyunsaturated (triene or greater unsatu-

ration) fatty acids that are oxidized and may not correlate with the degree of oxidation of the total lipids. For this reason the visibly more rapid change in meat color from pink to brown for the ground meat samples from the animals fed protected over those fed unprotected safflower oil may indicate differences in degree of lipid oxidation, but not TBA numbers.

The incorporation of 18:2 into the phospholipids appears to be more rapid than into the triglycerides of muscle tissues. However the phospholipids represent less than 1% of the weight of the wet muscle tissue and will not have much impact on increasing the polyunsaturated fatty acid content of the fat fraction of meat products. For the oxidative deterioration of freeze-dried and cooked meat products for which the phospholipids have been implicated, an increase in the 18:2 level of the phospholipids without a corresponding increase in the level of protection by antioxidants will result in decrease stability of the meat product.

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MICROFLORA OF FERMENTED KOREAN SEAFOODS

INTRODUCTION

SEAFOODS constitute a major source of protein in many oriental countries. Fermentation is one of the methods commonly used to preserve these foods. The importance of fermented seafoods in the orient has been reviewed by Amano (1962), Rao (1967), Mackie et al. (1971), and Kwon (1972).

In Korea, fermented seafoods are prepared in homes and village industries and are used extensively as condiments with rice. These fermented products, although not eaten in large portions, are considered to be staple items of the Korean diet. For example, *Kimchi*, a popular fermented vegetable dish, is prepared with radish root, chinese cabbage and fermented shrimp (in North Korea) or fermented anchovy (in South Korea).

The total production of fermented seafood in Korea is difficult to estimate. Rao (1967) reported that village industries produced 20,000 metric tons, 300 tons of which were exported. Since data on the amounts produced in homes were not available, Rao could not establish the total annual production of fermented seafoods.

The need for detailed information concerning the methods and equipment used in catching, handling and fermenting marine animals has been recognized (Kwon, 1972). Until this information becomes available and can be assessed for its microbiological significance in fermentations, investigations of fermented seafoods must be limited to laboratory analyses of the microflora present in the fermented products.

EXPERIMENTAL

SAMPLES of 10 Korean fermented seafoods were obtained from T.W. Kwon of the Korea Institute of Science & Technology, Seoul. They were prepared by adding marine salt to the seafood and allowing natural fermentation to occur. The samples were collected in sterile plastic bags and varied in size from an estimated volume of 5–10 cm³. The products sampled were in the final stage of fermentation (Table 1).

Representative portions of each sample were suspended in sterile saline solution and suspensions were streaked onto culture plates containing Brain Heart Infusion agar (BHI), Marine agar 2216, Malt agar containing 10% malt extract, and Potato Dextrose agar (PDA) containing 0.5% yeast extract. All media were obtained from BBL, Cockeysville, Md. except for the Marine agar, obtained from Difco, Detroit, Mich. and the Malt agar, prepared in this laboratory.

Inoculated plates of Marine, Malt Extract and BHI agars were incubated aerobically at 30°C for the isolation of bacteria and yeasts; PDA plates were incubated at 25°C for the isolation of yeasts and filamentous fungi. Isolations for anaerobic bacteria were made from plates of the same media incubated at 27–30°C in anaerobic jars containing GasPak® hydrogen + carbon dioxide generators (BBL).

Representative colonies on each medium inoculated with each sample were examined macroscopically and microscopically, and organisms exhibiting differences in growth characteristics or microscopical appearance were isolated into pure culture. Cultures were maintained on BHI agar (bacteria) or Malt Extract agar (yeasts).

Bacterial isolates were tested for Gram staining properties using 24 and 96-hr cultures and for catalase production with 10% hydrogen peroxide. Additional tests were conducted following procedures listed in the National Canners Association Manual (NCA, 1968): growth in 7% NaCl broth; hydrolysis of starch, casein and gelatin; and aerobic and anaerobic utilization of glucose. The lipolytic activity of several organisms was tested using a menhaden oil-nile blue assay procedure (Young, 1973). Anaerobic isolates were recultured under aerobic conditions at 27–30°C to further characterize their oxygen requirements.

Additional diagnostic tests were performed with specific groups of organisms: Bacillaceae: nitrate reduction and acetylmethylcarbinol (acetoin) production (NCA, 1968); Micrococcaceae: nitrate reduction in Indole Nitrate medium (INM), utilization of ammonium phosphate on Ammonium Phosphate agar (APA);

Lactobacillaceae: utilization of ammonium phosphate on APA, reactions in Litmus Milk, indole production and nitrate reduction in INM, growth in Lactose broth, growth in 4 and 10% NaCl broth (NCA, 1968), and acetylmethylcarbinol production in BHI broth containing 0.3% sodium citrate (Collins, 1974); and Enterobacteriaceae and other Gram negative rods: nitrate reduction in INM, indole production in Peptone water (Edwards and Ewing, 1972), methyl red reaction and acetylmethylcarbinol production in MR-VP broth, reactions in Litmus Milk, utilization of citrate on Simmons' Citrate agar, growth on Eosin Methylene Blue agar, and characteristics of growth on Triple Sugar Iron agar.

Bergey's Manual (Breed et al., 1957) was followed in identifying the families and genera of the bacterial isolates and the species of *Bacillus* and *Micrococcus*. The lactic acid bacteria were identified on the basis of their physiological characteristics (Günther and White, 1961; Sharpe et al., 1966). The data of Edwards and Ewing (1972) were followed in identifying the Enterobacteriaceae, and other Gram negative rods were identified following Lysenko (1961), De Ley (1964) and Baumann et al. (1972).

The yeast isolates were identified following the methods of Kreger-van Rij (1970) and Wickerham (1970).

RESULTS & DISCUSSION

52 BACTERIAL STRAINS, representing 15 species, and two species of yeasts were isolated from the fermented seafood samples. Neither filamentous fungi nor actinomycetes were found. In cases where intraspecific variants of a bacterial species

Table 1—Composition and protein content of fermented Korean seafoods studied

Seafood	Scientific name[a]	Protein content[b] Wet wt (%)	Dry wt (%)
Sea urchin, gonads	*Heliocidaris crassispina*	15.8	55.4[c]
Clam, muscle	*Cardium* spp., *Meretrix* spp., *Venus* spp.	14.5	44.6
Oyster, muscle	*Ostrea* spp.	15.9	54.5
Cuttlefish, muscle	*Sepia* spp.	13.8	59.5
Sea arrow, muscle and organs	*Ommastrephes solani pacificus*	35.2	57.2
Shrimp, whole animals	*Palaemon* spp., *Penaeus* spp.	11.7	37.4
Fish roe	Unidentified	–	–
Alaskan Pollack, roe	*Theragra chalcogramma*	26.0	72.2[c]
Alaskan Pollack, intestines	*T. chalcogramma*	11.5	60.2
Anchovy, whole animals	*Engraulis japonicus*	18.5	80.1[c]

[a] Rao and Polacchi, 1972
[b] Leung et al., 1972
[c] Unfermented; data on fermented product unavailable

were isolated, each isolate was treated as a separate strain and its unique characteristics were recorded (Table 2).

Although *Bacillus* spp. comprised 36 of the 52 bacterial isolates and occurred in each fermented seafood, some samples contained significantly greater numbers of different bacilli than did other samples. The microflora of seafoods which contained the largest variety of bacilli, e.g., no. 3–6, 9 and 10, also contained the fewest cocci. In other seafoods, however, the strains of cocci equalled or outnumbered the bacilli (no. 2, 7 and 8).

Micrococcus and *Pediococcus* were the second and third most commonly isolated genera. *Pediococcus halophilus*, occurring in six of the 10 samples, was the most widespread of the bacterial isolates. *Pseudomonas, Serratia,* and *Clostridium* were only isolated from single samples. This was interesting since species of *Pseudomonas* are often isolated in large numbers from marine animals (Baumann et al., 1972; Liston, 1957). *Debaryomces han-senii* and *Hansenula anomala* were the only yeasts isolated, and they were found exclusively in the two samples of fermented fish roe. Eleven of the organisms, representing 60% of the isolated cultures, have been reported from the marine environment or from fermented seafoods by other authors (Table 3).

Large numbers of bacteria and yeasts from diverse origins are likely to be present by the time a seafood has been prepared for fermentation and the process is ready to begin. Four possible sources of the fermentation inoculum are: organisms that occur naturally on or in the marine animals, themselves; organisms associated with the animal's environment, e.g., sea water or marine mud; terrestrial organisms not normally associated with the marine environment; and organisms associated with the natural microflora of the marine salt used in preparing the seafoods for fermentation.

Liston and Colwell (1963) reported significant variations in the bacterial populations associated with different marine animals. Our data suggest that fermented seafoods prepared from animals living in similar habitats exhibit similar natural microflora (Table 2). Fermented seafoods prepared from free swimming animals, such as cuttlefish, sea arrow, shrimp and anchovy, exhibited a composite microflora of seven strains of *Bacillus* which differed from the nine strains of *Bacillus* isolated from foods prepared from benthic animals, e.g., sea urchin and oyster. Although the clam and oyster are closely related, the microflora of fermented foods prepared from these animals showed little similarity. This may be explained on the basis of differences in their habitats within the benthic environment. Clams burrow into the marine mud and could be expected to harbor organisms associated with this habitat; marine muds are known to have a large and varied bacterial microflora (Shewan, 1949). Oysters, on the other hand, attach themselves to solid substrates above the level

Table 2—Microbiological composition of fermented Korean seafoods. Occurrence of specific strains in the microflora isolated from 10 samples

Isolate	Sea urchin (1)	Clam (2)	Oyster (3)	Cuttle-fish (4)	Sea arrow (5)	Shrimp (6)	Fish roe (7)	Pollack Roe (8)	Pollack Intestines (9)	Anchovy (10)	Unique reactions of variant strains
Bacillus cereus	−	−	−	+	+	−	−	−	+	−	typical
var. I	−	−	−	+	+	−	−	−	−	+	acetoin (±)
var. II	−	−	−	+	−	−	−	−	−	+	anaerob. glucose (−)
B. cereus var. *mycoides*	+	−	−	−	−	+	−	−	−	−	typical
B. firmus	−	−	−	−	−	−	−	−	+	−	typical
var. I	−	−	−	−	−	−	+	−	−	−	starch (−)
B. licheniformis	+	−	−	−	−	−	−	−	−	−	typical
var. I	+	−	−	−	−	−	−	−	−	−	starch (−)
var. II	−	−	−	−	−	−	−	−	+	−	casein (−)
B. megaterium var. I	+	+	+	−	−	−	−	−	−	−	lipase (−) on fish oil
var. II	+	−	−	−	−	−	−	−	−	+	NO$_3$ reduction (+)
var. III	−	−	+	−	−	−	−	−	−	−	lipase (+) on fish oil
B. pumilis	−	−	−	+	−	+	−	−	−	+	typical
var. I	−	−	−	−	−	+	−	−	−	−	spores (±) swollen
var. II	−	−	−	−	−	−	−	−	+	−	NO$_3$ reduction (+)
B. subtilis	−	−	+	−	−	−	+	+	−	−	typical
var. I	−	−	−	−	−	−	−	+	+	−	gelatin (−)
var. II	−	−	+	−	−	−	−	−	−	−	acetoin (±)
var. III	−	−	+	−	−	−	−	−	−	−	lipase: Tween 80 (−), fish oil (+)
var. IV	−	−	+	−	−	−	−	−	−	−	lipase: Tween 80 (+), fish oil (+)
var. V	−	−	+	−	−	−	−	−	−	−	starch (−)
B. subtilis var. *atterrimus*	−	−	+	−	−	−	−	−	−	−	typical
Clostridium setiense	−	−	−	−	−	−	−	−	−	+	lactose (±)
Micrococcus colpogenes var. I	−	+	−	−	−	−	−	+	−	−	casein (−)
var. II	−	+	−	+	−	−	−	−	−	−	lipase (+) on fish oil
M. flavus	−	−	−	−	−	−	−	+	−	−	typical
M. luteus	−	−	−	−	−	−	+	−	−	−	typical
M. varians	−	−	−	−	−	−	−	+	−	−	typical
Pediococcus halophilus	−	+	+	−	−	+	+	+	−	+	typical
Pseudomonas ovalis	−	+	−	−	−	−	−	−	−	−	catalase (±)
Serratia marcescens	−	−	−	−	−	−	−	−	−	+	typical
Debaryomyces hansenii	−	−	−	−	−	−	+	+	−	−	typical
Hansenula anomala var. *anomala*	−	−	−	−	−	−	−	+	−	−	typical

Table 3—Isolates reported from marine sources by other authors

Microorganisms	This study	Other studies	References
Bacillus cereus var. *mycoides*	sea urchin	squid	Nagao and Kimura (195·)
B. pumilis	anchovy, cuttlefish, shrimp, Pollack intestines	sea urchin, squid	Zenitani (1955)
B. subtilis	fish roe, Pollack intestines and roe, oyster	Korean seafoods sea urchin squid	Lee (1968) Zenitani (1955) Nagao and Kimura (1951)
Clostridium setiense	anchovy	oyster	Breed et al. (1957)
Micrococcus colpogenes	clam, cuttlefish, Pollack roe	marine mud, seawater	Breed et al. (1957)
M. flavus	Pollack roe	sea urchin, squid	Zenitani (1955)
M. luteus	fish roe	seawater	Shah and de Sa (1964)
M. varians	Pollack roe	sea urchin, squid seawater	Zenitani (1955) Breed et al. (1957)
Pediococcus halophilus	anchovy, clam, fish roe, oyster, Pollack roe, shrimp	anchovy Korean seafoods Soya sauce	Nakagawa and Kitahara (1959) Lee (1968) Sakaguchi (1958)
Debaryomyces hansenii	fish roe, Pollack roe	brined foods estuarine water seawater	Costilow et al. (1954) Norkrans (1966) Bhat and Kachwalla (1955)
Hansenula anomala	Pollack roe	estuarine muds fish intestines	Fell et al. (1960) Fell and van Uden (1963)

of the mud. Shewan (1961) suggests other environmental factors, such as water temperature and seasonal variations, which affect the normal microflora of marine animals.

MacLeod (1965) confirmed the existence of an indigenous marine microflora. Certain species of *Pseudomonas, Micrococcus, Clostridium, Bacillus, Debaryomyces* and *Hansenula* have been proposed as marine organisms (Kriss, 1961).

Terrestrial microorganisms contaminate the equipment used for catching and processing the animals and thereby constitute a large reservoir of inoculum. Seafoods that require extensive handling, such as fish roe, could therefore be expected to exhibit a microflora more characteristic of terrestrial sources of contamination. In this study, these seafoods showed yeasts and cocci not found in other samples.

The addition of marine salt to a fermentation could introduce halophilic microorganisms, such as *Bacillus subtilis* and *Pediococcus halophilus*, which have been isolated from several brined and fermented foods.

To understand the microbiological processes involved in seafood fermentation, it will be necessary to survey the microflora of additional samples. The natural microflora of the raw seafood and that existing at various stages of fermentation should be compared with the microflora found in completely fermented samples. Such data will indicate the succession of microbial populations that occur during fermentation. The contribu-

tion of specific microorganisms toward producing an acceptable product can be confirmed by duplicating the fermentation under controlled laboratory conditions.

Once the processes involved in the fermentation of seafood have been elucidated, they can be adopted for the development of new and more nutritional food products from underutilized marine resources.

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COMPUTATION OF INSULATION EFFICIENCIES OF FISH TRANSPORT CONTAINERS

INTRODUCTION

DESIGN of a suitable container for fish transport requires knowledge of possible ways of heat transfer through such containers under various environmental stresses. A great number of variables such as ambient temperature and humidity, moisture content of the container, size and shape of ice particles inside the container (void space), arrangement of ice and fish, transport vibration and air velocity around the container are involved in controlling the heat transfer rate in such a system. However, no attempt has yet been made to formulate a mathematical model to predict the insulation efficiency of fish transport container. So far, fish transport containers have been developed on the basis of extensive transportation trials (Wagner et al., 1969).

Our previous study has revealed (Chattopadhyay and Bose, 1972) that in conventional containers (e.g., bamboo basket, plywood box, wooden box) heat is transferred mainly by a transient conduction process due to the rapid change in moisture content of the container material due to direct exposure to drip water. As a result, the insulation efficiency of such containers was found to be improved by the application of moisture proof lining materials inside the container and a waterproof coating on outside surfaces. Transport variables such as storage temperature, vibration during transport, air velocity and void space inside the container were found to affect the overall heat transfer to and from the container.

An alternative approach for the prediction of insulation efficiency of fish transport containers is the development of a mathematical model of heat transfer for this system. The concept of the thermal time constant of a building fabric to assess its thermal characteristics when exposed to unsteady state heat flow conditions, has already been brought out (Raychaudhuri, 1961, 1963, 1965). The work was directed towards finding the thermal time constant values of individual building elements forming the wall of an enclosure having all walls of similar construction and all exposed to identical excitations (environmental stresses).

This paper presents a method for computation of thermal characteristics of fish transport containers in terms of a single parameter and their performance under various icing conditions.

Development of a mathematical model
of heat transfer

Wall transmission matrix. It has been shown (Raychaudhuri, 1965) that the one-dimensional heat conduction equation (1) for a homogeneous rectangular wall fabric of uniform thickness

$$\frac{\partial^2 t}{\partial x^2} = \frac{1}{\alpha} \frac{\partial t}{\partial \tau} \qquad (1)$$

and neglecting the heat losses through the edges may be solved by the use of a Laplace transform of temperature with respect to time; thus equation (1) may be expressed in the following

matrix form after considering the initial and final boundary conditions:

$$\begin{bmatrix} T_\ell \\ H_\ell \end{bmatrix} = \begin{bmatrix} \mathrm{Cosh}\beta\ell & -Z_0\,\mathrm{Sinh}\beta\ell \\ -(1/Z_0)\,\mathrm{Sinh}\beta\ell & \mathrm{Cosh}\beta\ell \end{bmatrix} \cdot \begin{bmatrix} T_0 \\ H_0 \end{bmatrix} \qquad (2)$$

On matrix inversion, equation (2) may be written as

$$\begin{bmatrix} T_0 \\ H_0 \end{bmatrix} = \begin{bmatrix} \mathrm{Cosh}\beta\ell & Z_0\,\mathrm{Sinh}\beta\ell \\ (1/Z_0)\,\mathrm{Sinh}\beta\ell & \mathrm{Cosh}\beta\ell \end{bmatrix} \cdot \begin{bmatrix} T_\ell \\ H_\ell \end{bmatrix} \qquad (3)$$

The square matrix containing the hyperbolic function in equation (3) is called the wall transmission matrix.

The fish container may be considered to be built up with a single wall or composite wall having multilayered fabric in series and the overall wall transmission matrix may be obtained by multiplying individual transmission matrices. Thus we may write,

$$\begin{bmatrix} E & F \\ G & H \end{bmatrix} = \begin{bmatrix} A_1 & B_1 \\ C_1 & A_1 \end{bmatrix} \cdot \begin{bmatrix} A_2 & B_2 \\ C_2 & A_2 \end{bmatrix} \cdot \begin{bmatrix} A_3 & B_3 \\ C_3 & A_3 \end{bmatrix} \qquad (4)$$

Where square matrix in the left-hand side of equation (4) represents the overall wall transmission matrix and the square matrices on the right-hand side represent individual transmission matrices of the wall fabrics along the positive direction of the x-axis. The elements A, B, C represent the corresponding elements of the square matrix in equation (3). The temperature of the ambient air in contact with the surface may be included in the system and should be considered as a layer of zero heat capacity and the square matrix $\begin{bmatrix} 1 & R \\ 0 & 1 \end{bmatrix}$ should be used in its respective location.

Each element of the individual transmission matrix may be expanded in polynomials of p as given below:

$$A = \mathrm{Cosh}\beta\ell = 1 + (\ell^2/2\alpha)p + (1/6)(\ell^2/2\alpha)^2 p^2 + (1/90)(\ell^2/2\alpha)^3 p^3 + \qquad (5)$$

$$B = Z_0\,\mathrm{Sinh}\beta\ell = (\ell/\kappa)\left[1 + (1/3)(\ell^2/2\alpha)p + (1/30)(\ell^2/2\alpha)^2 p^2 + (1/630)(\ell^2/2\alpha)^3 p^3 + \right] \qquad (6)$$

$$C = (1/Z_0)\,\mathrm{Sinh}\beta\ell = (2\kappa/\ell)\left[(\ell^2/2\alpha)p + (1/3)(\ell^2/2\alpha)^2 p^2 + (1/30)(\ell^2/2\alpha)^3 p^3 + \right] \qquad (7)$$

Thus the value of the coefficients of the polynomial of each element of wall transmission matrix may be calculated from the physical properties of the material.

Transfer function. Equation (4) for the overall wall transmission matrix was used for the conduction heat flow through fabrics for representation of a thermal circuit to study the mechanism of heat transmission (Raychaudhuri, 1965).

Let us consider the case of an unventilated (cubic) fish container with all the walls of similar construction. When such a container is exposed to a single excitation (temperature) on all the external surfaces, the transfer function of the container can be given by

$$TF(p) = \frac{1}{1/6Y_{ao}\left[\dfrac{E}{Y'_c} + \dfrac{F}{A_s}\right] + E} \qquad (8)$$

If Y_{ao} is neglected, the transfer function is reduced to $1/E$. Thus the value of the transfer function may be determined from the corresponding value of the element in the overall wall transmission matrix in equation (4).

Thermal time constant. As soon as the transfer function $TF(p)$ of the container is determined, the response of the container for any given external excitation may be obtained by first multiplying the $TF(p)$ with the Laplace transform of the excitation function and then obtaining the inverse Laplace transform of the end product, using partial fraction expansion. For example, the temperature response function due to unit step excitation may be given by,

$$\phi_\gamma(\tau) = 1 - \sum_{i=1}^{m} \kappa_i \exp(-\psi_i\tau) \qquad (9)$$

Where, ψ_i = roots of the polynomial in the denominator of $\frac{1}{p}\cdot TF(p)$; κ_i = constants obtained in the partial fraction expansion; and m = highest degree of p taken in the above expansion for certain accuracy.

The time required by the $\phi_\gamma(\tau)$ of the container—after application of external excitation—to attain 63.21% of the steady-state value is defined as the thermal time constant. Thus it is possible to compare the thermal characteristics of fish transport containers in terms of a single parameter, the thermal time constant.

EXPERIMENTAL

THERMAL CONDUCTIVITY of the container material was determined by the standard ASTM Method (C_{168-67}).

The mean specific heat of the material was determined by ASTM standard Method (C_{351-61}).

The value of the coefficients of the polynomials of each element of all wall transmission matrices were calculated from the physical properties of material as determined experimentally.

Table 1—Thermophysical properties of materials

Material	Thickness (ℓ) meter	Thermal conductivity (κ) Kcal/mhr°C	Density (ρ) Kg/m³	Specific heat (C_p) Kcal/kg°C	Thermal diffusivity (α) m²/hr
Plywood[a]	0.0040	0.0376	480	0.65	1.20 X 10⁻⁴
Polyethylene[a] film	0.0001	0.0280	940	0.55	0.41 X 10⁻⁴
Expanded polystyrene[b]	0.0100	0.0280	15	0.24	77.00 X 10⁻⁴
3-ply moisture proof corrugated board[a]	0.0032	0.0544	310	0.32	5.50 X 10⁻⁴
Fish[c]	0.0279[d]	0.4740	842	0.80	1.50 X 10⁻⁴

[a] Determined experimentally by ASTM standard method
[b] Ramachandran, V. 1973. Private communication. BASF India Ltd., Bombay, India
[c] Cutting (1969)
[d] The entire volume of fish (1 cu ft) was distributed to the six sides of the container (area 1 sq ft) to obtain equivalent thickness of fish layer.
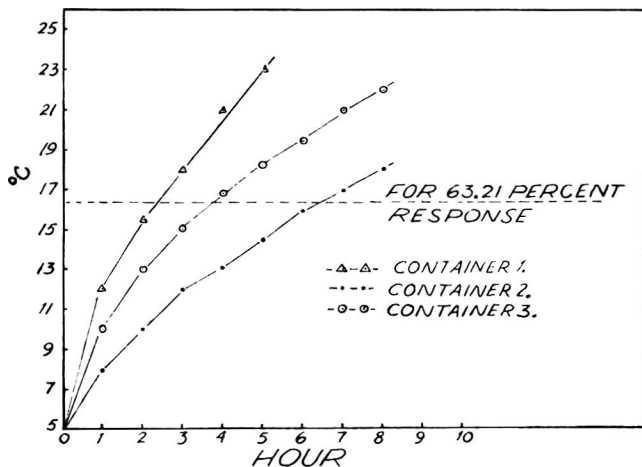

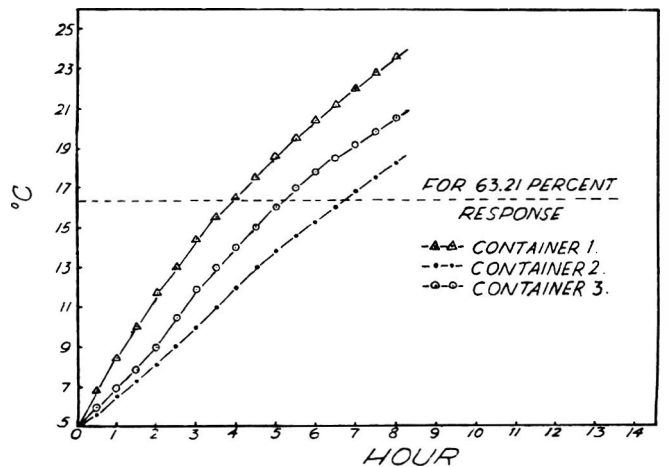Fig. 1—Thermal response of empty fish container stored at 31°C.

Fig. 2—Thermal response of fish containers packed with chilled fish at 5°C and stored at 31°C.

Table 2—Thermal time constant of various containers under different boundary conditions

Container no.	Material	Container description cubic: 30.48 cm X 30.48 cm X 30.48 cm	Thermal time constant of the container (hr)	
			With no internal mass	Packed with chilled fish
1	plywood	plywood box lined inside with polyethylene film	2.40	3.90
2	plywood	plywood box lined inside with 1 cm thick expanded polystyrene in polyethylene bag	6.50	6.70
3	paper	3-ply double walled corrugated moisture proof board with wood wool insulation in between the walls	3.80	5.25

Table 3—Comparison of experimental and predicted value of thermal time constant

Container no.	Thermal time constant of container with no internal mass (hr)		Thermal time constant of container packed with chilled fish (hr)	
	Experimental	Predicted	Experimental	Predicted
1	2.40	2.32	3.90	3.80
2	6.50	6.28	6.70	6.55
3	3.80	3.65	5.25	4.98

Table 4—Icing method for ice and fish in the ratio 1:1

Method	Arrangement of ice and fish	Fraction of the total weight of	
		Ice	Fish
1	1st layer (top)	1/4	—
	2nd layer	—	1/2
	3rd layer	1/4	—
	4th layer	—	1/2
	5th layer (bottom)	1/2	—
2	1st layer (top)	3/8	—
	2nd layer	—	1/2
	3rd layer	1/4	—
	4th layer	—	1/2
	5th layer (bottom)	3/8	—
3	1st layer (top)	1/2	—
	2nd layer	—	1/2
	3rd layer	1/4	—
	4th layer	—	1/2
	5th layer (bottom)	1/4	—

The matrix multiplication and addition was carried out in an IBM 1132 computer using a program developed for this purpose. The coefficients of the polynomial of the resulting matrix corresponding to the element E of the overall wall transmission matrix was found in the form $1 + bp + cp^2$ (neglecting terms from third power of p), where b and c are known constant coefficients.

Thus,
$$TF(p) = \frac{1}{1 + bp + cp^2} \tag{10}$$

The temperature response function can be written as.

$$\phi_\gamma(T) = L^{-1} \left\{ \frac{1}{p} \cdot \frac{1}{1 + bp + cp^2} \right\} \tag{11}$$

By partial fraction expansion we get,

$$\phi_\gamma(T) = 1 - \frac{b}{\sqrt{b^2 - 4c}} \cdot e^{-\frac{b}{2c}\tau} \operatorname{Sinh}\sqrt{D}\,\tau$$
$$- e^{-\frac{b}{2c}\tau} \operatorname{Cosh}\sqrt{D}\,\tau \tag{12}$$

where, $D = b^2/4c^2 - 1/c$ and $b^2 > 4c$.

To attain 63.21% of the steady-state value

$$\phi_\gamma(T) = 1 - \frac{1}{e} =$$
$$1 - \frac{b}{\sqrt{b^2 - 4c}} e^{-\frac{b}{2c}\tau} \operatorname{Sinh}\sqrt{D}\,\tau - e^{-\frac{b}{2c}\tau} \operatorname{Cosh}\sqrt{D}\,\tau \tag{13}$$

From equation (13) the temperature response function comes as,

$$\phi_\gamma(T) =$$
$$\frac{1}{e} - \frac{b}{\sqrt{b^2 - 4c}} e^{-\frac{b}{2c}\tau} \operatorname{Sinh}\sqrt{D}\,\tau - e^{-\frac{b}{2c}\tau} \operatorname{Cosh}\sqrt{D}\,\tau = 0 \tag{14}$$

The value of τ (thermal time constant) was determined by solving the transcendental equation (14) using the Newton-Rhapson Iteration process in an IBM 1132 computer.

Fish containers (30.48 cm × 30.48 cm × 30.48 cm) of different material were packed with prechilled (5°C) Talapia fish and stored at a temperature of 31°C. The midplane temperature of the fish container was recorded by a Negretti & Zambra automatic recorder (Bestobell India Ltd.). Fish containers were also packed with ice and fish in various arrangements of ice. The temperature of the top fish layer was recorded by automatic recorder.

RESULTS & DISCUSSION

THE THERMOPHYSICAL properties of containers as determined experimentally are given in Table 1. The thermal diffusivity values of the materials were used for computer prediction of thermal time constant values of the container. The

times required for attaining the temperature 16.43°C (63.21% of the steady-state temperature) were obtained from Figures 1 and 2. The difference in experimental and predicted values of Table 3 was found for neglecting the terms after the second power of p in the polynomial (equations 5, 6 and 7) corresponding to the element E of the overall wall transmission matrix (equation 4). However, the values of the thermal time constants predict that the 3-ply double-walled moisture proof corrugated container with wood-wool insulation between the walls is nearly as efficient as the expanded polystyrene-lined plywood box from the viewpoint of thermal behavior.

Since the maximum transport time was found to vary be-
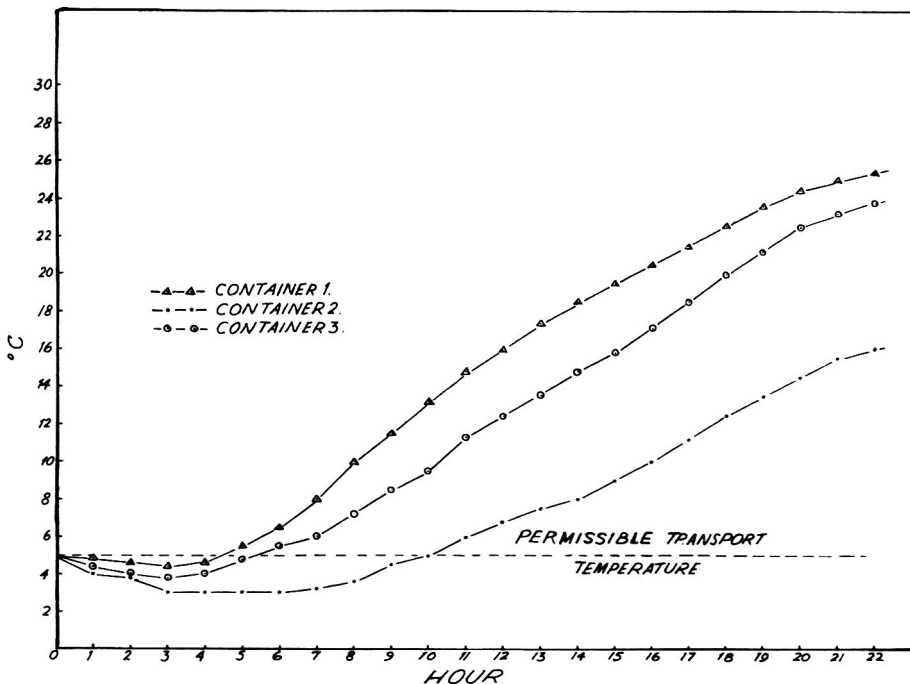
Fig. 3—Midplane temperature of fish containers for icing method 1.
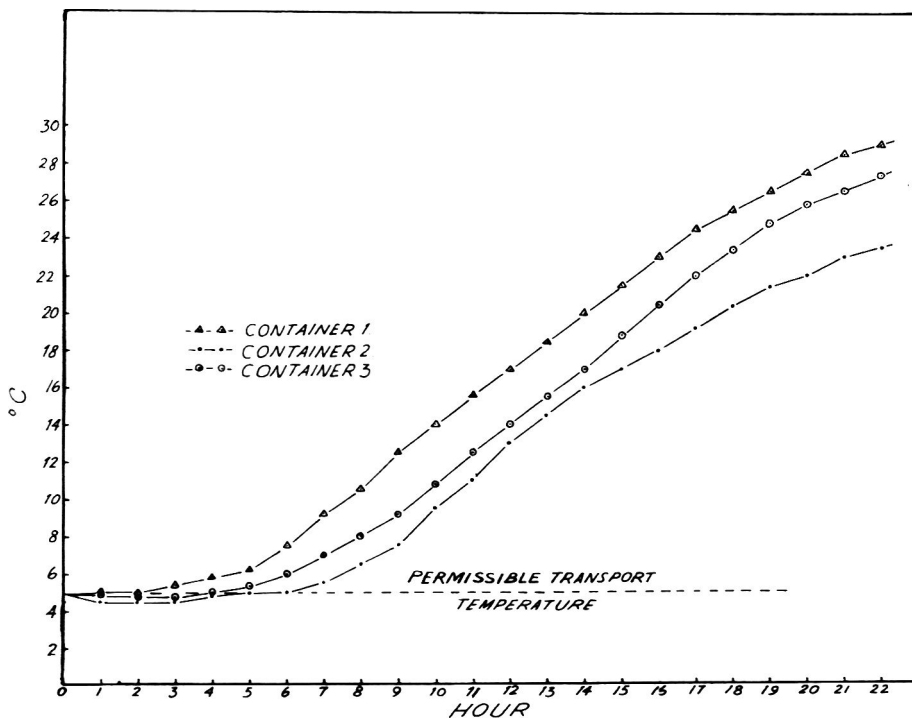
Fig. 4—Midplane temperature of fish containers for icing method 2.
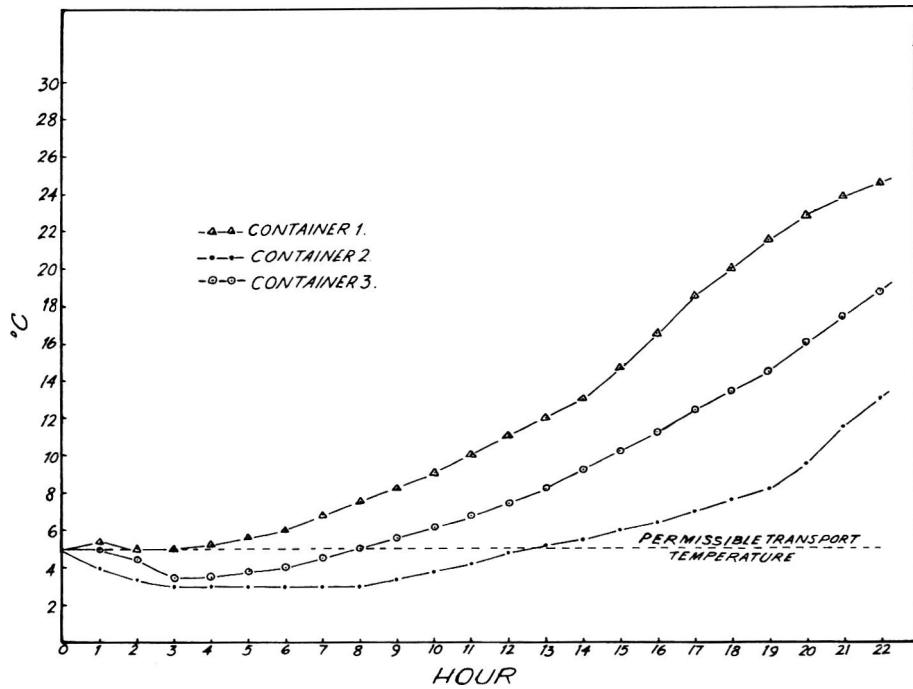
Fig. 5—Midplane temperature of fish containers for icing method 3.

tween 72 and 80 hr, a permissible temperature of 5°C may be suggested to keep fish at the acceptable quality level throughout the period of transport. The time required for attaining the permissible transport temperature of fish (5°C) packed in the three different containers with various icing arrangements as described in Table (4) may be obtained from Figures 3, 4 and 5. These results also agree well with the prediction of thermal characteristics of fish containers in terms of the thermal time constant.

As the increase in mass-to-surface ratio will not affect the computation process, it can be suggested that re-icing of fish at intermediate points will improve the final quality of fish transported in experimental containers (30.48 cm × 30.48 cm × 30.48 cm); otherwise, containers with better insulation would have to be developed to be suitable for transporting iced-fish over a period of 72–80 hr without re-icing.

This method, which was found capable of predicting insulation behavior of fish containers for a simple boundary condition (cubic), is expected to yield reliable results for generalized boundary conditions (any size) taking into account the variables controlling the heat transfer process which are sufficiently accurate for the design of fish transport containers.

NOMENCLATURE

Symbol	Definition	Units
t	temperature	°C
x	space coordinate measured along the width of the wall fabric	
α	thermal diffusivity	m²/hr
τ	time	hr
ϱ	thickness	m
p	Laplace transform parameter	

β	$= \sqrt{p/\alpha}$	
Z_0	$= 1/\kappa\beta$	
κ	thermal conductivity	Kcal/mhr°C
Y_{ao}	capacitive admittance of air inside the container	Kcal/hr°C
Y_c'	average surface admittance for the internal surfaces combining the effects of radiative and convective exchanges	Kcal/hr°C
A_s	surface area perpendicular to the heat flow path	m²
E, F, G, H, A, B, C	elements of the wall transmission matrix	
1/R	surface heat transfer coefficient	Kcal/m²hr°C

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ANALYSIS OF A VAPOR PRESSURE MANOMETER FOR MEASUREMENT OF WATER ACTIVITY IN NONFAT DRY MILK

INTRODUCTION

THE ESTABLISHMENT of water activity as a significant parameter in maintaining storage stability of dry and intermediate moisture foods has resulted in the need for rapid determination of the value. Traditional methods of determining the water activity are time consuming and require elaborate and expensive instrumentation. A method for water activity measurement, which will have commercial acceptability, must allow for rapid individual determinations as well as the capacity to measure several different samples in a relatively short period of time. In addition, the accuracy of the method should approach that of the available, more complex, methods.

The objectives of this investigation included the following: (a) to establish the optimum design for a vacuum manometer system used for direct water activity measurement of nonfat dry milk; (b) to investigate the influence of significant factors on design of the system; and (c) to evaluate the reproducibility and accuracy of the method.

Several previous investigations have investigated the use of direct water vapor pressure measurement as a means to determine water activity. The differential vacuum manometer described by Gibson and Adams (1933) is a standard apparatus used by physical chemists to measure the vapor pressure of solution. Makower and Myers (1943) designed a simple apparatus to measure the vapor pressure of foods. A freeze trap was introduced on the sample side to prevent the escape of moisture during evacuation of the apparatus. At the end of the evacuation period, the manometer was opened to the freeze trap and the moisture in the trap evaporated quickly and readsorbed on the food. Taylor (1961) modified the Makower and Myers (1943) apparatus to measure desorption isotherms for a variety of dehydrated foods. The freeze trap was eliminated; instead the sample was frozen to prevent escape of moisture during evacuation of the apparatus. The apparatus was expanded to allow vacuum distillation of moisture from the sample in steps, so that this same sample could be used for vapor pressure measurement at different moisture contents. Labuza et al. (1972) obtained more reproducible results with the vapor pressure technique compared to an electric hygrometer.

EXPERIMENTAL

THE APPARATUS was designed to measure the water vapor pressure of foods up to a temperature of 30°C, over the complete water activity range. The water activity of pure water is 1, and the vapor pressure of pure water at 30°C is 31.824 mm of mercury.

Apeizon B oil was used as the manometric fluid which gave a magnification factor of 15.7 compared to mercury (Taylor, 1961). The height of the manometer required was 31.824 × 15.7 = 499.637 mm. The manometer could be read accurately up to 0.2 mm, giving a sensitivity of 0.0004 water activity at 30°C. The

A. Manometer.
B. Three-way stopcock.
C. Three-way stopcock.
D. Sample tube.

Scale 1:5

Fig. 1—Vapor pressure apparatus for water activity measurement.

sensitivity of the apparatus could be increased by using higher temperatures, provided the water vapor pressure of the food did not exceed the vapor pressure of pure water at room temperature. This would result in local condensation of moisture in the manometer (Taylor, 1961).

The apparatus is shown in Figure 1. It is a simplified version of the design used by Taylor (1961). It had a 500 mm high oil manometer (A), connected to a three-way ground glass stopcock (B). One arm of the manometer was connected to another three-way ground glass stopcock (C). The bulb of the stopcock (C) was blown and joined to the inner part of a 10/30 ground glass joint to accept the sample tube (D). The manometer was fabricated from 5 mm outer diameter, thick wall glass tubing. The manometer was filled with oil through the stopcock (B) up to half the height. The stopcock (B) was connected to a two-stage vacuum pump of 21 liters per minute free air displacement and 0.1μ ultimate pressure attainable.

A mixture of acetone and dry ice in a Dewar flask was used as the freeze trap. The freezing mixture has a temperature of −80°C. Water has a vapor pressure of 0.4μ at −80°C, which is much lower than the pressure to which the apparatus was evacuated. This eliminated the possibility of moisture loss during evacuation, provided the sample was frozen for sufficient time to lower the temperature to this level.

Regular spray dried nonfat dry milk with 3.9% moisture content was used as a model system for the investigation. The sample was separated into three particle size ranges including (a) 44−63 micron, (b) 63−88 micron and (c) 88−125 micron. Samples from each particle size range were equilibrated at 30°C over saturated salt solutions which provided 11.2, 22 or 32.4% relative humidity. In addition, samples were equilibrated over phosphorus pentoxide to attain a zero water activity condition.

The influence of larger particle agglomerates on the various parameters investigated was accomplished using a commercial sample of nonfat dry milk. The original sample was separated into seven different samples according to particle sizes ranging from less than 125 micron to greater than 1000 micron.

RESULTS & DISCUSSION

IN GENERAL, the objectives of the investigation were achieved by determining the influence of operating parameters and procedures on water activity measurements. Each variable was investigated independently as often as needed to assure consistent results.
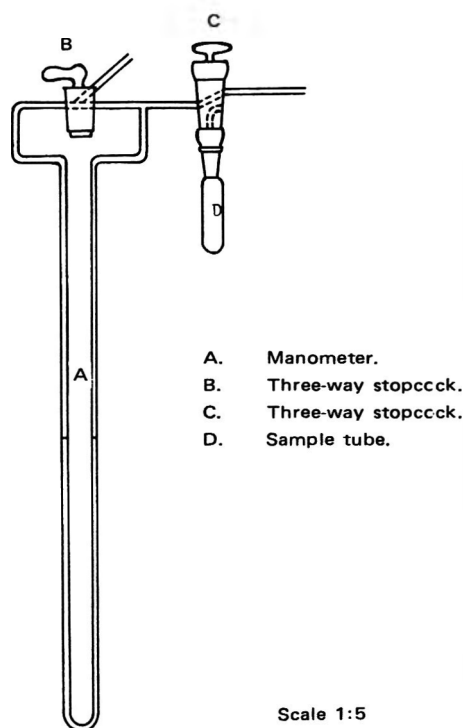
Table 1—Effect of freezing time on water activity, equilibration time and pressure due to desorbed gases on nonfat dry milk samples

Freezing time (min)	Temp of equilibration (°C)	Water activity	Equilibration time (min)	Pressure due to desorbed gases (mm of oil)
1	29.5	0.229	35	5.8
2	28.4	0.215	40	7.3
3	29.6	0.221	35	5.7
4	28.2	0.211	35	5.8
5	28.2	0.212	40	5.0
10	28.9	0.224	35	5.6
15	25.6	0.194	35	8.0

Table 2—Effect of sample size on water activity, equilibration time and pressure due to desorbed gases on nonfat dry milk samples.

Sample size (g)	Water Activity 25°C	Water Activity 30°C	Equilibration time (min)	Pressure due to desorbed gases (mm of oil)
1	0.216	0.225	25	6.5
2	0.210	0.228	30	9.0
3	0.215	0.230	45	11.5
4	0.213	0.232	45	15.0

Table 3—Effect of the volume of the sample tube on water activity of nonfat dry milk samples.

Sample no.	Water activity at 30°C 5 ml tube	Water activity at 30°C 10 ml tube	Water activity at 30°C 15 ml tube
1	0.205	0.198	0.205
2	0.207	0.211	0.212
3	0.212	0.207	0.208
4	0.213	0.214	0.214

Freezing time

The objective of freezing the sample was to prevent evaporative loss of moisture during subsequent evacuation of the apparatus (Taylor, 1961). The time required for freezing is a function of the thermal diffusivity and size of the sample. The time for freezing was varied from 1 to 15 min, and the samples were allowed to equilibrate to the room temperature in air. The results are presented in Table 1.

Freezing time did not have a consistent influence on water activity, equilibration time or pressure due to desorbed gases. The inconsistency in the results was probably due to a difference in the temperatures at which the samples were equilibrated. Water activity is temperature dependent, and the effect of temperature might have been greater than the effect of freezing time.

Sample size

In order to determine the optimum sample size for spray dried nonfat dry milk, the sample size was varied from 1–4g, while other experimental variables were held constant. The results are presented in Table 2.

A consistent influence of sample size on water activity was not apparent at 25°C, but the water activity at 30°C increased slightly with increasing sample size. The increase was, however, too small to be of any practical significance. A 1-g sample was, therefore, considered adequate for the water activity measurement

of spray dried nonfat dry milk. This confirmed the recommendation of Taylor (1961).

Sample tube volume

During equilibration, some moisture evaporates from the sample to fill the vacuum space above the sample. Taylor (1961) recommended that the volume of the sample side of the manometer should be kept to a minimum to minimize any possible reduction in water activity due to evaporation of moisture from the sam-

Table 4—Reproducibility of proposed water activity method

Replicate no.	Water activity Commercial sample	Water activity Equilibrated sample
1	0.204	0.223
2	0.209	0.225
3	0.205	0.218
4	0.209	0.227
5	0.199	0.223
6	0.204	0.223
7	0.204	0.226
8	0.206	0.226
9	0.198	0.225
10	0.202	0.227
Mean	0.204	0.224
Std dev	0.00365	0.0018

ple. This recommendation was kept in mind while designing the present apparatus. It was, however, considered important to evaluate the effect of sample tube volume on water activity of the samples.

Three sample tubes of approximately 5 ml, 10 ml and 15 ml were used, while the sample size and other process variables were held constant according to the procedure. The results are presented in Table 3.

The volume of the sample tube did not have a consistent or significant influence on the water activity of the samples. It was concluded that the evaporative loss of moisture during equilibration required to fill the vapor space above the sample was negligible.

Reproducibility

The ability of the proposed method to provide reproducible results was analyzed by repeated measurement of ten samples of nonfat dry milk. One sample with unknown water activity was measured with results as presented in Table 4. The second sample measured had been equilibrated over a saturated solution of potassium acetate (22% RH).

The results for the unequilibrated sample gave a mean water activity of 0.204 with standard deviation of 0.00365. The equilibrated sample had a mean water activity of 0.224 with standard deviation of 0.0018. The difference in standard deviation could be attributed to a uniform water activity of the equilibrated

sample. The mean value for the equilibrated sample is very close to the value expected for a sample equilibrated over potassium acetate (0.220).

Equilibration rate

Taylor (1961) recommended grinding product samples to increase rate of equilibration. The influence of particle size on the rate was evaluated by using different size fractions of regular spray dried and instant nonfat dry milk samples. The results in Table 5 illustrate that time required to reach equilibration decreased as particle size increased. There is an obvious difference between the regular nonfat dry milk and the instant product. Those results indicate that larger particles equilibrated more rapidly, possibly due to more void space within the sample. The influence of sample size on equilibration rate was indicated in Table 2.

An analysis of the equilibration rate results indicated that differences between times for equilibration for different particle size samples could be attributed to a lag in the change in vapor pressure during the measurement. These observations support the suggestion that more void space within the particles allowed more rapid release of the moisture vapor from the sample. The results confirmed that particle size did not influence water activity.

CONCLUSIONS

THE FOLLOWING conclusions were reached based on an experimental evaluation of the rapid water activity measurement technique.

1. The system evacuation time and sample freezing time are relatively short portions of the overall measurement procedure; about 1 min for each for a 1-g sample size.
2. The influences of sample size and sample container volume on water activity measurement were found to be insignificant.
3. Product particle size did not have a significant influence on the water activity value obtained by the proposed method.
4. The proposed method provided very reproducible results with standard deviation of 0.0018 for equilibrated samples and 0.00365 for commercial samples of nonfat dry milk.
5. Equilibration time was found to be a function of particle size with times ranging from 10 min for 1000 micron particles to 27 min for 44 micron particles; total measurement time would vary from 12–30 min per sample.

Table 5—Effect of particle size on equilibration time for regular and instant nonfat dry milk

Particle size (μ)		Equilibration time (min)
44–63		23–27
63–88	— Regular nonfat dry milk —	25
88–125		22–25
<125		14
125–177		14
177–250		14
250–500	— Instant nonfat dry milk —	10
500–707		11
707–1000		10
1000–1410		11

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TURBULENCE PROMOTERS IN ULTRAFILTRATION
OF WHEY PROTEIN CONCENTRATE

INTRODUCTION

PRESSURE DRIVEN membrane separation processes have comparatively recently reached the industrial stage. In processing diluted salt solutions, e.g., in desalination of water or recovery of valuable metals, the capacity of a given apparatus is generally given by the permeability of the membrane and the available driving force, i.e., the difference between the applied pressure and the osmotic pressure of the solution.

The phenomenon of concentration polarization causes increase of the effective osmotic pressure. However, concentration polarization is easily relieved by increased tangential velocity across the membrane and does not significantly alter the inherent characteristics of existing commercial membranes under proper operating conditions. The picture is completely different in ultrafiltration of solutes of higher molecular weight. The permeability of the membrane is as a rule high compared to the permeability of the layer of macromolecules that accumulates at the membrane interface. Thus, it is the behavior of this layer which determines the productivity of the separation equipment, both in terms of flux and in terms of separation characteristics. The problem was investigated by Blatt et al. (1970), Kozinsky and Lightfoot (1971), Merson et al. (1968), de Filippi and Goldsmith (1970), Strathman (1973) and Porter (1972).

In the food industry, the membrane processes are expanding particularly for separation of whey proteins and concentration of skim milk. The particular problems of dairy products were studied in detail by Lim et al. (1971), Fenton-May et al. (1971), Peri and Dunkley (1971) and Peri et al. (1973) and others.

In order to improve flux, measures must be taken to facilitate the transport of the accumulated solutes away from the membrane. The majority of commercial equipment today is making use of membrane-lined channels of constant cross-section. In addition, the channels used are long channels hydraulically speaking, i.e., the hydrodynamic entrance length is but a small fraction of the channel length. The hydraulic entrance length is estimated to be 0.03 · D · Re according to Kay (1963). Thus the existing equipment depends on high flow rates to induce turbulence or achieve high wall shear stress.

Potential performance of turbulence promoters has been evaluated for desalination applications by Thomas et al. (1971). They conclude that the advantage of TP lies in treatment of feeds with high fouling potential. Peri and Dunkley (1971) investigated the effect of detached turbulence promoters of Thomas (1967) in reverse osmosis of whey. Lowe and Durkee (1971) reported considerable success with dynamic turbulence promotion in processing of orange juice. Lately, Pitera and Middleman (1973) published data on the improvement of the mass transfer coefficient (j-factor) in reverse osmosis of dilute salt solutions with help of a Kenics Static Mixer as turbulence promoter.

In the present work the effect of a twisted tape insert of the same type is investigated in processing of simulated whey protein concentrate.

MATERIALS & METHODS

THE SOLUTIONS of whey from the production of "herrgård" cheese were concentrated in Havens 215 VDR ultrafiltration equipment to 11% DS (6.5% protein) and spray dried. For the experiments, the powder was reconstituted with distilled water to 11% DS or 5% DS. At 25°C the solutions had the viscosity of 3.3 and 1.6 centipoise, respectively, using the falling ball (Hoeppler) viscosimeter.

The modules

A membrane tube 52 cm long was cut from a Havens 215 VDR module. The volume displacement rod was removed. Two brass strips of 12 mm width were twisted to right-turn and left-turn spirals of 31
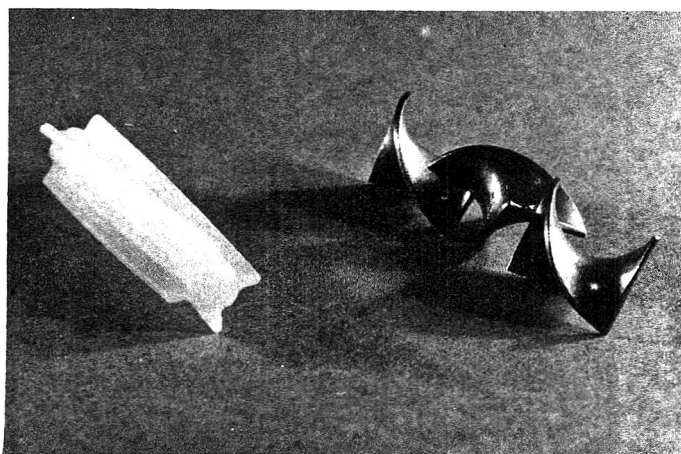

Fig. 1—Volume displacement rod and twisted strip turbulence promoter.
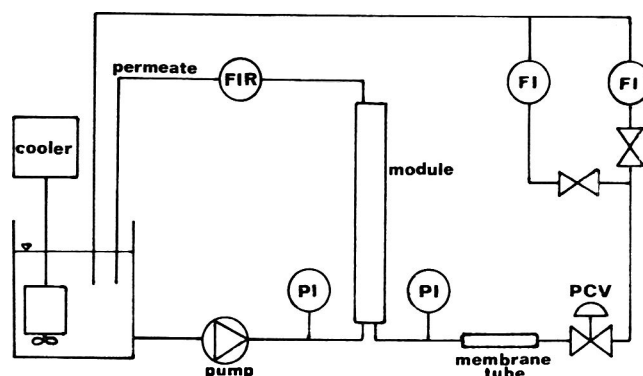

Fig. 2—Flow sheet of the apparatus.

turns/m. The spirals were cut into elements of 180° twist. Alternate elements were notched axially to a depth of 0.3 cm at each end to make proper assembly possible (Fig. 1). The elements were inserted in the membrane tube in such a way that an element with right twist was followed by a left-twisted one and the edges facing each other were at right angles. The tube with 32 turbulence promoter elements was mounted into a modified fixture provided for a Havens bench-top unit. For comparison, a complete Havens 215 VDR module consisting of 18 tubes 8 ft long coupled in series was used. The 1/2-in. tubes were equipped with volume displacement rods so that the effective cross-sectional flow area was 80 mm^2.

The loop

Figure 2 gives the flow diagram of the loop. A triplex plunger pump (model C, Cat Pumps) pressurized the feed. It was passed through the module and through the membrane tube with the promoters. A pneumatically controlled valve (Fisher Controls) maintained the pressure. Pressure gauges were provided at the inlet and outlet of the Havens module. One of the two rotameters (Brooks) alternatively could be used to follow the flow rate. The permeate outlet of the module was coupled to an electromagnetic flow meter (Kent Instruments). The permeate flux of the tube with turbulence promoters was followed manually with measuring cylinder and stopwatch.

The permeate was recirculated to the feed vessel. The temperature was controlled at 25 ± 1°C by a submerged cooler (Alfa Laval).

RESULTS

THE RESULTS of the experiments with solutions of 11% DS are given in Figure 3 and those of 5% DS in Figure 4. The circulation flows indicated on the curves were measured directly; the pressure drops indicated are approximate figures arrived at in the following way:

Pressure drops of a 52 cm long tube with the Havens end pieces were measured with a U-tube mercury manometer for water of 20°C. The pressure drops were determined for empty tube (Δp_e), a tube with volume displacement rods (Δp_{VDR}) and a tube with turbulence promoters (Δp_{TP}). The theoretical pressure drop of the tube itself is negligible in comparison to

other effects. Thus the value for an empty tube is expected to correspond mainly to pressure drop due to inlet and outlet effects. ($\Delta p_{VDR} - \Delta p_E$) and ($\Delta p_{TP} - \Delta p_E$), converted to 1m tube length are indicated in figures.

The Havens module consisted of 18 tubes of total length 40.5m. The expected pressure drop:

$$18\Delta p_E + 40.5 \cdot (\Delta p_{VDR} - \Delta p_E)/0.52$$

corresponds closely to data measured by Winge (1972) on whey at 25°C. The ultrafiltration experiments show convincingly that at the same recirculation rate, the twisted-tape turbulence promoters are far superior to the volume displacer rods as antipolarization devices, the flux being increased by a factor of two when the maximum flux for a given recirculation rate is reached. Actually, the results are slightly biased against the turbulence promoter tube; it was working at a higher average concentration than the module. For the lowest tangential flow used, the difference between the average concentrations amounted to 18%.

DISCUSSION

CONSIDER a concentrating system operating in a single pass. Assume that a constant linear tangential velocity must be maintained to guarantee satisfactory flux (assumed independent of concentration). Apparently, the channel dimensions should progressively decrease to compensate for the decreasing volume of the concentrated solution. The practical way of introducing this taper is to use a diminishing number of channels in parallel, which is common practice in continuous plants.

Let R = the required degree of concentration (ratio of inlet to outlet volumetric flow); c = linear tangential velocity; F = membrane flux; Q = concentrate flow at system outlet; A = crosssection of a channel; O = membrane-clad circumference; n
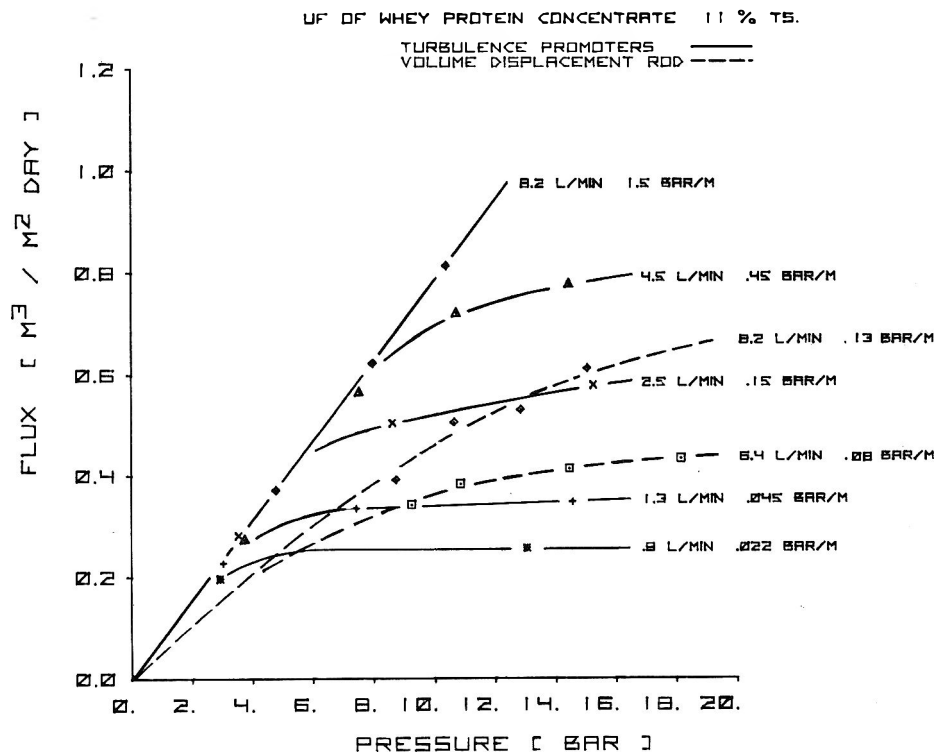

Fig. 3–Permeate flux vs. driving pressure for 11% DS concentrate.

= number of identical channels in parallel; and x = length dimension.

A mass balance of the system gives the necessary taper. If for simplicity a continuously changing number of channels and unchanging permeate flux is assumed, then approximately

$$c \cdot A \frac{dn}{dx} = -n \, OF \qquad (1)$$

$$\ln \frac{n}{n_{in}} = -\frac{OFx}{cA} \qquad (1a)$$

but

$$n_{in} = \frac{QR}{cA} \qquad (1b)$$

$$n = \frac{QR}{cA} \exp\left(-\frac{OFx}{cA}\right) \qquad (2)$$

The length of this ideal system narrowing down to only one tube is

$$L = \frac{cA}{FO} \ln R \qquad (3)$$

For R = 10 and a half-inch tube, L is of the order of 1000m. In a practical system, however, n can only be an integer and the permeate flux decreases along the channel with increasing concentration, which contributes to the length of the system. The total pressure drop along the ideally tapered system is approximately

$$\Delta p_{tot} = \left(\frac{\Delta p}{\Delta x}\right) \cdot L = \left(\frac{\Delta p}{\Delta x}\right) \frac{cA}{FO} \ln R \qquad (4)$$

if viscosity changes are neglected.

The power dissipated in friction losses along the channel using a single pump, E_1

$$E_1 = Q \cdot \left(\frac{\Delta p}{\Delta x}\right) \frac{cA}{FO} \cdot R \cdot \ln R \qquad (5)$$

The corresponding minimum power consumption, for an infinite number of booster pumps

$$E_\infty = \int_0^L \left(\frac{\Delta p}{\Delta x}\right) \cdot n \cdot c \cdot A \, dx = \left(\frac{\Delta p}{\Delta x}\right) \frac{cA}{FO} Q (R-1) \qquad (6)$$

Similarly it may be shown that in batch operation, the power required by the circulation pump is approximately

$$E_B = \left(\frac{\Delta p}{\Delta x}\right) \frac{cA}{FO} (R-1) \cdot Q = E_\infty \qquad (7)$$

In any membrane operation a maximum practical operating pressure exists, given by compaction of the membrane or pressure rating of the apparatus. At the same time, a minimum pressure capable of overcoming the osmotic pressure of the solution is necessary. Thus it is very likely that in the single pass operating mode, which is characterized by Δp_{tot}, a number of booster pumps will be necessary to keep inside the given pressure limits. An important point arises from Eq 4. For a given output and concentration ratio, the number of booster pumps is directly proportional to the parameter $\xi = (\Delta p/\Delta x) \cdot cA/FO$. Similarly, Eq 5–7 suggest that whatever the mode of operation, the energy loss due to friction is proportional to this parameter.

To compare membrane concentration operations properly, a complete economic calculation is necessary. The capital costs of membrane modules, turbulence promoters, pumps, piping and instrumentation and the running costs consisting of the costs of labor, membrane replacement, energy, cleaning and chemicals must be considered.

A turbulence promoter's design may affect essentially all of these. However, when the comparison is based on equal ξ-parameter, the cost of booster pumps is equal and so is the cost of the frictional energy. Neglecting the second order effects and comparing at the same level of driving pressure, the principal trade-off is between the flux and the membrane area dependent costs, i.e., the costs of membrane modules, membrane replacement and turbulence promoters.
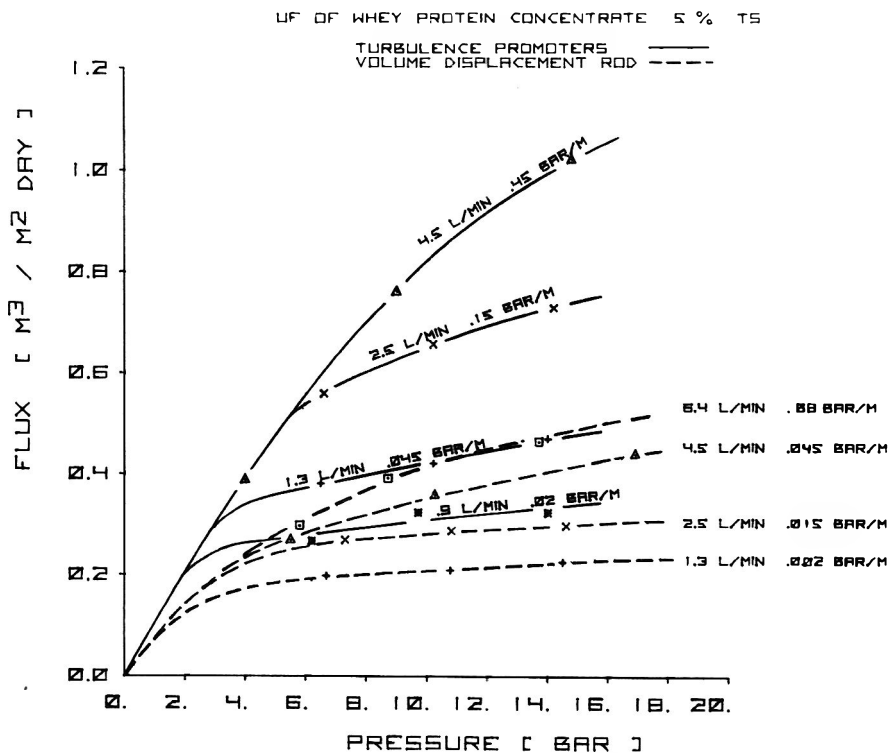
Fig. 4—Permeate flux vs. driving pressure for 5% DS concentrate.
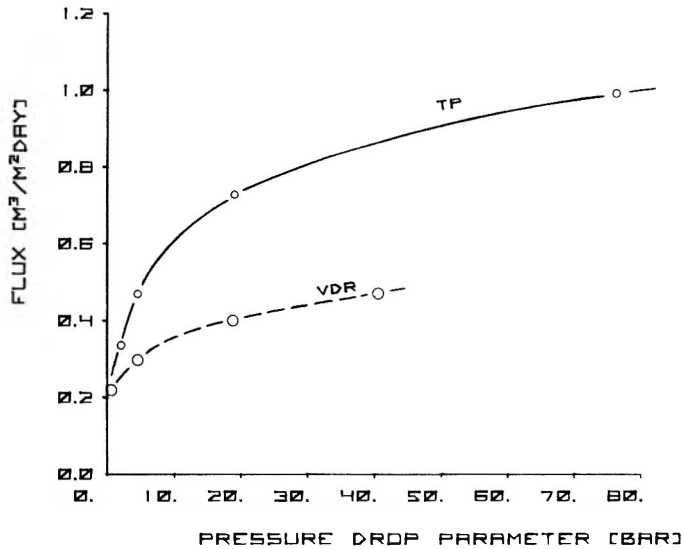
Fig. 5—Permeate flux vs. dissipational energy consumption.

It should be profitable to install a new type of turbulence promoter if the percent increase in cost of modules + replacement + promoters per square meter of membrane area is less than the percent increase in flux.

CONCLUSION

IN FIGURE 5 the results of Figure 4 are redrawn as a function of the parameter ξ for driving pressure of 14 bar.

It is apparent, that hydraulically, the twisted tape turbulence promoter is very superior to the volume displacement rod design. However, the question whether this type of turbulence promoter is economically feasible is a completely different one. The cost of the proprietary design of the twisted tape turbulence promoter seems to be several orders of magnitude too high for this application.

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GROWTH OF LACTIC ACID BACTERIA IN SOY MILKS

INTRODUCTION

SOYBEANS are an excellent source of low-cost protein. However, the soybean flavor is not as acceptable to the Western World as it is to the Asians. Beany flavors and indigestible components are obstacles to more widespread use of soybeans (Eley, 1968). Numerous processes have been developed to lessen development of undesirable flavors during processing (Wilkens et al., 1967; Mustakas et al., 1969; Steinkraus, 1973). Fermentation is known to enhance the appearance, flavor or aroma of some foods and has the possibility to further improve the acceptability of soybean products. Molds such as *Rhizopus oligosporus* (Steinkraus et al., 1960; Martinelli and Hesseltine 1964; Steinkraus et al., 1965a), *Neurospora* Sp. (Steinkraus et al., 1965b) and *Aspergillus oryzae* (Hesseltine and Wang, 1972) have been used to prepare fermented soybean foods. The action of these organisms or their preparations on soybean constituents have also been studied (Wagenknect et al., 1961; Sugimoto and Van Buren, 1970; Noguchi et al., 1970; Arai et al., 1970). However, products manufactured using molds are not widely accepted. Lactic acid bacteria which play an important role in many food fermentations have not been studied as extensively as molds in connection with the fermentation of soybeans.

Soybean milk serves as a suitable medium for propagation of lactic acid bacteria. However, acid production by *Streptococcus lactis*, *Streptococcus citrovorus (Leuconostoc citrovorum)* and *Streptococcus paracitrovorus (Leuconostoc dextranicum* is only about half as much in soy milk as in cow's milk (Gehrke and Weiser, 1947, 1948). *Streptococcus thermophilus* produces more acid in soy milk than does *Streptococcus diacetilactis* and it has been used for preparation of cheese (Hang and Jackson, 1967a, b). The suitability of *S. thermophilus* for acid production in soy milk has been confirmed by other workers (Matsouka et al., 1968; Yamanaka and Furukawa, 1970; Kim and Shin, 1971).

Lactobacillus bulgaricus (Ariyama, 1963; Wang et al., 1974) *Streptococcus faecalis* (Kenkyusho, 1965), *L. bulgaricus*

and *S. thermophilus* (Yamanaka et al., 1970), and *Lactobacillus acidophilus* (Hesseltine and Wang, 1972; Wang et al., 1974) have also been used to prepare fermented products from soybeans. Obara (1968) treated the curd obtained by salt precipitation of soy milk with proteolytic enzymes before inoculating with *Streptococcus cremoris* and *S. lactis* to prepare a cheese-like product. Soybean cheese has been prepared using *S. thermophilus* and inoculating the surfaces with *Rhizopus oligosporus* and *Penicillium camemberti* to improve its characteristics (Schroder and Jackson, 1971). Mold ripening resulted in desirable changes in texture but these were offset by development of bitter flavors. *S. thermophilus*, *Lactobacillus delbrueckii*, *Lactobacillus pentosus* and *L. dextranicum* produced greater amounts of acid in soy milk than nine other species of bacteria examined (Angeles and Marth, 1971). They observed that supplementation of soy milk with glucose or lactose enhanced acid production by *S. lactis*, *S. cremoris*, *S. diacetilactis*, *Lactobacillus casei* and *Lactobacillus helveticus* whereas sucrose addition was without effect. Incorporation of partially degraded proteins in the medium increased acid production by *S. thermophilus*, *L. pentosus* and *Leuconostoc* species while it appeared inhibitory to *L. delbruechii*.

The fermentable carbohydrates in soybeans and soybean products are low molecular weight oligosaccharides such as sucrose, raffinose and stachyose (Kawamura, 1967). Selection of lactic acid bacteria possessing the ability to utilize these oligosaccharides is essential if they are to be used in preparation of fermented products from soy milk. Consequently, this investigation was undertaken to study selected lactic acid bacteria for their growth characteristics in soy milks and their ability to utilize the carbohydrates found in soybeans and soybean products.

MATERIALS & METHODS

Cultures

Lactobacillus cellobiosis (NRRL-B-1840), *Lactobacillus buchneri* (NRRL-B-1837) and *Lactobacillus fermenti* (NRRL-B-585) were kindly supplied by Dr. William C. Haynes of the

Northern Regional Research Lab., Peoria, Ill; *Lactobacillus plantarum* (B-246) was given to us by Dr. J.R. Stamer (N.Y. State Agr. Exp. Station, Geneva, N.Y.). *S. thermophilus* (Marshall), *L. bulgaricus* (Marshall) and *L. acidophilus* ATCC No. 4356 obtained from Dept. of Microbiology, Cornell University, Ithaca, N.Y. were also included in the investigation. The stock cultures were grown and maintained in agar stabs of a medium containing Difco-tryptone 0.25%, Difco-yeast extract 0.05%, gelatin 0.25%, glucose 0.25%, sodium chloride 0.40%, sodium acetate 0.15%, ascorbic acid 0.05% and agar 1.2%. The cultures were grown in sterile litmus milk or soy milk and were maintained at 5°C between semimonthly transfers. Lactic broth (Elliker et al., 1956) was used to propagate the organisms in liquid medium.

Preparation of soy milk 1 (hot grind)

Dry, whole soybeans (variety Harasoy) were thoroughly washed and soaked in water at 60°C until the absorbed water was about 1 ml/g dry weight. The soak water was decanted and the beans were washed. The beans were ground in a Waring Blendor for 5 min (3 min at low, 1 min at medium and 1 min at high speed) with boiling water. The ratio of beans to water was 1:9 w/v and the temperature of water during grinding between 85–95°C. Use of boiling water inactivated the enzyme, lipoxygenase, during grinding (Wilkens et al., 1967). The resulting suspension was filtered under reduced pressure using a Buchner funnel equipped with a 7-in. standard Agway milk filter with one layer of coarse pad on top of a fine pad. The resultant soy milk was dispensed in 160 ml screw cap bottles, autoclaved for 15 min at 121°C and held at 5°C until used.

Preparation of soy milk 2 (defatted beans)

The process developed by Steinkraus (1973) was used for defatting the soybeans. Soy flour (40 mesh) prepared from dehulled soybeans was extracted with 95% ethanol (1:2 soy flour to solvent w/v) followed by ethanol (95%)-chloroform (1:1 v/v) mixture until a clear filtrate appeared. After each extraction the solvent was removed by filtration under reduced pressure using a Buchner funnel equipped with 6.5-in. Agway premium milk filters. The residue from the last extraction was dried at 40°C under vacuum to completely remove the solvent and powdered to uniform size in a Braun mixer (Type MX3, Max Braun, Frankfurt, Germany).

100g of defatted soy flour was comminuted with 1 liter of distilled water in a Waring Blendor for 10 min (6 min low, 2 min medium and 2 min high speed). The resulting slurry was centrifuged at 4080 × G for 20 min and the

supernatant fluid (extract 1) was collected by filtration under reduced pressure through a Buchner funnel equipped with 6.5-in. Agway premium milk filters. The residue was again comminuted with 1 liter of distilled water, centrifuged, filtered and the supernatant collected (extract II). Extracts I and II were combined, warmed to 70°C and 2.0% (w/v) sucrose and 2.5% (v/v) refined soybean oil (Proctor and Gamble, Cincinnati, Ohio) were added. The mixture was blended for 1 min and homogenized three times in a Model 15M homogenizer (Manton-Gaulin, Everett, Mass.) at 8000 PSI to obtain uniform distribution of fat. The soy milk thus prepared was dispensed in 160 ml screw cap bottles, autoclaved for 15 min at 121°C and held at 5°C until used.

Fresh whole homogenized cow's milk was purchased as required. It also was sterilized at 121°C for 15 min and cooled prior to inoculation.

Protein was determined by a slight modification of the semimicro Kjeldahl method (AOAC, 1970) replacing mercuric oxide and potassium sulfate with a Kjeldahl tablet containing sodium sulfate and selenium as the catalyst (The British Drug Houses, Ltd., Poole, England). A nitrogen to protein conversion factor of 5.71 was used (Joslyn, 1950). Fat, moisture and ash were determined by AOAC (1970) procedures.

Growth and acid production

The test inoculum was prepared by transferring the cultures from litmus or soy milk into the experimental medium and subculturing in the same medium twice at daily intervals. 100 ml of the experimental medium was brought to the temperature of incubation, inoculated with 1.0 ml of 16−18 hr culture as described earlier and incubated at 30°C for *L. plantarum* and at 37°C for the other organisms. 0.5 ml inoculum of each culture was used when growth of mixed cultures was studied. Samples were withdrawn from the experimental medium at selected intervals and analyzed for growth, acid production and changes in pH.

Plate counts on lactic agar (Elliker et al., 1956) were used to determine changes in viable counts. Duplicate plates were incubated for 24−48 hr at 37°C with the exception of *L. plantarum* which was incubated at 30°C. Titratable acidity was estimated by titrating 10.0g of sample with 0.1N NaOH using phenolphthalein as the indicator. Changes in pH were followed using a Beckman Zeromatic pH meter. The changes in both the soy milks were compared with the changes in cow's milk (protein 3.44%). The experiments were carried out in duplicate and the average values are reported.

Preparation of cell suspension

Lactic broth (Elliker et al., 1956) containing 1% glucose as the only energy source was used for preparation of cell suspensions of lactobacilli. For *S. thermophilus*, the medium contained (grams per liter of distilled water) Difco-tryptone 10.0, Difco-yeast extract 5.0, glucose 10.0, $K_2HPO_4 \cdot 3H_2O$ 1.3 and KH_2PO_4 1.0. 1-liter portions of the medium were inoculated and incubated at 30°C for *L. plantarum* and at 37°C for other organisms. The cells were harvested by centrifuging at 14,600 × G for 30

Table 1—Composition of soy milks[a]

Component	Soy milk 1 (hot grind) %	Soy milk 2 (defatted beans) %
Protein (N X 5.71)	3.31	3.04
Fat	2.20	2.41
Ash	0.49	0.33
Moisture	91.77	92.18
Carbohydrate (by difference)	2.23	2.04

[a] 2.5% refined soy oil v/v and 2.0% sucrose w/v were added to the aqueous extract of defatted soy bean flour

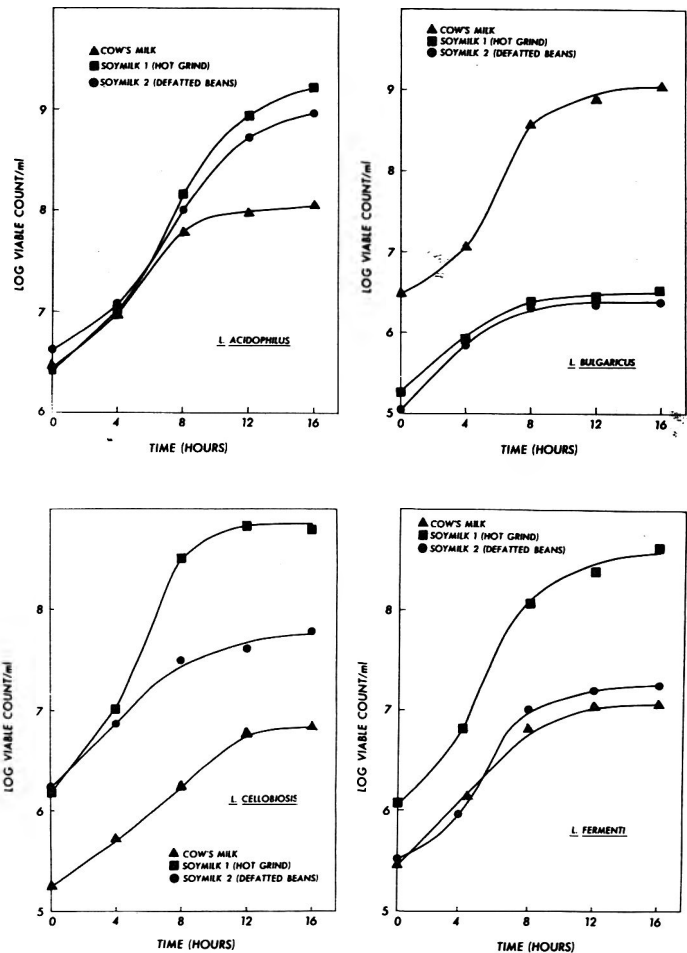Fig. 1—Growth of S. thermophilus in soy milk 1 (hot grind), soy milk 2 (defatted beans) and cow's milk.

Fig. 2—Growth of L. acidophilus, L. bulgaricus, L. cellobiosis and L. fermenti, in soy milk 1 (hot grind), soy milk 2 (defatted beans) and cow's milk.

min in Sorvall centrifuge model SS-3, washed twice with normal saline (0.85% NaCl) and centrifuged at 32,800 × G for 15 min to collect cells following each wash. They were then suspended in sterile saline (0.85% NaCl) and adjusted to 0.35–0.40 optical density at 525 nm in a Bausch and Lomb Spectronic-20. The inoculum used for studying the utilization of various carbohydrates consisted of 0.05 ml of cell suspension.

Utilization of carbohydrates

The basal medium for *S. thermophilus* contained (grams per liter distilled water) Difco-tryptone 10.0, Difco-yeast extract 5.0, $K_2HPO_4 \cdot 3H_2O$ 1.3 and KH_2PO_4 1.0. Lactic broth (Elliker et al., 1956) without carbohydrates was used for lactobacilli. Glucose, melibiose and raffinose (Nutritional Biochemicals, Cleveland, Ohio), sucrose (Mallinckrodt Chemical Works, St. Louis, Mo.) and stachyose (Sigma Chemical Co., St. Louis, Mo.) were sterilized by filtration through 0.45μ membrane filters (Millipore Corp., Bedford, Mass.) and added to the basal medium to give a final concentration of 0.5%. The medium containing carbohydrate to be tested was inoculated as described earlier and incubated at 30°C for *L. plantarum* and at 37°C for other organisms. Uninoculated broth containing the carbohydrate under study was

used for the blank. The growth of the organisms was measured in terms of optical density at 525 nm in a Bausch and Lomb Spectronic-20 colorimeter and changes in pH were followed using a Beckman Zeromatic pH meter. The average values of three pH determinations for each carbohydrate are reported.

RESULTS

THE PROXIMATE compositions of soy milk 1 and soy milk 2 show that both the milks contained similar quantities of major constituents (Table 1). However, differences in the kinds of carbohydrates existed between the two. Raw soybeans contain sucrose, raffinose, stachyose and polysaccharides (Kawamura, 1967) which are carried over in soy milk 1 while soy milk 2 contained only the 2% (w/v) sucrose which was added during the preparation. The natural soybean sugars were removed in the production of the original defatted, deflavored soy-flour base (Steinkraus, 1973).

S. thermophilus showed three- to fourfold higher population in cow's milk than in either soy milk (Fig. 1). However, rea-

sonably high numbers were also attained in both the soy milks. Lactobacilli, with the exception of *L. bulgaricus*, attained higher populations in soy milks than in cow's milk. Among the soy milks, soy milk 1 proved to be a better substrate than soy milk 2. *L. bulgaricus* population increased in cow's milk throughout the incubation period whereas the population in soy milks remained stationary after 8 hr (Fig. 2). *L. acidophilus*, *L. cellobiosis*, and *L. plantarum* attained higher maximum populations than other lactobacilli in soy milk 1 (Fig. 2 and 3). The growth of these organisms in soy milk 2 was less than in soy milk 1 but greater than in cow's milk. *L. fermenti* attained greater population in soy milk 1 than in either of the other two substrates (Fig. 2). *L. buchneri* showed slightly greater numbers in soy milk 1 than in soy milk 2 but appreciably greater numbers in both soy milks than in cow's milk (Fig. 3).

The mixed culture of *L. bulgaricus* and *S. thermophilus* exhibited maximum population in cow's milk (5.6 × 10^8/ml) as compared to soy milks (6.2–7.2 × 10^7/ml) (Fig. 3). *L. cellobiosis* and *S. thermophilus* in mixed cultures showed slightly greater population in cow's milk and soy milk 1 (1.7–2.4 × 10^8/ml) than in soy milk 2 (8.3 × 10^7/ml) (Fig. 3). Direct microscopic count (APHA 1972) of the soy milks fermented with mixed cultures revealed that *S. thermophilus* was the predominant organism throughout the incubation period.

S. thermophilus produced four times more acid than that initially present in cow's milk thereby lowering the pH to 4.46 (Table 2). Acid production in soy milk 1 (pH 4.56) and soy milk 2 (pH 4.65) was less than in cow's milk. The lactobacilli tested, with the exception of *L. bulgaricus* and to a lesser extent *L. acidophilus* exhibited little or no acid production and only slight pH changes in cow's milk. *L. bulgaricus* produced appreciable amounts of acid (0.67%) in cow's milk and appreciably lowered the pH while it caused no observable changes in soy milks (Table 2). *L. acidophilus*, *L. cellobiosis* and *L. plantarum* produced greater amounts of acid in soy milk 1 (0.43–0.49%) than in soy milk 2 (0.24–0.31%). *L. acidophilus* produced similar changes in pH in both the soy milks while *L. cellobiosis* and *L. plantarum* reached a lower pH (4.35–4.80) in soy milk 1 than in soy milk 2 (5.25–5.80, Table 2). *L. buchneri* and *L. fermenti* also produced more acid in soy milk 1 than in soy milk 2, but less than that produced by *L. acidophilus*, *L. cellobiosis* or *L. plantarum*. Mixed cultures of lactobacilli and *S. thermophilus* produced more acid in cow's milk than in soy milks with the exception of *L. acidophilus* which showed similar acid production in all three milks (Table 2).
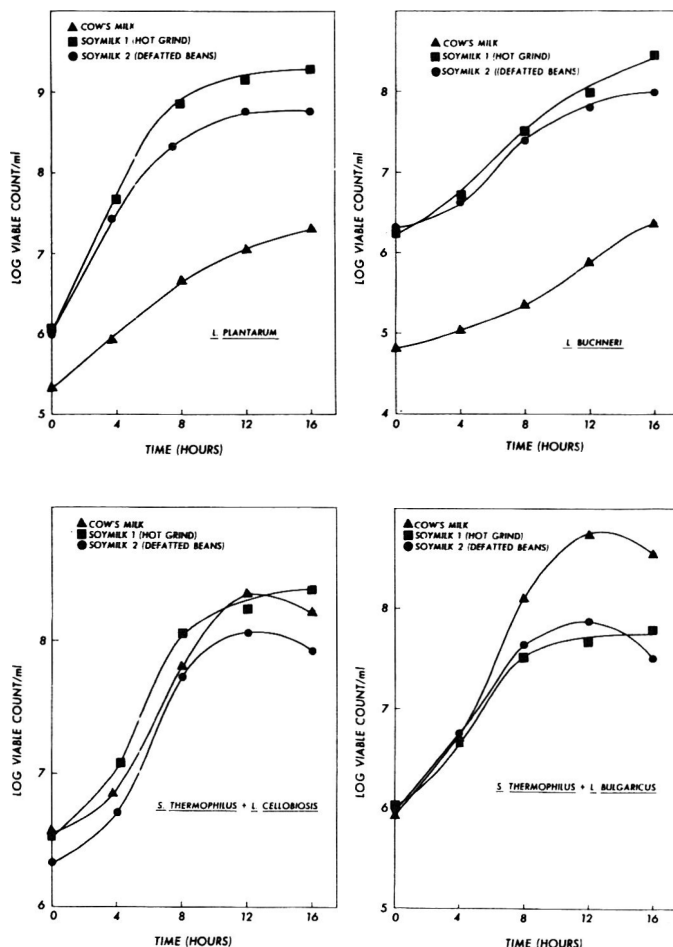

Fig. 3–Growth of L. plantarum, L. buchneri and mixed cultures of S. thermophilus and L. cellobiosis and S. thermophilus and L. bulgaricus in soy milk 1 (hot grind), soy milk 2 (defatted beans) and cow's milk.

Table 2—Growth and acid production by lactic acid organisms in cow's and soy milks[a]

Organism	Cow's milk			Soy milk 1 (hot grind)			Soy milk 2 (defatted beans)		
	Viable counts/ml[b]	pH[c]	%TA[c]	Viable counts/ml[b]	pH[c]	%TA[c]	Viable counts/ml[b]	pH[c]	%TA[c]
Initial range		6.40	0.16–0.18		6.40	0.16–0.18		6.65	0.10–0.12
S. thermophilus	4.30 X 10⁸	4.46	0.62	1.65 X 10⁸	4.56	0.47	1.06 X 10⁸	4.65	0.43
L. acidophilus	1.10 X 10⁸	5.95	0.26	1.74 X 10⁹	4.70	0.43	9.40 X 10⁸	4.90	0.31
L. bulgaricus	1.10 X 10⁹	3.98	0.67	3.40 X 10⁶	6.32	0.21	2.40 X 10⁶	6.28	0.12
L. buchneri	2.39 X 10⁷	6.30	0.18	2.80 X 10⁸	5.48	0.34	1.00 X 10⁸	5.95	0.20
L. cellobiosis	6.68 X 10⁶	6.34	0.18	6.30 X 10⁸	4.80	0.49	6.00 X 10⁷	5.80	0.25
L. fermenti	1.18 X 10⁷	6.35	0.19	4.40 X 10⁸	5.75	0.27	1.82 X 10⁷	6.20	0.14
L. plantarum	2.00 X 10⁷	6.28	0.19	1.84 X 10⁹	4.35	0.49	5.70 X 10⁸	5.25	0.24
S. thermophilus + L. acidophilus	2.02 X 10⁸	4.56	0.61	3.40 X 10⁸	4.28	0.63	1.64 X 10⁸	4.20	0.61
S. thermophilus + L. bulgaricus	5.60 X 10⁸	4.0	0.71	6.20 X 10⁷	4.26	0.47	7.20 X 10⁷	4.41	0.48
S. thermophilus + L. buchneri	1.78 X 10⁸	4.60	0.67	1.46 X 10⁸	4.42	C.42	1.54 X 10⁸	4.34	0.39
S. thermophilus + L. cellobiosis	1.70 X 10⁸	4.65	0.69	2.45 X 10⁸	4.50	C.49	8.27 X 10⁷	4.50	0.41
S. thermophilus + L. fermenti	1.73 X 10⁸	4.70	0.61	3.40 X 10⁸	4.50	0.44	2.50 X 10⁸	4.48	0.38

[a] Average of duplicate experiments
[b] The viable counts represent the maximum attained during the 16-hr incubation period.
[c] Titratable acidity and pH were determined after 16 hr.

The changes in pH in broth medium containing 0.5% of the carbohydrate under study as the only energy source are presented in Table 3. Glucose was included for comparison. Utilization of melibiose, an $\alpha(1\rightarrow6)$ galactoside, though not reported in raw soybeans, would indicate the capability of the organisms to sever α-D-galactosidic bonds present in raffinose and stachyose. *S. thermophilus* and *L. acidophilus* utilized only glucose and sucrose while *L. bulgaricus* utilized only glucose. *L. buchneri, L. cellobiosis* and *L. plantarum* utilized sucrose, melibiose, raffinose and stachyose. *L. fermenti* also utilized melibiose, raffinose and stachyose but no growth was observed in sucrose medium.

DISCUSSION

ACID PRODUCTION in the medium depends upon the growth of the organisms and their ability to ferment available carbohydrates. Green or immature soybeans contain substantial amounts of monosaccharides such as glucose, galactose, fructose and arabinose but they disappear as the beans approach maturity. The carbohydrates in mature soybeans are (1) polysaccharides — acidic polysaccharides, arabinogalactans and cellulosic material and (2) oligosaccharides — sucrose (5.0%), raffinose (1.1%) and stachyose (3.8%) (Kawamura, 1967). Since sucrose is the major fermentable sugar, organisms which utilize sucrose produce substantial amounts of acid in soy milk.

All the organisms exhibited more growth in soy milk 1 than in soy milk 2. The differences in the two soy milks are related to the loss of some of the growth factors during solvent extraction of the beans for the preparation of the latter milk. *S. thermophilus, L. acidophilus, L. cellobiosis* and *L. plantarum* showed appreciable growth and acid production in soy milk 1 because of their ability to utilize sucrose. Previous investigators have also reported substantial acid production by *S. thermophilus* in soy milk (Hang and Jackson, 1967a; Matsouka et al., 1968; Kim and Shin, 1971; Angeles and Marth, 1971). *L. acidophilus* and *L. bulgaricus* have also been used recently in preparation of fermented product from soy milk (Hesseltine and Wang, 1972; Wang et al., 1974). Although *L. buchneri* utilized sucrose, it exhibited a rather slow rate of growth in both the soy milks and would thus require longer time to produce suffi-

cient acid. *L. fermenti* showed slight acid production in soy milk 1. Angeles and Marth (1971) reported no acid production by this organism in a similar medium.

In general, mixed cultures of different lactobacilli and *S. thermophilus* showed similar acid production in both the soy milks. Yamanaka et al. (1970) utilized a mixed culture of *S. thermophilus* and *L. bulgaricus* in preparing a soy milk beverage.

The findings on utilization of various carbohydrates by different organisms are in agreement with previous reports (Rogosa and Sharpe, 1959), (Subcommittee of the International Committee on Nomenclature of Bacteria of the International Association of the Microbiological Societies, 1968). The inability of *L. bulgaricus* to utilize sucrose and galactooligosaccharides explains the poor growth and acid production by this organism in

Table 3—Changes in pH in different carbohydrate media inoculated with lactic acid bacteria and incubated for 72 hr

Organism	Glucose	Sucrose	Melibiose	Raffinose	Stachyose
Initial value	6.60–6.70	6.60–6.70	6.60–6.70	6.60–6.70	6.60–6.70
Streptococcus thermophilus	4.30	4.28	6.60	6.66	6.70
Lactobacillus acidophilus	4.45	4.52	6.70	6.68	6.62
Lactobacillus bulgaricus	4.37	6.65	6.50	6.60	6.65
Lactobacillus buchneri	4.56	4.96	4.60	4.75	5.20
Lactobacillus cellobiosis	4.44	4.51	4.30	4.72	4.85
Lactobacillus fermenti	4.62	6.48	4.70	4.91	4.98
Lactobacillus plantarum	4.48	4.20	4.28	4.68	4.75

soy milks. However, Ariyama (1963) used *L. bulgaricus* to prepare a synthetic yogurt from soy milks supplemented with 15% sucrose. Sixty two percent of the strains of *L. acidophilus* isolated by Wheater (1955) utilized melibiose and raffinose. A doubtful reaction for fermentation of these carbohydrates by *L. acidophilus* has been recorded by the Subcommittee of the International Committee on Nomenclature of Bacteria of the International Association of the Microbiological Societies (1968). Results showed that the strain of this organism used in this study does not utilize melibiose, raffinose or stachyose. *L. buchneri, L. cellobiosis, L. fermenti* and *L. plantarum* utilized melibiose, raffinose and stachyose. The results on utilization of melibiose and raffinose by these organisms are in agreement with previous findings (Rogosa and Sharpe, 1959; Subcommittee of the International Committee on Nomenclature of Bacteria of he International Association of the Microbiological Societies, 1968). Stachyose was, however, not included in these investigations. Anaerobic spore forming bacteria (clostridia) and human ileal and colonic microflora have also been shown to utilize stachyose (Calloway et al., 1966; Steggerda, 1968; Rackis et al., 1970). Previous investigations (Rogosa and Sharpe, 1959; Subcommittee of the International Committee on Nomenclature of Bacteria of the International Association of the Microbiological Societies, 1969) showed that some strains of *L. fermenti* utilize sucrose. However, the *L. fermenti* strain used in this study showed no growth in the medium containing sucrose as the only energy source.

The results of this investigation confirm that lactic acid bacteria possessing the ability to utilize sucrose can be successfully employed to manufacture fermented products from soy milks. Some of these organisms have also been shown to possess α-galactosidase, an enzyme necessary to split α-galactosidic bonds present in raffinose and stachyose (Mital et al., 1973).

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PHYTIC ACID IN SOY AND ITS HYDROLYSIS DURING BREADMAKING

INTRODUCTION

THE USE of soy preparations in foods as an economical source of protein has increased tremendously in recent years. Like most seeds, soy contains a substantial amount of phosphorus (Altman and Dittmer, 1968). However, most of this phosphorus is tied up in phytic acid complexes (Mollgaard et al., 1946). Due to reported absence of phytate-splitting enzyme system in man (Anonymous, 1967; Kon et al., 1973), phytate phosphorus is presumed to be poorly available physiologically. Evidence has been presented recently that man probably possesses phytase (Bitar and Reinhold, 1972) but that the lack of phytate cleavage may be caused by inhibitors of phytate destruction present in the food such as bread (Reinhold et al., 1973). The presence of undegraded phytates also renders less available for absorption some of the essential elements such as calcium, iron, magnesium and zinc (Anonymous, 1967; Kon et al., 1973; Prasad and Oberleas, 1973). Some of the phytate in soy probably undergoes hydrolysis during food preparation. In view of increasing significance of soy in human nutrition, present studies were undertaken to examine the extent of hydrolysis, during breadmaking, of phytates present in various commercial soy preparations.

EXPERIMENTAL

FIFTEEN soy preparations obtained from six different manufacturers and selected to represent variations in physico-chemical properties (particle size, protein and fat content, method of preparation, etc.) were used to fortify patent flour from hard red winter wheat; the flour was not necessarily from the same lot each time. 1-lb loaves were baked using the straight-dough procedure (fermentation time, 90 min; bench time, 30 min; intermediate proofing, 10 min; pan proofing to template height or a maximum of 75 min; and baking, 213°C for 25 min) based on the formula: flour or soy-flour blend, 300g; yeast, 9g; yeast food, 1.5g; sodium chloride, 4.5g; sugar, 15g; monoglycerides, 1.5g; emulsifier, 1.5g; fat including that from soy when soy-flour blends are used, 15g; and water 60–90% (flour basis) depending on the requirements of the dough as determined in preliminary studies.

Phytase activity in soy preparations was determined by the method of Gibbins and Norris (1963) and in wheat (flour, protein concentrate) by the method of Peers (1953) as

Table 1—Phytase activity and total and phytic acid phosphorus contents of various commercial soy preparations

| Sample no.		Phytase activity (unit/100g)	Phosphorus		
			Total (mg/100g)	Phytic acid (mg/100g)	Phytic acid (as % of total P)
1	Soy protein isolate	18.8	813.6	324.5	40
2	Soy concentrate	4.0	654.2	351.1	54
3	Soy concentrate	18.6	899.0	611.5	68
4	Soy flour (full-fat)	5.2	562.6	424.3	75
5	Soy flour (full-fat)	22.9	676.2	509.1	75
6	Soy flour (high-fat)	0.0	814.8	514.1	63
7	Soy flour (high-fat)	0.9	698.0	439.3	63
8	Soy flour (defatted)	13.2	733.0	504.1	69
9	Soy flour (defatted)	27.2	719.2	479.0	67
10	Soy flour (defatted)	9.7	664.6	456.7	69
11	Soy flour (defatted)	4.9	712.0	489.2	69
12	Soy flour (defatted)	11.8	738.2	501.6	68
13	Soy flour (defatted)	5.5	727.2	499.2	69
14	Soy flour (defatted)	2.4	752.8	521.6	69
15	Whey-soy blend	52.0	706.4	364.4	52
16	Wheat flour	70.7	99.9	39.6	40
17	Wheat protein concentrate[a]	515.5	840.6	763.7	91

[a] Values reported earlier (Ranhotra, 1972)
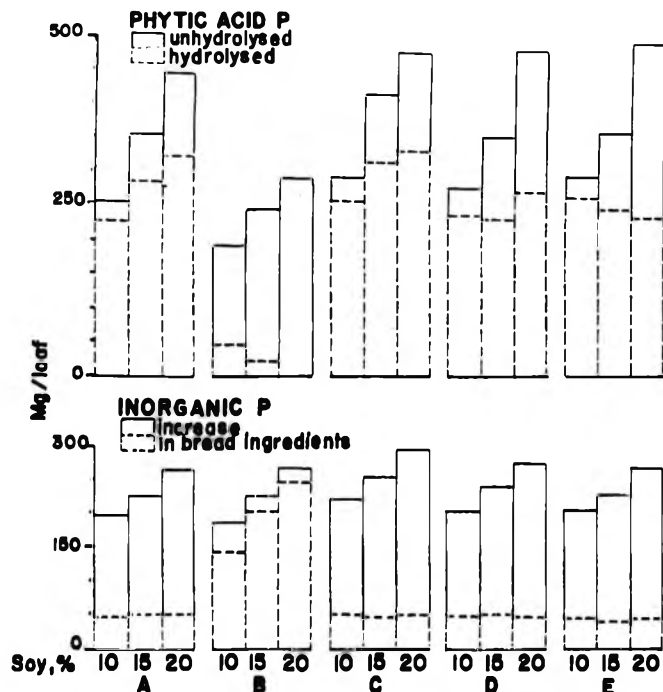
Fig. 1—Effect of increasing addition of soy to wheat flour on the hydrolysis during breadmaking of phytic acid and on the increase of inorganic P. A, soy flour (full-fat); B, whey-soy blend; C, soy flour (defatted); and D and E, soy flours (high-fat)

Table 2—Hydrolysis of phytic acid in bread baked from soy-fortified or whey-fortified wheat flour[a]

Supplements added to wheat flour	Phytic acid phosphorus		Inorganic phosphorus		Residual phosphorus	
	In breading [b] (mg/loaf)	Hydrolysed (%)	In breading [b] (mg/loaf)	Increase (mg/loaf)	In breading [b] (mg/loaf)	Increase (mg/loaf)
Soy protein isolate	318.1	79.8	43.7	194.8	253.7	53.7
Soy protein concentrate	293.6	100.0	46.7	185.6	199.9	108.0
Soy protein concentrate	336.9	89.2	45.3	251.1	241.8	43.1
Soy flour (full-fat)	261.1	84.4	53.9	157.3	177.0	66.6
Soy flour (full-fat)	308.0	84.2	45.0	175.8	205.1	80.5
Soy flour (high-fat)	290.4	78.2	46.4	171.6	273.1	53.6
Soy flour (high-fat)	333.6	86.7	44.1	166.4	197.0	116.3
Soy flour (defatted)	319.3	86.4	43.5	189.5	178.6	88.3
Soy flour (defatted)	332.9	88.5	52.5	211.0	197.3	79.1
Soy flour (defatted)	303.7	57.6	43.2	169.5	201.5	1.2
Soy flour (defatted)	320.2	84.1	44.5	219.8	194.3	45.1
Soy flour (defatted)	322.3	87.1	45.0	184.1	213.0	96.7
Soy flour (defatted)	301.5	81.7	51.0	176.2	229.7	68.2
Soy flour (defatted)	336.7	85.0	38.9	172.7	206.5	109.1
Whey-soy blend	202.6	22.2	161.3	67.7	212.9	−22.8[c]
None	134.4	100.0	44.8	70.4	162.7	66.4

[a] 1-lb loaves were baked from soy-fortified (soy, 10%) wheat flour.
[b] In bread ingredients
[c] Indicates a decrease during breadmaking

described earlier (Ranhotra, 1973). One unit of phytase activity is defined as the amount of enzyme releasing 1 mg of inorganic phosphorus per hour from sodium phytate under specified conditions. Phytase activity in freshly obtained yeast (compressed) was determined by the method used by Shieh and Ware (1968) for microorganisms. Sodium phytate, instead of calcium phytate, was used and incubation, under standard conditions, was carried out for 1 hr; unit of activity is accordingly defined as the amount of enzyme that liberates 1 mg of inorganic phosphorus per hour. The method of Pons and Guthrie (1946) was used to determine inorganic P in soy, in wheat and in resultant breads. The method of Fiske and Subbarow (1925) was used for determining inorganic P in the assay of phytase activities. This method permitted a rapid analysis of a large number of samples freshly obtained from incubation mixtures. Phytic acid was determined by the method of Makower (1970); it is based on the content of iron precipitated as ferric phytate. Calculation of P content was based on a 4:6 iron to P molecular ratio. Total P was determined by the volumetric method of AOAC (1970). Residual P, representing nonphytate organic P including that from lower phosphoric esters of inositol, was calculated by difference from the determined values for inorganic, phytic acid and total P.

RESULTS & DISCUSSION

THE RESULTS in Table 1 show that all soy products tested were high in P, with most of it in the form of phytic acid. Phytate P ranged from 54–75% of the total P present in all the soy preparations tested except one (isolate contained 40%); a value of 68% in soy-bean-meal was quoted by Mollgaard et al. (1946). In contrast to wheat (Ranhotra, 1973; Table 1), all soy preparations tested showed little phytase activity. In fact, no activity was measurable by the method used for

wheat (Peers, 1953). Low phytase activity would be expected from the work of Mollgaard et al. (1946) which indicated that most oilseeds including soybeans contain no naturally present phytase.

When breads were made with soy-fortified wheat flour (soy, 10%; wheat, 90%), with one exception, more than 3/4 of the phytate was hydrolysed (Table 2). Complete phytate hydrolysis occurred in the bread made with wheat flour (control). This is in agreement with previous observations (Ranhotra, 1972) and with the work of deLange et al. (1961) who indicated that destruction of phytate during breadmaking was complete in low-extraction wheat flours. In all soy-fortified breads, a three to fivefold increase in the level of inorganic (available) P also occurred simultaneously. Residual P levels

also increased suggesting that only a part of the degraded phytate underwent complete hydrolysis. When whey was added to soy, in spite of enhanced phytase activity (Table 1), only a small fraction of the phytate underwent cleavage. As would be expected, the increase in inorganic P level was also small. Residual P level even decreased suggesting that, during breadmaking, reactions favored rephosphorylation more than they did hydrolysis.

Since little phytase activity is present in soy, the phytate hydrolysis that occurred (Table 2) was probably due to phytases of wheat and yeast. Relative to the amount of phytate present, phytase activity in wheat is quite high (Table 1). Yeast is also quite high in phytase (Table 3). When soy-fortified bread was made

Table 3—The effect of yeast on the hydrolysis during breadmaking of phytic acid in soy-fortified wheat flour[a]

	Yeast (g/pound loaf)			
	0	3	9	15
Phytic acid P [b]				
In bread (mg/loaf)	167.0	77.7	37.2	42.4
Hydrolysed (%)	45.6	74.7	87.9	86.2
Inorganic P [b]				
In bread (mg/loaf)	184.7	226.4	215.4	231.3
Increase[c] (%)	439.7	539.0	512.8	550.7
Residual P [b]				
In bread (mg/loaf)	211.3	269.7	315.8	300.1

[a] Phytase activity in yeast (unit/100g) 135 ± 7
[b] Values (mg) in bread ingredients representing 1-lb loaf: Phytic acid P, 307.2 ± 12.4; Inorganic P, 42.0 ± 1.1; Residual P [Total P − (Phytic acid P + Inorganic P)], 224.5 ± 10.7.
[c] Over values in bread ingredients

without yeast, less than half of the phytate was hydrolysed (Table 3). Hydrolysis increased substantially when yeast was added; and at levels normally used in bread formulation (9g yeast), phytate hydrolysis was maximum. Increased hydrolysis was accompanied by increase in the level of available (inorganic and residual) P. It is possible that some non-enzymatic hydrolysis might have also occurred.

When a full-fat or a defatted soy flour was added to wheat flour at increasing levels, both the amount of phytate hydrolysed and the level of inorganic P, increased (Fig. 1); apparently phytase activity in yeast and/or wheat was still available. Similar results were earlier obtained when wheat protein concentrate was used to fortify wheat flour (Ranhotra, 1972). On the contrary, in the case of two high-fat soy flours which contained virtually no phytase activity, phytate hydrolysis either decreased or was inconsistent. Inorganic P levels, however, increased suggesting that some of the residual P was probably hydrolysed. When whey-soy blend was used at increasing levels, phytate cleavage was increasingly inhibited. This inhibition

might have occurred due to initial high levels of inorganic P in the system (Fig. 1). In wheat (Ranhotra, 1973) as in microorganisms (Shieh and Ware, 1968) phytase activity and thus phytate hydrolysis is strongly depressed by inorganic P. The high level of calcium, a potent inhibitor of phytate hydrolysis (Ranhotra, 1972), in whey might have also contributed to the inhibition observed.

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INFLUENCE OF VACUUM SOAKING ON
YIELD AND QUALITY OF CANNED MUSHROOMS

INTRODUCTION

PREVIOUS RESEARCH in this laboratory demonstrated that yield of canned mushrooms was increased significantly when the fresh mushrooms were soaked in water for 20 min, stored at 2°C for 18 hr, and then soaked in water a second time for 2 hr before processing (Beelman et al., 1973). This set of treatments, termed the PSU-3S process, has been difficult to integrate into present commercial mushroom processing systems because of the time-consuming second soak operation.

Studies were undertaken to determine the effectiveness of a vacuum-soaking process, similar to that employed in preparing apples for freezing (Rasche, 1932), to increase the efficiency of the soaking process. Vacuum-soaking treatments were also investigated for combined effects with other post-harvest and processing operations which have been shown to influence yield and quality of canned mushrooms (Dommel, 1964; Beelman et al., 1973).

EXPERIMENTAL

Equipment

Research was conducted in the pilot-plant at University Park and in several commercial mushroom processing plants in the vicinity of Kennett Square, Pa. Equipment for applying vacuum-soak and blanching treatments was transported from one experimental site to another. Other processing equipment such as slicers, closing machines and retorts varied with the experimental site.

Vacuum-soaking equipment, specially designed and fabricated for this research (Lee Metal Products Co., Philipsburg, Pa.), consisted of a stainless steel kettle with lock-down cover (Fig. 1). Vacuum was supplied by a microvac pump (Stokes model 900-146-11, Pennwalt Corp., Philadelphia, Pa.). Treatments were applied to experimental samples submerged in water in a perforated stainless steel basket with a lid designed to hold the product beneath the water surface.

Raw product

Mushrooms for all experiments were selected from uniform lots of known strain harvested at one time from a commercial production facility. After harvest, the mushrooms were transported to the experimental site where caps with a diameter range of 2.8–4.1 cm were selected. The caps were hand trimmed either as buttons with 0.3 cm stem or as whole mushrooms from which only the stem bases were removed. The trimmed product was pooled, accurately weighed into 1 kg experimental samples and placed in numbered Kraft bags. Four bagged samples were selected at random for each treatment.

Processing conditions

All factors influencing product yield and quality during processing were controlled by standardizing processing procedures and comparing treatment results with those of the normal control process. All samples were spray washed, blanched to a center temperature of 77°C in boiling water, cooled for 2 min in cold water, drained and weighed. Mushrooms were filled into plain tin cans (211 × 212) using a fill weight between 110 and 130g depending upon the weight of the blanched sample. A 20-grain salt tablet was added to each can; the cans were filled with hot water and closed. Thermal processing was applied in a still retort at 121°C for 18 min. The canned products were held for 4 wk in the pilot-plant before evaluations were made.

Processed product evaluation

The percent yield of canned products, based on weight of the raw product sample, was determined by the following formula:

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

Drained weights were determined from the pooled sample of all cans within each treatment repetition after the sample had been drained for 2 min in a perforated stainless steel tray. Color of the canned mushrooms was determined with an Agtron M-30-A Reflectance Color Meter. Duplicate readings from each repetition were made immediately after the drained weight procedure. The meter was standardized with the M-00 and M-44 discs using the green mode. Solids content of canned products was determined by freeze-dehydration of pre-weighed samples.

Vacuum-soaking process

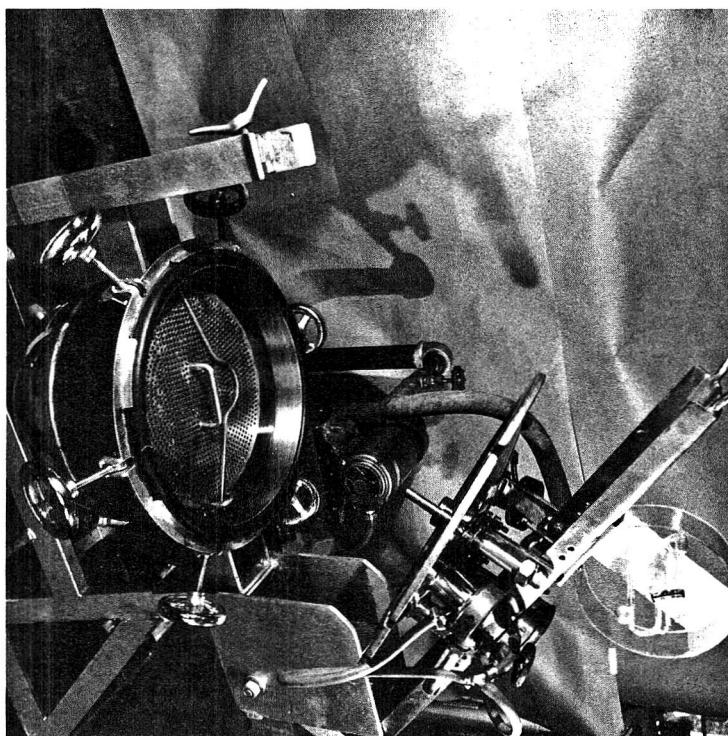The optimum vacuum-soaking procedure was determined empirically on the basis of

Fig. 1—Vacuum-soaking equipment employed in canned mushroom yield studies.

maximum water absorption by the mushroom tissues. Mushrooms were submerged in water in the vacuum chamber, the chamber pressure was reduced to 2 mm Hg (optimum pressure maintained by the system) and held for 5 min. After the chamber was returned to atmospheric pressure by admitting air into the headspace, the mushrooms were held in the water for 10 min. Total time required for the process was 16 min, allowing 1 min for the system to reach 2 mm Hg pressure.

Vacuum soaking compared with other processes

A series of experiments was performed in commercial mushroom canneries to compare the yield and quality of canned mushrooms produced by the vacuum-soaking method with those produced by the standard processing practice and the PSU-3S process. Effectiveness of vacuum soaking when integrated with other methods was also determined. The treatments applied to the fresh product prior to canning were: (1) Control, storage at 2°C for 18 hr; (2) VAC, vacuum soaking at harvest; (3) PSU-3S process, soaked 20 min, storage at 2°C for 18 hr, soaked 2 hr; (4) SSV, vacuum-soaking treatment was substituted for the second soak of the PSU-3S process; (5) VS, vacuum soaking at harvest followed by storage at 2°C for 18 hr.

Combined effects of vacuum soaking and storage

A series of experiments, performed in commercial mushroom canneries was designed to determine the combined effects of vacuum soaking and cold storage of raw product on the yield and quality of canned mushrooms. Treatments applied to the raw product before canning were: (1) Storage, stored at 2°C for 0, 24, 48 and 72 hr; (2) VS, vacuum soaking applied at harvest followed by storage at 2°C for 0, 24, 48 and 72 hr; (3) SSV, vacuum soaking substituted for the second soak period of PSU-3S process after storage at 2°C for 0, 24, 48 and 72 hr.

Slicing performance of vacuum-soaked mushrooms

Influence of vacuum soaking on yield of canned mushroom slices was of particular interest to processors because many canned products are sliced or chopped. A series of experiments was performed in commercial processing plants to compare sliced product yield from vacuum-soaking treatments with other processes. Treatments applied to the raw product prior to processing were: (1) Control, processed at harvest; (2) Storage, stored at 2°C for 18 hr; (3) PSU-3S process, soaked 20 min, stored at 2°C for 18 hr, soaked 2 hr; (4) SSV, vacuum soaking substituted for the second soak in the PSU-3S process; and (5) VAC, vacuum soaking applied at harvest.

Slicing treatments were applied to blanched mushroom samples using production-line equipment (A.K. Robins, Baltimore, Md.) with 0.5 cm blade spacings. Sliced samples were collected in perforated stainless steel trays, transferred to perforated plastic containers and weighed. Products were canned as described previously.

Sensory evaluation

Canned button mushrooms, processed by four experimental methods in four repetitions were presented to a trained panel for sensory evaluation. A commercially canned product was included as a fifth treatment. At one sitting judges were asked to rate the products for flavor on a 9-point hedonic scale. At a second sitting they were asked to rate the products for texture on a similar scale. Products presented to the panel were: (1) Control, canned at harvest; (2) Storage, canned after storage at 2°C for 24 hr; (3) PSU-3S process; (4) SSV, vacuum soaking substituted for the second soak in the PSU-3S process; and (5) Commercial sample.

Data analyses and presentation

Data were analyzed statistically by means of single classification ANOV. Significant differences between means were determined using an unpaired "Student" t-test. Data are presented as the average of four repetitions for each treatment.

RESULTS & DISCUSSIONS

Vacuum soaking compared with other processes

Comparative effects of vacuum-soaking and other post-harvest treatments on yield and color of canned mushrooms are shown in Table 1. Data from two experiments are given, each performed on different dates with different sources of raw product; therefore, statistical comparison of data between experiments is not valid. Differences in canned product yields between the two experiments was due to crop variations which are usually experienced by the industry.

It is significant that in spite of the variability in raw product relative performance of the treatments was the same in both experiments. Similar differences had been observed previously in pilot-plant experiments. Vacuum soaking of mushrooms at harvest (VAC) increased yield significantly over that of the control, but VAC alone was not as effective as the PSU-3S process. However, when vacuum soaking was combined with storage, either as SSV, where vacuumization replaced the 2-hr soak, or as VS, in which vacuumization was followed by 18-hr storage, the yield increases were greater than obtained by the PSU-3S process. In-

Table 1—Yield and color of canned button mushrooms as influenced by various raw product treatments

Treatment[a]	Canned Yield (%)		Color (reflectance)[b]	
	Exp 1	Exp 2	Exp 1	Exp 2
Control	60.8 A[c]	71.9 A	34.0 A	33.1 A
VAC	64.6 B	77.8 B	43.3 B	38.1 B
PSU-3S	67.8 C	79.7 C	33.0 A	33.4 A
VS	68.4 CD	83.2 D	34.5 A	33.2 A
SSV	69.4 D	83.2 D	35.5 A	33.0 A

[a] Code: Control—storage at 2°C for 18 hr; VAC—vacuum soaking at harvest; PSU-3S—soaked 20 min, storage at 2°C for 18 hr, soaked 2 hr; VS—vacuum soaking at harvest, storage at 2°C for 18 hr; SSV—soaked 20 min, storage at 2°C for 18 hr, vacuum soaking.
[b] High number indicates greater whiteness
[c] Means followed by the same letter do not differ significantly (P=0.05).

Table 2—Yield and color of canned button mushrooms as influenced by vacuum soaking and other post-harvest treatments

Process/treatments	Hr at 2°C	Canned yield (%)	Color (reflectance)[a]
Storage	0	66.9 A[b]	32.8 A
	24	69.1 B	26.9 B
	48	70.1 B	27.8 B
	72	71.7 C	25.1 B
VS	0	71.9 C	35.3 A
(vacuum-soaking	24	77.9 D	26.8 B
at harvest followed	48	80.0 E	27.6 B
by storage)	72	81.8 F	25.0 B
SSV	0	73.2 G	33.8 A
(soaked 20 min,	24	79.9 E	32.9 A
stored, vacuum-	48	80.6 E	31.4 A
soaking)	72	82.4 F	28.8 B

[a] Higher number indicates greater whiteness
[b] Means followed by the same letter do not differ significantly (P=0.05).

creased yield combined with the time-saving feature of the vacuum-soaking process offers improvements over the PSU-3S process. Since the VS procedure eliminates the need for an additional soaking period it might offer an advantage over the SSV method in spite of the slightly higher yields obtained with the latter.

The best canned mushroom color resulted from the VAC treatment. This was the only treatment which gave an improved color throughout the experiment. The color effect probably resulted from the canning operations being performed soon after harvest of the mushrooms, thus eliminating storage. None of the treatments resulted in a significant decrease in color quality of the canned products compared to the control.

Combined effects of vacuum soaking and storage

When vacuum-soaking and storage treatments were combined the increases in yield were greater than the sum of yield increases for the treatments taken separately (Table 2). For example, adding the yield increases obtained from the un-stored vacuum-soaking treatments to the yield increase obtained by 72 hr storage, the sums of the increases are 9.8% for VS and 11.1% for SSV. However, the yield increases obtained experimentally were 14.9% and 15.4% for VS and SSV treatments, respectively, when they were applied in combination with 72 hr storage. Similar combined effects were observed for all vacuum-soaking and storage combinations within the treatment range employed. Beelman et al. (1973) also reported interacting effects between treatments which combined soaking and various storage periods as applied in the PSU-3S process.

The influence of vacuum soaking and storage treatment combinations on color of canned mushrooms was not very pronounced (Table 2). The three treatments at 0 storage time produced canned mushrooms with the best color; none of the three treatments differed significantly from each other. Only SSV-24 and SSV-48 treatments resulted in finished product with color equal to the unstored treatments. Color quality for both vacuum-soaking methods was reduced as the storage time increased, but the changes were not statistically significant except for the SSV-72 treatment. Reductions in color quality of canned mushrooms due to storage of the raw product were ob-

Table 3—Yield of canned sliced button mushrooms as influenced by vacuum soaking and other post-harvest treatments

Treatment[a]	Slicing loss (% wt)	Canned yield (%)
Control	8.5 A[b]	70.3 A
Storage	9.2 B	73.8 B
PSU-3S	9.9 B	77.8 C
VS	10.8 C	79.9 D
SSV	12.2 D	82.0 E

[a] Code: Control—canned at harvest; Storage—stored at 2°C for 18 hr; PSU-3S—soaked 20 min, stored at 2°C for 18 hr, soaked 2 hr; SSV—soaked 20 min, stored at 2°C for 18 hr, vacuum soaking; VS—vacuum soaking at harvest followed by storage at 2°C for 18 hr.

[b] Means followed by same letter do not differ significantly (P=0.05).

Table 4—Solids content of canned mushrooms as influenced by various post-harvest treatments

Treatment[a]	Solids (%)
Control	10.6 A[b]
Storage	10.3 A
PSU-3S	9.6 B
SSV	9.2 B

[a] Code: Control—canned at harvest; Storage—stored at 2°C for 18 hr; PSU-3S—soaked 20 min, storage at 2°C for 18 hr, soaked 2 hr; SSV—soaked 20 min, storage at 2°C for 18 hr, vacuum soaked.

[b] Means followed by the same letter do not differ significantly (P=0.05).

served by Dommel (1964). Similar storage effects are apparent in these data, but the SSV treatment controlled the color changes during the 24- and 48-hr storage periods.

Slicing performance of vacuum-soaked mushrooms

Total yield of canned sliced mushrooms processed after various raw product treatments (Table 3) were similar to those obtained with whole canned products which had received the same raw product treatments in previous experiments (Table 1). This similarity indicates that the experimental treatments did not complicate the commercial slicing operation. Examination of the products for defects due to slicing indicated equally high quality products were obtained from all treatments.

Percent weight lost as a result of the slicing operation increased significantly for all treatments compared to the control; the vacuum-soaking treatments showed the highest losses. In all cases slicing loss increased directly with canned product yield. Mushrooms which had absorbed water during soaking operations showed a greater loss of weight during slicing, but the soaked samples retained a higher percentage of water during the thermal process and produced the highest canned product yields. The data indicate that treatments which combine soaking and storage alter the water-binding and water-holding capacities of the mushroom tissues. The increased capacity of the tissues to absorb water and retain it during subsequent heat treatment processes results in increased canned product yield. Vacuum-soaking processes combined with storage, either as VS or SSV appeared to be more effective than the PSU-3S process in increasing water retention by the canned product.

Solids content of soaked mushrooms

Solids content of canned mushrooms processed by various methods (Table 4) constitutes further evidence that the yield increases resulting from storage-soaking combination treatments are due to increased water retention by the tissues. Treatments which combined soaking and storage produced canned mushrooms with about 1% less solids than the control, a 10% reduction. Yield increases for the PSU-3S and SSV treatments were about 10% (Table 3), evidently as a result of increased water retention.

Sensory evaluation

No significant differences were observed in flavor or texture of the canned products produced from the various raw product treatments.

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Ms received 2/1/74; revised 3/22/74; accepted 3/28/74.

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INFLUENCE OF POST-HARVEST STORAGE AND CANNING ON THE SOLIDS AND MANNITOL CONTENT OF THE CULTIVATED MUSHROOM AND THEIR RELATIONSHIP TO CANNED PRODUCT YIELD

INTRODUCTION

SHRINKAGE, or loss of weight during canning, is a problem of major concern to the mushroom processing industry. Weight losses as high as 40% are common in commercial canning operations. This shrinkage results from the loss of both water and solids during processing with water representing the bulk of the material lost. Any reduction in total shrinkage may represent a substantial economic gain to the processor by increasing canned product yield (Coale and Butz, 1972). Efforts have been initiated to alleviate some of this weight loss through a more efficient utilization of known growing and processing practices and through the development of new methods and processing equipment (Beelman et al., 1973; Bradley, 1970; Dommel, 1964; McArdle and Curwen, 1962).

Several studies (McArdle and Curwen, 1962; Beelman et al., 1973) have demonstrated that post-harvest storage at 2°C for a period of at least 1 day prior to canning significantly decreased shrinkage and thus increased canned product yields. While this knowledge is valuable from a practical standpoint to the mushroom processor, few studies have been conducted relating to the chemical or physical changes within the mushroom during storage that influence shrinkage. Lee (1969) hypothesized that a transformation of simple sugars to starch might occur during storage that could lead to reduced shrinkage. Although such a sugar to starch transformation was not demonstrated, the possibility of an influence upon the water-holding capacity exerted by other changes within the solids constituents was not disproved.

The dry matter of the cultivated mushroom has been shown to contain up to 20% mannitol in the cap tissue (Hughes, 1961; Rast, 1965) and up to 40% of the sporocarp, (Rast, 1965). Since mannitol is a dominant constituent of mushroom dry weight, Holtz (1971) suggested that

mannitol might exhibit humectic properties or influence the osmotic balance within the mushroom (Holtz, 1971; Lewis and Smith, 1967). This investigation was conducted to explore the possibility that mannitol might influence the shrinkage of mushrooms during canning by influencing the water-holding capacity of mushroom tissue.

EXPERIMENTAL

Processing operations

A cream strain (324) and a brown strain (337) of the cultivated mushroom, *Agaricus bisporus* (Lange) Sing, grown commercially for processing were used in this study. The mushrooms were obtained from a single harvest at two different commercial mushroom farms near Kennet Square, Pa. Uniform sampling procedures were used in the selection of mushrooms for experimental use. Only mushrooms with unstretched veils ranging in size from 1-1/8 to 1-3/4 in. were selected for the experiment. 1 kg samples (initial weight) of mushrooms trimmed to 1/8 in. from the cap were placed into individual Kraft paper bags. A smaller bag containing 12 mushrooms selected from the same source was placed inside the larger bag. These mushrooms were used for the solids and mannitol analyses.

Treatments consisted of 0, 1, 2 and 3 days of post-harvest storage at 2°C and 90% RH. Each treatment contained four repetitions. After storage treatments the mushrooms were blanched by immersion in boiling water in a steam-jacketed kettle for 5 min, cooled 2 min in cold water, drained and weighed. The mushrooms were then filled into 4 oz 211 × 212 plain tinned cans using a uniform fill weight of approximately 120g. A 20-grain NaCl tablet

was added to each can, the can was then filled with boiling water leaving about a 3/16 in. headspace. The cans were sealed in an automatic can closer and thermally processed in a still retort at 121°C for 18 min.

Canned product yield determination

After processing, the mushrooms were held at 18°C for 2 months. After this period the cans were opened and drained weights were determined by allowing the mushrooms to drain for 2 min in a perforated tray at 30° angle, then weighing the drained product. The canned product yield was calculated by the following formula:

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

Solids determination

Approximate 20-g samples were obtained from the 12 mushrooms held separately by quartering the mushrooms and placing one-quarter of each mushroom into a preweighed aluminum moisture dish. The samples were freeze dried to constant weight and the solids were determined by weight difference.

Mannitol determination

A second quarter of the same mushrooms used for solids determinations were used for mannitol analyses. 20-g samples were extracted using a method similar to that of Holtz (1971). The samples were ground in a Waring Blendor for 5 min using 100 ml of 20% methanol containing 300 mg of rhamnose for use as an internal standard for the quantitation of mannitol. The extracts were filtered through Standard Gray Sugar filter paper by suction. The filtrate was taken to 200 ml with 20% methanol. A 2-ml aliquot of extract was freeze dried to constant weight in a 10-ml vial.

Trimethylsilyl (TMS) derivatives of the

Table 1—Influence of post-harvest storage on the yield of canned mushrooms

Storage time (Days)	Canned product yield (%)	
	Cream strain (324)	Brown strain (337)
0	64.9 A[a]	63.4 A
1	67.5 B	67.2 B
2	68.0 B	68.6 C
3	68.1 B	69.5 C

[a] Means in the same column followed by the same letter are not significantly different with k = 100.

freeze-dried extracts were prepared by the addition of 1 ml of Tri-Sil Z (Pierce Chemical Co., Rockford, Ill.). The sample was thoroughly mixed in the 1 ml of derivitizing agent and refrigerated overnight.

The TMS derivatives were chromatographed on a Varian Aerograph 1840-4 gas chromatograph equipped with a flame-ionization detector. Separation of TMS derivatives was accomplished by using a 5 ft × 1/8 in. stainless steel column packed with 3% SE-30 on 100/120 mesh Variport #30. The injection port temperature was maintained at 250°C. The column temperature was programmed from 150 to 210°C at a rate of 2°/min for 2 min and 4°/min for 14 min. The detector temperature was 300°C. The carrier gas (N_2) flow rate was 30 ml/min.

Quantification of mannitol by GLC was accomplished by using a standard curve formed using standard solutions of mannitol and rhamnose. The rhamnose concentration was maintained at 150 mg/100 ml while mannitol concentration was varied from 50 mg/100 ml to 300 mg/100 ml. The response factor was obtained by comparing relative peak heights.

Statistical analysis

An analysis of variance was performed using a completely randomized design. Mean separations were performed using the modified least significant difference procedure of Waller and Duncan (1969) with a k value of 100.

RESULTS & DISCUSSION

Effect of storage on canned product yield

In both strains an increase in canned product yield was observed with each successive day of post-harvest storage (Table 1). This yield increase was statistically significant for the 1-day storage period in both strains. In the brown strain, 337, the yield increase was also significant between 1 and 2 days of storage. In the cream strain, 324, no significant yield increase was observed beyond 1 day of storage.

Effect of storage on solids content

No significant change in the solids content of the fresh mushrooms of either strain was detected over the 3-day storage period (Table 2). Mushrooms can lose weight during the storage period due to evaporation of water and loss of solids as CO_2 during post-harvest respiration. The

fact that no significant change in solids content occurred during storage indicates that weight losses due to evaporation and respiration were nearly proportional to the water:solids ratio of the mushroom tissue.

Total solids in the processed mushrooms (Table 2) of the two strains were approximately 1–2% greater than that in the corresponding fresh mushrooms which represented increases of about 7–19%. This finding agreed with the results reported by Dommel (1964) and was probably due to salt uptake and water loss during canning. Of interest was the trend for the total solids content of the processed mushrooms to decrease with increasing storage time. Since no change in total solids content occurred in the fresh mushrooms, this decrease in total solids content (or increase in the water content) in the processed mushrooms with increasing storage time could be attributed to the increased water-holding capacity of the mushrooms that developed during post-harvest storage.

Effect of storage on mannitol content

In general, the mannitol content of the fresh mushrooms decreased during post-harvest storage (Table 2). This decrease was statistically significant in the cream strain, 324, after 2 days of storage. The decrease in mannitol content was not significant in the brown strain, 337 although a decreasing trend was evident. Decrease in mannitol content during storage might be attributed to the utilization of mannitol as a carbon source by the mushrooms during this post-harvest period. Rast (1965) and Dutsch and Rast (1972) indicated that mannitol was not normally utilized by *Agaricus bisporus* except under conditions of extreme stress. However, when mushrooms are harvested, they are separated from their external nutrient source. Since the mushrooms continue to metabolize during the post-harvest period, carbon sources from within the mushroom must be utilized. Our study indicates that mannitol probably represents a carbon source which is catabolized by the mushroom during this period.

Parrish (1973) reported that the rate of the synthesis of mannitol in different mushroom strains might be directly related to the metabolic activity of the mushrooms before harvest. The inverse relationship might apply to mushrooms during post-harvest storage. The degradation of mannitol during this period may be indicative of post-harvest metabolic activity. The fact that mannitol losses in the cream strain were greater than those observed in the brown strain might indicate differences in metabolic activity, with the "creams" catabolizing mannitol more rapidly than the "browns" due to a more rapid inherent respiratory rate. However, the mannitol content of the fresh (unstored) mushrooms was lower in the brown strain which could effect its susceptibility to post-harvest metabolism. The mannitol content of both strains was lower than reported earlier (Hughes, 1961; Rast, 1965) but Parrish (1973) found that mannitol content varied substantially among the flushes of a crop cycle.

The mannitol content of the mushrooms was greatly reduced during canning with losses ranging from about 57–66%. This is in agreement with the report of Dommel (1964) who demonstrated by proximate analysis that carbohydrates represented the bulk of the solids fraction lost during canning. No significant effect of storage was observed on the mannitol content in the processed mushrooms of either strain. However, the percentage loss in mannitol during processing decreased with increased time of storage. In the cream strain the percentage loss dropped from 64.3 to 56.7 between 0 and 3 days storage while the loss in the brown strain dropped from 65.7 to 59.3 during the same period.

CONCLUSIONS

THE RESULTS of this investigation revealed that post-harvest storage at 2°C and 90% RH for a period of at least 1 day significantly increased canned product yield in agreement with past results. A substantial decrease in mannitol content was observed after the canning process, while total solids content increased

Table 2—Influence of post-harvest storage on the solids and mannitol content of fresh and processed mushrooms

Storage time (Days)	Fresh				Processed			
	Solids (%)		Mannitol (% dry wt.)		Solids (%)		Mannitol (% dry wt.)	
	Cream (234)	Brown (337)	Cream (234)	Brown (337)	Cream (234)	Brown (337)	Cream (234)	Brown (337)
0	8.34 A[a]	8.59 A	10.26 A	8.70 A	10.34 A	10.03 A	3.66 A	2.98 A
1	8.53 A	8.57 A	9.39 AB	8.61 A	10.18 A	9.70 B	3.70 A	3.19 A
2	8.22 A	8.55 A	9.02 B	8.18 A	10.14 A	9.28 C	3.59 A	3.43 A
3	8.56 A	8.59 A	8.68 B	8.42 A	9.93 A	9.26 C	3.76 A	3.43 A

[a] Means in the same column followed by the same letter are not significantly different with k = 100.

markedly after canning. An increase in the water-holding capacity of the mushrooms over the post-harvest storage period was demonstrated by a decrease in total solids content of the processed mushrooms with each successive day of storage. Mannitol content of the unprocessed mushrooms generally decreased during the post-harvest storage period possibly due to its utilization as a carbon source. Since mannitol decreased or remained unchanged during post-harvest storage, it can be concluded that mannitol has no direct influence on the increased water-holding capacity of mushrooms that occurs during post-harvest storage.

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USE OF RECYCLED SALT IN FERMENTATION
OF CUCUMBER SALT STOCK

INTRODUCTION

SALT reclaimed from spent pickling brine by submerged combustion and incineration contains carbon residue from the incineration and potassium in excess of the amount to be found in the fresh salt (Lowe and Durkee, 1971; Durkee et al., 1973). A 4% brine solution made from reclaimed salt has a pH of 10.0 (Lowe and Durkee, 1971). The pH of brine made from fresh salt ranges from 7.2−8.6 depending on the quality of the water used.

Differences in the chemical composition of the pickling brine can make a difference in the quality of the salt stock that is produced. This is a report on studies that were made to determine the quality difference between cucumber salt stock produced with fresh kiln-dried salt and salt stock produced with salt that has been recycled once by submerged combustion and incineration.

EXPERIMENTAL

Salt

The recycled salt was reclaimed from brine drained from large (7−10,000 gal) commercial storage tanks at the end of the 1971−72 season. The salt was reclaimed in the manner described by Lowe and Durkee (1971) and Durkee et al. (1973). The original or fresh salt was a kiln dried coarse grade obtained from the Leslie Salt Co., Newark, Calif. Chemical composition of both the precursor and the reclaimed salt are shown in Table 1.

Preparation of salt stock

Ohio-grown cucumbers with diameters in the range of 1-1/2 in. to 1-3/4 in. were placed in 48 gal wood casks and covered with brine initially containing between 6.3 and 7.1% salt (24−27 Salometer degrees). The brine made from reclaimed salt was prepared in one of four different ways: (1) as received; (2) pH adjusted; (3) clarified; and (4) pH adjusted and clarified. A control brine was prepared with the fresh kiln-dried salt. Three 48-gal casks of brine were prepared for the control and each of the four test batches, for a total of 15 casks. Each cask contained about 225 lb of cucumbers.

Each cask was fitted with a 1-in. diam polyvinyl chloride pipe perforated with numerous 1/4-in. diam holes in the section below the brine surface. The pipe protruded through the center of the cask head or cover and served as the port through which brine samples were withdrawn for analyses.

Brine made with reclaimed salt was clarified, when called for, by allowing the solution to settle overnight in stainless steel drums. The supernatant brine was then decanted so as to remove an estimated 95% of the carbon suspended in the original solution. Make-up or feeder salt was added to the casks in flannelette sewn sacks which retained the carbon impurities while allowing the salt to dissolve.

Feeder salt was added at the rate of approximately 5 lb per week per cask beginning when the lactic acid concentration in the brine reached 0.6% and continuing until the brines reached a final concentration of 13.7−16.1% NaCl (52−61 Salometer degrees). The salt was placed on top of the salt stock and allowed to diffuse down into the cask. There was no pH

Table 1—Composition of salts

	Kiln-dried coarse	Reclaimed
NaCl, %	98.5	97.0
H_2O insol, %	0.03	0.27
Moisture, %	0.13	0.28
K, ppm	13.1	3990
Fe, ppm	<0.1	<0.1
Cu, ppm	0.1	0.2
$CaSO_4$, %	0.19	0.18
$CaCl_2$, %	0.03	0.24
$MgCl_2$, %	0.06	0.02
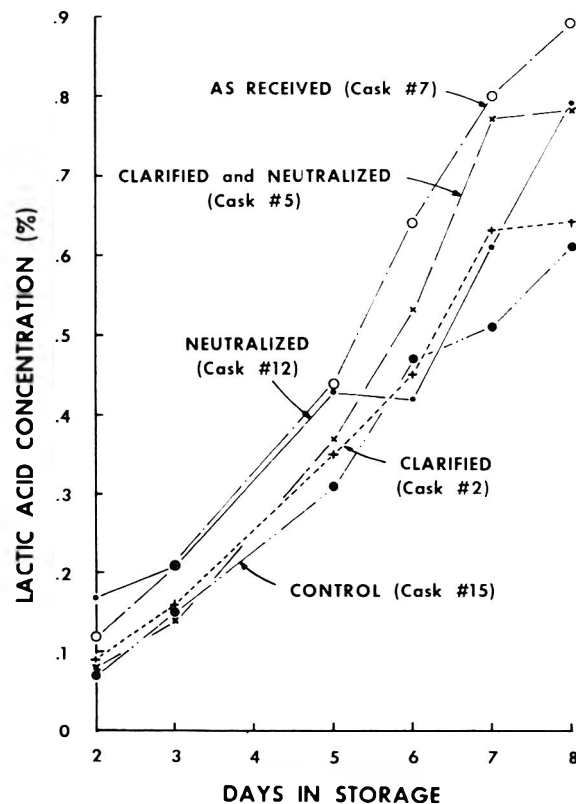
Fig. 1—Lactic acid build-up.

Table 2—Brine and salt stock data

Exp	Cask no.	Brine clarification	pH adj	Initial NaCl conc ° Salometer	NaCl conc at termination of feeder salt addition ° Salometer	Days to 0.6% lactic acid	% Hollow centers	Texture
A	1	Settled	As is (9.0)	25	57	8	33	Exc
	2	Settled	As is (9.0)	25	57	8		
	3	Settled	As is (9.0)	24	56	7		
B	4	Settled	Adj to 7.2	25	57	7	34	Exc
	5	Settled	Adj to 7.2	24	53	7		
	6	Settled	Adj to 7.2	24	52	7		
C	7	None	As is (9.0)	25	53	6	23	Exc
	8	None	As is (9.0)	27	54	7		
	9	None	As is (9.0)	26	56	7		
D	10	None	Adj to 7.2	25	56	6	29	Exc
	11	None	Adj to 7.2	25	57	7		
	12	None	Adj to 7.2	25	56	7		
E	13	Control	Control (7.2)	25	58	7	39	Exc
	14	Control	Control (7.2)	26	61	7		
	15	Control	Control (7.2)	25	58	8		

re-adjustment. Salt concentration increased at the rate of about 5 Salometer degrees per week.

When called for, pH of the starting brine was adjusted with HCl to match the pH of the control (7.2).

The casks were initially located outdoors (Aug.–Sept., Holland, Mich.) to prevent scum yeast formation. When the brines reached 50° Salometer (13.2% NaCl) the casks were moved indoors to prevent weather dilution.

Brine samples were withdrawn daily for lactic acid determination until a peak concentration of not less than 0.6% was reached. After that, samples were taken weekly and analyzed for salt concentration. A weekly record was also kept of salt addition and the temperature at the center of the cask.

After 37-1/2 wk, the salt stock was considered fully cured and ready for harvest. Final brine and stock samples were taken from each cask for chemical analyses and organoleptic evaluation.

Test methods

Total acid concentration in the brines was determined by titration with NaOH and expressed as lactic.

Salt concentrations were determined using standardized hydrometers calibrated in Salometer degrees.

Cation salt concentrations were determined by the standard AOAC atomic absorption method using a Perkin-Elmer Model 303 instrument.

Texture was determined organoleptically using a subjective crunch test.

Moisture was determined by vacuum oven.

Hollow centers were determined by sampling 100 cucumbers from each test group and the control. The samples were cut in half lengthwise and visually inspected.

RESULTS & CONCLUSIONS

EXPERIMENTAL RESULTS are summarized in Figure 1 and Tables 2 and 3. In general, the results indicate substantially no difference in fermentation or in the quality of the salt stock produced with either fresh kiln-dried salt or salt reclaimed by submerged combustion and incineration. Fermentation proceeded

normally in all cases (See Fig. 1) and all brines reached the 0.6% lactic acid level within 6–8 days, with a mean of 7 days. Fully 2/3 of the casks were grouped at the mean (See Table 2).

The dominant quality factor for salt stock, texture, was judged to be excellent for all samples (See Table 2). No off-flavors or gross appearance changes were noted. The high percentage of hollow centers was judged to be due to factors other than brine composition (i.e., weather conditions during storage, varietal and other raw material variations) since the control group had a greater percentage of hollow centers than did the test groups.

The test data showed no advantages in clarifying or neutralizing the starting brines. Eliminating these two operations naturally reduces the cost of salt recovery.

The final brine containing once recycled, twice used salt showed a potassium concentration of 1900–2000 ppm which is 500–600 ppm greater than the concentration in the final control brine (See Table 3), and approximates the concentration of potassium in fresh cucumber (Watt and Merrill, 1963).

Table 3—Final brine samples

	Cask no. 2	Cask no. 5	Cask no. 7	Cask no. 12	Cask no. 15
$CaSO_4$, %	0.051	0.054	0.071	0.061	0.056
$MgSO_4$, %	0.048	0.035	0.018	0.024	0.038
$MgCl_2$, %	0.011	0.025	0.049	0.043	0.007
K, ppm	1960	1900	1960	2000	1400
Fe, ppm	5.8	6.1	8.5	10.8	8.2
Cu, ppm	0.31	0.32	0.27	0.23	0.25
H_2O Insol, %	1.3	1.6	1.4	1.5	1.7
Acid as lactic, %	0.23	0.23	0.23	0.23	0.23
NaCl, %	14.0	14.0	14.0	13.8	14.4

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EFFECT OF PROCESSING AND STORAGE ON PROVITAMIN A AND VITAMIN C IN APRICOTS

INTRODUCTION

THE UNITED STATES produces a greater quantity of apricots than any other single country in the world, with over 140,000 tons being marketed in 1973, returning over $20 million to the grower. Like other agricultural products this fruit is perishable and must either be consumed rapidly or treated in some manner to retard spoilage. About 12% of the apricots that reach the market are consumed fresh within a few weeks of the harvest season. The other 88% is preserved for later consumption, by canning, drying or freezing. This preservation is essential for two reasons: (1) to provide the grower a market for his product, since all of his crop cannot be consumed at harvest time; and (2) to provide a nutritious flavorful fruit for world-wide customers on a year-round basis.

Apricots are nutritionally recognized for their vitamin A content, with about 1/2 lb fresh, or 1/8 lb of dried providing 100% of the Recommended Daily Allowance. In addition, apricots contain other vitamins and minerals which the body needs. Processing and storage can have a detrimental effect on some of these nutrients. Aczél (1973) found that the β-carotene content of canned apricots was reduced 26% during storage. Moyer (1943) also indicated that carotenoids, as well as vitamin C, could be degraded gradually during storage at temperatures ranging from −30°C to 25°C. Light has also been shown to affect nutrient breakdown in foods. Bolin et al. (1964) found that illumination caused dried apricots held at 32°C for 20 wk to lose β-carotene 20% faster than unilluminated samples. Hollingsworth (1970) also indicated that vitamin A is sensitive to light, as well as heat, and that ascorbic acid stability is influenced by sulfur dioxide in the fruit. However, Heikal et al. (1972) indicated that additives, such as sulfur dioxide, did not have any effect on the stability of vitamin C. Morgan and Field (1930), in their work using biological testing procedures on fruits, indicated that vitamin A stability varied widely among fruits.

Since a large tonnage of apricots is processed and stored for later consumption, a systematic study was undertaken to determine the effects various processing methods and storage conditions have on their nutrient retention.

EXPERIMENTAL

BLENHEIM APRICOTS (600 lb) were mixed and divided into four lots. The apricots in the first lot were halved and placed cup up on wooden trays. This lot was divided further into three sublots, two of which were sulfured with burning sulfur. One of these sulfured sublots was sun dried and the other dehydrated. The remaining unsulfured sublot was sun dried. In the second lot the apricots were halved, dipped in a 2% sodium bisulfite solution for 7 min, and then put through a 0.045-in. screen pulper-finisher to produce a fresh apricot puree. This puree was then dried to 12% moisture apricot sheets, using a double-drum drier operating at 132°C with a drum clearance of 0.008 in. and speed of 45 sec per revolution. For the third lot, the apricots were halved, steam blanched for 5 min until a negative catechol test was obtained (Ponting, 1944), and then put through a pulper finisher to give a 13.7° Brix puree. This material was then concentrated in a stirred vacuum evaporator to 28° Brix. The concentrate was divided into two sublots, one of which was dried to 12% moisture on a double-drum drier directly to produce a sulfur-free product. Sodium bisulfite was added to the other sublot to give 0.2% sulfur dioxide in the puree before it was dried. The fourth lot was canned as halves in 40° Brix syrup, with 5 min exhausting and 15 min processing in 100°C water.

Commercial 31° Brix apricot concentrate was also used for preparing drum-dried apricot sheets. Sodium bisulfite was added to this concentrate to give 0, 0.03, 0.10 and 0.30% sulfur dioxide. One batch of commercial concentrate was also divided into two lots: one was left sulfur-free and the other was sulfured by adding potassium metabisulfite to give 0.3% sulfur dioxide in the puree. Each of these lots was divided into three sublots which were spread 2 mm thick on cellophane sheets. One of each of the sulfured and unsulfured sublots was dried to a leather by dehydration, sun drying and shade drying.

All samples were analyzed. Moisture was determined by the AOAC (1970) procedure. The β-carotene was determined by the AOAC (1970) procedure where a 2–10g sample was soaked in water until rehydration, after which it was blended with the organic solvents. Ascorbic acid was analyzed by the AOAC (1970) titrimetric procedure. Where the end point was difficult to determine, the results were checked using the Loeffler and Ponting (1942) colorimetric method. In these procedures, 1–2 ml of formaldehyde was added to tie up the free SO_2 in the sulfured samples. Color was determined by measuring sample reflectance through a 5.6 cm diameter hole with a Gardner Automatic Color Difference Meter using a standard tile, with L = 48.8, a = +19.9 and b = +29.5.

Fig. 1—Vitamin A and C content of processed apricots.

Table 1—β-Carotene lost during sulfured apricot leather drying

Drying method	Moisture	β-carotene IU/100g (MFB)[a]	β-carotene Lost %
None	68.8	19,000	
Dehydrated	12.6	17,390	9.2
Shade dried	17.1	17,080	10.1
Sun dried	16.0	13,300	30.0

[a]Moisture-free basis

Table 2—β-Carotene in apricot concentrate and dried sheets (IU/100g, Moisture-free basis)

Sulfur dioxide level	Concentrate	Sheets Time 0	Sheets 13 wk @ 32°C
0	15,100	13,300	9,100
0.03	15,100	—	10,100
0.10	15,100	—	10,800
0.30	15,100	14,400	11,800

RESULTS & DISCUSSION

PROCESSING and drying methods both influenced carotenoid retention in apricots. In preparing dried apricot halves, the method of drying had the greatest effect. No carotenoid degradation occurred in halves which were sulfured and dehydrated. However, sun drying of the sulfured and unsulfured halves resulted in a 10% loss (Fig. 1). A loss gradient was also noticed in producing leather, where the sulfured sun-dried product had 20% less β-carotene than the sulfured dehydrated or shade-dried materials, Table 1. Similar effects were noticed in unsulfured samples. The carotenoid loss was more pronounced in these leathers because of the larger surface area exposed to the sun. These findings are in agreement with Salem and Hegazi (1973), who found that carotenoids were degraded during processing and sun drying of apricot "juice" samples.

Sulfuring also had an effect on carotenoid loss during drying. Only a slight difference was noticed in dried halves, but a more pronounced effect was observed in the drum-dried products. Apricot sheets prepared by drum drying sulfur-free concentrate lost 12% of their β-carotene during drying, compared to 5% which was lost in the sample prepared from the 0.3% sulfured puree, Table 2.

Sulfur dioxide treatment also reduced the rate of carotenoid degradation during storage. Sheets made from apricot concentrate to which bisulfite had been added before drying lost 18% of their β-carotene after 13 wk storage at 32°C, Table 2. However, the unsulfured product lost 32%. After 13 wk storage only the highest sulfured sample contained any detectable residual sulfur dioxide, and that only amounted to 130 ppm. As well as losing sulfur dioxide during storage, these apricot sheets gradually turned dark (Fig. 2, reflectance). This darkening is caused by a combination of at least two different reactions. The greatest effect is undoubtedly from the nonenzymatic Maillard-type browning reaction, which occurs between amino acids and sugars and results in the production of dark-colored compounds (Song and Chichester, 1967). The loss of some of the pigmented carotenoids also is important, as determined by the hue shift that occurred during storage. The saturation values ($a^2 + b^2$)$^{1/2}$ of all the apricot sheets at time 0 was 30−31. However, after 13 wk storage at 32°C, these values shifted down to 21 in the sulfured products and to 11 in the unsulfured. This hue shift indicates an increased diminution of pigmentation for the product that did not contain sulfur dioxide. The same product had lost 1/3 of its β-carotene.
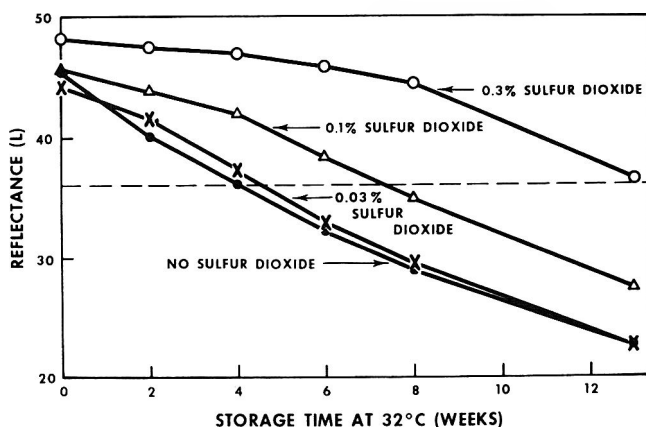
Specific processing and drying methods did not have as great an effect on ascorbic acid loss as they had on β-carotene loss; however, the sulfur dioxide treatment was important. Halves which were dried without sulfuring lost 95% of their ascorbic acid during drying, compared to a 74% loss from the sulfured samples, Figure 1. The drum-dried sulfured puree also retained more ascorbic acid than the unsulfured product. Drying methods did have some effect, however, which was noticed in apricot leather production where sun-dried, shade-dried and dehydrated samples lost 29%, 19% and 12%, respectively.

Besides sulfur dioxide reducing the vitamin C loss rate during processing, it also gave added protection during storage. Some vitamin C preservative action was obtained when as little as 0.03% sulfur dioxide was incorporated into the apricot concentrate before drying. At this level, the amount of ascorbic acid present in apricot sheets which had been stored at 32°C for 13 wk was 8.4 mg/100g, compared to 5.0 mg/100g which was in the unsulfured products. When 0.1% sulfur dioxide was added, the amount retained was 10.0 mg/100g, an increase of 100%; when 0.3% was added, 14.7 mg/100g was retained. This indicates that, even though a large percentage of this vitamin is degraded during processing and storage, the loss can be minimized by the incorporation of sulfur dioxide into the product.

Overall, sulfur dioxide treatment of fruits and fruit purees before drying is an economical treatment procedure for insuring that these products will lose less of their natural vitamin A and C during drying and subsequent storage.

Fig. 2—Darkening of apricot sheets during storage at 32° C.

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CLOUD LOSS DURING STORAGE OF PASTEURIZED CITRUS JUICES AND CONCENTRATES

INTRODUCTION

MAXIMUM CLOUD content is one of the main requirements in the different types of citrus products which are used by reprocessors and bottling plants as raw material for preparation of consumer beverages. Adequate heat treatment of the raw materials is necessary to prevent cloud loss during storage or subsequent clarification. This was already established by Cruess (1914), and applied by Irish (1928) who used flash pasteurization of citrus bases in order to retain cloud in beverages prepared from them. Cloud retention and heat treatment was systematically studied first by Joslyn and Marsh (1933, 1934). Even after heat processing, some residual pectinesterase activity may remain in pasteurized products, due to minor pasteurization deficiencies. Since usually many months elapse from the time of manufacture of the product until reprocessing or consumption, the present study was conducted to investigate the eventual loss of turbidity caused by traces of residual pectinesterase activity in juices and concentrates prepared from different Israeli citrus fruits throughout prolonged storage at room temperature or in the cold. Although, a very comprehensive literature exists on cloud loss during storage (see review by Joslyn and Pilnik (1961)) most of the publications refer to frozen, cutback, or not fully pasteurized concentrates (mainly orange) having considerable pectinesterase activity.

EXPERIMENTAL

Test materials and storage conditions

Samples of juices of varying concentrations were prepared from fourfold or sixfold hotpack concentrates, undiluted or diluted with varying amounts of distilled water. The final concentrations were one- to fourfold for lemon and one- to sixfold for grapefruit and orange as referred to single strength juice. Sodium benzoate and/or sodium bisulfite were added as preservatives, irrespective of juice concentration, in amounts corresponding to 1,250 ppm benzoic acid plus 1,250 ppm sulfur dioxide, or 2,000 ppm sulfur dioxide only. The test samples were held in a large number of small brown glass bottles, so that for each subsequent examination, a previously unopened bottle could be used.

Samples having zero pectinesterase activity consisted of:
(A) Reference samples used for control determinations;
(B) Factory products, i.e., juices, concentrates and comminuted materials of all varieties which were stored for 9 months in a factory shed; and
(C) Concentrates of equal Brix values (45.7° Brix, acid-corrected), but different acidities, which had been prepared by mixing shamouti with lemon and grapefruit with

lemon, in varying proportions. The test series was stored for a period of 5 months at room temperature and in a refrigerator at 5°C.

Samples of different pectinesterase activity were prepared by adding to pasteurized juices of zero activity varying amounts of unpasteurized macerated fruit cells of known pectinesterase activity. The samples of different °Brix values and fruit varieties possessed pectinesterase activities ranging from PEu × 10⁴ = 0.02–1.0 per gram of single strength juice or in

Table 1—The influence of acidity on cloud stability of fully pasteurized products

Variety	Product	pH	Acidity (% citric)	Room temp Cloud loss (%)	Cold storage (5°C) Cloud loss (%)
				Storage temperature	
Reference samples after 9 months' storage					
Shamouti	conc juice		up to 4.6	0	
Lemon	juice		4.6	0	
Valencia	conc juice		up to 4.9	0	
Grapefruit	conc juice		up to 5.7	0	
Grapefruit	conc juice		7.6	35	0
Lemon	conc juice		9.2	7	0
Lemon	conc juice		13.8	49	0
Factory products after 9 months' storage					
Shamouti	comminuted		1.95	0	
Shamouti	base		3.45	4	
Valencia	conc juice		4.60	0	
Shamouti	conc juice		up to 4.96	0	
Grapefruit	base		up to 5.69	1	
Lemon	comminuted		7.62	8	
Lemon	comminuted		7.74	10	
Lemon	base		14.00	26	
Lemon	conc juice		24.30	59	
Lemon	conc juice		25.80	69	
Lemon	conc juice		27.20	80	
Laboratory prepared mixtures after 5 months' storage					
Shamouti	conc juice	3.2	4.30	4	
Shamouti + lemon	conc juice	2.9	6.05	6	
Grapefruit	conc juice	2.85	7.10	10	0
Shamouti + lemon	conc juice	2.7	8.90	16	4
Grapefruit + lemon	conc juice	2.7	9.40	30	7
Shamouti + lemon	conc juice	2.5	12.30	27	6
Shamouti + lemon	conc juice	2.4	14.80	47	8
Grapefruit + lemon	conc juice	2.4	15.10	53	5
Grapefruit + lemon	conc juice	2.3	17.90	64	8
Shamouti + lemon	conc juice	2.2	19.60	76	10
Grapefruit + lemon	conc juice	2.3	20.80	77	7
Grapefruit + lemon	conc juice	2.15	23.60	81	10
Lemon	conc juice	2.1	26.10	81	4

terms of unpasteurized juice content from 0.1–5%. (Throughout this study, the enzyme activity of an unpasteurized single strength juice was taken as PEu $\times 10^4$/g = 20.)

Test samples were placed in cartons and stored in the dark for a maximum of 2 yr either at room temperature or in a refrigerator. In cold storage, a constant temperature of 5°C ± 1° was maintained, but considerable fluctuations occurred during room storage, where fairly constant temperatures of ± 2°C could be achieved for periods of 3 months only. Laboratory temperatures varied from an average low in winter of 20°C to a high of 26°C in the summer with extremes of 18° and 28°C. Although storage in the laboratory, i.e., at temperatures between 18–28°C provided a fair simulation of factory conditions, actual fluctuations in a factory storeroom may be even more pronounced with shade temperatures varying between 10–30°C throughout the year. As pointed out by Porter and Greenwald (1971), storage areas in a large warehouse or depot show considerable temperature variations, depending on the time of day, season of the year, location of the warehouse and location within the warehouse.

Analytical methods

Pectinesterase activity of the unpasteurized fruit cells was determined by titration of liberated carboxyl groups due to the enzyme action on a synthetic pectin substrate according to the procedure of Rouse and Atkins (1955).

Pasteurized test materials were checked for absence of pectinesterase activity by the following modified version of the method of Pilnik and Rothschild (1960), i.e., by a qualitative test having a sensitivitiy limit at PEu $\times 10^4$ = 0.02/g single strength juice (Gerda Rothschild, unpublished data).

Procedure. A test sample, containing 0.3–0.35g citric acid, is placed in a 250 ml beaker. Neutralization is carried out, using a magnetic mixer, with 1N NaOH against phenolpthalein, and thereafter, 5 ml of 1N $CaCl_2$ and 100g of a 1% pectin solution prepared from 55% esterized pectin are added with mixing. The pH is adjusted to pH 7 with 0.1N NaOH and distilled water added to a total weight of 200g. After mixing, the samples are transferred to small sterilized bottles and a few drops of toluene added as a preservative. The bottles are stored in the dark at room temperature and examined daily for gel formation or increased viscosity. If, after 3 days, the mixture remains free flowing, pasteurization is considered as having been adequate.

Turbidity examinations were carried out at the start of the test and thereafter usually at monthly or quarterly intervals. Experiments were interrupted either when severe cloud loss occurred, or in cases of browning which was found to interfere with the analysis. Analyses were performed as described by Loeffler (1941) on single strength juices, and concentrated materials were diluted accordingly with distilled water prior to analysis.

Test samples were centrifuged for 15 min at 370 × G in a Hettich Universal Centrifuge and the optical transmittance of the supernatant was measured at a wavelength of 540 mμ in a Klett Summerson Colorimeter which had been standardized against distilled water. Results were expressed in terms of percent cloud loss or as periods of cloud stability which refers to that duration of storage during which cloud losses were negligible, i.e., below 10%. From the difference in optical transmittance before and
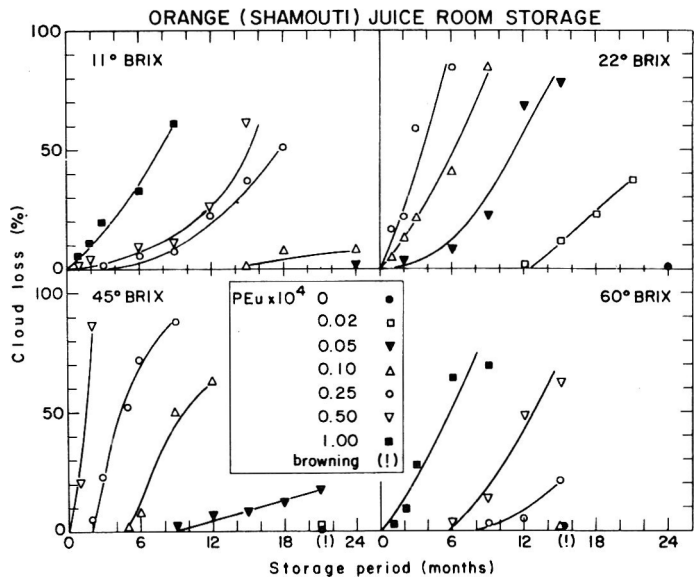

Fig. 1–Influence of residual pectinesterase activity on cloud loss in orange (shamouti) juice.
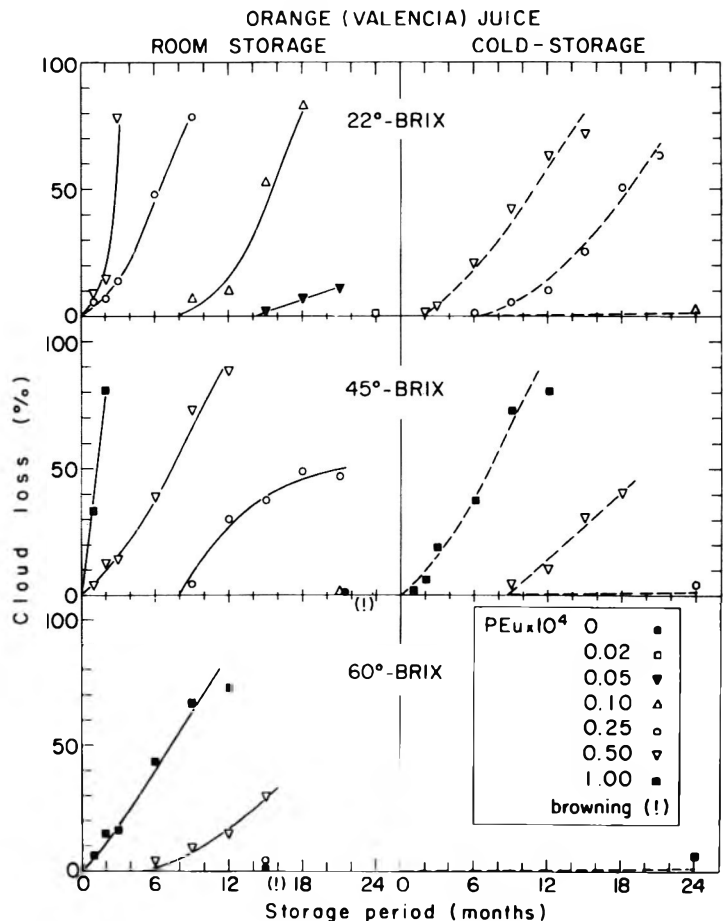

Fig. 2–Influence of residual pectinesterase activity on cloud loss in orange (valencia) juice.

after storage, cloud retention was calculated and percent cloud loss was computed by deducting percent cloud retention after storage from the initial value of 100%.

RESULTS

TABLE 1 shows that cloud losses occurred during storage at room temperature in some faultlessly pasteurized test materials. This was due to high acidity, the rate of clarification increasing with increasing acidity or decreasing pH. However, in cold storage, cloud remained practically stable even in fourfold lemon concentrates having an acid content of 26% (pH 2.10).

In Figures 1–4, loss of turbidity caused by residual pectinesterase activity is shown. The extent of cloud loss, which depended on pectinesterase content, as well as on temperature and duration of

storage varied also for different concentrations of the same juice. Thus, for equal enzyme activities and under the same storage conditions, the extent of cloud decrease was inversely proportional to the juice concentration (an exception being single strength orange juice), i.e., cloud loss increased for orange in the order 60°–22°Brix, grapefruit 60°–11°Brix, and for lemon juice 21°–7°Brix. Cloud stability was the same for both the 21° and 28°Brix lemon juice over 18 months of observation in cold storage; thereafter clarification occurred in the 28°Brix concentrate due to high acidity. Cloud loss occurred in control samples (zero PE activity) of 45° and 60°Brix grapefruit and 7°–28°Brix lemon juices when kept at room temperature, but in cold storage only the 28°Brix lemon juice showed some clarification after more than 1½ yr.

Cloud remained stable in all orange control samples; however, browning occurred in the 45° and 60°Brix concentrates after 21 and 15 months of storage at room temperature, respectively.

Periods of cloud stability throughout storage at room temperature or in the cold for the different varieties and degrees of juice concentration are presented in Table 2. At identical concentration, enzyme activity and storage temperature, periods of cloud stability differed for the different citrus varieties, in decreasing order for valencia and shamouti orange, lemon and grapefruit. When single strength juices of PEu × 10^4/g = 0.02 were stored at room temperature, cloud remained stable in the valencia and shamouti varieties for 24 months or more, but only for 7 and 5 months respectively in the lemon and grapefruit samples.
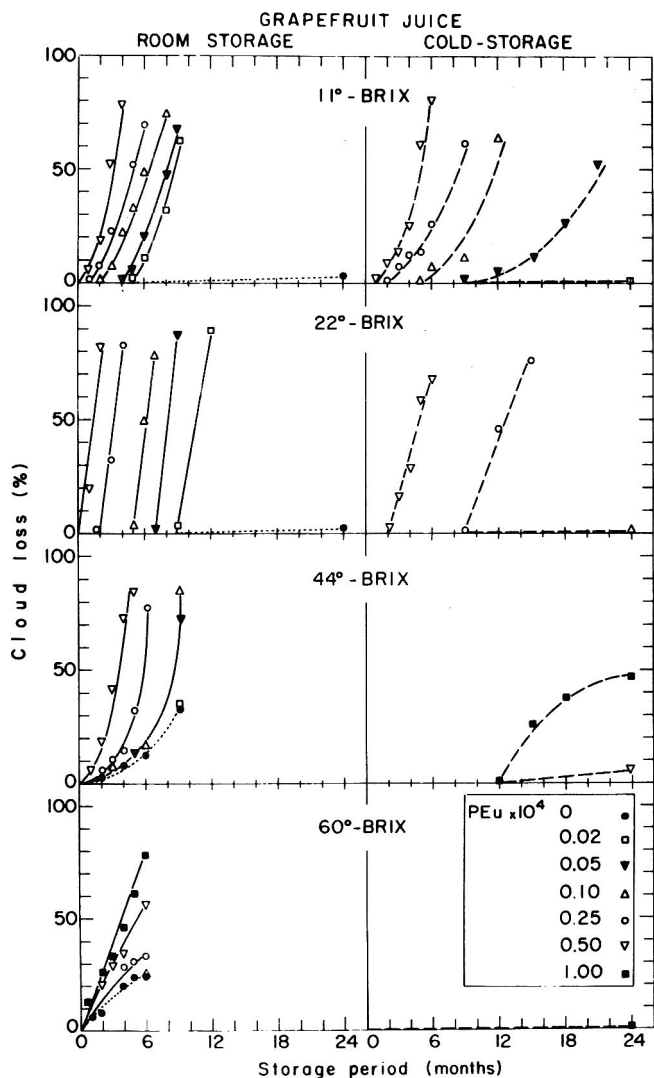

Fig. 3—Influence of residual pectinesterase activity on cloud loss in grapefruit juice.
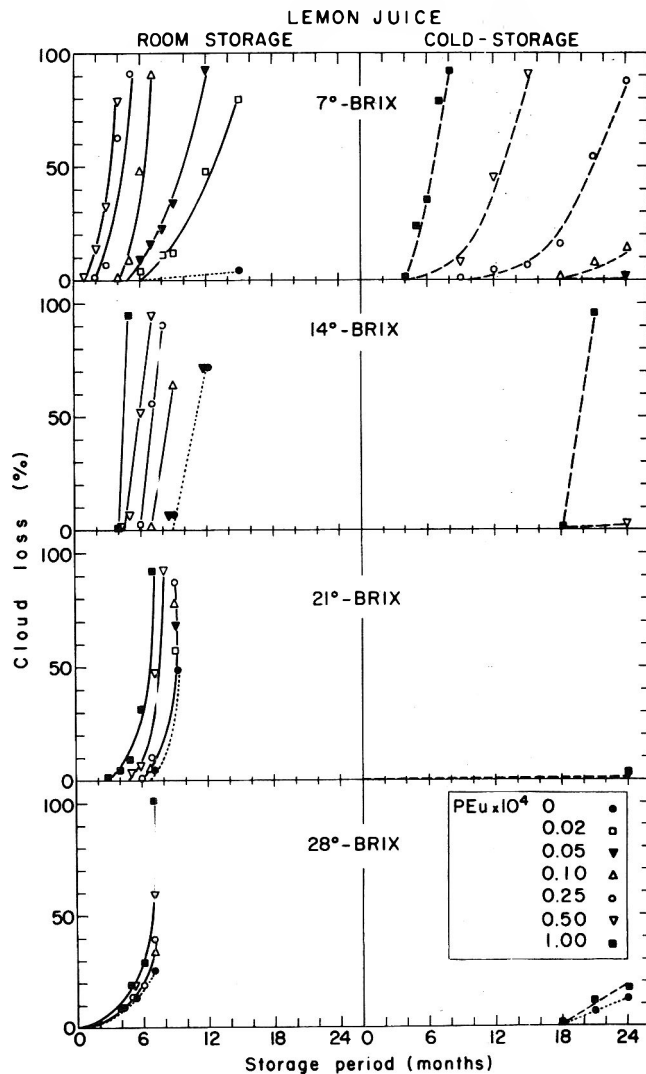

Fig. 4—Influence of residual pectinesterase activity on cloud loss in lemon juice.

Table 2—Duration of cloud stability[a] in different varieties of citrus juices and at different acidities, pectinesterase activities and degrees of concentration

Variety and storage conditions	Acidity range (%)	Pectinesterase activity PEu X 10⁴ (g/single strength)	Concentration (1-fold = single strength)			
			1-fold	2-fold	4-fold	6-fold
				Stability (months)		
Shamouti						
Room temp	0.86–4.60	0	24	24	21	15
		0.02	24	12	21	15
		0.05	24	6	15	15
		0.10	24	2	6	15
		0.25	9		2	12
Valencia						
Room temp	0.90–4.90	0	24	24	21	15
		0.02	24	24	21	15
		0.05	24	18	21	15
		0.10	15	9	21	15
		0.25	15	2	9	15
Cold storage		0	24	24	24	24
		0.10	24	24	24	24
		0.25	24	9	24	24
		0.50	24	3	10	24
Grapefruit						
Room temp	1.90–10.40	0	24	24	4	2
		0.02	5	9		
Cold storage		0	24	24	24	24
		0.02	24	24	24	24
		0.05	12	24	24	24
		0.10	6	24	24	24
		0.25	3	9	24	24
		0.50		2	24	24
		1.00			12	24
Lemon						
Room temp	4.60–18.60	0	15	9	3	
		0.02	7	9		
Cold storage		0	24	24	21	
		0.10	21	24	21	
		0.25	15	24	21	
		0.50	8	24	21	

[a] Cloud stability is considered as < 10% loss of cloud

DISCUSSION

LOSS OF TURBIDITY during storage was found to be due to either high acidity or residual pectinesterase activity or both; in either event, the extent of cloud loss could be reduced or periods of cloud stability extended by keeping test materials in cold storage. Already in 1939, Heid showed that cloud may be precipitated by excess acid even in the absence of pectic enzymes; cloud loss due to high acidity at unfavorable storage temperatures was mentioned also by Swisher and Higby (1961). The extent of cloud loss, when due to residual pectinesterase activity, was more severe at the lower juice concentrations (with the exception of single strength shamouti and valencia juice). The influence of degree of concentration on clarification was studied before by Cotton et al. (1947) who found that

cloud stability decreased in unpasteurized orange concentrate kept at 4.4°C in the order of 12°, 52°, 42° and 25° Brix. We observed practically the same trend for pasteurized orange concentrate stored at room temperature and possessing some residual pectinesterase activity in which the extent of cloud stability decreased in the order 11°, 60°, 45° and 22° Brix. Huggart et al. (1951) compared 42° Brix concentrate from three orange varieties with reconstituted single strength juice made from these concentrates and found that at the same storage temperature, the concentrate of all three varieties clarified much more quickly than the corresponding juice. This is in accordance with our findings for valencia and shamouti orange concentrates and reconstituted single strength juices. McColloch et al. (1956) found in one- to sixfold orange concentrates a clarification optimum at the

three- and fourfold concentration. They were able to prove (from simulated two- to sixfold concentrates 'prepared by adding sugars and citrates to single-strength and double-strength juices, which showed the same storage behavior' as the real concentrates,) that variations of cloud stability with variation of concentration of orange juices is associated with the changing concentrations of sugars and citrates. Cloud stability of juices and concentrates was found to be affected differently in the different citrus varieties, which was observed also by Moore et al. (1950) and by Bisset et al. (1957) for frozen concentrates. Wenzel et al. (1951) who investigated five different citrus varieties, stated that degree of concentration, fruit variety and temperature of storage are factors of practical significance in relation to the problem of clarification, which was confirmed by our findings.

CONCLUSIONS

DETERMINATION of acidity and residual pectinesterase activity in pasteurized citrus products can be used as a means to predict cloud loss which may occur after many months of storage. Thus it should be possible to guard against clarification by choice of optimal storage conditions, by reprocessing, or by marketing products within their respective periods of cloud stability.

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INFLUENCE OF HOLDING TIME BEFORE PASTEURIZATION, PASTEURIZATION AND CONCENTRATION ON THE TURBIDITY OF CITRUS JUICES

INTRODUCTION

TURBIDITY is a desired characteristic of citrus juices and beverages, and cloud content is one of the criteria of quality. Inactivation of the pectinesterase enzyme by adequate pasteurization is, therefore, of primary importance in order to achieve cloud stability during shelf life. Although heat treatment is generally one of the first steps in citrus processing, holding times prior to pasteurization may differ from several minutes to several hours (depending on the size of the storage tanks and also on the capacity of the evaporators, speed of the filling procedure, and on stoppages due to breakdowns along the manufacturing line). With the aim of preserving or enhancing to as great an extent as possible the turbidity of freshly squeezed citrus juices, we investigated the effect of duration of exposure in the unpasteurized state, of pasteurization and of concentration on the cloud content of juices from Israeli citrus fruits. Cloud increases in fresh juices were observed by Loeffler (1941a), Huggart (1952), Pilnik and Rothschild (1954), Blau (1957) and Mizrahi and Berk (1970). Huggart (1952) for example, reported that standing for 5 hr increased the turbidity of valencia juices. Mizrahi and Berk (1970) in their very interesting study on the nature of orange juice cloud, observed that a clear serum prepared by centrifugation from fresh shamouti orange juice became turbid after 2 hr standing and that turbidity increased with time during 48 hr. Loeffler (1941a, b) found an increase in the cloud index on pasteurization, and Joslyn and Pilnik (1961) expressed the view that pasteurization not only stabilizes cloud but also increases or forms it where turbidity before pasteurization is negligible, pointing out with regard to cloud increase, that a mechanical dispersion of cloud particles must also be considered. Huggart (1952) found that concentrated, reconstituted valencia juice has greater turbidity than the original juice.

EXPERIMENTAL

Materials

Juices tested were from Israeli shamouti and valencia orange, grapefruit and lemon varieties, before and after various treatments, as follows:

Designated as U. Unpasteurized juice extracted by an FMC in-line juice extractor, and placed in storage vessels of about 2000–3000 liter capacity. Samples used for determination of the initial turbidity were analyzed within 15 min after the filling procedure was completed, which took for orange and grapefruit juice from 10–20 min and for lemon juice between 40–60 min.

Designated as UT. As above, but kept in the laboratory prior to analysis for periods of from 1–6 hr for valencia, grapefruit and lemon juices and from 30 min to 24 hr for shamouti juice at temperatures between 10–17°C, i.e., during the early part of the citrus season (winter) or during night-shifts.

Designated as UMH. As U, after flash pasteurization in the factory, which consisted of passing the unpasteurized juice via a stainless steel pump (APV puma) through a heat exchanger (APV paraflow type X), i.e., applying combined mechanical and heat treatment. Pasteurization temperatures were 80°C for lemon, 85°C for grapefruit and 90°C for orange juices at 45 sec holding time for all varieties. The time between extraction and pasteurization which differs considerably for factory-line juices, was unknown for these samples.

Designated as UM. As U, plus mechanical treatment, that is, equivalent to UMH without heat treatment. Unpasteurized juice was passed through an *unheated* heat exchanger, employing the same equipment as in the UMH process.

Designated as UH. As U, plus heat application, equivalent to UMH without mechanical treatment. Test samples were pasteurized in the laboratory as follows: 50-ml portions of unpasteurized juice were quickly brought to 90°C in an electrically-heated waterbath, kept for 45 sec and cooled to room temperature in a Liebig glass condenser. The entire procedure, including coming up time, took 2½–3 min.

Designated as UTH. As UH, after having been kept previously for controlled periods in the unpasteurized state (at temperatures between 10–17°C).

Designated as UMHC. As UMH, after six-fold concentration in an APV triple effect vacuum plate concentrator and reconstitution to single strength juice with distilled water.

Experiments U, UT, and UMH were carried out for all varieties, UMHC for shamouti and valencia, and UM, UH and UTH only for the shamouti variety.
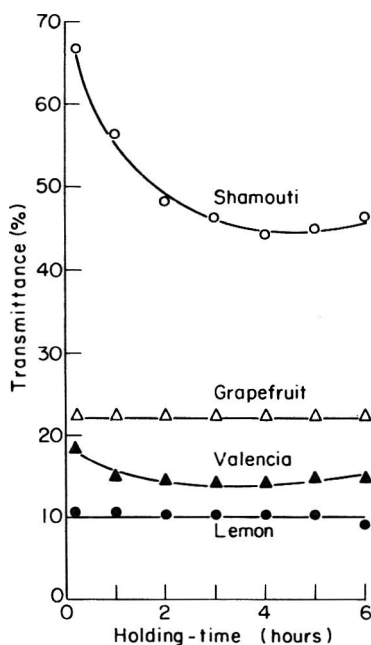

Fig. 1—Influence of holding time on cloud content of unpasteurized juices (UT). (Values are averages of 9–38 determinations.)
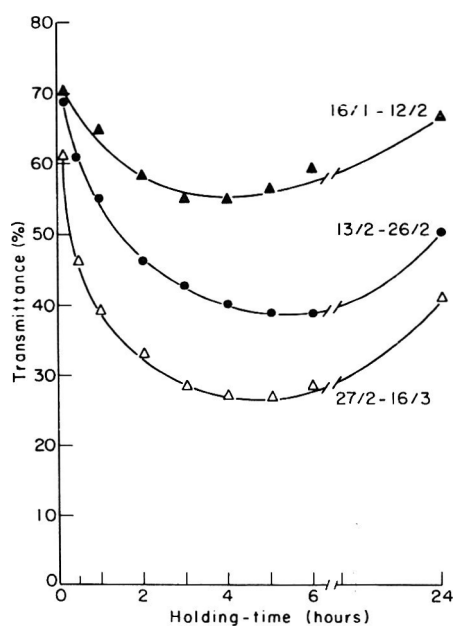

Fig. 2—Influence of duration of holding time and of season on cloud content of unpasteurized shamouti orange juice. (Values are averages of 8–19 determinations.)

Analytical methods

The terms turbidity or cloud are terms which have been used synonymously for a parameter which can be quantitated by the optical transmittance. The method of Loeffler (1941a) was adopted in principle by measuring light transmittance in the supernatant liquid after centrifugation of single strength juice, as follows:

The juice was adjusted to a temperature of 20°C and centrifuged at 370 × G for 15 min in a Hettich Universal Centrifuge. Percent transmittance of the supernatant was measured at 540 mμ in a Klett Summerson Colorimeter, standardized against distilled water. At this wavelength color variations of the differently treated samples did not interfere with the results.

RESULTS

Holding time

Values for initial turbidity differed considerably for the different varieties; on keeping juices designated UT up to 6 hr, turbidity remained unchanged in lemon and grapefruit, but increased in orange and especially in shamouti juice (Fig. 1).

Results from experiments on holding time, UT, were recorded separately for the early, middle and end of the shamouti season. Whereas initial turbidity of shamouti juice varied only slightly throughout the whole period (Jan. 16th to March 16th), cloud increase due to prolonged exposure became more pronounced with the advancing season (Fig. 2). Cloud increase was measurable after 30 min of standing time, progressed to a maximum

at approximately 4 hr and declined gradually thereafter. However, even after 24 hr standing time the cloud index was still higher than the original value.

Holding time before pasteurization

By comparing transmittance values obtained from experiments involving holding time (UT), or pasteurization following different holding times (UTH), it was found that cloud content increased either due to prolonged keeping in the unpasteurized state, or by heat treatment, but that duration of holding time prior to heat treatment had no effect on the turbidity of the pasteurized juice (Fig. 3).

Pasteurization and concentration

While initial transmittance values differ somewhat among the varieties, the turbidity of fresh shamouti juice is exceptionally low (Fig. 1 and Table 1). After pasteurization, the transmittance values decreased; the turbidity of shamouti juice increased greatly, to a value equal to that of the turbidity of pasteurized grapefruit juice. After sixfold concentration, a further cloud increase occurred in shamouti but not in valencia juice, and the same transmittance values were obtained for reconstituted juice from both varieties (Table 1).

Combined treatment

The contributions of each of the types of treatment on turbidity can be observed in Figure 4. Each of the treatments, mechanical treatment, heat treatment and concentration contribute to the cloud content of pasteurized juice or of juice reconstituted after concentration.

Table 1—The influence of pasteurization and concentration on turbidity of citrus juices

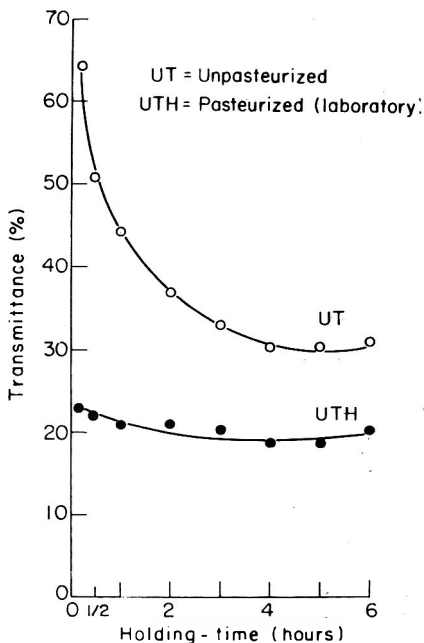| Variety	Unpasteurized juice (U)		Pasteurized juice (UMH)		Reconstituted juice from sixfold conc (UMHC)	
	Transmittance (%)		Transmittance (%)		Transmittance (%)	
	Range	Avg	Range	Avg	Range	Avg
Shamouti	56—77	68	12—23	18	7—10	9
Grapefruit	19—25	22	17—22	19		
Valencia	16—22	18	9—11	10	8—11	10
Lemon	9—13	11	6—8	7		

Fig. 3—Comparison of holding time before pasteurization on cloud content of pasteurized and unpasteurized shamouti orange juice. (Values are averages of 16 determinations.)
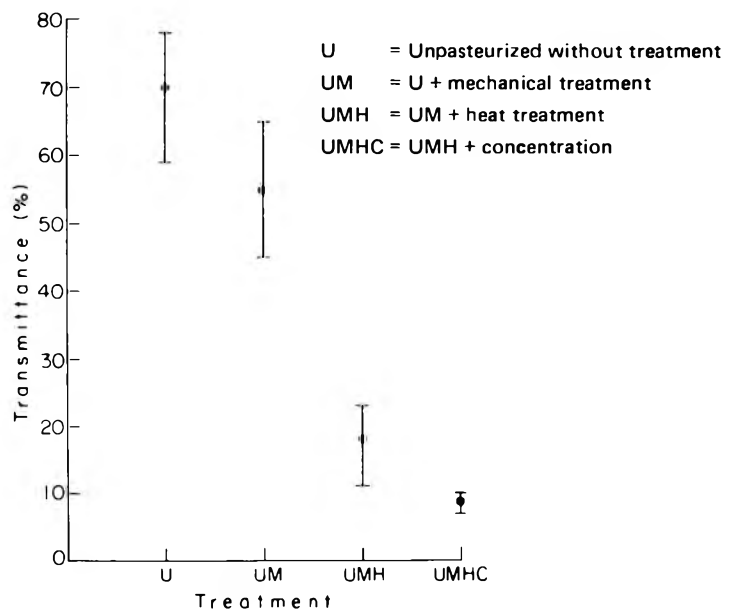

Fig. 4—Influence of various treatments on cloud content of shamouti orange juice. (Values are averages of 6—12 determinations.)

DISCUSSION

THE JUICE of the shamouti variety was the most affected by all of the treatments applied; each of the treatments enhanced the slight turbidity originally present in freshly squeezed juice. Cloud increase due to pasteurization was found to be caused to some extent by mechanical, but mainly by heat treatment. The considerable differences in the initial cloud content of juices from the different varieties diminished after pasteurization or concentration; indeed the very low turbidity of unpasteurized shamouti juice was so much enhanced by pasteurization and concentration that reconstituted shamouti juice possessed the same turbidity as pasteurized valencia juice. As prolonged keeping of unpasteurized juice prior to pasteurization had no effect on cloud content of pasteurized juice, for storing freshly squeezed juice until pasteurization, the use of very large containers in which holding times are necessarily longer, is feasible.

The considerable cloud increase which was found to occur throughout prolonged standing in unheated shamouti juice may be attributed to crystallization of hesperidin, formed according to Mizrahi and Berk (1970), immediately after juice extraction. Goren (1965) found that shamouti orange juice is saturated with hesperidin, while Mizrahi and Berk (1970) reported that freshly pressed shamouti orange juice is heavily supersaturated with the bioflavonoid. The latter succeeded in isolating needle-like crystals from a centrifuged serum, and identified them as hesperidin; they concluded that crystallization of flavonones may be a significant factor in overall juice cloudiness.

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EXTRACTION OF A HIGH-PROTEIN LAYER
FROM OAT GROAT BRAN AND FLOUR

INTRODUCTION

OAT GROATS (dehulled oats) contain protein that is high in quality and quantity for a cereal crop (Clark, 1972; Robbins et al., 1971). Cooperative efforts are aimed at further improvement of this protein (Youngs et al., 1973). Also, efforts have been made to isolate and concentrate this protein for use as a food supplement. Cluskey et al. (1973) isolated a high-protein fraction from oat groats by solubilizing the protein in dilute alkali and freeze drying the solution. Amino acid composition of this fraction differed only slightly from that of the original groats (Wu et al., 1973). Wu and Stringfellow (1973) also isolated a high-protein fraction from oat groats by air classification.

In this study we fractionated oat groat flour and bran by centrifuging a water slurry of each, then removed the resulting layers or fractions. Besides a protein-rich layer, we also obtained layers of starch, residue bran and water solubles. These layers were analyzed for ash, protein and lipid concentration.

EXPERIMENTAL

FROKER AND GOODLAND, medium- and high-protein concentration oat cultivars from Wisconsin, were used. The oats were dehulled in a Quaker Oats experimental dehuller. The groats were tempered with 1% water for 1 hr, then milled into bran, and a low- and a high-ash flour with a Brabender Quadrumat, Jr. flour mill. The two flour streams obtained from this mill were blended into a single stream; thus two mill streams, flour and bran, were fractionated.

The technique used to fractionate groat flour and bran was similar to that of Gilles et al. (1964), who separated wheat flour into water-soluble, sludge, gluten and starch fractions. Groat flour was mixed with water (1:3, flour:water) 4 min in a Waring Blendor. The slurry was centrifuged for 20 min at 12,000 × G. The experimental conditions of mixing and centrifuging were arbitrarily chosen, and do not represent minimum conditions. Figure 1 is a drawing of the resulting layers. In this study, no sludge layer appeared, as it did in the fractionation of wheat flour (Gilles et al., 1964), and the oat groat protein-rich layer did not have the properties of wheat flour gluten. Residue bran appeared as an impurity in the flour fractionation. The water solubles were decanted, and the other layers were removed individually with a spatula. Each layer was again mixed with water in a Waring Blendor for 1 min, recentrifuged and separated as before. All layers were freeze dried.

Groat bran was fractionated the same way, except that a bran:water ratio of 1:9 was necessary to produce water solubles that were fluid enough to pour. Bran was apparently rich in gums, which increased viscosity. Layers obtained are shown in Figure 1. Starch was an impurity in this fractionation.

No adjustments for pH were made in this study. The pH of the flour:water mixture was 5.9; that of the bran:water mixture, 6.5. In a protein-solubility study, Wu et al. (1973) stated that oat groat protein was least soluble around pH 5.

Attempts were made to fractionate ground oat groats by the technique described here. A high-protein layer was obtained, although the yield was only half that obtained by fractionating bran and flour separately. Also, the residue bran and starch did not separate by centrifugation.

Fig. 1—Drawings of fractions obtained after centrifuging water slurries of oat groat flour and bran.

All layers were analyzed for nitrogen and ash concentration by AACC Approved Methods 46-11 and 08-01 (1962). Nitrogen values were converted to protein concentration by the formula N × 6.25. Free, or unbound, lipids were extracted with petroleum ether (bp 39°C) for 6 hr on a Goldfisch extractor. The solvent was removed by roto-evaporation under vacuum, and the lipid was weighed. The petroleum ether-extracted fractions were re-extracted with water-saturated n-butanol (1/2 hr, intermittent shaking) to remove the bound lipids. The lipid extract was roto-evaporated to dryness under vacuum, taken up in chloroform, filtered and evaporated again, and the lipid was weighed.

All results are expressed on a dry basis.

RESULTS & DISCUSSION

THE PERCENT bran and flour obtained by milling oat groats is shown in Table 1. Recovery in the milling process was 97%. Also shown are the ash, protein and lipid concentrations of the oat groats and the two mill streams. Froker groats, which were lower in protein percent, produced more flour than Goodland groats. Protein, ash and lipid concentrations were higher in bran than in flour in both samples. Between 65–80% of the lipids were free in all samples.

Table 2 shows the distribution of the layers obtained after the mill streams of Froker and Goodland had been mixed with water and centrifuged, and the layers had been separated and freeze dried. Average recovery in the fractionation of flour was 90.2%; in bran 90.0%. The residue bran is a product of the fractionation procedure, and should not be confused with the original bran obtained as a mill stream from the flour-milling process.

Table 1—Milling yield, ash, protein and lipid analysis of two oat groat cultivars[a]

	% of Total wt[b]	Ash (%)	Protein (%)	Lipid %Free	Lipid %Bound
Froker groats	100.0	2.36	16.2	6.0	2.4
Flour	58.9	1.02	12.4	5.0	2.7
Bran	41.1	4.52	20.0	7.7	1.9
Goodland groats	100.0	2.66	21.5	6.3	2.5
Flour	53.5	1.13	16.8	5.3	2.3
Bran	46.5	4.39	26.8	7.8	3.4

[a] All results reported on a dry basis
[b] Based on recovered material

Table 2—Distribution, protein, ash and lipid analysis of oat groat bran and flour layers[a]

Layer	% of Total wt[b]	Protein (%)	% of Total Protein	Ash (%)	Lipid %Free	Lipid %Bound
Froker bran						
Water solubles	25.2	24.1	28.0	7.79	5.4	3.2
High protein	15.3	49.9	35.2	2.43	16.5	4.0
Residue bran	43.8	16.4	33.1	3.52	4.1	2.0
Starch-res. bran	15.7[c]	5.0	3.7			
Froker flour						
Water solubles	6.2	22.8	10.7	8.10	6.9	2.9
High protein	17.0	57.2	73.8	1.72	20.9	3.7
Res. bran-starch	13.0[d]	11.4	11.2			
Starch	63.8	0.9	4.3	0.41	0.8	0.8
Goodland bran						
Water solubles	25.9	24.6	25.2	7.50	8.4	4.4
High protein	18.7	54.8	40.5	2.36	15.9	5.5
Residue bran	37.5	18.9	28.0	3.64	3.0	2.6
Starch-res. bran	17.9[c]	8.9	6.3			
Goodland flour						
Water solubles	7.0	26.4	11.6	8.18	5.2	2.3
High protein	18.8	57.3	67.8	1.87	18.4	4.0
Res. bran-starch	16.4[d]	15.4	15.9			
Starch	57.8	1.3	4.7	0.34	1.0	0.3

[a] All results reported on a dry basis
[b] Based on recovered material
[c] Starch was an impurity in the bran. Some residue bran was removed with the starch.
[d] In milling, bran was an impurity in the flour. Some starch was removed with the residue bran.

Also, the residue bran-starch, and starch-residue bran layers shown in Table 2 are mixtures. When the residue bran was removed from the flour fractionation (see Fig. 1), a small amount of the high-protein layer above it and a small amount of the starch layer below it were also taken to insure purer high-protein and starch layers. Similarly, in bran fractionation, a small amount of residue bran remained with the starch.

The high-protein layer comprised 15.3–18.8% of the total weight of bran or flour in all fractionations, with a protein range of 49.9–57.3%. In the flour fractionations, this represented 73.8% and 67.8% of the total protein available in oat groat flour of Froker and Goodland, respectively. Starch, the. largest layer in this fractionation, was relatively free of protein. In bran fractionation, the high-protein layer contained 35.2% and 40.5% of the total protein of the oat groat bran in Froker and Goodland, respectively. Residue bran was the largest single layer in bran, and it contained approximately 30% of the total protein in oat groat bran.

Ash values were highest in the water solubles and lowest in starch, with averages 7.89 and 0.37%, respectively.

Lipids in the layers were mostly free; i.e., soluble in a nonpolar solvent—petroleum ether in this case. Wheat flour and flour fractions that had been mixed with water and freeze dried contained a high percentage of bound lipids (Youngs et al., 1970). Preliminary work in the Oat Quality Laboratory has shown that oat lipids contain fewer phospholipids than wheat lipids. This observation may partially explain the difference in binding between oat and wheat lipids. The high-protein layers extracted from bran and flour contained the highest percent of lipid, and starch the lowest.

Since the high-protein layer can be separated from oat groat flour and bran simply by centrifugation, it may be an economical source of protein for use as a food supplement. Freeze drying is an ideal method of drying the layer, but this is expensive, and air-drying at controlled temperatures to prevent destruction of heat-labile amino acids may be more feasible. Also, the high-protein layer can be easily defatted, because most of the lipids are not bound. This would also increase the protein concentration.

Residue bran and starch should be usable by-products in this fractionation. Residue bran still contains about 16–19%

protein, and should find uses in feeds or bran cereals. Oat starch granules generally are between 3 and 10μ in their largest dimension, and have the smallest starch particles of all cereal grains except rice (Matz, 1969). Oat starch has received little attention in industry, probably because of unavailability. Little is known about its properties, but if oat starch were available, it should be useful to the food industry.

The water-soluble layer contained over one-fourth the total protein in bran, but only about 11% of the total flour protein was found in the flour water solubles. Although amino acid analysis was not performed on the layers, one would speculate that the water solubles would be rich in lysine, probably at the expense of the protein-rich layers. The economic feasibility of extracting protein from the water solubles is not known. Finley et al. (1973) have recently described a procedure for removing protein from effluents resulting from washing wheat flour doughs. Condensed phosphates were used to complex and precipitate the proteins. The protein was resolubilized, and phosphate was removed by ion exchange.

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Ms received 1/25/74; revised 3/13/74; accepted 3/20/74.

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AN ANALYSIS OF HIGH TEMPERATURE/SHORT TIME STERILIZATION DURING LAMINAR FLOW

INTRODUCTION

A POTENTIAL advantage of continuous flow sterilization of fluid food products over conventional (batch) sterilization methods for canned foods is that product quality can be improved due to higher nutrient retention (Burton, 1972; Leniger and Beverloo, 1969; Blakebrough, 1968). The continuous process takes advantage of the difference in magnitude of the Arrhenius activation energies for denaturation of bacterial spores and of nutrients. The ratio of these quantities is in most cases significantly greater than unity, i.e., $E_{a,Spores}/E_{a,Nutrients} > 1.0$ (Feliciotti and Esselen, 1957; Burton and Jayne-Williams, 1962). Now the criterion for any sterilization process is a particular survival level of a selected microorganism at the point of "minimum treatment." Thus by increasing the process temperature from T_1 to T_2 (Fig. 1) a larger fractional increment in the reaction rate constant for denaturation of bacterial spores is observed compared to that for nutrients. In consequence less nutrient denaturation is permitted, while maintaining the required probability of sterility at the point of minimum treatment, during higher temperature (and consequently shorter time) processing.

Food processors appear to have been reluctant to change from batch processing methods despite the possible advantages (including economic) of continuous sterilization. Mechanical difficulties associated with the aseptic processing required subsequent to continuous sterilization are in part responsible for this situation. Another reason is that adequate rational design criteria have not been established by which food processors can assess the true utility of the type of continuous process in which they would be most interested.

Food products which may conveniently be sterilized continuously are liquids such as soups, purées, sauces and concentrates. The majority of these products are particulate suspensions of high apparent viscosity exhibiting non-Newtonian behavior (Holdsworth, 1969). Using rheological data typical of these food products (Harper, 1960; Saravacos and Moyer, 1967) it can readily be shown that pumping pressures required to obtain fully turbulent conditions are not economic for production rates of interest. In consequence, the constraint of laminar flow is imposed upon the process.

Several analyses have been made of the continuous sterilization process. The analysis of Deindoerfer and Humphrey (1959) and its simplification by Richards (1965) are appropriate only to turbulent flow, relying upon concepts of bulk fluid motion and the corresponding predictions of overall heat transfer coefficients. Analyses applicable to laminar flow sterilization (Aiba et al., 1965; Leniger and Beverloo, 1969; Charm, 1966) have been subject to the constraint of isothermal operation, and so illustrate only the effect of longitudinal dispersion due to the laminar velocity profile during the holding phase of the process.

Batch sterilization and the continuous process are similar in the nature of the temperature cycle imposed on an element of material (Fig. 2). The residence time required for the holding phase decreases almost exponentially as the holding temperature is increased. At a sufficiently high maximum temperature, a holding period is not required and sterilization is completed during the heating and cooling cycles. It is apparent that processes which do not require a holding section are necessarily the "highest-temperature/shortest-time" processes attainable and consequently cause the least nutrient denaturation, as recognized by Leniger and Beverloo (1969) and evidenced by Figure 1. Thus design for continuous sterilization requires analysis of
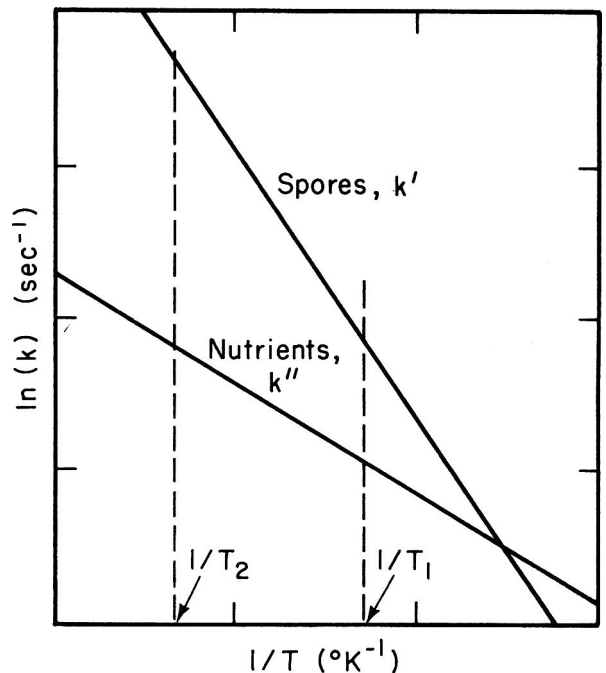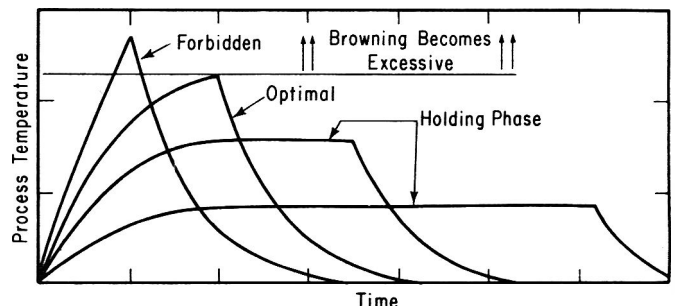

Fig. 1—Reaction rate constants.

Fig. 2—Equivalent time-temperature combinations (schematic). By using higher process or wall temperatures, the holding period can be reduced. The upper limit for temperature is established by product browning reactions.

the heating and cooling cycles alone, leading to prediction of operating temperatures necessary to ensure a "safe" sterilization at the point of minimum treatment in a laminar flow process. This is the *first* design objective. The analysis requires:

(1) An idealized physical model of the process;
(2) Mathematical description of:
   (a) The flow characteristics of the foods to be processed, i.e., a rheological model;
   (b) The heat transfer problem; and
   (c) The temperature-dependent kinetics of nutritional degradation and of denaturation of a selected species of bacterial spore.

The *second* objective is a three-part evaluation of the resulting product and process, determining:

(3) Probability of sterility of the entire product;
(4) Total retention of nutrients in their natural form; and
(5) Equipment investment and operating costs.

This is intended to facilitate comparison of continuous processing with "in-can" processing in several ways, leading to rational choices between the two alternatives in specific cases.

DESIGN AND MODELING OF THE PROCESS

Physical model

The process model is shown in Figure 3. In practice, heat exchange equipment of both shell-and-tube and plate type is used. The former is chosen here for illustrative purposes. A model based upon a single tube is useful to elucidate design criteria for both plate and tube exchangers, since the transport equations involved are of a similar nature for both systems. The fluid food, at a uniform temperature T_0, is pumped in fully developed laminar flow into a heating section where the tube wall temperature, $T_w (> T_0)$, is maintained constant by condensing steam. Immediately following the heating section, the food enters the cooling section where again the wall temperature is maintained approximately constant by means of cooling water at 20°C (arbitrarily selected temperature).

Analytical development

Fluid food rheology. The majority of viscometric data for isothermal laminar flow of food products have been correlated using the Ostwald-deWaele powerlaw model (Holdsworth, 1969):

$$\tau = K(\dot{\gamma})^n = K\left(-\frac{du_z}{dr}\right)^n \tag{1}$$

The velocity profile derived from this model for isothermal flow is of the form:

$$u_z(\rho) = \bar{u}\left(\frac{3n+1}{n+1}\right)\left(1 - \rho^{(n+1)/n}\right) \tag{2}$$

It is apparent, however, that isothermal conditions do not occur at any

point in the process considered here. The most important effect of the nonisothermal nature of the process is that radial temperature gradients will cause radial viscosity gradients leading to velocity profiles considerably distorted from Eq (2).

Only a limited amount of temperature-dependent rheological data has been taken for fluid foods. However, data for aqueous suspensions of consistency and characteristics similar to soups, purées and pastes, have been correlated extensively by Christiansen and Craig (1962) and Christiansen et al. (1966) using a temperature-dependent powerlaw model of the form:

$$\tau = K(\dot{\gamma}\exp[\Delta H^+/RT])^n \tag{3}$$

which is equivalent to Eq (1) in the isoviscous case. This has also been used to correlate the limited nonisoviscous data available for food products (Charm and Merrill, 1959). The velocity profile derived from this model is of the form:

$$u_z(\rho) = (\bar{u}/2)(I_1/I_2) \tag{4}$$

where

$$I_1(\rho) = \int_\rho^1 \rho^{1/n}\exp[-\Delta H^+/RT]\,d\rho \tag{5}$$

$$I_2 = \int_0^1 \rho\, I_1\, d\rho \tag{6}$$

Eq (4) reduces to Eq (2) when $\exp[\Delta H^+/RT]$ is a constant which can be absorbed in K and thus also in $\bar{u}$.

Heat transfer. An energy balance made on the fluid under the assumptions that (1) properties κ, $\hat{C}_p$, d are temperature-independent and (2) heat conduction is important only in the radial direction, leads to the dimensionless temperature relationship (Graetz, 1883; Skelland, 1967)

$$\frac{\partial\theta}{\partial\zeta} = \frac{2I_2}{I_1(\rho)}\left(\frac{1}{\rho}\frac{\partial\theta}{\partial\rho} + \frac{\partial^2\theta}{\partial\rho^2}\right) \tag{7}$$

where $\zeta \equiv z\alpha/R^2\bar{u}$, $\rho \equiv r/R$, and $\theta \equiv (T-T_0)/(T_w-T_0)$, with boundary conditions $\theta(0,\rho) = 0$, $\theta(\zeta,1) = 1$, and $(\partial\theta/\partial\rho)|_{\rho=0} = 0$. This is coupled to the temperature dependence of viscosity through I_1 and I_2, the strength of this coupling being characterized by the parameter

$$\psi(H) = \frac{\Delta H^+}{R}\left(\frac{1}{T_0} - \frac{1}{T_w}\right) \tag{8}$$

The absence of coupling ($\psi=0$) is designated as the "isoviscous case," for which fluid flow is taken to be unaffected by temperature variation. Data of Harper (1965) and of Saravacos and Moyer (1967) show that $\psi=1.71$ for pear purée (16% solids) and $\psi=3.91$ for tomato purée (30% solids).

Isoviscous solutions for a number of rheologies are well known and generally accessible–e.g., Lyche and Bird (1956), pseudoplastic, tube; Brown (1960), Newtonian, tube and flat plate; Tien (1962), pseudoplastic, flat plate; Wissler and Schechter (1959), Bingham, tube. Non-
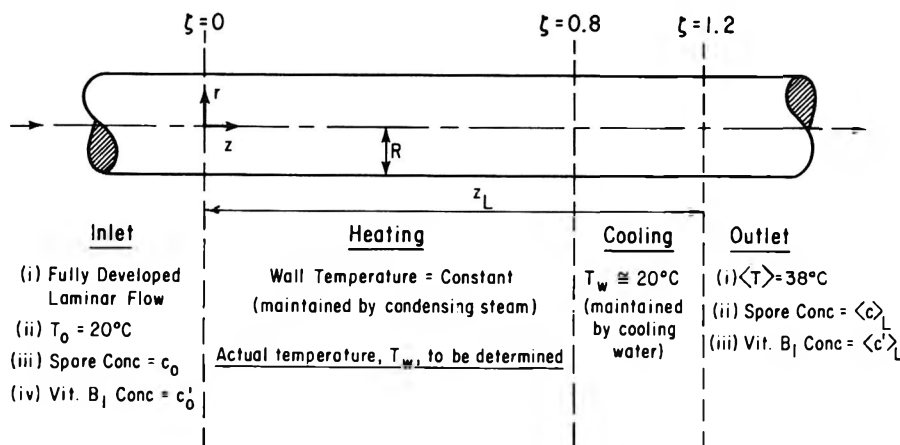

Fig. 3–Model for a continuous tube sterilizer, without holding section.

isoviscous solutions are also available but are usually published in a form less detailed than will be needed here—e.g., Christiansen and Craig (1962), pseudoplastic, tube (limited temperature profiles); Christiansen et al. (1966), pseudoplastic, tube; Christiansen and Jensen (1969), pseudoplastic, tube; Kwant et al. (1973), pseudoplastic, tube.

Kinetics of denaturation. *Bacterial spores:* In food processing, the "minimum thermal process" for low-acid foods is based upon denaturation of the spores of *Cl. botulinum* (Ball, 1928; Charm, 1963). The most convenient description of the denaturation kinetics of *Cl. botulinum* spores is in terms of the Arrhenius rate expression (Lawrence and Block, 1968). Use of basic data for denaturation of *Cl. botulinum* spores (Ball and Olson, 1957) and the simple algebra outlined by Charm (1958), together with the knowledge that these kinetics are first order, leads to

$$k' = A' \exp[-E_a/RT] = 2 \times 10^{40} \exp[-3.73 \times 10^4/T] \ \text{sec}^{-1} \quad (9)$$

for $373°K \leqslant T \leqslant 423°K$.

Nutrients: The prototype chosen for the evaluation of nutrient retention is vitamin B_1 (thiamin) which also exhibits first order kinetics of denaturation (Feliciotti and Esselen, 1957). This is a heat-labile, water soluble vitamin, which occurs naturally in many foods and has an activation energy for denaturation which is representative of most common nutrients. Since it is the difference in magnitude in the activation energies for denaturation of spores and nutrients that makes continuous sterilization attractive, this single nutrient is a useful prototype in studying the effect of sterilization on a wide range of nutrients. Again an Arrhenius expression is used to describe the rate constant for denaturation (Feliciotti and Esselen, 1957).

$$k'' = A'' \exp[-1.18 \times 10^4/T] \ \text{sec}^{-1} \quad (10)$$

The factor A'' varies for different foods (Feliciotti and Esselen, 1957). Values used here represent the greatest likely range, from $A_1'' = 2.19 \times 10^9$ (a low-pH food) to $A_2'' = 4.39 \times 10^{10}$ (a neutral food), which spans values for all the puréed meats and vegetables studied.

RESULTS & DISCUSSION

Qualitative interpretation of the model

Typical velocity profiles occurring during the process, as predicted by Eq 4–6, are shown in Figure 4 for conditions of equal flow rate. It can be seen that the nonisothermal nature of the process has a considerable influence on the laminar velocity profile, which in turn controls the residence time distribution of the food in the sterilizer.

The maintenance of a constant wall temperature is feasible in the steam heating section where heat transfer is tube-side controlled. There is, of course, a small error made in assuming an isothermal wall in the water-cooled section. Because of the illustrative nature of these calculations no corrections will be
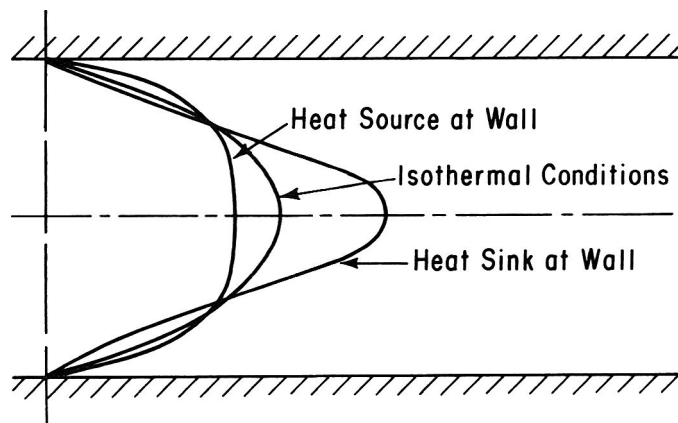

Fig. 4—Velocity profiles for laminar flow of a Newtonian fluid. The heated-wall and cooled-wall cases are contrasted with the isothermal case for equal flow rates.

made, and in any event it is useful to note that the error here will make these sterilization predictions conservative.

The sterilization analysis will ensure that the minimum required probability of sterility is achieved at the point of minimum thermal treatment. Then all other parts of the food receive a treatment which is more than adequate. It will later be shown—at least in the case of isoviscous fluids—that the centerline region marginally receives the least treatment. Consider the experience of material moving along the tube centerline. This material experiences the greatest thermal lag in the heating section and also in the cooling section. However, in the cooling section this thermal lag is coincident with the highest temperatures being retained at the centerline. Thus the centerline fluid receives the least treatment in the heating section, but not in the cooling section, and detailed calculations are needed to verify that it indeed receives minimum treatment. It is reasonable to believe this result holds for the nonisoviscous case also.

Evaluation of the minimum thermal process (centerline conditions)

Initially, evaluation has been made using isoviscous $u_z(r,z)$ solutions to the non-Newtonian Graetz problem. Subsequently, by comparing the results of these calculations with those in which the effect of thermal viscosity variation is taken into account, appropriate correction factors have been developed, thus enabling extensive practical use of the more comprehensive isoviscous results of the Graetz problem.

The assumptions made in the following analysis are: Bacterial spores remain on streamlines throughout the process; fluid at the centerline receives the least thermal treatment; sterilization achieved at the centerline must be equivalent to a conventional batch sterilization carried out at 121.1°C of duration 2.45 min for which $\log(c/c_0) = -11.5$ for *Cl. botulinum;* and the heating section is terminated when $T_{\mathfrak{C}} - T_0 = 0.95(T_w - T_0)$ and the cooling section then commences immediately.

This last assumption is a compromise of the features of the process and is essential to the analysis that follows. A 95% approach to temperature uniformity was taken to be sufficiently great to be able to assume (for analytical purposes) a uniform entry temperature to the cooling section without needing an excessively long heating section. If $(T_{\mathfrak{C}} - T_0)/(T_w - T_0)$ were appreciably less than 0.95 – implying a higher T_w – then even shorter sterilization times and consequent better nutrient retention would be possible in principle. However, the gain is superficial, since results discussed later in this work show that these T_w would lead to browning reactions (dependent upon food type and other factors) at the wall, causing quality loss and possible tube blockage (Desrosier, 1970).

Applying these assumptions to the nonisothermal kinetics of *Cl. botulinum* denaturation as the spores move along the centerline, we can write:

$$\frac{dc}{dt} = -k'(T)c \quad (11)$$

Integrating,

$$\ln\left[\frac{c_L}{c_0}\right] = -\int_0^{t_L} k'(t)dt \quad (12)$$

Eq (12) cannot be evaluated easily since $k'(t)$ dependence on process time at the centerline, t, is not known until $T(t)$ of the material element is evaluated. Evaluation here is effected by converting from t to the reduced length variable $\zeta = z\alpha/R^2\bar{u}$. At the centerline the *total* time of processing in isoviscous fluid flow is

$$t_L = \frac{L}{u_{\mathfrak{C}}} = \frac{z_L}{u_M} \quad (13)$$

where $u_{\mathbb{C}}$ is taken to be independent of z; in terms of ζ,

$$t_L = \zeta_L \frac{R^2}{\alpha} \left(\frac{\bar{u}}{u_M} \right) \qquad (14)$$

For the isoviscous powerlaw velocity profile, Eq 2 gives

$$t_T = \zeta_L \frac{R^2}{\alpha} \left(\frac{n+1}{3n+1} \right) \qquad (15)$$

Then Eq (12) becomes

$$\int_0^{\zeta_L} k' d\zeta = -\frac{\alpha}{R^2} \left(\frac{3n+1}{n+1} \right) \ln \left[\frac{c_L}{c_o} \right] \qquad (16)$$

A centerline process equivalent to the batch criterion $F_{121.1°C} = 2.45$ min must reduce the final spore concentration, c_L, to $c_o \times 10^{-11.5}$ (Ball and Olson, 1954) whence $\ln(c_L/c_o) = -26.5$ and Eq (16) becomes

$$\int_0^{\zeta_L} k' d\zeta = \frac{26.5}{R^2} \alpha \frac{3n+1}{n+1} \qquad (17)$$

Eq (17) is in a convenient form for numerical integration and is employed as follows:

(1) Results to the Graetz problem are expressed on the centerline ($\rho = 0$) as $\theta = \theta(\zeta,n)$ from which can be found, for each particular heating section wall temperature, the centerline temperature $T_{\mathbb{C}} = T_{\mathbb{C}}(\zeta,n)$. Combining this with Eq (9) gives $k'_{\mathbb{C}} = k'_{\mathbb{C}}(\zeta,n)$. Thus $k'_{\mathbb{C}}$ can be evaluated as a function of ζ for any particular powerlaw index, n, and so the correct heating section wall temperature (buried in θ) is determined by forcing Eq (17) to balance.

(2) The total dimensionless tube length, ζ_L, is

$$\zeta_L = \zeta(\text{heating}) + \zeta(\text{cooling}) = \zeta_H + \zeta_C \qquad (18)$$

From the results to the Graetz problem, under the constraints of (1) 95% temperature gain at the centerline in the heating section; and (2) a cup-mixing (average) temperature $<T> = 38°C$ at the exit of the cooling section (chosen as the temperature normally required in food canning operations), it is found that $\zeta_H \cong 0.8$ and $\zeta_C \cong 0.4$. Hence $\zeta_L = 1.2$. for all powerlaw fluids, even in nonisoviscous flow. This is valid within $\pm 2\%$ for $0.3 \leqslant n \leqslant 1.0$ and $-4 < \psi < 4$, and represents a special case of the more general observation that *centerline* temperatures realized at any particular distance down the tube are only a very weak function of the velocity profile that exists. This observation is important to this work, and is used extensively later. It results from the fortuitous trade-off of the radial heat transfer driving force with the centerline residence time of the fluid food. For example, the blunted velocity profile of Figure 4 (n < 1, or hot wall) shows that centerline residence time in the heating section is increased, but simultaneously the accelerated fluid near the hot wall has reduced residence time and thus fails to get hot enough to transfer more heat to the centerline.

Figure 5 displays $k'_{\mathbb{C}}(\zeta)$ for spore denaturation for a fluid food with n = 0.33, for various heating section wall temperatures. It is apparent that for only one wall temperature will the area under the curve balance Eq (17), in which case the requisite design (i.e., identification of T_W) is achieved. Before discussing the integration of the curves in Figure 5, it is convenient to consider modifications arising from radial viscosity variation due to radial temperature gradients (the nonisoviscous case).

Viscosity-temperature effect

As stated in Eq (3), the viscosity has an exponential sensitivity to temperature. In the sterilization process temperatures vary considerably and large variations in viscosity occur, so initially one might expect this to invalidate any analysis which

does not account for these changes and necessitate a far more complex analysis. However, due to the unique conditions of the sterilization process, remarkable simplifications can be made, and the effect of viscosity variation can be approximately accommodated in the isoviscous analysis through a single correction factor.

This is illustrated for a hypothetical powerlaw fluid with $\Delta H^+/R = 2017°K$ and n = 0.33. Velocity profiles were obtained for this nonisoviscous case by numerical integration of Eq (5) and (6), using values of $\theta(\rho,\zeta; n,\psi)$ taken from Christiansen et al. (1962, 1966). Results, in terms of the maximum (centerline) velocity variation with axial position, are shown in Figure 6.
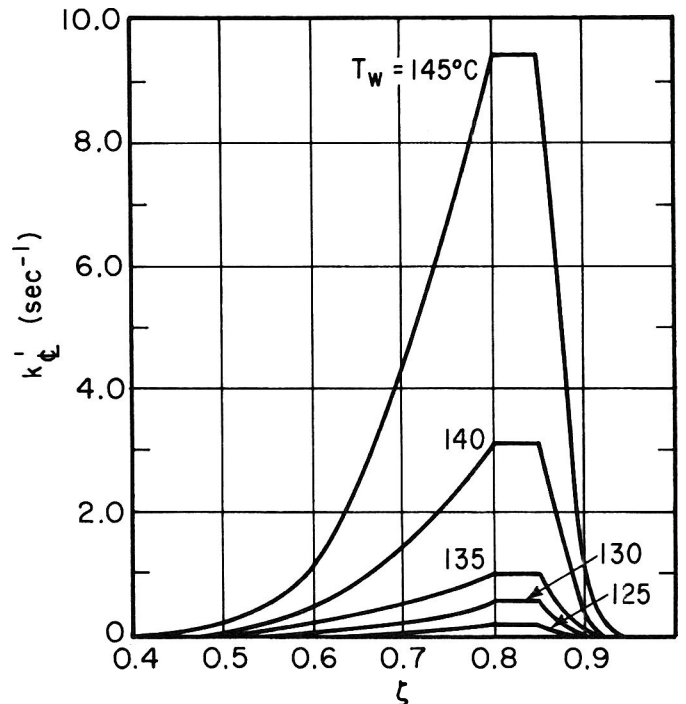

Fig. 5–Centerline sterilization rate constant for various heating-section wall temperatures, using n = 0.33.
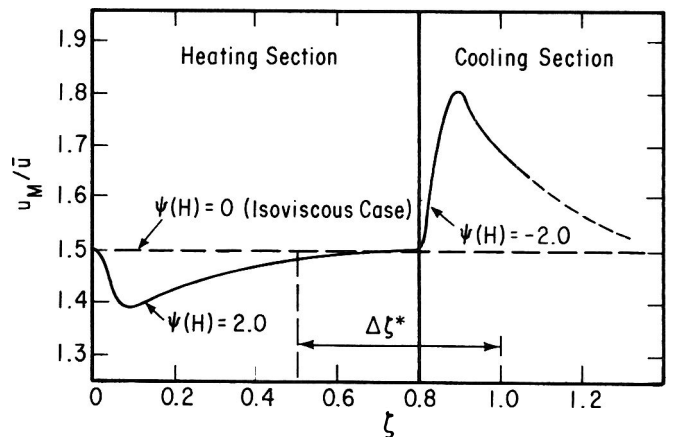

Fig. 6–Centerline velocity influenced by temperature-dependent viscosity (nonisoviscous case) for a non-Newtonian fluid. The range designated $\Delta\zeta^*$ is the region over which sterilization is effective at the centerline. (n = 0.33 and $\Delta H^+/R = 2017°K$)

Heating section design is facilitated by the fact that k'_ℓ does not assume a significant value until the fluid food has travelled a dimensionless distance $\zeta \cong 0.5$ (see Fig. 5). For values of $\zeta \lesssim 0.5$ the centerline temperature has not been raised sufficiently high to produce significant sterilization. In the remainder of the heating section $(0.5 \leqslant \zeta \leqslant 0.8)$ where centerline sterilization is actually occurring, Figure 6 shows that the centerline velocity is no longer affected by radial viscosity gradients, and is predicted sufficiently accurately by the isothermal model, Eq (2). Thus the isoviscous heat transfer model can be used to design the least thermal treatment for the heating section.

Application of the isoviscous model to the cooling section, however, does lead to error. A simple correction factor can be applied in order to make use of the isoviscous solutions, by means of the following argument. First, it is assumed that distortion of the velocity profile, which from Figure 6 is most significant in the initial part of the cooling section where the sterilization reaction is still effective (Fig. 5), does not greatly affect the centerline *temperatures* realized at any particular distance down the tube (as demonstrated previously). Then the correction factor is generated as follows, beginning with $t = \zeta(R^2\bar{u}/\alpha u_M)$. Previously, in developing Eq (15)–(17) for the isoviscous velocity profile, u_M was treated as a constant such that $\bar{u}/u_M = (n+1)/(3n+1)$. In the general nonisoviscous case, however, $u_M = u_M(\zeta)$ and thus Eq (12) gives,

$$\bar{u}\int_0^{\zeta_L} \frac{k'}{u_M(\zeta)} d\zeta = \frac{26.5}{R^2}\alpha \qquad (19)$$

analogous to Eq (17). The left hand side of Eq (19) is conveniently divided as follows:

$$\bar{u}\left\{\int_0^{0.5} \frac{k'}{u_M(\zeta)} d\zeta + \int_{0.5}^{0.8} \frac{k'}{u_M(\zeta)} d\zeta + \int_{0.8}^{1.2} \frac{k'}{u_M(\zeta)} d\zeta\right\}$$
$$= \frac{26.5\alpha}{R^2} \qquad (20)$$

Next, use is made of the approximations $k' \cong 0$ for $0 \leqslant \zeta \leqslant 0.5$ and $u_M(\zeta) \cong$ constant for $0.5 \leqslant \zeta \leqslant 0.8$. Then, with little error, Eq (20) becomes

$$\int_{0.5}^{0.8} k'd\zeta + \int_{0.8}^{1.2} k'\frac{\bar{u}}{u_M(\zeta)}\left(\frac{3n+1}{n+1}\right)d\zeta = 26.5\frac{\alpha}{R^2}\left(\frac{3n+1}{n+1}\right) \quad (21)$$

The factor $\frac{\bar{u}}{u_M(\zeta)}\left(\frac{3n+1}{n+1}\right)$ in the integral for the cooling section leads to the required correction factor. Ideally the appropriate average value of this factor should be evaluated exactly over the range $0.8 \leqslant \zeta \leqslant 1.0$, where $k' \geqslant 10^{-2}s^{-1}$; however, this would be laborious, and unnecessary. Since the sterilization predictions must by convention be conservative, and since from Figure 6 the velocity profile distortion is greatest in the initial part of the cooling section where the centerline temperature is high (and hence k' is almost at its greatest value), then the obvious safe choice for the correction factor is its smallest value. This choice is then both conservative and approximately equal to the exact value, due to the weighting caused by the variation of k'. Using Figure 6(n = 0.33) as an illustration we have $\frac{\bar{u}}{u_M(\zeta)}\left(\frac{3}{2}\right) \geqslant \frac{1}{(u_M/\bar{u})_{max}}\left(\frac{3}{2}\right) = 0.83$ for $0.8 \leqslant \zeta \leqslant 1.2$. However, since $u_M(\zeta)/\bar{u}$ could not be determined with great accuracy for $0.8 < \zeta < 0.9$, a more conservative value of 0.7 was chosen in order to ensure "safe" sterilization predictions:

$$\int_{0.5}^{0.8} k'd\zeta + 0.7\int_{0.8}^{1.2} k'd\zeta \cong 26.5\frac{\alpha}{R^2}\left(\frac{3n+1}{n+1}\right) \qquad (22)$$

Figure 7 illustrates the use of Eq (22) for two fluids, one

Newtonian (n = 1) and one pseudoplastic (n = 0.33). First, the integrals on the left side of Eq (22) are evaluated from curves such as in Figure 5 and plotted against tube wall temperature as the solid curves in Figure 7. Next, physical properties are chosen as typical of food products ($\kappa = 0.692$ W/m°K, d = 1.2 × 10^3 kg/m³, $\bar{C}_p = 3.77$ kJ/kg°K) and, with these assumed independent of temperature, the right side of Eq (22) is evaluated. These values for the two fluids are displayed as dashed lines in Figure 7, corresponding to different possible choices of tube radius R. The intersection of the dashed and solid lines (for the same n) then determines, from the abscissa, the T_w required to achieve safe sterilization at the centerline. This is the first design objective.

Figure 7 highlights certain key features of this analysis. It demonstrates that R is the only independent design parameter for sterilizing a given fluid (fixed n, κ, d, $\bar{C}_p$) within the rather conventional temperature constraints assumed here. It also shows the importance of accurate characterization of the rheology (n) of the food.

This general scheme must now be evaluated in terms of product quality, and operating and investment costs, to complete the design.

Product quality

Product quality is interpreted here in terms of (1) total probability of sterility of the final product; and (2) fractional retention of nutrients in the final product. This requires evaluation of the thermal treatment received by all parts of the food while flowing through the sterilizer. The concentration of spores (or nutrients) at the end of the sterilizer can be expressed as

$$<c>_L = \left.\frac{\int_0^R c(r)\cdot u_z(r)\cdot 2\pi r dr}{\int_0^R u_z(r)2\pi r dr}\right|_{z_L} \qquad (23)$$

or, using Eq (12),

$$<c>_L = \frac{2\pi c_0}{Q}\int_0^R \exp\left[-\int_0^{t_L} k'(T)dt\right]u_z(r)\cdot r dr \qquad (24)$$

Eq (24), as with Eq (12), is not readily evaluated since $k'(t,r)$ is not known until $T(t,r)$ is established. Analytical integration of Eq (24) is not thought feasible, and approximate numerical methods must be sought.
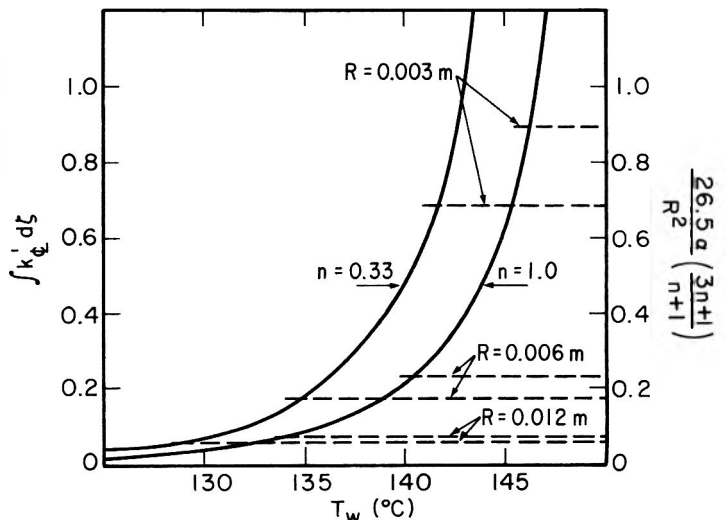

Fig. 7–Evaluation of heating section wall temperature.

Leniger and Beverloo (1969) have bypassed exact solution by making the isothermal simplification, $T \neq f(t,r)$. Far less restrictive is the solution presented here, where the fluid is assumed isoviscous. This assumption enables usage of the isoviscous solutions to the Graetz problem presently available. The few nonisoviscous results in the literature do not, unfortunately, supply the temperature profile information required here. The error made by using isoviscous velocity distributions cannot therefore be assessed quantitatively. This particular use of the isoviscous assumption is justified, however, since evaluation of Eq (24) is carried out merely to determine the order of magnitude of the effects of various parameters upon the product quality in order to optimize the design. It will be shown that a highly accurate evaluation of Eq (24) is not necessary.

Generalizing Eq (14),

$$t_L(r) = \zeta_L \frac{R^2}{\alpha} \frac{\bar{u}}{u_z(r)} \qquad (14a)$$

Then introducing Eq (2) and (14a) into Eq (24), and noting that $Q = \pi R^2 \bar{u}$, we have upon rearranging and introducing reduced variables

$$\frac{<c>_L}{c_o} \cong 2 \left(\frac{3n+1}{n+1}\right) \int_0^1 \rho(1-\rho^{(n+1)/n}) \exp \qquad (25)$$

$$\left[-\left(\frac{n+1}{3n+1}\right) \int_0^{\zeta_L} \frac{k'(\rho,\zeta)R^2}{\alpha(1-\rho^{(n+1)/n})} d\zeta \right] d\rho$$

Next consider the tube to be composed of I incremental concentric annuli, each of a radius increment $\Delta\rho_i$ so small that k' does not vary appreciably across it, so that $k'(\rho,\zeta) \cong k'(\zeta)$ for

$\rho_i \leqslant \rho \leqslant (\rho_i + \Delta\rho_i)$, in which case Eq (25) can be approximated by

$$\frac{<c>_L}{c_o} = 2 \left(\frac{3n+1}{n+1}\right) \sum_{i=0}^{i=I} \bar{\rho}_i \left(1 - \bar{\rho}_i'^{(1+n)/n}\right) \exp$$

$$\left[-\left(\frac{n+1}{3n+1}\right) \int_0^{\zeta_L} \frac{k_i'(\zeta)R^2 d\zeta}{\alpha(1-\bar{\rho}_i'^{(1-n)/n})} \right] \Delta\rho_i \qquad (26)$$

where

$$\bar{\rho}_i = (\rho_i + \rho_{i+1})/2 \qquad (27a)$$

$$\bar{\rho}_i' = \left(\frac{2n}{3n+1}\left[\frac{\rho_{i+1}^{(1+3n)/n} - \rho_i^{(1+3n)/n}}{\rho_{i+1}^2 - \rho_i^2}\right]\right)^{\frac{n}{n+1}} \qquad (27b)$$

Eq (27a) assures a correct approximation to cross-sectional area of differential annulus, and Eq (27b) assures a correct approximation to the average velocity through it. Axial temperature profiles, and subsequently reaction rate constants, are then evaluated for each incremental annulus and plotted against the reduced distance ζ through the sterilizer (Fig. 8). The appropriate tube wall temperature for Figure 8 was selected by the method outlined earlier and corresponds to R = 3×10^{-3} m. The area under each of the curves in Figure 8 is then computed and the I terms summed under the conditions of Eq (26)–(27b). Identical procedures are used to evaluate nutrient reduction and total probability of sterility. The results of several case evaluations are shown in Tables 1 and 2.

In Table 1 the effect of doubling the radius of the sterilizer is shown. For all radii considered the total probability of sterility of the product is of the order of 10^{-13}, which compares favorably with conventional sterilization, making the continuous process satisfactory from this standpoint. Fraction-
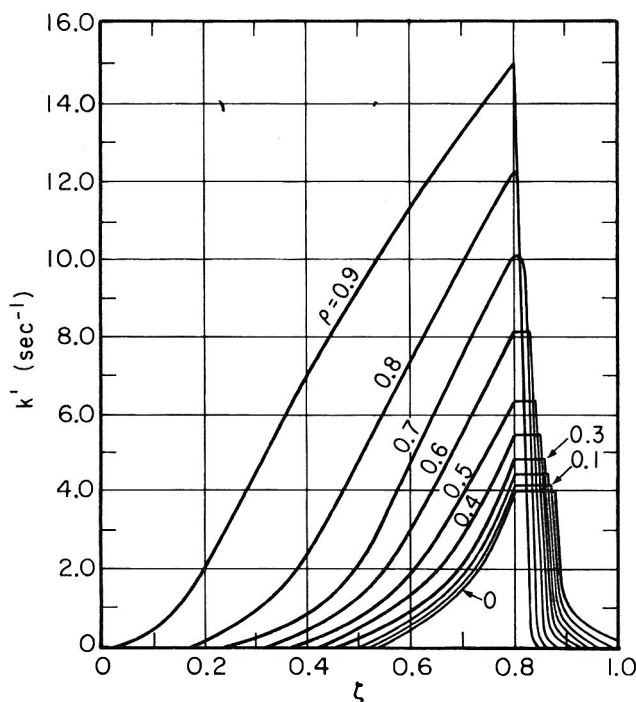
Fig. 8–Sterilization rate constants during fluid passage through the tube, for various radial positions. Here, n = 0.33 and T_W = 141°C.

Table 1—Effect of tube radius

Tube radius (m)	Heating wall temperature (°C)	Probability of sterility ($<c>_L/c_o$)	Nutrient retention ($<c'>_L/c_o$) A_1''	Nutrient retention ($<c'>_L/c_o$) A_2''
3.0 X 10⁻³	141.0	7 X 10⁻¹³	0.99	0.75
6.0 X 10⁻³	134.5	1 X 10⁻¹³	0.95	0.47
12.0 X 10⁻³	129.0	5 X 10⁻¹⁴	0.85	0.12

Table 2—Effect of accomodating pseudoplastic index, with R = 3.0 X 10⁻³ m

n	Heating wall temperature (°C)	Probability of sterility ($<c>_L/c_o$)	Nutrient retention ($<c'>_L/c_o$) A_1''	Nutrient retention ($<c'>_L/c_o$) A_2''
0.33	141.0	7 X 10⁻¹³	0.99	0.75
1.0[a]	146.0	2 X 10⁻³⁶	0.98	0.68

[a] This is the case of incorrectly assuming Newtonian behavior for a fluid which actually exhibits powerlaw rheology.

al nutrient retention, however, can be adversely affected by increasing the sterilizer tube radius, depending upon the actual rate of nutrient degradation that occurs. For case A_2'' this is particularly severe, and no minimum limit for $<c'>_L$ is predicted within reasonable operating conditions.

It is thus concluded that the radius of the tubes in the sterilizer is a most important variable in establishing the viability of continuous processing. Smaller diameter tubes lead to better nutrient retention, without compromising the total probability of sterility of the product.

In Table 2, the effect of neglecting the pseudoplastic nature of the food is demonstrated for a fluid with n = 0.33. It is seen that the incorrect assumption that the food is Newtonian results in selection of too high a heating section wall temperature. In this case nutrient retention is also compromised, with the correct rheological characterization always being somewhat better. The real danger, however, is that neglect of the pseudoplasticity of the fluid increases the total probability of sterility by some twenty orders of magnitude, providing a safety factor which is far too conservative. The designer would be tempted, on the basis of these (incorrect) predictions, to select a lower wall temperature, but would clearly be driven in the direction of choosing one which was too low for safety (e.g., 130°C).

It is suggested that for optimal design the pseudoplastic index of the food be characterized accurately, and the design completed on this basis. Subsequently, safety factors may be incorporated as required.

Investment and operating costs

A direct consequence of the result $\zeta_L \equiv z_L/R^2 \bar{u} = 1.2$ is that the total length of tubes required to complete sterilization increases as R^2. Thus for small tube radii ($R = 3 \times 10^{-3}$ m) very compact equipment can be designed, but for large tube radii ($R = 12 \times 10^{-3}$ m) huge equipment is required. This must be expected to have a significant effect upon process costs.

The purpose of the economic analysis here is not to attach specific costs to pieces of equipment, nor to compare directly the cost of continuous processing with "in-can" methods. The purpose is to determine whether the use of smaller tubes in the sterilizer, thereby improving product quality, will increase or decrease the cost of the process.

It is common practice in engineering design of heat exchange processes to characterize operating costs in terms of tube-side pressure drop, and investment costs in terms of metal heat-transfer surface area requirements, these being the dominant cost factors in each case. Consider a bundle of N tubes delivering a *"constant continuous production rate Q"* specified by the food processor,

$$Q = N\pi R^2 \bar{u} \qquad (28)$$

The total heat transfer surface area required, S_H, is

$$S_H = 2\pi N R z_L \qquad (29)$$

With elimination of N, Eq (28) and (29) lead to

$$S_H = \frac{b_1 z_L}{R\bar{u}} \qquad (30)$$

(where b_1 and subsequent b_i are constants).

The pressure gradient can be approximated by

$$\frac{dP}{dz} \cong 2K_w \left(\frac{3n+1}{n}\right)^n \frac{\bar{u}^n}{R^{n+1}} \qquad (31)$$

where K_w is the consistency index evaluated at τ_w. [Kwant et al. (1973) show that such a relationship is accurate to

within 15% (high for heating, low for cooling) for $\zeta > 0.1$. Here, accuracy of pressure gradient prediction is less important than showing the functionality of pressure gradient with $\bar{u}$ and R as displayed in Eq (31).] Integrating Eq (31) for the isoviscous case,

$$\Delta P \cong 2K_w \left(\frac{3n+1}{n}\right)^n \frac{\bar{u}^n}{R^{n+1}} z_L \cong b_2 \frac{\bar{u}^n}{R^{n+1}} z_L \qquad (32)$$

Now the total length of the exchanger tubes, z_L, can be eliminated from Eq (30) and (32) by employing

$$z_L = \zeta_L \frac{\bar{u} R^2}{\alpha} = b_3 \bar{u} R^2 \qquad (33)$$

since $\zeta_L = 1.2$. From this, Eq (30) and (32) become

$$S_H = b_4 R \qquad (34)$$

$$\Delta P = b_5 \bar{u}^{n+1} R^{1-n} \qquad (35)$$

Eq (34) and (35) show that decreasing tube radius to improve product quality also improves the economics of the process, in that investment costs ($\sim S_H$) and operating costs ($\sim \Delta P$) decrease. It is also apparent that operating with a lower $\bar{u}$ permits a smaller pressure drop in the system, further reducing operating costs without detriment ot product quality or investment cost. These advantages related to reducing the tube radius and average velocity are accomplished by increasing N in Eq (28) and (29). It would appear that the limiting factor in the reduction in tube size and processing velocity is tube blockage by food clumps or later fouling. These limits need to be determined experimentally.

CONCLUSIONS

A SIMPLE generalized method has been developed for the design of continuous sterilizers operating in laminar flow, the only flow regime in which most fluid food products can economically be processed. It is shown that product quality considerations ard economic factors all require the smallest diameter tubes and most compact design possible. Virtually all design parameters (i.e., wall temperature, tube length) are linked together and directly related to the size of tube (or plate spacing) chosen. The limit to channel size reduction in the sterilizer will be established by the practical limitation of tube blockage.

NOTATION

A', A''	:	Preexponential factor in Arrhenius expression (s^{-1})
b_1, b_2, b_3, b_4, b_5	:	Constants in Eq (30) – (35)
$\hat{C}_p$	:	Specific heat of fluid food (kJ/kg°K)
c(c')	:	Concentration of spores (nutrients) (m^{-3})
d	:	Density of fluid (kg/m^3)
E_a	:	Activation energy in Arrhenius expression (kJ/kg mol)
$F_{121.1°C}$	:	Time in min. required at 121.1°C to denature *Cl. Botulinum* spores to a level $c/c_0 = 10^{-11.5}$
ΔH^+	:	Activation energy for flow (kJ/kg mol)
I_1	:	$= \int_\rho^1 \rho^{1/n} \exp[-\Delta H^+/RT] d\rho$
I_2	:	$= \int_0^1 I_1 \rho d\rho$
K	:	Consistency of fluid food (kN (s) $^n m^{-2}$)
k', (k'')	:	Reaction rate constants for denaturation of spores (nutrients) (s^{-1})
n	:	Pseudoplastic index
N	:	Total number of tubes in sterilizer

P	:	Fluid food pressure (kN/m^2)
Q	:	Continuous production rate (m^3/s)
r	:	radial coordinate (m)
R	:	Tube radius (m)
R	:	Gas constant (kJ/kg (mole) °K)
S_H	:	Total heat transfer surface area in sterilizer (m^2)
T	:	Temperature (°C, °K)
<T>	:	Cup-mixing temperature = $^R\!\int_0 T\,u\,rdr\,/\,^R\!\int_0 u\,rdr$
t	:	Time (s)
u	:	Fluid velocity (m/s)
$\bar{u}$	:	Average fluid velocity (m/s)
u_M	:	Maximum (centerline) velocity (m/s)
z	:	Axial coordinate (m)

Greek symbols

α	:	Thermal diffusivity = $\kappa/\hat{C}_p d$
$\dot{\gamma}$	:	Strain rate (s^{-1})
ζ	:	Reduced axial dimension = $z(\alpha/\bar{u}R^2)$
θ	:	Reduced temperature = $(T-T_0)/(T_w-T_0)$
κ	:	Thermal conductivity of fluid (kJ/m.s. °K)
ρ	:	Reduced radial dimension = r/R
τ	:	Shear stress
$\psi(H)$	:	$(\Delta H^+/R)(1/T_0-1/T_w)$

Subscripts

o	:	Inlet values
L	:	Outlet (or total) values
w	:	Wall values
i	:	Streamtube i.

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A Research Note
CAFFEINE DISTRIBUTION IN C. acuminata, T. cacao and C. arabica

INTRODUCTION

THE MAJOR nonalcoholic beverages of Nigeria are derived mainly from tea, coffee, cocoa and cola. Of these, only cola and cocoa are cultivated extensively. They owe their popularity to their refreshing and stimulating properties which result from the presence of caffeine and or theobromine. Caffeine acts on the central nervous system, and if taken in small amounts will increase mental activity and reduce fatigue; however, if taken in excesses it has markedly harmful effects such as nausea, nervousness, delirium and sleeplessness (Bowman et al., 1971; Wilson et al., 1969).

The leaves of tea and mate, kolanuts, coffee beans and cocoa beans have been reported to contain caffeine (Bienfait, 1956; Cobley, 1962; Kamp, 1960; Tango and Carvalho, 1964; Borker and Sloman, 1965). In this paper, a study on the caffeine contents of the bark, beans, leaves, roots and pods of *C. acuminata, T. cacao* and *C. arabica* is reported.

EXPERIMENTAL

Materials

The bark, beans, leaves and pods of *C. acuminata* and *T. cacao* were collected from the botanical garden of Mayflower School, Ikenne. The bark, beans, leaves and roots of *C. arabica* were obtained from the Agriculture Department of the Lagos State Ministry of Agriculture and Natural Resources at Ikorodu. The roots of *C. acuminata* and *T. cacao* were obtained as gifts from a herbalist, Alhaji Shewu. The bark, beans, leaves, pods and roots were separately grated into coarse particles, dried to a constant weight in an oven at 105°C and ground to a fine powder.

Extraction

250g powdered bark samples, 250g powdered leaf samples, 250g powdered root samples, 250g powdered pod samples, and 250g of powdered bean samples of *C. acuminata, C. arabica* and *T. cacao* were separately shaken vigorously in a 2-liter flask with 1 liter of pet-ether (100–120°C). Each mixture was mechanically stirred for 12 hr and filtered on 32 cm Whatman Grade I filter paper. The residue was extracted for caffeine by shaking it vigorously in a 2-liter flask with 1 liter of chloroform and 100 ml of 25% aqueous ammonia for 1 hr. The mixture was magnetically stirred for 24 hr and filtered on 32.0 cm Whatman Grade I filter paper. The residue was washed thoroughly with about 250 ml of chloroform and the washing added to the filtrate. The filtrate was gently evaporated to dryness, so as not to sublime the ingredients present.

Preparation of calibration curve for caffeine in chloroform

The absorption peak for pure caffeine (BDH Chemical Ltd., England) in spectroscopic chloroform (BDH Chemical Ltd., England) was determined by observing the ultraviolet spectrum recorded with a Unicam SP 800 ultraviolet spectrophotometer. It was found to be 276 mμ.

Very pure caffeine was obtained by placing several grams of the crystalline commercial caffeine in an evaporating dish covered by a watch glass and heating over a hot plate. The sublimed caffeine was collected from the watch glass and dried at 110°C for 1 hr. Chloroform solutions containing from 5–25 mg per liter of the pure sublimed caffeine were examined with a Unicam SP 500 Series 2 ultraviolet and visible spectrophotometer. Readings were made at 276 mμ. A plot of absorbancy vs. concentration gave a straight line showing excellent conformity with Beer's Law.

Purification and spectrophotometric estimation of caffeine in the various extracts

The procedure used for purification and spectrophotometric estimation of caffeine in the various extracts was essentially that reported by Somorin (1973).

Recovery studies

Fractions with optical density values greater than 0.025 were combined and gently evaporated to dryness, to avoid sublimation of the ingredients. The dry crystalline material obtained was washed with small amounts of petroleum ether (60–80°C) to remove colored impurities which contaminated it, then recrystallized from benzene/petroleum ether 100–120°C, (20:80 v/v) mixture, weighed and its melting point determined.

Thin-layer chromatography

Silica gel G Merck (Brinkman Co., New York) was used to prepare the thin-layer chromatographic plates according to the procedure outlined by Randerath (1966). The thin-layer chromatography plates were spotted with chloroform solution of the recovered material and eluted separately with petroleum ether (60–80°C); 96% ethanol; benzene; chloroform; and 96% ethanol/chloroform (1:9 v/v) mixture. The plates were dried and developed in a tank of iodine vapor.

Spectral studies

The ultraviolet spectrum of the recovered material was recorded in water using a Unicam SP 800 ultraviolet spectrophotometer. The infrared spectrum was examined as a mull, using Nujol as the mulling oil. The spectra was recorded with a Unicam SP 1200 infrared spectrophotometer. The nuclear magnetic resonance spectrum of caffeine dissolved in deutero-chloroform was recorded with a Varian T60 NMR spectrophotometer.

RESULTS & DISCUSSION

ALKALOIDS are probably formed in the green tissues such as the leaves and are carried to various parts of the plant for storage. Storage of alkaloids is not localized in specific organs. In some plants, during the first year of growth of the plant, alkaloids seem to be evenly distributed amongst the various organs, but with increasing age there appears to be a localization in a few organs. Thus, in the case of trees, the bark generally becomes richer in alkaloids than the leaves and shoots, owing to accumulation year after year (Swan, 1967). Caffeine has been iso-

Table 1—Caffeine contents of the bark, beans, leaves, roots and pods of C. acuminata, T. cacao and C. arabica

Sample	Wt of caffeine[a] per 100g of powdered sample	Wt of recovered recrystallized caffeine after chromatography per 100g of powdered sample
Beans of *C. arabica*	1.558 ± 0.001g	1.430 ± 0.005g
Beans of *T. cacao*	0.132 ± 0.001g	0.105 ± 0.005g
Leaves of *C. acuminata*	0.007 ± 0.001g	impure
Leaves of *C. arabica*	0.010 ± 0.001g	impure
Leaves of *T. cacao*	0.003 ± 0.001g	impure
Pods of *C. acuminata*	0.015 ± 0.001g	impure
Pods of *T. cacao*	0.010 ± 0.001g	impure
Bark of *C. acuminata*	None	None
Bark of *C. arabica*	None	None
Bark of *T. cacao*	None	None
Roots of *C. acuminata*	None	None
Roots of *T. cacao*	None	None
Roots of *C. arabica*	None	None

[a] Determined spectrophotometrically

lated from the leaves of tea and mate and has also been isolated from the coffee beans, cocoa beans and kolanuts.

The results presented in Table I showed that the caffeine content of 0.132% for cocoa beans is in good agreement with the values of 0.06–0.17% reported by Bienfait (1956). The caffeine content of 1.558% for coffee beans is higher than the values of 1.34% and 1.43% reported by Tango and Carvalho (1963) and Borker and Sloman (1965) respectively.

The recrystallized recovered caffeine from the extracts of coffee beans and cocoa beans was demonstrated to be homogenous as indicated by the presence of only a single spot in the thin-layer chromatogram after eluting the plate with various solvents. The infrared, ultraviolet, nuclear magnetic resonance spectral data and the melting point of the recovered material were found to be in excellent agreement with the spectral data and melting point reported for caffeine (Bhancca et al., 1962; Ikan, 1966; Leal et al., 1961), hence the recrystallized recovered material is pure caffeine.

The recovered caffeine from the leaves and pods was impure due to the presence of colored impurities which could not be removed even on recrystallization. The bark, despite the speculation that it might be the site of accumulation of alkaloids (Swan, 1967), and the roots do not contain caffeine.

CONCLUSION

THE MAIN SITE of caffeine storage in *C. acuminata*, *C. arabica* and *T. cacao* are the kolanuts, coffee beans and cocoa beans respectively.

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A Research Note
THE DETERMINATION OF HYPOGLYCIN A IN ACKEE

INTRODUCTION

HYPOGLYCIN A (L-α-amino-2-methyl-enecyclopropane-propionic acid), commonly referred to as hypoglycin, is a constituent of the fruit and seed of ackee (*Blighia sapida*), although recently it has been shown to occur also in other plants (Hassall and Reyle, 1955; Eloff and Fowden, 1970; Fowden and Pratt, 1973). The fleshy arillus of the ackee fruit is a common component of the diet in Jamaica. There are limited sales of canned ackee in North America. Consumption of unripe ackee fruit is believed to be responsible for a "vomiting sickness" in Jamaica, and toxicity studies on hypoglycin A provide evidence that this is the causative agent of the disease (Bressler et al., 1969; Feng, 1969). Persaud and Kaplan (1970) found that hypoglycin A was teratogenic when administered intraperitoneally (30 mg/kg) to rats, and their report included a suggestion that ackee might be a factor in the production of certain human birth defects observed in Jamaica. Ellington (1961) showed that hypoglycin A was present in low concentration (0.008%) even in the ripe arillus, the portion of the ackee fruit normally eaten, as well as at much higher concentration (0.111%) in the unripe arillus. It therefore appeared to be desirable to determine the concentration of hypoglycin A in commercial canned ackee, particularly in view of the possibility that less mature fruit might be packed. Available methods for the analysis of hypoglycin A in ackee are lengthy and involve paper chromatography (Abrahams and Kean, 1969; Ellington, 1961; Hassall and Reyle, 1955). We have therefore developed the following rapid method for determination of hypoglycin A using an amino acid analyzer.

EXPERMENTAL

ACKEE FRUIT (arillus) in brine was drained and blotted dry between layers of paper towels. A 5-g sample was blended with 15 ml distilled water for 1 min using a high-speed mixer emulsifier (micro model, Silverson Machines Ltd., London, England). The mixture was centrifuged for 5 min at about 2000 rpm, and 5 ml was withdrawn by pipette from the middle layer. Protein was precipitated by mixing with one half volume of 5% trichloroacetic acid and the mixture was then centrifuged at about 3000 rpm for 5 min. A portion of the cloudy solution was taken up in a syringe and filtered through a 0.20μ membrane filter (Gelman Metricel GA-8, 25 mm) to obtain at least 0.5 ml filtrate for analysis.

Brine was preferably centrifuged at about 2000 rpm for 5 min, then a 5-ml volume was withdrawn from the aqueous layer by pipette. This was deproteinized as described above for ackee extract.

Deproteinized ackee extracts (0.15 or 0.2 ml) or brine (0.05 ml) were diluted to 1.5 ml with 0.2N sodium citrate buffer solution pH 2.2 and injected into a Beckman model 116 Amino Acid Analyzer. Operating conditions were as follows: column, 56 cm × 0.9 cm Beckman type AA-15 resin; buffer, 0.75N sodium citrate pH 5.25; buffer flow rate 70 ml per hr;

ninhydrin flow rate 35 ml per hr; and temperature, 53.5°C. Known amounts of standard hypoglycin A (gift from H.L. Borison) in distilled water (136 μg/ml) were diluted with Beckman type 1 amino acid calibration mixture (containing 2.5 μmoles per ml of individual amino acids) and analyzed using the amino acid analyzer. The elution time of hypoglycin A was 37 min. Absorbance of standard (mean of 2 determinations) was compared with that of hypoglycin A in ackee extracts at 570 nm.

The concentration of hypoglycin A in the ackee fruit was calculated from the formula:

$$\text{hypoglycin A}\ \mu g/g(ppm) = \frac{M \times (V_1 + 0.75\ W) \times 1.5}{W \times V_2}$$

Where M = μg hypoglycin A found in the final aliquot (V_2 ml) of filtered trichloroacetic acid treated extract; V_1 = volume of water (ml) used to extract the ackee; and W = fresh weight (g) of ackee. Abrahams and Kean (1969) assumed the water content of unripe ackee arillus pulp to be 75%. We have used this figure in the calculation to correct for water contributed by the ackee to the volume of the extract. A comparable estimate of 77.5% water was found by drying canned ackee fruit for 3 hr at 128°C. The factor 1.5 allows for the 50% dilution of the extract with trichloroacetic acid solution.

The concentration (μg/ml) of hypoglycin A in brine was obtained by multiplying by 1.5 the concentration found in the deproteinized brine.

RESULTS & DISCUSSION

THE METHOD developed for analysis of amino acids in ackee extracts separates hypoglycin A from leucine to an extent sufficient to permit determination of the hypoglycin A. Apparently, previous attempts to effect this separation by ion exchange chromatography were unsuccessful (Abrahams and Kean, 1969). A typical chromatogram of an ackee extract is shown in Figure 1. Although leucine was not separated from norleucine in our system, the latter has not been reported in ackee, while L-leucine is a known constituent (West, 1968).

By plotting absorbance versus weight of hypoglycin A added to the mixture of standard amino acids, a standard curve could be obtained which was linear at least between 2 and 16 μg (on the column). The lowest amount (2 μg) was determined in the presence of an excess of leucine similar to that observed in sample
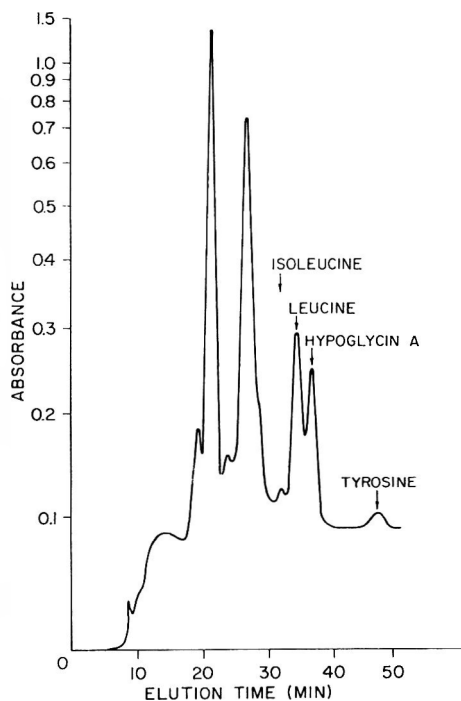

Fig. 1—Chromatogram of typical ackee extract (sample 4).

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2 (Table 1). This sample gave a measured amount of 2.8 μg hypoglycin A on the column and had the lowest hypoglycin A: leucine ratio of the seven samples of commercial ackee analyzed. Only two ackee samples (1 and 5) gave leucine peaks smaller than those of hypoglycin A.

An experiment was carried out to study the possible effect of boiling the ackee extract after blending. One-half of the mixture obtained by blending 50g ackee in a Waring Blendor with 150 ml water was boiled under reflux for 1 hr, then both parts were analyzed for hypoglycin A using the amino acid analyzer. There was no increase in the recovery of hypoglycin A due to boiling.

As a further check on the extraction procedure, a 5-g sample of ackee (mean hypoglycin A content 260 ppm found from two analyses on 50-g samples from the same can) was spiked with 750 μg hypoglycin A (150 ppm). The total estimated hypoglycin A concentration in the sample was found to be 376 ppm, a 77% recovery of the added hypoglycin A.

Mean concentrations of hypoglycin A found in seven different samples of commercial canned ackee (four brands, packed in brine in Jamaica) ranged from 108–260 ppm (Table 1). These results may be compared with previous reports of hypoglycin A concentrations of 80 ppm in the ripe arillus and 900 and 1,100 ppm in unripe arilli (Ellington, 1961; Hassall and Reyle, 1955; Abrahams and Kean, 1969).

Table 1—Hypoglycin A concentration in commercial canned ackee fruit and brine

Sample	Hypoglycin A concentration	
	Fruit (μg/g)[a]	Brine (μg/ml)[b]
1	108 ± 3	122
2	119 ± 1	153
3	135 ± 3	182
4	162 ± 5	214
5	187 ± 8	234
6	235 ± 16	287
7	260 ± 14	–

[a] Mean ± SD of duplicate determinations on 5-g (50-g for sample 7) portions of ackee from the same can
[b] Single determinations only

Amino acid patterns found in the brines were almost identical to those found in the corresponding canned fruit, and it is readily apparent (Table 1) that the concentration of hypoglycin A found in the brine increases with the concentration in the fruit. Except for sample 1, which unlike the others contained less ackee (g) than brine (ml) in the can, the total weight of hypoglycin A calculated to be in the brine of any one can was somewhat lower than the total amount calculated to be in the ackee fruit in that

can. On average, data from six cans revealed that 45% of the total hypoglycin A present had leached into the brine.

The question remains as to what amounts of hypoglycin A may be harmful to humans, and teratogenic studies in animals using oral administration have yet to be undertaken. The method of analysis developed for hypoglycin A in ackee should permit further data on amounts that are consumed by humans to be obtained if needed.

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A Research Note

SOME OBSERVATIONS ON THE OCCURRENCE OF CHLOROPHYLL AND SOLANINE IN POTATO TUBERS AND THEIR CONTROL BY N^6-BENZYLADENINE, ETHEPHON AND FILTERED LIGHTS

INTRODUCTION

THE PRODUCTION of potatoes is important as an economic commodity and as a food source of carbohydrate energy, vitamin C and minerals. When potatoes are exposed to light during post-harvest handling and marketing, a green pigment develops at the surface. Often, a bitter taste or an off-flavor is associated with this pigmentation. The green color arises from the synthesis of chlorophyll, while the bitterness is due to an excess formation of solanine (Hilton, 1951). Currently, the term solanine represents a mixture of naturally occurring glycoalkaloids in the potato plant, of which α-solanine and α-chaconine are the predominant forms. Although these glycoalkaloids exhibit poisonous characteristics, their accumulations in potato tubers are usually too small to be harmful, unless certain environmental conditions favor an increase in their syntheses. Though the syntheses of both chlorophyll and solanine in potatoes are activated by light, their processes of formation are independent of one another (Conner, 1937).

These experiments were undertaken to investigate possible methods of reducing solanine and chlorophyll in light-exposed potato tubers. Solanine distribution in the outer layers of the potato tubers was studied. The effects of N^6-benzyladenine, Ethephon and filtered lights on chlorophyll and solanine syntheses were also examined.

MATERIALS & METHODS

SHURCHIP POTATO plants which were growing at the Utah State University farm in Farmington, Utah received foliar applications of N^6-benzyladenine (N6BA) in a 500 ppm solution and 2-chloroethylphosphonic acid (Ethephon) in a 50,000 ppm solution. A few drops of Triton B-1956 were added to each solution as a surfactant. Control plants were sprayed with distilled water and surfactant only. Four plants were used per treatment and each plant was sprayed twice until run-off. The potatoes were harvested 2 wk following the spraying, washed, towel dried and exposed to 200 ft-c at 15.5°C and 60% RH for 7 days. There were two

replications for each treatment.

Fluorescent tube lights (F40 CW) were used as the source of illumination in all experiments. Different colors were obtained through Dennison colored cellophane filters comprised of red, orange, yellow, green, blue and violet. A clear cellophane filter served as the control. Russet Burbank potatoes, which were used in this phase of the research, were selected from a local supermarket. The tubers were isolated from extraneous filtered light by placement in individual cardboard boxes with the colored cellophane stretched across the opening at the top. The boxes were vented for air circulation. All tubers were adjusted below their filters to receive 50 ft-c of light intensity regardless of the color of the filter. Intensities of light were measured with a Weston Illumination Meter (Model 603). There were two replications for

each treatment. Each replication contained 12 tubers. Tubers were exposed to each color of light for 10 days at 15.5°C and 60% RH.

Chlorophyll contents in the potatoes from the foliar sprays and colored cellophane experiments were measured by a colorimetric method described in AOAC (1965). Solanine was extracted and determined colorimetrically using a method devised by Gull and Isenberg (1960). The least significant difference (LSD) test was employed to determine the significance of the effects.

For the histochemical study, small peridermal sections were cut from the tubers, quick-frozen on dry ice, and stored in labeled vials at −10°C. Thin ribbons were sliced on a Model CTD-International Harris Cryostat. The ribbons were mounted on glass slides and stained for alkaloids according to a photo-

Table 1—Mean values of total chlorophyll, chlorophyll a, chlorophyll b and solanine determined in potato tubers which received foliar spray applications prior to harvest and illumination

Chemical treatment	Total chlorophyll	Chlorophyll a	Chlorophyll b	Solanine
	mg/100g fresh peel			
Control	2.106	1.300	0.806	50.0
N6BA	1.459**	0.881**	0.578**	36.5*
Ethephon	2.132	1.278	0.854	31.5**

* Significant at 0.05 level
** Significant at 0.01 level

Table 2—Mean values of total chlorophyll, chlorophyll a, chlorophyll b and solanine determined in potato tubers which were illuminated through colored cellophane filters

Color of cellophane filter	Total chlorophyll	Chlorophyll a	Chlorophyll b	Solanine
	mg/100g fresh peel			
Clear	4.360	2.290	2.070	49.5
Red	3.704	1.962	1.742	42.5*
Orange	2.996**	1.902	1.074*	52.0
Yellow	2.588**	1.624	0.964*	47.0
Green	4.384	2.227	2.157	35.8*
Blue	4.618	2.422	2.196	45.5
Violet	3.983	2.359	1.624	42.5*

* Significant at 0.05 level
** Significant at 0.01 level

graphic procedure described by White and Spencer (1964).

RESULTS & DISCUSSION

TABLE 1 shows the results of preharvest foliar spraying on chlorophyll and solanine formations in illuminated tubers. Only N6BA significantly reduced chlorophyll formation, while both Ethephon and N6BA significantly inhibited the synthesis of solanine. Cherry and Anderson (1972) suggested that N6BA acts to preserve the integrity of cytokinin-containing transfer-RNA groups, and thus, promotes a continuation of normal protein syntheses. Regarding this, one would expect chloroplast functions also to be maintained. It is possible that light exposure may deter N6BA from its normal function in regard to chlorophyll production. Ethylene-induced growth inhibition may also be altered by light (Lieberman and Kunishi, 1972). This may explain why Ethephon, which metabolizes into ethylene, maintained a chlorophyll production similar to that of the control. Jadhav et al. (1973) found that compounds which release ethylene upon metabolic breakdown may inhibit solanine formation.

Preliminary tests have shown that N6BA may exhibit differential responses in regard to solanine synthesis. Some experiments with thin-layer chromatography have shown nonlinear changes in solanine production with variations of light exposure and N6BA concentrations (Jeppsen, 1974). Further experimentations investigating N6BA metabolites and the photoreceptor(s) responsible for synthetic initiation of solanine would help to explain our findings.

Figure 1 indicates the sites of alkaloid concentrations found in the peridermal and cortical regions of the potato tubers. The speckled gray areas indicate solanine can be more easily detected against a colored background, but as seen here in black and white, they resemble small accumulations of iron filings. The glycoalkaloids were distributed mainly in, and just below, the compact phellem cells. Secondary deposits were found in the cortical parenchyma cells just below the skin layers of the tuber.

Table 2 lists the results of chlorophyll and solanine analyses from potato tubers receiving different colors of light. Tubers which were illuminated through red, orange, yellow and violet filters all exhibited a decrease in total chlorophyll production as compared to the control. In each of these four colors the concentrations of chlorophyll b were reduced by greater amounts than were those of chlorophyll a. Yamaguchi et al. (1960) also found reductions in total chlorophyll syntheses following exposure of tubers to amber and yellow wavelengths. One might expect red light to yield a greater amount of the chlorophylls than that indicated here. The discrepancy may be due to the low intensity of light used. Salisbury and Ross (1969) noted some differential effects of red and blue wavelengths on phytochrome activation when the light intensities were manipulated over a low to high range.

All of the colors of light tested were capable of reducing solanine concentrations except orange light which produced a slight increase of the alkaloid. Next to orange, the yellow filter induced the least inhibition of solanine formation. The greatest reduction occurred in tubers beneath the green filter; red and violet exhibited a moderate inhibition. Conner (1937) reported similar findings in that green and green with infra-red wavelengths were the most limiting to solanine production. Since neither chlorophyll nor solanine responded to the same wavelengths of light, Conner concluded that their synthetic pathways must be dissimilar. The effectiveness of green light in decreasing solanine production may reveal a phytochrome involvement in the

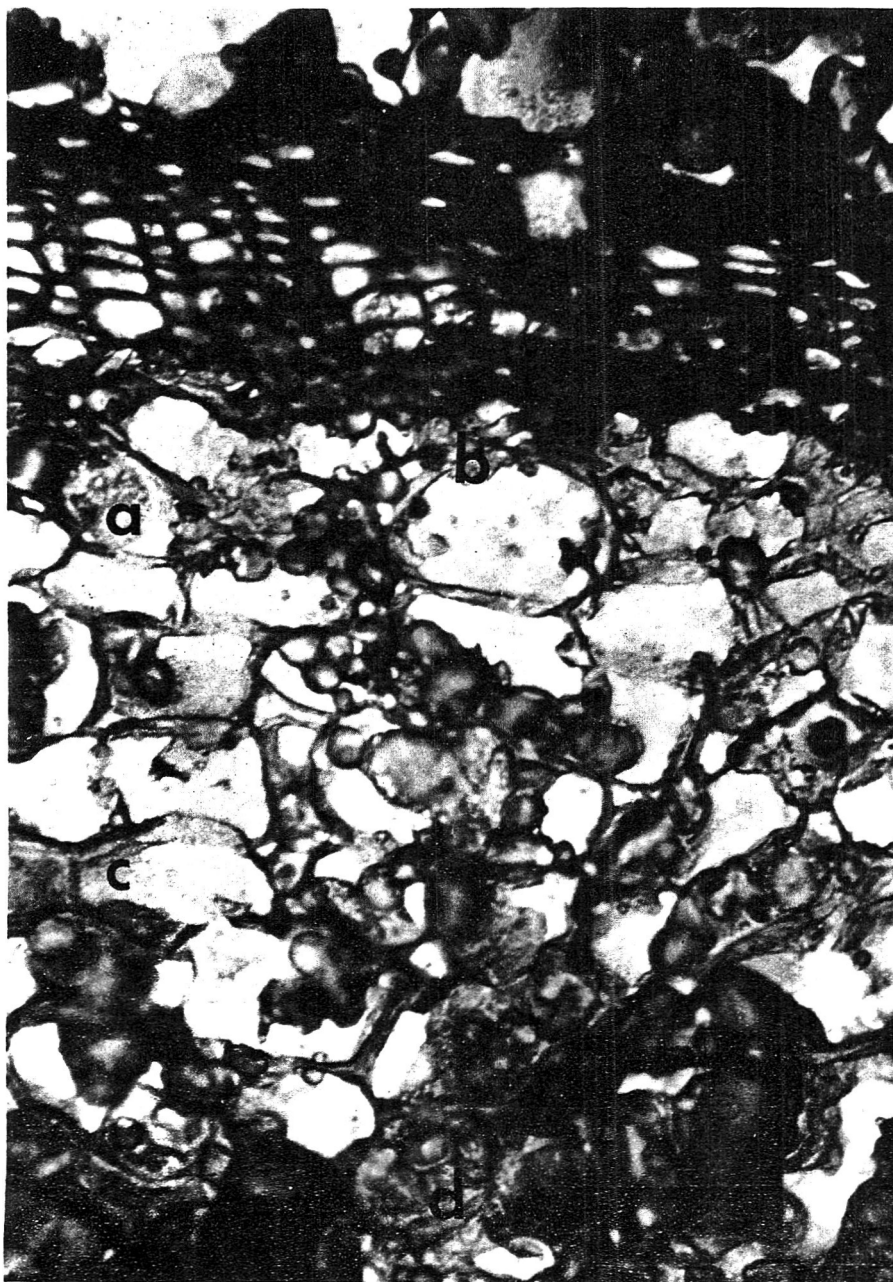*Fig. 1–Sites of glycoalkaloid depositions in a microscopic section of the peridermal region of a potato tuber (Russet Burbank cv.). The speckled deposits are indicated in and just under the phellem cell layer (a, b) and also further down in the cortical parenchyma (c, d).*

syntheses of glycoalkaloids, since the energy absorbing forms of this photoreceptor (phytochrome-660 nm and phytochrome-730 nm) are themselves green. Also, far-red light converts the active form of phytochrome at 730 nm to the inactive form at 660 nm. Thus, both green light and far-red or infra-red light could inhibit solanine formation if it were monitored by the phytochrome system.

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A Research Note

THE QUANTITATIVE DETERMINATION OF GLUCOSE, FRUCTOSE AND SUCROSE IN FRUITS AND POTATOES

INTRODUCTION

IN PROCESSED FRUITS and vegetables the brown coloration, off-flavors, excessive hygroscopicity and other undesirable properties often result from the interaction of natural sugars with other reactive components in these foods. Consequently, in food processing studies, determination of the major sugars is essential at various stages, e.g., as the fresh product before and after storage conditioning, and in the final processed form.

Most sugar analyses are based on the oxidation-reduction reaction involving alkaline solutions of metal salts. Ting (1956) utilized the reduction of alkaline ferricyanide to determine total reducing sugars in citrus juices. Furuholmen et al. (1964) modified Ting's method and applied it to the determination of glucose and fructose in potatoes. The difference in the rates of reaction at 55° and 100°C of the monosaccharides with potassium ferricyanide was utilized. Although this method is satisfactory for measuring the total monosaccharide concentration when reacted at 100°C, it does require rigid control of temperature at 55°C to differentiate between glucose and fructose. Therefore, the highly specific glucose oxidase method described by White (1964) for determining glucose was used to supplement the total reducing sugar analysis.

Jacobs (1951) and Schwimmer et al. (1954) have indicated that the major sugars in fruits and potato tubers are glucose, fructose and sucrose. Therefore, the fructose content can be estimated as the difference between total reducing sugar and glucose concentration. Sucrose has been determined by measuring the increase in glucose content after acid hydrolysis of the disaccharide.

MATERIALS & METHODS

A HITACHI Perkin-Elmer, Model 139, UV-Visible Spectrophotometer and a Beckman, Model B, Spectrophotometer were used to measure absorbance.

The alkaline ferricyanide and arsenomolybdate (A.R. Grade) solutions were prepared as described by Ting (1956).

Glucose oxidase-peroxidase reagent was prepared (White and Subers, 1961) using either crude or the purified-Type II glucose oxidase (Sigma Chemical Co.) and Type I, horseradish peroxidase (Sigma Chemical Co.).

Neutral lead acetate solution was prepared by dissolving 20g $Pb(OAc)_2 \cdot 3H_2O$ in deionized water to a final volume of 100 ml.

Reagent grade glucose, fructose and sucrose dried under vacuum at 70°C for 18 hr were used as standards. A standard stock solution containing 5.000g of glucose per liter was standardized iodometrically (Browne and Zerban, 1941). Working standards, ranging from 50–500 μg glucose per ml, were used for standardization of both methods.

Total reducing sugars (glucose plus fructose) were determined by reacting with alkaline ferricyanide at 100°C for 10 min (Furuholmen et al., 1964). Absorbance was measured at 720 nm 40 min after addition of arsenomolybdate complexing agent. Both sugars in the concentration range 0–400 μg gave highly significant linear functions (r = 0.9996) with an absorptivity of 17.20 for a 1% solution. Periodic checks showed excellent reproducibility of the absorptivity value.

Samples were then analyzed specifically for glucose using the glucose oxidase procedure as described by White (1964). 200 μg of glucose had an absorptivity of about 0.423 when reacted under the above conditions. Although a highly significant linear function (r = 0.9995) was obtained in the concentration range studied (0–400 μg), the absorptivity varied sufficiently to necessitate a daily check of the standard value. This value was used to calculate the glucose concentration. The fructose content was calculated as the difference between the total reducing sugar value and the glucose content.

Finally, the sucrose in a measured aliquot of each sample was hydrolyzed with 5 ml 12N HCl at 70°C for 10 min, cooled and neutralized with 5N NaOH. The solution was then reassayed for glucose by the glucose oxidase reaction. The increase in glucose was used to calculate the concentration of sucrose.

Fresh products, e.g., apples, peaches and potatoes were prepared for analysis by homogenization and filtration of the resultant puree. The filtrate (juice) was clarified with neutral $Pb(OAc)_2$, diluted and analyzed as described. Partially dried or dehydrated fruits and fruit juice powders were rehydrated with hot deionized water to their original moisture level and treated as the fresh product. With processed potatoes ethanol extraction was utilized.

RESULTS & DISCUSSION

THE RELIABILITY of the analytical procedure was tested with a series of ternary mixtures containing varying amounts of glucose, fructose and sucrose. The total sugar content of each mixture was determined and the results (Table 1) were compared to the known composition. The average overall recovery of the added sugars was satisfactory. The glucose content averaged 100.19% (+1.99 to −0.70%) of the amount added. The fructose accounted for averaged 100.81% for all five mixtures, but showed a slightly greater range (+3.83 to −1.30%) than did glucose. Sucrose content from the five mixtures averaged only 97.66% with considerably more variation (+8.25 to −9.50%) than the two monosaccharides. The generally good overall recovery of added sugars indicates this to be a workable method for determining glucose, fructose and sucrose simultaneously.

The suitability of hot water rehydration for the quantitative extraction of sugars from dried fruits was ascertained. In this experiment the sugar content of a partially dried apple was compared to the sugar content of the fresh product from which it was prepared. The results

Table 1—The recovery of sugars from ternary mixtures

	Glucose mg/100 ml		Fructose mg/100 ml		Sucrose mg/100 ml	
Mixture	Added	Found	Added	Found	Added	Found
1	601	602	1000	1025	201	190
2	201	205	600	623	200	181
3	1000	993	1000	1000	100	100
4	201	201	200	198	2000	2165
5	1000	995	1000	987	1001	951

Table 2—Efficiency of extraction procedure for removing sugars from potatoes

Sample	Extraction method	Glucose	Fructose	Sucrose
			Percent[a]	
Fresh potato	Squeezed juice	1.64 ± 0.03	1.70 ± 0.07	2.50 ± 0.03
	Soxhlet	1.49 ± 0.04	2.23 ± 0.01	2.65 ± 0.11
	AOAC	1.49 ± 0.01	1.98 ± 0.13	2.34 ± 0.15
Dried potato	Soxhlet[b]	1.24 ± 0.00	4.02 ± 0.26	1.52 ± 0.01
	AOAC[b]	1.26 ± 0.01	3.46 ± 0.25	1.34 ± 0.14
	AOAC[c]	1.22 ± 0.10	3.50 ± 0.33	1.04 ± 0.01

[a] Uncorrected for moisture content
[b] Potato rehydrated prior to extraction with 95% ethanol
[c] Potato extracted directly with 80% ethanol without rehydration

showed that 97% of the glucose and slightly more than 100% of the fructose and sucrose were recovered. Hot water rehydration was found equally reliable when applied to processed peaches and various fruit juice powders.

When applied to processed potatoes hot water extraction was not feasible because of water retention by the gelatinized starch. Ethanol extraction has been utilized for the removal of sugars from plant materials (AOAC, 1970). However, the suitability of this method for quantitatively extracting the total sugars from processed potatoes at any moisture level was uncertain. The efficiency of ethanol extraction was therefore evaluated by comparing the sugar content of fresh potato analyzed "as is" and the same potato extracted with ethanol. In this study a Soxhlet extractor was employed. For comparison the AOAC extraction method, although somewhat troublesome because of bumping and occasional boil-over, was also used. In each extraction sufficient 95% ethanol was added to fresh potatoes to yield a final ethanol concentration of 80% when diluted with the sample moisture. After extracting for 90 min the mixture was cooled and filtered into a volumetric flask. The extracted potato sample was washed several times with 80% ethanol and the washings added to the original extract. Clarification of this solution was unnecessary. Due to the inhibitory effect of ethanol on the glucose oxidase reagent, however, it was necessary to remove all traces of alcohol from the samples using a rotary vacuum evaporator prior to glucose determina-

tion. The results of this comparative study are shown in Table 2. The sugar content of the potato juice, assumed to represent the true sugar content of the sample, was used as the basis for comparison with the ethanol extracts. The average concentration of glucose, fructose and sucrose in the fresh potato samples extracted with ethanol are of the same order of magnitude as the concentration of these sugars in the control (potato juice) sample. Thus, the two procedures appear equally reliable for extracting sugars from fresh potato.

Although the Soxhlet extraction was suitable for removing sugars from fresh potato, erratic results were obtained when tried on dried potato dice (4% moisture). The problem was the absence of sufficient sample moisture to dilute the alcohol-water azeotrope (95%–5%) which is formed during reflux. The solubility of sugars is greatly reduced in 95% ethanol, thus limiting extraction efficiency. Prior to extraction, therefore, sufficient de-ionized water was added to the ground dried potato sample increasing the moisture level to that of fresh potato (85–90%). Sufficient water is then present to dilute the azeotrope, especially at the early stages of refluxing, thereby insuring maximum solubility of the sugars.

Table 2 also shows the glucose, fructose and sucrose concentrations of a typical sample of dried potato dice extracted by the modified Soxhlet procedure described above. Also shown are the sugar concentrations of the same sample that was extracted by the direct method (AOAC, 1970) both with and without

prior rehydration. The glucose content of the dried potato extracted by the three procedures is identical. Fructose and sucrose levels are slightly higher in the Soxhlet extract. It appears that sucrose solubility may be enhanced by preliminary rehydration of the dried sample prior to extraction by either procedure.

SUMMARY

THE UTILITY of the Soxhlet extraction technique for removing sugars from fresh potato with 80% ethanol has been shown. The procedure is equally effective for extracting sugars from processed potato provided the potato sample is rehydrated prior to ethanol extraction.

Hot water extraction of dried fruits and fruit juice powders has been shown to be adequate for extracting sugars from these products.

Ternary mixtures of glucose, fructose and sucrose can be analyzed by a two-step procedure utilizing the determination of total reducing sugars coupled with the use of the glucose oxidase reaction for measuring glucose specifically.

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A Research Note

SEPARATION OF COMMERCIAL SOYBEAN-MILK WHEY PROTEIN BLENDS BY ELECTROPHORESIS AND ISOELECTRIC FOCUSING

INTRODUCTION

SPRAY-DRIED blends of soybean protein and bovine milk whey protein have been introduced commercially as functional replacers for nonfat dry milk in conventional and new fabricated foods. The combining and processing of plant and animal proteins creates questions relating to possible protein-protein interactions or other changes occurring and the contribution of such changes to the overall functional properties of the finished blended product. In addition, blends may create regulatory problems relative to component quantitation and adulteration.

The purpose of this paper is to report on the electrophoretic and isoelectric focusing behavior of soybean-milk whey protein blends as a means of resolving and identifying the major protein components and evaluating process-induced protein changes. Acidic and alkaline urea starch gel electrophoresis and urea polyacrylamide gel isoelectric focusing were run and the patterns of protein blends were compared to those of nonblended soybean globulin and bovine milk whey and casein protein preparations.

EXPERIMENTAL

SPRAY-DRIED samples of isoelectric-type iso-lated soy protein, two types of sodium soy proteinate, sweet cheese whey, a sweet cheese whey-sodium caseinate blend, a soy protein-sweet whey-buttermilk solids blend, and two soy protein-sweet whey blends were obtained from commercial sources. The 7S and 11S protein rich fractions were prepared from the isoelectric-type isolated soy protein by a calcium ion precipitation method (Saio and Watanabe, 1973) and then lyophilized. Fresh fluid sweet cheese whey was supplied by a commercial cheese manufacturing plant. Lyophilized preparations of bovine β-lactoglobulin A (β-Lg A), bovine serum albumin (BSA) and bovine IgG_1 immunoglobulins were purchased from Miles Labs, Inc., Kankakee, Ill. The α-lactalbumin (α-La) rich fraction was prepared from fresh bovine milk whey by ultrafiltration using an
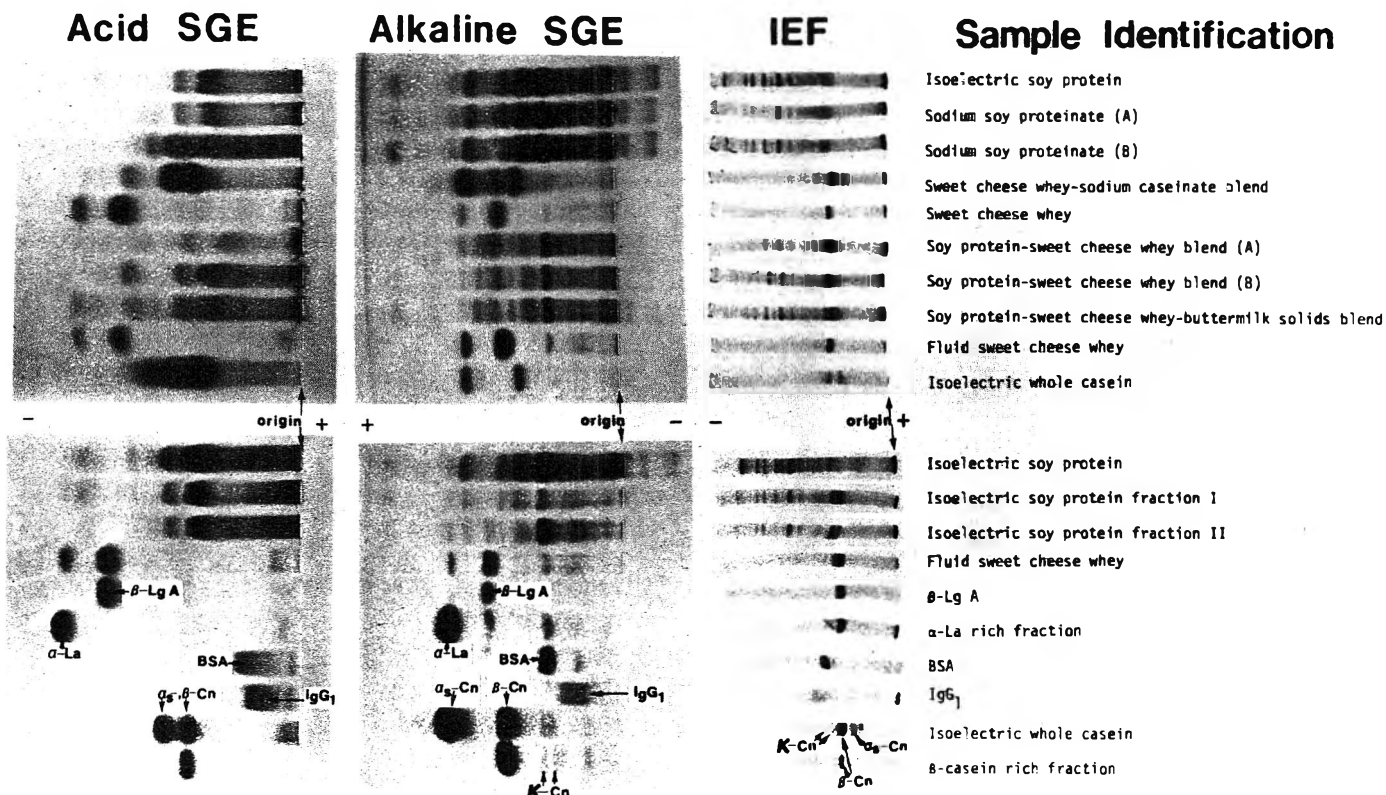
Fig. 1—Acid and alkaline urea starch gel electrophoresis (SGE) and urea polyacrylamide gel isoelectric focusing (IEF) patterns of various soybean protein and milk whey protein and casein preparations and commercial blends.

Amicon PM-30 membrane (Sinha and Mikolajcik, 1972), and acid-precipitated isoelectric whole casein and a crude casein fraction rich in β-casein were prepared as described previously (Josephson, 1972).

The acid urea starch gel electrophoresis (SGE) method developed by Puski and Melnychyn (1968) for soybean globulin separation was used. All protein samples were dispersed in a 1N acetic acid/6M urea/0.1M β-mercaptoethanol solution at pH 3.5 prior to electrophoresis by this method. Also employed was the alkaline urea starch gel electrophoresis (SGE) method of Morr (1971) originally developed for bovine milk casein separation. For this method, all protein samples were dispersed in a 0.076M Tris citrate/7M urea/0.1M β-mercaptoethanol buffer solution at pH 8.6 prior to electrophoresis. Urea polyacrylamide gel isoelectric focusing (IEF) was carried out as before (Josephson, 1972) using a final ampholyte (LKB, pH 3–10) concentration of 1% in the gels. All protein samples were dispersed in a solution containing 7M urea, 25% sucrose and 0.1M β-mercaptoethanol prior to focusing.

All methods reported here incorporated urea in the gel systems because it was found in the present study that standard alkaline disc polyacrylamide gel electrophoresis or gel isoelectric focusing run in the absence of urea separated soy globulins and bovine milk caseins into poorly resolved and smeared bands.

RESULTS & DISCUSSION

FIGURE 1 shows typical patterns from acid and alkaline urea starch gel electrophoresis (SGE) and urea polyacrylamide gel isoelectric focusing (IEF) of several standard soybean and milk protein preparations and commercial blends. The results are presented here in such a way as to allow direct comparison of each sample pattern by the three methods.

Soy protein samples

Patterns for isoelectric and sodium soy protein products and the prepared 7S and 11S rich fractions (Fractions I and II) by any one method were quite similar. However, the methods varied considerably in their ability to resolve the heterogeneity of soy globulins. IEF was clearly superior to the electrophoretic methods, revealing over 15 distinct protein bands. The number of protein bands separated by alkaline urea SGE was more than by acid urea SGE but both revealed less distinct and somewhat blurred zones.

The various soy products were described by the manufacturer as containing immunochemically distinct glycinin and α-, β- and γ-conglycinins. Therefore, stated in terms of the classical ultracentrifuge nomenclature (Catsimpoolas, 1969), these soy globulin products should contain 2S, 7S, 11S and 15S fractions. Since the 7S and 11S rich fractions prepared here yielded similar patterns as compared to each other and the original isoelectric soy globulin preparation, we will not attempt to identify the specific globulin

bands. However, one can conclude on the basis of the broad distribution of bands in alkaline urea SGE and urea gel IEF patterns that soybean globulin species range from slightly acidic to basic in apparent isoelectric points (pI's). The pH gradient in the gels ranged from about pH 4.6 to 9.1 in the IEF gel (+ to − end) using pH 3–10 ampholytes which was due in part to the effect of urea (Josephson, 1972).

Milk whey protein samples

The results for fresh fluid and spray dried sweet cheese whey samples and various individual whey protein standards indicated that alkaline urea SGE best resolved all major species. IgG_1 and BSA were poorly resolved by acid SGE. The major whey proteins have similar pI's (Rose et al., 1970) and consequently were inadequately resolved by urea gel IEF in the wide pH gradient created by pH 3–10 ampholytes. In other trials, use of narrow pH range ampholytes (pH 3–6) in the presence or absence of urea did resolve whey protein species quite well, but because comparison to the widely spread soy globulin patterns is of critical importance here, these IEF patterns will not be presented.

Milk casein samples

The alkaline urea SGE and urea IEF patterns of isoelectric whole casein and β-casein rich fractions were typical of those reported earlier (Josephson, 1972). Separation by acid urea SGE was not as complete.

Relative mobilities of soy and milk proteins

A prerequisite to positive identification of blended components is relative band location differences of standard soy globulins and milk whey proteins and caseins for each method. Based upon position differences in patterns, separation of the major whey proteins (β-Lg A and B and α-La) from caseins or soy globulins was best achieved by acid urea SGE. Although position differences were slight, alkaline urea SGE also allowed for identification of β-Lg bands from those of soy globulins or caseins. Milk caseins and whey proteins exhibited similar band locations by urea gel IEF and no differentiation of β-Lg and α-La was possible because of their similar apparent pI values. The soy globulins had pI positions starting at a pH slightly above those for the milk proteins and therefore were readily identifiable.

Blended products

Regarding the patterns for specific product blends the following observations were made. The sweet cheese whey-sodium caseinate blend had as its major components, $α_s$- and β-caseins and β-Lg. The somewhat less distinct major bands

and streaky zones between the major bands (see the alkaline SGE pattern) could be indicative of whey protein-casein interactions The gel patterns for soy protein-sweet cheese whey blends A and B and that with added buttermilk solids were similar by any one method and indicated that soy globulins were the primary components and β-Lg and α-Lg were the major and minor whey protein components, respectively. For the most part the soy globulins in the blends exhibited quite similar band resolution as in the soy globulin standards. The β-Lg and α-Lg bands in the blends appear more diffuse (see acid and alkaline urea SGE patterns) than in the fresh wheys or whey standards which is presumptive evidence of denaturation and/or interaction with soy globulins.

From the present study one can conclude that: (1) no one urea starch gel electrophoretic method or urea gel isoelectric focusing pH gradient was effective in resolving all soy globulins, milk whey proteins and caseins simultaneously, but combining the results from these methods allowed for identification of the proteins in complex blends; (2) application of these methods may provide a means for quantitating blended components; (3) soy globulins and milk whey proteins in commercial blends exhibited relatively similar electrophoretic and isoelectric focusing patterns to those in non-blended samples, although some change in β-lactoglobulin and α-lactalbumin was observed in the processed blends; and (4) alkaline urea starch gel electrophoretic and urea polyacrylamide gel isoelectric focusing methods developed for milk protein fractionation and identification have shown promising application for soy globulin separation and characterization.

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A Research Note
IMPORTANCE OF SURFACE HEAT TRANSFER
DURING STEAM HEAT AND HEAT/HOLD PROCESSES

INTRODUCTION

HEATING and heating-holding equations for food products have recently been published (Lenz and Lund, 1973; Lund et al., 1972). These have assumed that external resistance was negligible and internal resistance was the only factor limiting heat transfer.

Ling (1973), however, has shown that for steam heating of carrot dice, outgassing of the piece can cause considerable external resistance by forming a layer of noncondensable gas and therefore impeding steam condensation. Carrot dice saturated with nitrogen at 1500 psi or vacuum degassed and heated in steam showed that there was indeed an outgassing effect on heat transfer which varied with the amount of dissolved gas in the substance. It is significant that the external resistance seemed to be present even in the vacuum degassed sample. This was probably due to the difficulty of removing CO_2 arising from tissue metabolism by vacuum degassing as reported by Denny (1946). This note attempts to show the magnitude of the error introduced by assuming negligible external resistance.

THEORY

THE EQUATIONS for heat transfer with external resistance can be developed for various geometries by the methods of Carslaw and Jaeger (1959). For a slab, the partial differential equation for conduction of heat is:

$$\frac{\partial T}{\partial \tau} = \kappa \frac{\partial^2 T}{\partial X^2} \tag{1}$$

For the heating period, the boundary conditions are:

(1) $\quad T = T_0 @ \tau = 0, -L \leqslant X \leqslant L$

(2) $\quad \frac{\partial T}{\partial X} = H(T_\infty - T) @ X = \pm L$

For the holding period we have:

(1') $\quad T = f(X, \tau)$

(2') $\quad \frac{\partial T}{\partial X} = 0 @ X = \pm L$, all τ

where $f(X, \tau)$ is the temperature distribution at the end of the heating period. The equations and boundary conditions for an infinite cylinder and a sphere are similar. Carslaw and Jaeger (1959) present solutions for Equation (1) with the two sets of boundary conditions. These can be combined to give:

$$\theta = \frac{T_\infty - T}{T_\infty - T_0} = 2 \sum_{k=1}^{\infty} \frac{H^2 \exp(-\alpha_k^2 \kappa \tau)}{\alpha_k^2 [LH(LH+1) + \alpha_k^2 L^2]}$$

$$+ 4 \sum_{n=1}^{\infty} \sum_{k=1}^{\infty} \frac{(-1)^n L^2 H^2 \cos \frac{n\pi X}{L} \exp(-\kappa(\frac{n^2 \pi^2 t}{L^2} + \alpha_k^2 \tau))}{(\alpha_k^2 L^2 - n^2 \pi^2)[LH(LH+1) + \alpha_k^2 L^2]} \tag{2}$$

where α_k are the roots of
$$\alpha_k \tan(\alpha_k L) - H = 0 \tag{3}$$

for the cylinder:

$$\theta = \frac{T_\infty - T}{T_\infty - T_0} = \frac{4H^2}{a^2} \sum_{n=1}^{\infty} \sum_{k=1}^{\infty} \frac{J_0(\alpha_n r) \exp[-\kappa(\alpha_n^2 t + \alpha_k^2 \tau)]}{(H^2 + \alpha_k^2)(\alpha_k^2 - \alpha_n^2) J_0(\alpha_n a)} \tag{4}$$

where α_k are the roots of

$$\alpha_k [J_1(\alpha_k a) - H[J_0(\alpha_k a)] = 0 \tag{5}$$

α_n are the roots of

$$J_1(\alpha_n a) = 0 \tag{6}$$

for the sphere

$$\theta = \frac{T_\infty - T}{T_\infty - T_0} = \frac{6H^2}{a^2} \sum_{k=1}^{\infty} \frac{a^2 \alpha_k^2 + (aH-1)^2}{\alpha_k^4 [a^2 \alpha_k^2 + aH(aH-1)]} \sin^2(\alpha_k a) \exp(-\kappa \alpha_k^2 \tau)$$

$$+ \frac{4H^2}{ar} \sum_{n=1}^{\infty} \sum_{k=1}^{\infty} \frac{(a^2 \alpha_n^2 + 1)[a^2 \alpha_k^2 + (aH-1)^2] \sin^2(\alpha_k a)}{a^2 \alpha_n^2 \alpha_k^2 (\alpha_k^2 - \alpha_n^2)} \tag{7}$$

$$\times \frac{\sin(\alpha_n a) \sin(\alpha_n r) \exp[-\kappa(\alpha_n^2 t + \alpha_k^2 \tau)]}{a^2 \alpha_k^2 + aH(aH-1)]}$$

where α_k are the roots of

$$a\alpha_k \cot(a\alpha_k) + aH - 1 = 0 \tag{8}$$

and α_n are the roots of

$$a\alpha_n \cot a\alpha_n = 1 \tag{9}$$

To evaluate the equation for the sphere at $r = 0$, one simply notes that $\lim_{r \to 0} \sin \alpha_n r / r = \alpha_n$ by L'Hopital's rule. A similar substitution must be made for $\lim_{\alpha_{n_1} \to 0} \sin(\alpha_n a) \sin(\alpha_n r) / \alpha_n^2 = ar$ during calculations because $\alpha_{n_1} = 0$. The equations herein presented can also be used for simple heating processes by putting $t = 0$. The equations for the cylinder and slab are useful even if external resistance is negligible simply by taking the limit as H approaches infinity. For the sphere in this case, one can use the equations of Lenz and Lund (1973).

It should be noted that heat profiles in geometries such as rectangular solids and finite cylinders can also be evaluated using these equations. For a rectangular solid, for example, one evaluates θ for each of the three half thicknesses, then multiplies them together. For a finite cylinder, one evaluates θ for an infinite cylinder of the radius of the cylinder and for a slab of thickness equal to the length of the cylinder and multiplies them together.

RESULTS

EQUATIONS (2) and (3) for a slab were applied to the data of Lien (1973) for the heating of a rectangular solid carrot (moisture content of 87%) assuming that the thermal diffusivity of the carrot piece was equal to that of water (1.71×10^{-6} ft^2/sec). The thermal diffusivity is an average value in the range 70–212°F. The surface heat transfer coefficient was found to be 100 BTU/hr-ft^2-°F for the sample saturated with nitrogen, 190 BTU/hr-ft^2-°F for the untreated sample, and 280 BTU/hr-ft^2-°F for the vacuum degassed sample. A graph of the fitted data with the untreated sample is shown in Figure 1.

The effect of surface heat transfer coefficients on blanching times in heat hold systems such as IQB (Lazar et al., 1971) was calculated. In each case an adiabatic holding time of 45 sec after the steam heating was assumed. Figure 2 shows the heating time necessary so that the final piece-center temperature is
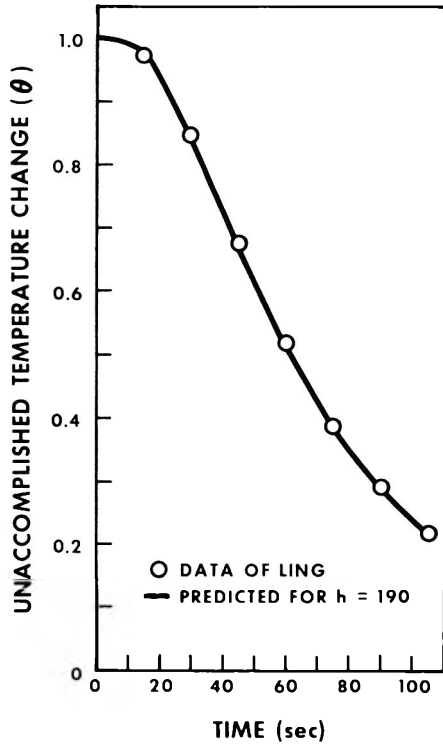
Fig. 1—Results of Ling (1973) compared to results predicted by theoretical model for h = 190 BTU/hr-ft²-°F.
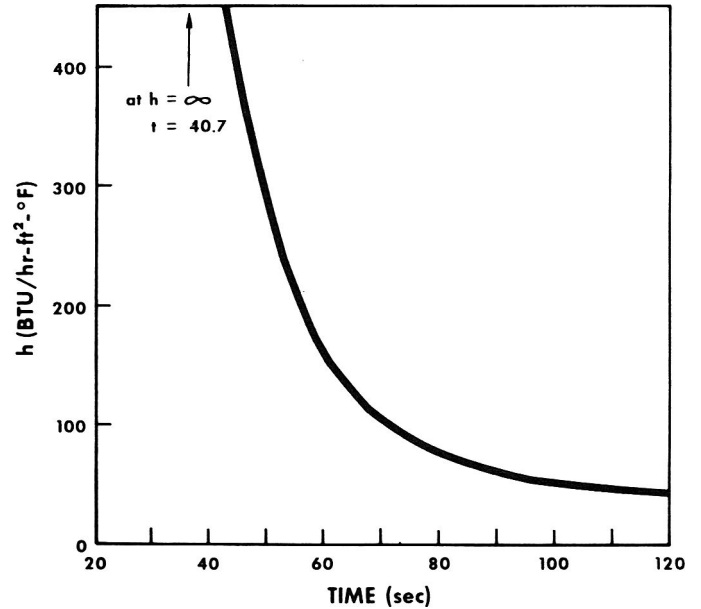

Fig. 2—Heating time necessary to achieve 180°F center temperature with different surface heat transfer coefficients. 45 sec adiabatic hold assumed in all cases.

180°F for a 7/16 in. diameter × 1 in. long cylinder. These dimensions were the average dimensions of a commercial green bean sample.

DISCUSSION

THE MATHEMATICAL model herein proposed, coupled with the data of Ling (1973), shows the importance of surface heat transfer resistance during steam heating of food pieces and a method of calculating its effect. An examination of Figure 2 shows that foods could easily require actual blanching times 40% longer than those predicted neglecting external resistance. These results point up the need for further experimental evaluations of heat transfer coefficients to particulates in steam heating systems such as blanching and HTST sterilization. Processors going to high temperatures to shorten process times for particulates should be especially aware of the outgassing phenomena.

It should be pointed out that the equations presented herein can be useful in other situations than steam heating. External heat transfer resistances in water systems are not negligible and these equations can be used for water heating. The equations can also be used to calculate cooling curves by using T_∞ as the cooling medium temperature and T_0 as the initial temperature of the piece to be cooled. By using the methods of Lenz and Lund (1974), one can also use these equations for thermal property calculations, for kinetic calculations, or for predicting times necessary for blanching or thermal death.

NOMENCLATURE

a	Radius of sphere or cylinder (ft)
C_p	Heat capacity (BTU/lb-°F)
d	Thermal conductivity (BTU/hr-ft²-°F)
h	Surface heat transfer coefficient (BTU/hr-ft²-°F)
H	h/d (ft⁻¹)
J_0	Bessel function of the first kind of order 0
J_1	Bessel function of the first kind of order 1
k, n	Indexes of summation
L	Half-thickness of a slab (ft)
r	Radial variable for sphere & cylinder (ft)
t	Adiabatic holding time (sec)
T	Temperature (°F)
T_0	Initial temperature (°F)
T_∞	Heating medium temperature (°F)
X	Dimension variable for slab (ft)
α	Eigenvalue (ft⁻¹)
κ	$d/\rho\ C_p$
ρ	Density (lb/ft³)
θ	Unaccomplished temperature change = $(T_\infty - T)/(T_\infty - T_0)$
τ	Heating time (sec)

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Ms received 9/18/73; accepted 3/28/74.

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A Research Note
USE OF CARBON DIOXIDE PELLETS FOR SHIPMENT
AND STORAGE OF LAMB CARCASSES

INTRODUCTION

IDENTIFICATION of a more effective system for maintaining acceptable quality during transportation, storage and subsequent display in the retail case would greatly enhance the marketing and distribution of lamb carcasses. Reductions in the quality of wholesale and retail cuts are most often associated with microbial spoilage, and such spoilage is largely the result of the growth and metabolism of aerobic psychrotrophs (Ayres, 1960). Incorporation of inert gas into meat packages has been demonstrated to reduce microbial growth. King and Nagle (1967) reported that *Pseudomonads* were inhibited by an atmosphere of CO_2, but found that the incidence of off-colored lean detracted from shelf life. Varnadore (1972) reported that the use of CO_2 gas in lamb packaging produced very inconsistent results, thus little confidence could be placed in its use for prolonged storage. Adams and Huffman (1972) found that use of a gas mixture (CO_2, O_2 and N_2) inhibited the growth of aerobic bacteria on pork cuts during periods of 9 days of storage.

In the meat packing industry, solidified CO_2 (in solid, pelleted, flaked or dispersed forms) has been used for a number of years as a chilling medium. More recently, CO_2 snow and/or pellets have been used to facilitate chilling and/or to retard deteriorative changes during shipment of beef wholesale cuts and lamb carcasses. The first report comparing carbon dioxide chilling with vacuum packaging as methods for maintaining acceptable quality during shipment and storage of beef (Smith and Carpenter, 1973) suggested that CO_2 could not be used for periods of storage in excess of 10 days without encountering substantial reductions in product quality. Lamb carcasses and beef wholesale cuts are presently being chilled with CO_2 pellets for shipment from packers to the retail trade, with claims of "reduced shrinkage," "improved appearance," "enhanced bloom," "retarded bacterial growth" and "longer shelf life." The present study was designed to determine the actual storage life of carbon dioxide-chilled lamb which was

shipped and stored for periods of 7 to 19 days.

EXPERIMENTAL

24 LAMB CARCASSES were weighed (to the nearest 0.1 lb), placed in polyethylene bags with 1.4 kg of CO_2 pellets, boxed in cardboard containers and shipped 1287 km by common carrier. The boxes were unloaded at a distribution center, stored for 3 days at 1°C, loaded, shipped 322 km in a refrigerated container, unloaded and stored in a 2°C cooler. Six lambs were evaluated at each of four postmortem intervals (7, 11, 15 and 19 days following packaging). At the appropriate times, the cardboard boxes were opened and a three-member trained panel evaluated the external carcass surface and the surface of the inside flank muscles for freshness (by use of a 6-point scoring system), surface appearance (by use of a 6-point scoring system) and odor (by use of a 4-point scale). Microbial samples were obtained from the surfaces of the leg, rack and shoulder by swabbing 1 sq in. areas with sterile cotton swabs. Each carcass was removed from the cardboard box, weighed (to the nearest 0.1 lb) and cut to provide one leg roast (American-style) and 4 loin

chops (3.2 cm in thickness). Two of the loin chops from each carcass were cooked in a 177°C oven to an internal temperature of 75°C and presented to an eight-member trained taste panel. Each member of the sensory panel scored each chop for flavor, juiciness, tenderness and overall satisfaction by use of an 8-point hedonic scale. The leg roast and two of the loin chops were sampled for bacterial counts, placed on plastic backing boards, overwrapped with polyvinyl chloride film (Goodyear Choice-Wrap) and placed in a 2°C retail display case illuminated with 82 ft-c of incandescent light. Each retail cut was scored daily by a three-member trained panel for surface discoloration (by use of a 7-point scale) and consumer acceptance (by use of an 8-point scale). The panel evaluated each retail cut for odor (by use of a 4-point scale) on the final day of retail display. Microbial samples were obtained from a 1 sq in. area of the subcutaneous fat surface of the leg roast and from a 1 sq in. area of the longissimus muscle surface of the loin chops. All microbial samples were enumerated by preparing appropriate dilutions in sterile phosphate buffer blanks. The total psychrotrophic counts were determined from pour plates of standard plate count agar incubated at 3°C for 7 days.

Table 1—Comparison of weight losses, microbial counts and scores for freshness, surface appearance and odor for lamb carcasses at each storage interval

Trait	Storage interval (days)			
	7	11	15	19
Weight loss[a], %	0.3[f]	0.7[f]	0.9[f]	0.9[f]
Freshness[b], external surface	4.8[fg]	5.0[f]	4.3[g]	2.8[h]
Freshness[b], inside flank	6.0[f]	6.0[f]	4.8[g]	4.2[g]
Surface appearance[c], external surface	5.8[f]	5.8[f]	1.2[g]	1.0[g]
Surface appearance[c], inside flank	5.2[f]	5.2[f]	5.0[f]	3.5[g]
Odor[d], external surface	4.0[f]	3.8[f]	1.3[g]	1.2[g]
Odor[d], inside flank	4.0[f]	4.0[f]	3.8[f]	2.8[g]
Microbial count[e], leg surface	3.2	5.3	>7.5	>7.5
Microbial count[e], rack surface	3.4	5.4	>7.5	>7.5
Microbial count[e], shoulder surface	3.2	5.1	>7.5	>7.5

[a] Percent shrinkage determined as loss in carcass weight during transit and storage.
[b] Means based on a 6-point scoring system (6 = very fresh, 5 = fresh, 4 = normal, 3 = slight discoloration, 2 = moderate discoloration, 1 = extreme discoloration).
[c] Means based on a 6-point scoring system (6 = dry—not slimy, 5 = wet—not slimy, 4 = slightly slimy, 3 = moderately slimy, 2 = slightly beaded surface, 1 = beaded surface).
[d] Means based on a 4-point scale (4 = no off-odor, 3 = slight off-odor, 2 = moderate off-odor, 1 = extreme off-odor).
[e] Psychrotrophic plate counts from a 1 sq in. area expressed as $\log_{10}$ values.
[fgh] Means in the same line bearing different superscripts differ significantly (P < 0.05).

Table 2—Comparison of microbial counts, palatability ratings and scores for surface discoloration, consumer acceptance and odor for retail cuts from lamb carcasses at each storage interval

Trait	Storage interval (days)			
	7	11	15	19
Surface discoloration[a], loin chops—day 2	4.7[f]	4.5[fg]	3.4[gh]	3.2[h]
Surface discoloration[a], leg roasts—day 2	5.3[f]	4.6[fg]	3.6[g]	3.3[g]
Surface discoloration[a], leg roasts—day 4	4.6[f]	4.7[f]	3.1[g]	2.8[g]
Consumer acceptance[b], loin chops—day 2	5.0[f]	4.3[fg]	3.6[g]	3.3[g]
Consumer acceptance[b], leg roasts—day 2	5.4[f]	4.6[fg]	4.3[fg]	4.0[g]
Consumer acceptance[b], leg roasts—day 4	4.2[fg]	4.5[f]	3.7[g]	2.8[h]
Odor[c], loin chops—day 4	3.4[f]	2.5[g]	1.0[h]	1.0[h]
Odor[c], leg roasts—day 4	3.0[f]	3.0[f]	1.4[g]	1.3[g]
Microbial count[d], loin chops—day 2	4.1	5.5	>7.5	>7.5
Microbial count[d], leg roasts—day 4	5.2	>7.5	>7.5	>7.5
Flavor rating[e], loin chops	6.2[f]	5.8[fg]	5.5[g]	5.5[g]
Juiciness rating[e], loin chops	6.2[f]	6.2[f]	6.5[f]	6.0[f]
Tenderness rating[e], loin chops	6.5[fg]	6.3[g]	6.9[f]	7.2[f]
Overall satisfaction rating[e], loin chops	6.1[f]	5.9[f]	6.0[f]	5.9[f]

[a] Means based on a 7-point scale (7 = no surface discoloration; 1 = 100% surface discoloration).
[b] Means based on an 8-point scale (8 = extremely desirable; 1 = extremely undesirable).
[c] Means based on a 4-point scale (4 = no off-odor, 1 = extreme off-odor).
[d] Psychrotrophic plate counts from a 1 sq in. area expressed as log_{10} values.
[e] Means based on an 8-point scale (8 = like extremely, 1 = dislike extremely).

RESULTS & DISCUSSION

MEAN VALUES for weight loss, microbial counts and scores for freshness, surface appearance and odor for lamb carcasses at each storage interval are presented in Table 1. Although carcasses stored for 15—19 days shrank 0.6% more than those stored for 7 days, the values were not consistent enough for statistical significance. Differences in freshness, surface appearance and odor were evident (P < 0.05) on the external surfaces of the intact carcasses between storage intervals of 11 and 15 days. Significant differences (P < 0.05) in surface appearance and odor of the inside flank muscles were noted between the 15 and 19 day storage intervals, while freshness differed at this location after 11 days of storage. Microbial counts had attained or exceeded the incipient spoilage level suggested by Reagan et al. (1971) after storage intervals of 15 days. Previous research involving the use of polyvinyl chloride film for wrapping lamb cuts (Varnadore, 1972) and beef quarters or cuts (Rea et al.,

1972) has suggested that PVC film should not be allowed to remain on meat cuts for periods longer than 7 days following application because of its effect in enhancing conditions for bacterial growth. The data of the present study suggest that carbon dioxide chilling can be used to facilitate shipment and storage for periods of 11 days without markedly affecting the freshness, appearance or odor of intact lamb carcasses.

Microbial counts, palatability ratings and scores for surface discoloration, consumer acceptance and odor for retail cuts from lamb carcasses at each storage interval are presented in Table 2. Retail cuts from lamb carcasses stored for 15 days were significantly (P < 0.05) less desirable than those from carcasses stored for 7 days in all three determinations of surface discoloration, one of three evaluations of consumer acceptance, both comparisons of off-odor incidence and one of four palatability ratings. Odor scores for loin chops and microbial counts from leg roasts evidenced substantial increases in off-odor and bacterial

growth as the storage interval increased from 7 to 11 days. Retail cuts from lambs stored for 11 days had numerically lower ratings for surface discoloration, consumer acceptance and flavor in five of seven comparisons, but the differences were not consistent enough for statistical significance. The data suggest that the added stress of 2—4 days of retail display in combination with 11 days of storage in carcass form, detract from the ultimate salability in acceptability of consumer cuts.

Data in the present study reveal that lamb carcasses can be adequately protected during distribution (shipping and storage) periods involving intervals of approximately 11 days by use of a carbon dioxide chilling system. Use of the CO_2 chilling system and the protection provided by polyethylene bags and cardboard containers resulted in carcasses which maintained acceptable freshness, appearance and odor after 11 days of storage and retail cuts which were acceptable in appearance, odor and palatability. Storage for intervals of 15 or 19 days following packaging resulted in markedly reduced freshness, appearance and odor of intact carcasses, microbial counts indicative of incipient spoilage and these carcasses produced retail cuts which were unacceptable in appearance, odor, microbial counts and flavor.

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A Research Note
THE ROLE OF LEAN AND ADIPOSE TISSUE ON THE FORMATION OF NITROSOPYRROLIDINE IN FRIED BACON

INTRODUCTION

FRIED BACON has been reported to contain nitrosopyrrolidine (NO-Pyr) in concentrations as high as 108 ppb (Crosby et al., 1972; Fazio et al., 1973; Pensabene et al., 1974; Sen et al., 1973). This is of concern from a public health standpoint since NO-Pyr has been shown to be carcinogenic when administered to rats (Druckrey et al., 1967; Greenblatt and Lijinsky, 1972a; Greenblatt et al., 1973) and mice (Greenblatt and Lijinsky, 1972b). Some investigators have reported a larger concentration of NO-Pyr in the cooked-out fat than in the fried bacon (Fazio et al., 1973; Pensabene et al., 1974; Sen et al., 1973). This may be accounted for by the fact that NO-Pyr is very fat-soluble and partitions into the fat phase, but the possibility should be considered that larger concentrations of nitrosamine are formed in the adipose tissue. The role of lean and adipose tissues of bacon as precursors for NO-Pyr was investigated and the results reported herein.

EXPERIMENTAL

BACON was purchased from three different manufacturers a few days after processing. A portion of the bacon was visually separated into adipose and lean components. Three brands of Canadian bacon (back bacon); ham, including a cured pork shoulder; and breakfast beef (bacon-like products) were purchased at local retail markets. The samples were fried in a preheated Presto teflon-coated electric frying pan (Model PC4AT) for 6 min at a calibrated thermostat setting of 177°C. The lean portions of bacon, ham and Canadian bacon samples were fried in Crisco, a hydrogenated vegetable oil product, under the same conditions.

The samples were assayed for dimethylnitrosamine, methylethylnitrosamine, diethylnitrosamine, nitrosopiperidine, nitrosopyrrolidine and nitrosomorpholine. The fried bacon samples were analyzed using the multidetection method as described by Fazio et al. (1971) and the drippings as reported by White et al. (1974) except for a modification of the column chromatographic clean-up step in which a 4-g layer of Silica Gel was used on top of 1g of Florisil acidified with 1 ml of 6N HCl. The sample was washed with 200 ml of CH_2Cl_2/pentane (1/1) and eluted with 125 ml of ether/CH_2Cl_2 (1/5). The nitrosamines were determined by GLC using an alkali flame ionization detector (Howard et al., 1970). Concentrations as low as 0.5 ppb nitrosamine could be detected. Only NO-Pyr was found in confirmable concentrations. The average recovery of 20 ppb added NO-Pyr was 75% in the fried samples and 50% in the drippings. When a peak was observed at the same retention time as an authentic sample of NO-Pyr, its identity was confirmed by mass spectrometry in the peak-matching mode using the parent peak of m/e 100.06366 at a resolution of 1 in 12,000. The GLC and GLC-MS systems and conditions have been described previously (Pensabene et al., 1974).

RESULTS & DISCUSSION

THE RESULTS of analyses for NO-Pyr in fried whole bacon and in the lean and adipose tissue portions fried separately are shown in Table 1. Results from samples of three different producers were similar. In the case of whole bacon, NO-Pyr was found in the fried product and its drippings. No NO-Pyr was found in uncooked adipose tissue which had been separated from the lean portion of raw bacon. However, after frying, more NO-Pyr was found in the cooked out fat than in the solid residue remaining after frying. The lean portion did not contain NO-Pyr when uncooked, pan-fried alone, or when fried in Crisco under the same conditions as the bacon adipose tissue. No NO-Pyr was detected in heated Crisco alone or in Crisco remaining after the lean was fried in it. It appears that NO-Pyr is derived from the adipose tissue and not the lean portion of bacon. Some components in the adipose tissue therefore must serve as precursor(s) for NO-Pyr. While the exact mechanism for NO-Pyr formation in bacon is not known, several pathways have been proposed, as shown in Figure 1.

Bills et al. (1973) have reported NO-Pyr can be formed in the highest yield from nitrosoproline and in lesser quantities from nitrite reacted with pyrrolidine, spermidine, proline and putrescine at 170°C. In addition, NO-Pyr has been formed by decarboxylating nitrosoproline at different temperatures with the maximum formation occurring at 185°C, close to the recommended temperature for frying bacon, 177°C (Fiddler et al., 1973; Pensabene et al., 1974). Therefore, proline indirectly appears to be the most probable of the above-mentioned precursors. The amount of free proline in bacon and ham is approximately the same (Lakritz, 1973). The fact that NO-Pyr is found only in cooked bacon and not in other cured pork products suggests that NO-Pyr is not formed via a simple mechanism. Fazio et al. (1973) found no NO-Pyr in fried ham or Canadian bacon and theorized that the nitrosamine, being fat soluble, is protected from volatilization during frying and is retained on the fried bacon strips. We examined ham, Canadian bacon (back bacon), and beef bacon-like products. Samples of ham and Canadian bacon were fried in Crisco. Nitrosopyrrolidine was not found in either the fried product, its cooked-out fat, or the Crisco in which it was fried. However, it is possible that the composition of pork belly is different than either the ham, shoulder or loin portions, particularly with respect to the amount of collagen or connective tissue present. Collagen is known to contain large concentrations of bound proline and hydroxyproline and has recently been claimed to produce NO-Pyr at elevated temperatures in a model system (Huxel et al., 1973). Nitrosopyrrolidine could form from the action of nitrite on collagen itself or its pyrolytic decomposi-

Table 1—Effect of lean and adipose tissue on nitrosopyrrolidine formation in fried bacon

Product	Nitrosopyrrolidine,[a] ppb Samples		
	A	B	C
Whole bacon	2	28	13
Drippings	6	24	22
Fat residue	5	n.d.	11
Drippings	14	58	24
Lean (in Crisco)	n.d.[b]	n.d.	n.d.
Drippings (Crisco)	n.d.	n.d.	n.d.
Fat—unfried	n.d.	n.d.	n.d.
Lean—unfried	n.d.	n.d.	n.d.

[a] Confirmed by high resolution mass spectrometry
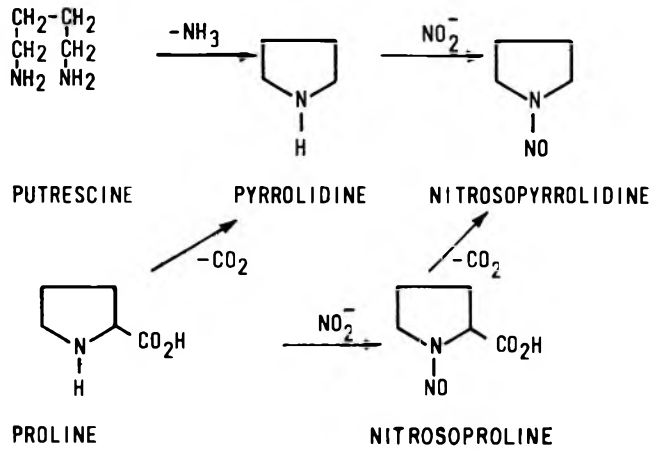[b] n.d. = none detected

Fig. 1—Possible pathways for the formation of nitrosopyrrolidine in fried bacon.

tion products. Collagen might also hydrolyze to provide a higher concentration of free proline than is normally available for nitrosation in nonbelly cuts of pork. While the latter explanation is the most reasonable, the exact role of collagen and other components present in bacon adipose tissue regarding nitrosamine formation needs to be investigated.

(Precautions should be exercised in the handling of nitrosamines, since they are potential carcinogens.)

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A Research Note
AN IDEA FOR PRECISELY CONTROLLING THE WATER ACTIVITY IN TESTING CHAMBERS

INTRODUCTION

PRECISE CONTROL of environmental temperature and humidity is essential in studies of the viability of microbes under various conditions of water activity. The range of test conditions required by one such study, 5–40°C, 50–95% RH, is well beyond the capability of commercially available environmental test chambers. The precision of humidity control in such chambers is also inadequate for the purpose primarily because of limitations imposed by state-of-the-art humidity sensing devices.

A unique system has been proposed to control the environment in chambers designed to expose microbial cells in food materials or on inert surfaces such as stainless steel strips or glass slides to constant and uniform conditions of water activity. Significantly, operation of the system does not depend on measurements of the amount of moisture in the conditioned air. In this respect, the approach is similar to the one used by the National Bureau of Standards in its Two-Pressure Humidity Generator (Wexler, 1968).

EXPERIMENTAL

THE PROPOSAL is shown diagrammatically in Figure 1. Basically the idea involves the addition of exactly the right amount of water vapor to air previously standardized at a known level of humidity. This is done on a continuous basis by saturating air at 0°C, 5 psig, allowing the air to expand to atmospheric pressure to establish a base line dew point of −4.51°C, heating the moisture stabilized air stream to the desired dry bulb temperature, and then adding enough water vapor to bring the final humidity to the desired level.

The added moisture is metered into the system not as a vapor but as a liquid, using an infusion syringe pump of the type commonly used to continuously and precisely meter the flow of extremely small amounts of liquid into chromatographic analyzers. The water is vaporized prior to its addition to the air stream.

In Figure 1 the hyperbaric saturator is preceded by a precooler simply as a matter of convenience. Ice cubes were used to cool the saturator in the experiments that have been performed and the precooler extended the operating period between replenishments of the ice in the saturator.

The saturator was operated at 0°C and 5 psig for several reasons. The ice point is a stable, easily attained and easily maintained reference temperature. Saturation at 5 psig is necessary to deliver air at 5°C, 50% RH.

The saturator is designed to remove moisture from the incoming air to reach saturation at the ice point. The saturator can also add moisture to the incoming air, but with reduced effectiveness. Operating the saturator at 5 psig insures a condition of moisture removal so long as the entering air is at or above 18% RH when measured at normal room temperature (20°C).

The experimental equipment included a Gast Model 0211-P103-G8 oilless air compressor, a Fischer and Porter Model 3F-3/8-25-5 Flowrator, a Fenwal Model 56105-2 air temperature controller, a Fenwal Model 19-404005-200 vaporizer temperature controller, a Cheminert Model CMP-1 infusion pump, and a small blower-mixer of the type normally used to cool electronic equipment.

Initially a Beckman Model ESMPF hygrometer was used to record the relative humidity of the conditioned air. Later a Cambridge Systems Model 992 hygrometer was used to monitor the dew point. Air temperature was indicated by a M-H Model 15 Electronik thermocouple instrument.

RESULTS & DISCUSSION

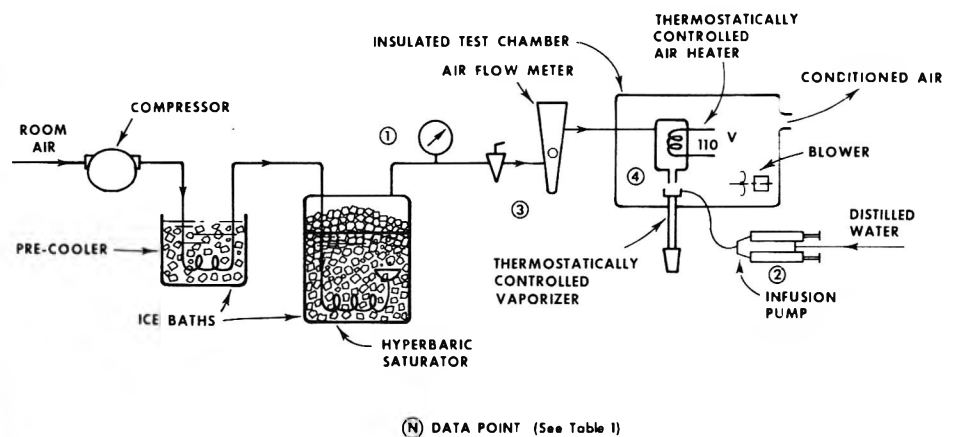TEST RESULTS are shown in Table 1. Calculated dew point is based on the

Fig. 1—Controlled humidity generator.

Table 1—Experimental data[a]

(1) Psig	(2) Ml/Hr	(3) cc/Min	(4)		% RH (Hygrometric)
			°C	%RH	
5.0	12	18,600	48.5	17	16 1/2
5.0	24	18,600	48.6	28	28
0	24	23,000	48.6	25 3/4	26
					Calc
				DP–°C	DP–°C
5.0	12	18,200	48.1	16.6–16.7	16.6

[a] See Figure 1 for location of data points.

The chart is also used for an alternate mode of operation possible when the dew point is between −4.51°C and 0°C. Dew points in this range can be generated simply by adjusting the pressure in the hyperbaric saturator without of course, any addition of moisture to the air stream.

For microbial viability studies, the conditioned air can be brought into contact with the test samples in several ways. The simplest approach involves an insulated sample chamber through which the conditioned air is circulated. A more sophisticated system involves the use of air and water-tight capsules in which the test samples are stored. Conditioned air is circulated through the capsules until the samples are at equilibrium with the circulating air stream, at which time the capsules are isolated and disconnected from the source of conditioned air. The encapsulated environment is kept at the proper level of water activity by immersing the capsules in water baths maintained at the proper temperature. The capsule approach frees the humidity generator for multi-duty use and eliminates the necessity for continuous operation for any given test condition.

Work is continuing on the development of the humidity control system described in this research note. Improved implementation of the concept will include the use of a refrigerated coil in place of ice cubes in the hyperbaric saturator, the use of a continuously variable discharge infusion pump in place of the step-wise adjustable pump used in the earlier work, and more precise determinations of the water activity of the conditioned air over the full range of operating conditions.

REFERENCE

Wexler, A. 1968. Calibration of humidity-measuring instruments of the National Bureau of Standards. ISA Trans. 7(4): 356.

Fig. 2—Relative humidity (water activity) vs. water injection rate.

measured air flow rate, the water injection rate and the amount of moisture in air saturated at 0°C, 5 psig. The hygrometrically determined RH takes into account the measured dry bulb temperature and the calculated dew point.

Recordings of the water activity in the insulated test chamber indicate the system has the ability to rapidly adjust to new test conditions and to maintain the new condition with good stability.

Figure 2 shows a chart prepared to assist in the operation of a system handling 18,000 cc of air a minute. The ordinate scale shows the water injection rate for any conditon of water activity or A_w, where $A_w = \%RH/100$.

The smaller chart in Figure 2 shows an enlargement of the water injection data for temperatures between 5° and 10°C.

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1. TITLE OF PUBLICATION

Journal of Food Science

2. DATE OF FILING

January 3, 1974

3. FREQUENCY OF ISSUE

7 Times a year-bimonthly except monthly January & February

4. LOCATION OF KNOWN OFFICE OF PUBLICATION *(Street, city, county, state, ZIP code) (Not printers)*

Institute of Food Technologists, 221 N. LaSalle St., Chicago, Ill. 60601 (Cook County)

5. LOCATION OF THE HEADQUARTERS OR GENERAL BUSINESS OFFICES OF THE PUBLISHERS *(Not printers)*

Same as above

6. NAMES AND ADDRESSES OF PUBLISHER, EDITOR, AND MANAGING EDITOR

PUBLISHER *(Name and address)*

C. L. Willey, 221 N. LaSalle St., Chicago, Ill. 60601 (Cook County)

EDITOR *(Name and address)*

J. B. Klis " " " "

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